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PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

ARSENIC

April 2004

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Public Health Goal for

ARSENIC

in Drinking Water

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PREFACE

Drinking Water Public Health Goals
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that can cause chronic disease shall be based upon currently available data and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.

8. The PHG may be set at zero if necessary to satisfy the requirements listed above.

9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.

10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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SUMMARY

A causal association between human arsenic exposure, usually in the form of inorganic compounds containing trivalent arsenite (As III) or pentavalent arsenate (As V), and various forms of human cancer has been established for many years. The International Agency for Research on Cancer (IARC) evaluated arsenic in 1980 and classified "arsenic and arsenic compounds" in Group 1, which includes "chemicals and groups of chemicals, which are causally associated with cancer in humans." Arsenic is also known to be atherogenic, genotoxic, teratogenic, and may cause other adverse developmental effects in exposed children.

Quantitative risk assessments of arsenic from inhalation exposures include the U.S. Environmental Protection Agency (EPA) Health Assessment Document for Inorganic Arsenic (U.S. EPA, 1984), and the California Department of Health Services' Health Effects of Inorganic Arsenic Compounds (CDHS, 1990). OEHHA's Arsenic Recommended Public Health Level for Drinking Water Draft (OEHHA, 1992a) provided a quantitative risk assessment for arsenic in drinking water. The Special Report on Ingested Inorganic Arsenic: Skin Cancer; Nutritional Essentiality (U.S. EPA, 1988) also provided a quantitative risk assessment applicable to drinking water exposure. Recently the National Research Council's Subcommittee on Arsenic in Drinking Water also conducted evaluations and quantitative risk assessments deriving theoretical lifetime excess risk estimates up to 23 in 10,000 for bladder cancer in males and up to 18 in 10,000 for lung cancer in males at 10 ppb arsenic (NRC, 1999, 2001). The U.S. EPA's final rule on arsenic in drinking water (U.S. EPA, 2001) developed an MCLG of zero. The MCLG is the functional equivalent of the California public health goal (PHG) for drinking water. The U.S. EPA also established a national primary drinking water regulation or MCL for arsenic of 10 ppb. U.S. EPA's upper bound (90^{th} percentile) estimates of lifetime cancer risk at 10 ppb ranged up to 6.1 in 10,000. This federal regulation does not become fully effective until 2006. In California the MCL for arsenic will be determined by the Department of Health Services to be as close to the PHG as possible considering other factors such as cost and analytical feasibility. All of these assessments recognize the relatively high cancer risks associated with chronic exposure to inorganic arsenic. The current assessment refines and extends our earlier arsenic risk assessment (OEHHA, 1992a).

OEHHA has developed a public health goal (PHG) of 0.004 µg/L (4 ppt) for arsenic in drinking water based on the mortality of arsenic-induced lung and urinary bladder cancers observed in epidemiological studies of populations in Taiwan, Chile, and Argentina. For lung and bladder tumors combined, and both sexes combined, the estimated unit risk was 2.7×10^{-4} $(\mu g/L)^{-1}$. Similar unit risks were derived from a mouse bioassay using prenatal exposure to arsenic. The risk estimates were based on a low-dose linear extrapolation approach although the mode of carcinogenic action is not fully

understood. The actual risks of low-level exposure are unlikely to exceed these risk estimates but could be lower or zero. It should be noted that at low water concentrations of arsenic food-borne arsenic would become the dominant source (Yost *et al*., 1998).

A noncancer health protective value of 0.9 µg/L (0.9 ppb) was developed based on vascular effects in a human epidemiological study of cerebrovascular disease (Chiou *et al*., 1997b). A benchmark dose approach was used to derive this value, with an uncertainty factor of 10 for inter-individual variation and three for extrapolation from the lower bound on the effect levels (LEDs) to negligible effect levels.[1]

INTRODUCTION

Arsenic is a naturally occurring element in the earth's crust and is very widely distributed in the environment. All humans are exposed to microgram quantities of arsenic (inorganic and organic) largely from food (25 to 50 µg/day) and to a lesser degree from drinking water and air. Some edible seafood may contain higher concentrations of arsenic which is predominantly in less acutely toxic organic forms.

In certain geographical areas, natural mineral deposits may contain large quantities of arsenic and this may result in higher levels of arsenic in water. Waste chemical disposal sites may also be a source of arsenic contamination of water supplies. The main commercial use of arsenic in the U.S. is in pesticides, mostly herbicides and in wood preservatives. Misapplication or accidental spills of these materials could result in contamination of nearby water supplies. Burning of fossil fuels also produces low levels of arsenic emissions. Arsenic may also be found in low levels in tobacco smoke.

Most ingested arsenic is quickly absorbed through the gastrointestinal tract into the blood stream. Most of the organic arsenic (e.g., arsenobetaine) is excreted unchanged or metabolized (e.g., arsenocholine → arsenobetaine). The inorganic arsenic which is absorbed is converted by the liver to methylated forms which may be more toxic (i.e., trivalent MMA^{III} and DMA^{III}) and more efficiently excreted in the urine (e.g, DMA). Arsenic does not have a tendency to accumulate in the body at low environmental exposure levels.

In humans, while ingestion of larger doses of arsenic may be lethal, lower levels of exposure may cause a variety of systemic effects including irritation of the digestive tract,

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[1] This document is based in part on an assessment conducted by Smith and Lopipero (2001) under an interagency agreement between OEHHA and the University of California. It represents an updating of our earlier draft “Arsenic Recommended Public Health Level” (OEHHA, 1992a), which in turn was based on information in the report "Health Risk Assessment for Arsenic Ingestion" prepared by A.H. Smith *et al.* (1990) under contract to the California Department of Health Services. Additional details can be found in these earlier documents. Common abbreviations used in this document: As, arsenic, form unspecified; As^{V}, arsenate; As^{III}, arsenite; MMA^{V}, monomethylarsinic acid; MMA^{III}, monomethylarsonic acid; DMA^{V}, dimethylarsinic acid; DMA^{III}, dimethylarsonic acid; Asi, inorganic arsenic oxidation state unspecified; MMA, monomethylarsenic oxidation state unspecified; DMA, dimethylarsenic oxidation state unspecified.

nausea, vomiting, and diarrhea. Other effects of ingested arsenic include decreased production of erythrocytes and leukocytes, abnormal cardiac function, blood vessel damage, liver and/or kidney damage, and impaired nerve function in hands and feet (paresthesia). Characteristic skin abnormalities are also seen appearing as dark or light spots on the skin and small "corns" on the palms, soles, and trunk. Some of the corns may ultimately progress to skin cancer. In addition, arsenic ingestion has been reported to increase the risk of cancer at internal sites, especially lung, urinary bladder, kidney, and liver (Gosselin *et al*., 1984; ATSDR, 2000).

The amounts of arsenic required to cause adverse health effects depend on the chemical and physical form of the arsenic that is ingested. Inorganic forms are generally more acutely toxic than organic forms and more water-soluble forms tend to be more toxic than those that dissolve poorly in water. Also, the oxidation state of arsenic affects its toxicity, with As III being more toxic than As V. Recent evidence indicates that trivalent methylated metabolites of inorganic arsenic can be more toxic than arsenite in both in vitro and in vivo tests (Styblo *et al*., 2002).

Studies in humans have shown considerable individual variability in arsenic toxicity (Chen CJ *et al*., 2001). A number of recent studies have associated chronic intake of arsenic in drinking water with a number of serious health effects including heart attack, stroke, diabetes mellitus, and hypertension. In some of these and other studies, effects were noted at arsenic concentrations less than 100 µg/L. The duration of arsenic exposure appears to be a key factor in determining the extent of toxic effects. The levels of arsenic that most people ingest in food and water (ca. 50 µg/day) have not usually been considered to be of health concern for non-cancer effects. However, in view of the recent lowering of the federal MCL for arsenic to 10 µg/L, and continuing reports of serious chronic health effects associated with arsenic ingestion via drinking water in a number of countries, the risks of low-level non-cancer effects need to be reassessed.

Arsenic contamination of ground water in Bangladesh is associated with the largest poisoning of a population in history with 35 to 77 million people at risk of arsenic induced adverse health effects (Smith *et al*., 2000). Of over 2000 water wells analyzed in 1998, 35 percent had arsenic concentrations >50 µg/L and 8.4 percent had arsenic concentrations >300 µg/L. In 1997 200 villages were surveyed and 1802/469,424 people were found to have arsenic-induced skin lesions. A more detailed analysis of four villages gave 430/1,481 with skin lesions. Due to the latency for effects from chronic arsenic exposure, a number of more serious health effects are expected to occur in the exposed population in the future, including skin and internal cancers, neurological effects, hypertension and cardiovascular disease, peripheral vascular disease, and diabetes (Smith *et al*., 2000). Arsenic exposures and populations at risk of adverse effects in over a dozen countries have been summarized in a recent review by Ng *et al.* (2003).

The U.S. EPA’s final rule on arsenic in drinking water (U.S. EPA, 2001) established an MCL of 10 ppb and an MCLG of zero. This rule does not become fully effective in all water systems covered until 2006. U.S. EPA’s upper bound (90^{th} percentile) estimates of lifetime cancer risk at 10 ppb ranged up to 6.1×10^{-4}. The OEHHA assessment employs an alternative analysis of risks of internal cancers, particularly lung and urinary bladder.

CHEMICAL PROFILE

Chemical Identity

Arsenic is a naturally occurring metalloid element (atomic number 33). Table 1 lists the common names, CAS numbers, molecular formulas, and synonyms for arsenic and a number of arsenic salts, oxides, and organic derivatives. These compounds were selected because of their toxicity and/or environmental occurrence (ATSDR, 1997).

Table 1. Chemical Identity of Arsenic and Selected Arsenic Compounds

				RTECS
Arsenic	Arsenic-75, metallic arsenic, arsenic black, colloidal arsenic	As	7440-38-2	CG0525000
Arsenic acid	Orthoarsenic acid	H_3AsO_4	7778-39-4	CG0700000
Arsenic trioxide	Arsenic oxide, arsenous acid anhydride, white arsenic, arsenolite	As_2O_3	1327-53-3	CG3325000
Arsenic pentoxide	Arsenic (V) oxide, arsenic acid anhydride, diarsenic pentoxide	As_2O_5	1303-28-2	CG2275000
Sodium arsenate	Disodium arsenate, sodium biarsenate	Na_2HAsO_4	7778-43-0	CF0875000
Sodium arsenite	Sodium metarsenite	$NaAsO_2$	7784-46-5	CG3675000
Arsine	Arsenic hydride, arsenic trihydride	AsH_3	7784-42-1	CG6475000
Arsenobetaine	Fish arsenic	$(CH_3)_3As^+CH_2CO_2^-$	64436-13-1	NA
Dimethylarsine acid	Cacodylic acid. dimethylarsinic acid, DMA, DMA^{V}	$(CH_3)_2HAsO_2$	75-60-5	CH7525000
Methanearsonic acid	Methylarsenic acid, monomethylarsonic acid, MMA, MMA^{III}	$CH_3H_2AsO_3$	124-58-3	PA1575000
Sodium dimethyl arsinate	Sodium cacodylate	$(CH_3)_2NaAsO_2$	124-65-2	CH7700000
Sodium methane arsonate	MSMA	$CH_3NaHAsO_3$	2163-80-6	PA2625000
Trimethylarsine	Arsenic trimethyl, Gosio gas	$(CH_3)_3As$	593-88-4	NA

Physical and Chemical Properties

The inorganic arsenic compounds are solids at normal temperatures and are unlikely to volatilize. The water solubility of these compounds varies from quite soluble (sodium arsenite and arsenic acid) to practically insoluble (arsenic trisulfide). Some organic arsenic compounds are gases or low-boiling liquids at ambient temperatures. Except for the organic arsenic acids, they are not readily water-soluble (ATSDR, 1997). Selected physical and chemical properties are summarized in Table 2.

Table 2. Physical and Chemical Properties of Arsenic and Selected Arsenic Compounds

				Water Solubility (g/100 mL)
Arsenic	74.92	0	Solid	Insoluble
Arsenic acid	141.95	+5	Solid	302
Arsenic trioxide	197.82	+3	Solid	2.1
Arsenic pentoxide	229.84	+5	Amorphous solid	Freely soluble
Sodium arsenate	185.91	+5	Solid	Very soluble
Sodium arsenite	130.92	+3	Solid	Freely soluble
Arsine	77.93	+3	Gas	20 mL/100g
Dimethylarsinic acid	138.01	+5	Solid	Soluble
Methanearsonic acid	139.98	+5	Solid	Freely soluble
Sodium dimethyl arsinate	159.98	+5	Solid	ND
Sodium methane arsonate	161.96	+5	Solid	57
Trimethylarsine	120.03	+3	Liquid	NA

Production and Uses

In the U.S. in 1987 about $23x10^6$ kg of arsenic was used in commerce. Seventy-four percent was used in wood preservatives, 19 percent in agricultural chemicals (mainly herbicides and desiccants), three percent in glass manufacture, two percent in nonferrous alloys, and two percent in other uses. The use of gallium arsenides in semiconductors is increasing but, at about five tons/year, this use is still small compared to other industrial uses (ATSDR, 1997).

Sources

Chilvers and Peterson (1987) estimated the ratios of natural to anthropogenic global As emissions as 60:40. Natural sources were mainly from low-temperature volatilization from soil (60 percent) and volcanic activity (about 40 percent). Anthropogenic emissions were dominated by metal production, particularly copper smelting (40 percent) (Cullen and Reimer, 1989). Pirrone and Keeler (1996) observed trends in anthropogenic trace element emissions in urban areas of the Great Lakes region. They found that 69 percent of total arsenic emissions were derived from coal combustion, 13 percent from iron-steel manufacturing, and 17 percent from nonferrous metals production (ATSDR, 2000).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Arsenic ranks 20th in abundance (0.0001 percent) among elements in the earth's crust, but is widely distributed and commonly associated with ores of metals like copper, lead, and gold (Cullen and Reimer, 1989; Oremland and Stoltz, 2003). In 1979 the total amount of arsenic released into the environment as a result of anthropogenic activities was estimated to be 53.4×10^6 kg (U.S. EPA, 1982). Eighty-one percent of this quantity was deposited on land. According to the Toxic Chemical Release Inventory (TRI97, 1999), in 1997, the total releases of arsenic into the environment including air, water, soil, and underground injection from 52 large industrial facilities in the U.S. was 60,700 pounds. In addition, another 989,000 pounds of arsenic were transferred offsite (ATSDR, 2000). The primary sources of As for human exposure are usually food and drinking water. Certain individuals may have additional occupational and related exposures (e.g., miners, smelter workers and nearby residents, semiconductor workers).

Air

Mean levels of arsenic in ambient air in the United States range from <1 to 3 ng/m^3 in remote areas and from 20 to 100 ng/m^3 in urban areas. This arsenic is usually a mixture of arsenite and arsenate except in areas where methylated arsenic pesticides are used (ATSDR, 1997). Urban areas often have higher airborne arsenic due to coal-fired power plants but maximum concentrations are usually less than 100 ng/m^3. The highest reported arsenic air levels were near nonferrous metal smelters, with concentrations up to 2,500 ng/m^3. According to the Toxic Chemical Release Inventory (TRI97, 1999), the estimated releases of arsenic to the air totaled 50,500 pounds in 1997. This figure does not include all important sources of airborne arsenic such as coal combustion facilities and metal and coal mining (ATSDR, 2000). Arsenic is also emitted from volcanoes, and may occur naturally in airborne dust (ATSDR, 2000).

Soil

Weathering of rocks and minerals appears to be a major source of arsenic found in soils and drinking water sources (U.S. EPA, 1987). Due to its ubiquitous nature, low concentrations of arsenic are present in almost all foods and drinking water, which are the primary sources of human exposure.

Soil also receives arsenic from a variety of anthropogenic sources including fly ash from power plants, smelting operations mining wastes, and municipal and industrial waste (ATSDR, 2000). Arsenic is released to soil from wood treated with chromated copper arsenate (CCA), and the use of CCA-treated wood in playground equipment has been a concern with respect to child exposures to arsenic (CDHS, 1987; CPSC, 1990).

The Toxic Chemical Release Inventory for 1997 (TRI97, 1999) reported releases of 10,000 pounds of arsenic to soil from 52 facilities accounting for 17 percent of total environmental releases (ATSDR, 2000), not including releases from CCA-treated wood.

A biological methylation/demethylation cycle exists for arsenic. This may result in the presence of various organic forms of arsenic (e.g., methylarsonic acid, MMA, and dimethylarsinic acid, DMA), as well as the inorganic forms arsenite and arsenate, in the environment (Fowler, 1983; Irgolic *et al.*, 1983). Under normal environmental conditions arsenate is the most stable form of arsenic and is therefore the main exposure form of inorganic arsenic (Marafante *et al.*, 1985; Willhite and Ferm, 1984). The average level of As in soil is about 5000 ppb (ATSDR, 1997). Arsenic residues in areas surrounding former copper smelters may be of concern with respect to child exposure. Hwang *et al.* (1997) studied a population of 414 children less than 72 months of age in the vicinity of a former smelter in Anaconda, Montana. Speciated urinary arsenic was observed to be significantly related to soil arsenic in bare areas of residential yards ($P < 0.0005$). The geometric mean urinary arsenic was 8.6 µg/L (GSD = 1.7, N = 289). The average arsenic concentration in different types of soil ranged from 121 to 236 ppm.

Water

Water is the major means of transport of arsenic under natural conditions (Bencko, 1987). Sedimentation of arsenic in association with iron and aluminum represents a major factor in environmental transport and deposition of this element. It has generally been assumed that surface waters, including the sea, are "self-purifying" with respect to arsenic, i.e., that the arsenic is removed from solution by deposition with sediments (Woolson, 1983).

Arsenic is present in all sources of water (Woolson, 1983). Water devoid of living organisms will very likely contain only inorganic arsenic in the form of arsenate and/or arsenite. Studies examining the form of arsenic in water supplies have largely reported only arsenate and arsenite in varying ratios (U.S. EPA, 1984; Irgolic *et al.*, 1983).

The arsenate/arsenite ratio is not only dependent on the source of water but also redox conditions in the supply (Woolson, 1983). Pentavalent arsenic (As^{V}), which is the stable oxidation state in oxygen-containing waters, can be reduced to trivalent arsenic (As^{III}) in anoxic or reducing systems (Turner, 1987). For example, As^{III} has been observed in estuarine waters and seawater. The proportion of As^{III}, however, is low in these waters and even in anoxic interstitial waters, complete reduction of arsenic to As^{III} has not been observed. As^{III} released to oxygenated waters can be reoxidized to As^{V} within a time scale of days.

In most municipal water supplies, particularly surface reservoirs, the chief form of arsenic is As^{V} due to aeration and chlorination. In chlorinated drinking water supplies, all arsenic forms have been found to be pentavalent as a result of oxidation by free chlorine (U.S. EPA, 1988). The major form of arsenic in well waters relatively rich in arsenic also appears to be As^{V} (U.S. EPA, 1984). In freshwater sources often more than 80 percent is As^{V} while the remaining 20 percent or less is composed of As^{III}, MMA and DMA (Braman, 1983). In both groundwater and surface water, the arsenic concentration is normally less than 0.01 mg/L (Pershagen, 1986).

Anthropogenic sources of arsenic in water include mining, nonferrous metals, especially copper, smelting, waste water, sewage sludge, coal fired power plants, urban runoff and atmospheric deposition. Annual arsenic global input estimates ranged from 11,600 to 70,300 metric tons with a median of 41,800 metric tons (Pacyna *et al*., 1995; ATSDR, 2000).

A 1995 survey of arsenic in California drinking water covered 180 water agencies, utilities and cities from 27 counties throughout the state (ACWA, 1995). The median value of arsenic in more than 1500 samples analyzed was 0.002 mg/L (2 ppb). Of the 1378 groundwater wells sampled, detectable As (≥ 1 ppb) was seen in 65 percent of the wells sampled. Eighteen percent of the wells had As concentrations between 1-2 ppb, and 12 percent had 5 ppb or greater. The concentrations ranged from less than 1 ppb (undetectable) to 52 ppb As. Of the water systems sampled, 118 had 10,000 or more connections, whereas only 55 had fewer than 10,000 connections. In this study report, individual water systems were not identified.

A recent survey by the U.S. EPA (U.S. EPA, 2000) compared arsenic occurrence data from a number of data bases including the Safe Drinking Water Information System (SDWIS), the National Arsenic Occurrence Survey (NAOS), the U.S. Geological Society (USGS) ambient ground water arsenic databases, the National Inorganics and Radionuclides Survey (NIRS), and the Metropolitan Water District of Southern California Radionuclides Survey (MWDSC). The SDWIS is based on compliance monitoring data for ground and surface water community systems (CWS) and non-transient, non-community water supply systems (NTNCWS). According to the analysis of the SDWIS data, 11,873 ground water CWS systems were estimated to have mean arsenic concentrations that exceeded 2 μg/L, 5,252 systems that exceed 5 μg/L, and 2,303 systems that exceed 10 μg/L. Arsenic concentrations were projected to be much lower in surface water systems, e.g., 1,052, 325, and 86 systems exceeding the 2, 5, and 10 μg/L levels, respectively. For the MWDSC database the percent of ground water systems exceeding 2, 5, 10, and 20 μg As/L were 19.2, 13.5, 5.8, and 1.9 percent, respectively. For surface water systems, only eight percent exceeded 2 μg As/L. A comparison of three other surveys of California arsenic occurrence (Tables 6-9ab in the U.S. EPA report) indicates somewhat higher levels in ground water (3 to 9.5 percent exceedance at 20 μg/L; 0.6 to 1.9 percent at 50 μg/L) and surface water (<1 to 3.5 percent at 20 μg/L; 0.5 to 2.0 percent at 50 μg/L).

Food

Food arsenic values taken from U.S. FDA surveys indicate an average daily dietary intake of approximately 50 μg As (U.S. EPA, 1988; Gartrell *et al.*, 1985). The Total Diet Study (1991-1997) (FNB, 2002) reported percentiles of arsenic intake distributions for different age groups in the U.S. population. Individuals consumed an average of 37.9 μg As/day. The highest-consuming subgroup was males aged 51-70 yr with a mean intake of 63.2 μg/d, followed by females of the same age with a mean intake of 54 μg/d. Meacher *et al*. (2002) using a Monte Carlo approach estimated intakes of inorganic arsenic in the U.S. population from food, drinking water, air, and soil. The 90^{th} percentile of total Asi intake was 11.4 μg/day for males and 9.4 μg/day for females, approximately 55 percent derived from food. The authors noted that regional differences in inorganic As exposure were due mostly to consumption of drinking water with varying arsenic levels rather than to food preferences. For example, the mean intakes from all sources in the western U.S. region, with higher drinking water arsenic, were 10.6 μg/day in males and 9.26 μg/day in females, approximately 32 percent derived from food.

Most food contains low levels of arsenic, normally less than 0.25 mg/kg (Ishinishi *et al.*, 1986). ATSDR (1997) gives a typical range of As in food as 20-140 ppb. Generally, the meat, fish and poultry group is the predominant source of arsenic intake for adults and has been estimated to account for about 80 percent of arsenic intake (U.S. EPA, 1988). Of this group, fish and seafood consistently contain the highest concentration of arsenic. The amount of arsenic ingested daily in food by humans is greatly influenced by the amount of seafood in the diet (Ishinishi *et al.*, 1986). The concentration of arsenic in fish and seafood (particularly shellfish and marine foods) is generally 1-2 orders of magnitude higher than that in other foods. Freshwater fish contain much lower levels of arsenic than marine fish (Woolson, 1983). Approximately 5-10 percent of the arsenic in seafood is inorganic (GESAMP, 1986; Edmonds and Francesconi, 1993). The remainder is present in lipid- and water-soluble organic compounds (Pershagen, 1986). Arsenobetaine appears to be the major water-soluble organoarsenic compound in lobsters and shrimp. Yost *et al*. (1998) estimated the intake of inorganic arsenic in North American diets at 8.3 to 14 μg/d in the United States and 4.8 to 12.7 μg/d in Canada for various age groups.

Shoof *et al*. (1999) conducted a market basket survey of arsenic in 40 commodities collected in Bryan and Tyler, Texas in 1997. The commodities were anticipated to provide at least 90 percent of the dietary inorganic arsenic intake. Total arsenic concentrations (ng/g, wet weight) were higher in seafood (160 to 2360), rice (303), chicken (86), grape juice (58), and beef (52), than in the other commodities analyzed. The highest inorganic arsenic concentration was found in raw rice (74 ng/g), followed by flour (11 ng/g), grape juice (9 ng/g), and cooked spinach (6 ng/g). In fruits and vegetables inorganic arsenic comprises about half, while in grains, sugars, and oils it comprises only about one quarter of the total arsenic. Lower percentages of Asi were seen in meat, poultry, fish, and eggs. MMA and DMA were generally at very low concentrations or undetectable. The highest DMA concentrations were found in rice and shrimp (91 and 34 ng/g, respectively), followed by sugar (7 to 8 ng/g), seafood (1 to 6 ng/g), meat, fruits, and fruit juices. MMA was repeatedly detected in apple juice.

Other Sources

Some occupations involve the use of arsenic and can result in exposures (e.g., copper or lead smelting, wood treating, pesticide application). Sanding wood treated with the chromated copper arsenate (CCA) preservative can result in inhalation of small sawdust particles, as can inhaling smoke from burning CCA-treated wood. Most household uses of arsenic containing pesticides have ended, so exposure to As via these products is less likely now than previously (ATSDR, 1997). Child exposure to arsenic in playground equipment treated with CCA wood preservative was estimated to range from 24 to 630 µg/visit, typically 60 µg/visit (CDHS, 1987).

METABOLISM AND PHARMACOKINETICS

Several comprehensive reviews of the absorption, distribution, metabolism and elimination of arsenic have been published (Marcus and Rispin, 1988; U.S. EPA, 1988; Vahter, 1983; Thompson, 1993). It has been suggested that the failure to demonstrate carcinogenicity of inorganic arsenic in experimental animals is possibly due to metabolic and/or distribution differences between humans and the animal models presently utilized. These differences will be addressed below. Arsenic and arsenic compounds also cross the placenta. A separate subsection addressing the transplacental transfer of arsenic will be included at the end of this section. The kinetics of arsenic vary depending on the chemical form of arsenic and on the animal species. The following discussion is limited to the forms found in water and forms that are ingested via the aquatic food chain. These include the inorganic, soluble forms of arsenite (As^{III}) and arsenate (As^{V}), as well as the organic methyl arsonate (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), and or arsenobetaine (in fish). Discussion is also limited to studies involving oral or parenteral administration.

Absorption

In general, investigations that have monitored arsenic excretion of experimental animals following parenteral administration have demonstrated that only a small fraction of the administered arsenic is excreted in the feces. Thus, to estimate the amount of inorganic arsenic absorbed following oral administration, most kinetic and metabolic studies have monitored the urine. Soluble compounds of inorganic arsenic, whether in the trivalent or pentavalent form, are readily absorbed (80-90 percent) in most animal species following oral administration (Charbonneau *et al.*, 1978; Vahter, 1981; Freeman *et al.*, 1995, Hughes *et al.* 1994). However, only about 40-50 percent absorption has been reported in hamsters (Marafante and Vahter, 1987; Yamauchi and Yamamura, 1985). Absorption of orally administered inorganic arsenic in humans has been shown to range between 54-80 percent (Buchet *et al.*, 1981a,b; Tam *et al.*, 1979; Kurttio *et al.*, 1998).

Organic forms of arsenic are also extensively absorbed from the gastrointestinal tract. Experimental studies examining the absorption of MMA, DMA, TMA and arsenobetaine in humans have demonstrated 75-92 percent absorption.

At low-level exposures, excretion of arsenic and its metabolites seems to balance absorption of inorganic arsenic. With increasing arsenic intake, there is suggestive evidence that methylation appears less complete. Studies that examine the effect of dose on excretion patterns have been conducted in mice and humans (Vahter, 1981; Buchet *et al.*, 1981a,b). As the dose of inorganic arsenic increases, the percent of arsenic excreted as DMA decreases, accompanied by an increased excretion in the percent as inorganic arsenic. The percent excreted as MMA remains virtually unchanged. *In vitro* metabolism studies on the methylation of inorganic arsenic have demonstrated that the liver is the site of methylating activity and that S-adenosylmethionine and reduced glutathione are required as methyl donors (Buchet and Lauwerys, 1985a,b).

Foa *et al.* (1983) measured the level of arsenic and its metabolites in blood and urine of 148 subjects drawn from the general population. These subjects were apparently healthy males, who had no occupational exposure to arsenic and had not ingested seafood within the preceding week. Based on the broad range and standard deviation of values found for arsenic and each metabolite, the methylation capacity appeared to be quite variable within the study population.

As stated above, methylation occurs enzymatically in the liver by methyl transfer from S-adenosylmethionine. Therefore, methylation is influenced by hepatic methyltransferase activity. Choline-, methionine- or protein-deficiencies affect hepatic concentrations of S-adenosylmethionine and thereby affect transmethylation reactions (Marafante and Vahter, 1986; Vahter and Marafante, 1987; Marafante *et al.*, 1985). It is interesting to note that severe health effects due to chronic ingestion of arsenic via drinking water have largely been reported in populations of low socioeconomic status and poor nutrition (U.S. EPA, 1988).

The ratio of DMA to inorganic arsenic in urine is different for As^{V} and As^{III} (Vahter, 1981). The ratio is usually 2-3 times higher following exposure to As^{III} than an equivalent amount of As^{V}. Two reasons contribute to this observation: 1) As^{V} must be reduced to As^{III} before it can be methylated to DMA; and 2) As^{V} itself is excreted more quickly than As^{III}.

The fate of organic arsenicals has also been examined in experimental animals and humans. All organic forms of arsenic, which have been studied, are excreted rapidly, for the most part unchanged. All studies to date indicate that no *in vivo* demethylation occurs (Vahter, 1981, 1983; Vahter and Marafante, 1983, 1985, 1987; Vahter and Envall, 1983; Vahter *et al.*, 1984; Yamauchi and Yamamura, 1984a,b; Yamauchi *et al.*, 1988; Crecelius, 1977; Buchet *et al.*, 1981a,b; Tam *et al.*, 1982).

While absorption from the gastrointestinal tract is the most important route of exposure for waterborne arsenic, some potential for dermal absorption has been reported. Rahman *et al.* (1994) conducted *in vitro* studies with sodium [^{74}As] arsenate and clipped full-thickness mouse skin in a flow-through system. Doses of 5, 50, 500, or 5000 ng were applied to 0.64 cm^2 of skin as a solid, in aqueous vehicle, or in soil. Absorption of sodium arsenate increased linearly with applied dose from all vehicles. The maximum absorption of 62 percent of applied dose was obtained with the aqueous vehicle and the least (0.3 percent) with soil. Wester *et al.* (1993) evaluated the percutaneous absorption of [^{73}As] arsenate from soil or water *in vivo* in Rhesus monkeys and *in vitro* in human cadaver skin. Water solutions of [^{73}As] arsenate at low (0.024 ng/cm^2) or high (2.1 µg/cm^2) surface concentrations were compared. With topical administration for 24 hr, *in vivo* absorption in the Rhesus monkey was 6.4 ± 3.9 (SD) percent from the low dose and 2.0 ± 1.2 (SD) percent from the high dose. *In vitro* percutaneous absorption of the low dose from water in human skin was 0.93 ± 1.1 percent in receptor fluid and 0.98 ± 0.96 percent in the washed skin; the total was about 1.9 percent. Absorption from soil (0.4 ng/cm^2) was less, at 6.4 percent in the monkey *in vivo* and 0.8 percent in human skin *in vitro*.

Distribution

The retention and distribution patterns of arsenic are in part determined by its chemical properties. Arsenite (As^{III}) reacts and binds to sulfhydryl groups while arsenate (As^{V}) has chemical properties similar to those of phosphate. As^{V} also has affinity for sulfhydryl groups; however, its affinity is approximately 10-fold less than As^{III} (Jacobson-Kram and Montalbano, 1985). The distribution and retention patterns of As^{III} and As^{V} are also affected by species, dose level, methylation capacity, valence form, and route of administration.

Vahter *et al.* (1984) studied tissue distribution and retention of ^{74}As-DMA in mice and rats. About 80 percent of an oral dose of 0.4 mg As/kg was absorbed from the gastrointestinal tract. In mice >99 percent of the absorbed dose was excreted within three days compared to only 50 percent in rats, due largely to accumulation in blood. Tissue distribution in mice showed the highest initial (0.5-6 hr) concentrations in kidneys, lungs, intestinal mucosa, stomach, and testes. Tissues with the longest retention times were lungs, thyroid, intestinal walls, and lens.

The effect of dose on arsenate disposition was evaluated in adult female B6C3F$_1$ mice dosed orally with 0.5 to 5000 µg/kg [^{73}As]-arsenate in water (Hughes *et al.*, 1994). Urine was collected at several time points over a 48-hr period, and feces at 24 and 48 hr postexposure. The recovery of As-derived radioactivity in excreta and tissues ranged from 83.1 to 89.3 percent of dose. As-derived radioactivity was detected in several tissues (urinary bladder, gall bladder, kidney, liver, lung) although the sum for each exposure level was very low (<0.5 percent of dose). The principal depot was the liver, followed by the kidneys. As the dose of arsenate increased there was a significant increase in the accumulation of radioactivity in the urinary bladder, kidney, liver, and lungs. The greatest concentration of As radioactivity was in the urinary bladder.

Metabolism

Most studies of arsenic metabolism have involved administration of inorganic arsenic (Asi) as arsenate (As^{V}) or arsenite (As^{III}) to an experimental animal or a human, and detection of Asi and the methylated metabolites methylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) in urine, feces, and tissues. In an extensive review and analysis of the mammalian metabolic data on arsenic, Thompson (1993) made the following observations:

- Glutathione (GSH) is required for the reduction of As^{V} to As^{III} in preparation for enzyme-catalyzed oxidative methylation;
- GSH is not involved in monomethylation once arsenite is formed, but GSH is involved in dimethylation by reducing MMA^{V} to methylarsonous acid (MMA^{III});
- GSH is also required in the methylation of arsenic by stabilizing the reductive nature of the cell;
- A different methyl transferase is used in each methylation step (i.e., MMTase, DMTase);
- Dithiols (either a cofactor or the MTases) are required for both mono and dimethylation.

The metabolism of arsenate can be viewed as a cascade of reductive and oxidative methylation steps leading successively to As^{III}, MMA^{V}, MMA^{III}, DMA^{V}, DMA^{III}, $TMAO^{V}$, and TMA. MMA^{III} and DMA^{III} have only recently been detected as stable urinary metabolites in human subjects (Aposhian *et al.*, 2000a,b; Le *et al.*, 2000a,b), and trimethylarsine oxide (TMAO) and trimethylarsine (TMA) are rarely seen and are very minor metabolites in most mammals if found at all. Few data are available on the tissue concentrations of trivalent methylated As species (Kitchin, 2001). Gregus *et al.* (2000) found that in bile duct-cannulated rats, As^{III} and its metabolites were preferentially excreted into bile (22 percent) versus eight percent into urine in two hr. Arsenite appeared in bile rapidly and constituted the large majority in the first 20 min. Thereafter As^{III} declined and MMA^{III} output gradually increased. From 40 min after i.v. As^{III} administration, MMA^{III} was the dominant form of biliary arsenic. Within two hr 9.2 percent of the dose was excreted in the bile as MMA^{III}. Injection of arsenate produced a mixture of As^{V}, As^{III} and MMA^{III} in the bile. Curiously, rats injected with MMA^{V} did not excrete MMA^{III}.

Arsenate appears to employ phosphate transporters for uptake into cells since foscarnet (phosphonoformic acid), a phosphate antagonist, blocked to some extent arsenate uptake in rats but was ineffective on arsenite. Also treatment of rats with periodate-oxidized adenosine, an inhibitor of S-adenosylmethionine-dependent methyltransferases, nearly abolished the appearance of methylated As metabolites in bile and urine. Similar treatment with inhibitors of catechol-*O*-methyltransferase or methylcobalamin had little effect on the biomethylation of arsenite (Csanaky and Gregus, 2001).

The metabolism results of Styblo *et al.* (1995) in rat liver cytosol *in vitro* seem to support the overall metabolic scheme noted above with MMA^{III} and MMA^{III}-diglutathione complex being more rapidly methylated to the dimethyl forms than MMA^{V}. Thompson also suggests that the data support the presence of two inhibitory loops:

- Competitive inhibition by MMA^{III} of the $As^{III} \rightarrow MMA^{V}$ step catalyzed by MMTase;
- Possibly noncompetitive inhibition by As^{III} of the $MMA^{III} \rightarrow DMA^{V}$ step catalyzed by DMTase.

Styblo *et al.* (1996) observed 50 μM arsenite inhibition of DMA^{V} production in rat liver cytosol *in vitro*. Healy *et al.* (1998) studied the activity of MMTase in tissues of mice. The activity was determined with sodium arsenite and *S*-[methyl-^{3}H]-adenosyl-L-methionine by measuring the formation of [methyl-^{3}H] monomethylarsonate. The mean MMTase activities (units/mg ± SEM) measured in cytosol of mouse tissues were: liver, 0.40±0.06; testis, 1.45±0.08; kidney, 0.70±0.06; and lung, 0.22±0.01. When mice were given arsenate in drinking water for 32 or 92 d at 25 or 2500 µg As/L, the MMTase activities were not significantly increased compared to controls. MMTases and DMTases have been partially purified from the livers of rabbits (Zakharyan *et al.*, 1995), rhesus monkeys (Zakharyan *et al.*, 1996) and hamsters (Wildfang *et al.*, 1998). All of the enzyme preparations exhibited Michaelis-Menten enzyme kinetics with Km values ranging from 8×10^{-4} M for hamster DMTase to 1.8×10^{-6} M for hamster MMTase. Vmax values ranged from 0.007 pmol/mg protein/hr for hamster DMTase to 39.6 pmol/mg protein/hr for rabbit MMTase. Comparative studies have shown several species to be deficient in MTase activities, notably New World monkeys, marmosets, tamarin, squirrel, chimpanzee, and guinea pig (Vahter *et al.*, 1995b; Aposhian, 1997).

While the reduction of arsenate and MMA^V can be accomplished nonenzymatically *in vitro*, and arsenate reduction by glutathione occurs in mammalian blood *in vivo* (Vahter and Envall, 1983; Winski and Carter, 1995), these reductive steps are most likely enzymatically mediated *in vivo*. An arsenate reductase has been partially purified from human liver and described (Radabaugh and Aposhian, 2000). The approximate mass of the enzyme is 72,000, it is specific for arsenite (i.e., does not reduce [^{14}C]MMA^V), and exhibits substrate saturation at about 300 µM. The human arsenate reductase requires a thiol and a heat-stable cofactor and is apparently distinct from those isolated from bacteria (Ji and Silver, 1992; Gladysheva *et al.*, 1992; Krafft and Macy, 1998).

The arsenate reductases of unicellular prokaryotes and eukaryotes were described by Mukhopadhyay and Rosen (2002). They noted that at least three families of arsenate reductases have arisen, apparently by convergent evolution. These include a family typified by the *Escherichia coli* plasmid R773 ArsC that uses Grx and GSH as reductants, a family represented by the *Staphylococcus aureus* plasmid p1258 ArsC that uses thioredoxin (Trx) as a reductant, and Acr2p, the only eukaryotic arsenate reductase which belongs to the superfamily of PTPases that includes the Cdc25a cell-cycle phosphatases. Both the *E coli* ArsC reductase and the *Saccharomyces cerevisiae* Acr2p reductase use glutaredoxin (Grx) and reduced glutathione (GSH) as reductants. The structure of the bacterial enzyme has been solved at 1.65 Å. The active site consists of Cys12, several Arg residues, including Arg60, and Arg94. The region between the Cys76 and Arg82 in the Acr2p yeast enzyme is likely to be the active site since mutations in either residue result in loss of activity. It is not known if the mammalian arsenate reductase described by Radabaugh and Aposhian (2000) is related to Acr2p or either of the bacterial enzymes. However, since the *S. aureus* and yeast enzymes are homologues of protein phosphotyrosine phosphatases, and the yeast enzyme is also homologous to rhodanases, which are thiosulfate sulfurtransferases, it seems likely that all arsenate reductases share a common evolutionary lineage with phosphatases (Mukhopadhyay and Rosen, 2002).

Monomethyl arsonate (MMA^V) reductases have been isolated and described for rabbit (Zakharyan and Aposhian, 1999) and hamster (Sampayo-Reyes *et al.*, 2000). In the latter study the distribution of MMA^V reductase activity ranged from brain (91.4 nmol MMA^{III}/mg protein/hr) and bladder (61.8 nmol MMA^{III}/mg protein/hr) to skin > kidney > testis (all < 15 nmol/mg/hr). Spleen > liver > lung > heart were all between 15 and

62 nmol/mg/hr. The high activity of MMA^V reductase in brain is curious and may help explain some of the neurotoxic effects of arsenic. Due to relatively low affinity of the MMA^V reductase (K_M=2.2x10^{-3} M) compared to the methyl transferases (K_M=5-9x10^{-6} M), the MMA^V reduction is thought to be the rate-limiting step in arsenic metabolism (Zakharyan and Aposhian, 1999). The partially purified human liver MMA^V reductase has been shown to be identical with human glutathione S-transferase Omega class hGSTO 1-1 (Zakharyan *et al*., 2001).

Methylation of Asi has also been observed *in vitro* in mouse intestinal cecal contents incubated anaerobically (Hall *et al*., 1997). MMA was the predominant metabolite from arsenate or arsenite over a concentration range of 0.1 to 10 µM Asi. About three percent of either substrate was converted to DMA. The significance of microbial methylation of ingested arsenic is uncertain at present. An As^{III} methyltransferase (41 kDa) has been purified by Lin *et al*. (2002) from rat liver. The enzyme methylates As^{III} in two steps in which MMA is an intermediate and DMA is the final product. $MAs^{III}O$ is also a substrate for the enzyme yielding DMA (Km 250 nM, Vmax 68 pmol/mg protein/min). *S*-adenosyl-L-methionine is the methyl group donor. The enzyme requires a dithiol for activity and is inhibited by $MAs^{III}O$ concentrations above five µM. Protein and cDNA sequences for the rat As^{III} methyltransferase show a high degree of homology with a putative human methyltransferase CYT19, indicating that CYT19 is a human As^{III} methyltransferase (Styblo *et al*., 2002).

De Kimpe *et al.* (1999) observed effects of metal ions on the *in vitro* methylation of arsenate by rabbit liver cytosol. The methylation of carrier-free ^{74}As-arsenate was increased by supplementation with essential trace elements particularly zinc (Zn^{+2}), vanadium (V^{+5}), iron (Fe^{+2}), copper (Cu^{+2}) and selenate. Trivalent metal ions (e.g., Al^{+3}, Cr^{+3}, and Fe^{+3}), Hg^{+2}, Tl^{+} and SeO_3^{-2} had inhibitory effects. Some ions exhibited differential effects for MMA or DMA formation, e.g., VO_3^- inhibited MMA formation but stimulated DMA formation, whereas the reverse was observed for Cr^{+3} and Hg^{+2}. Overall the findings suggest a co-factor role for a specific divalent metal ion, possibly zinc.

DMA is the main metabolite found in the tissues and urine of most experimental animals administered inorganic arsenic. Humans are also somewhat unique in that MMA has been found to be an important metabolite of inorganic arsenic in addition to DMA. Studies conducted on human volunteers given a single oral dose of inorganic arsenic demonstrated that within 4-7 days, 46-62 percent of the dose was excreted in the urine (Buchet *et al*., 1981a,b; Tam *et al*., 1979; Pomroy *et al*., 1980). Approximately 75 percent of the excreted arsenic is methylated, about one-third as MMA and two-thirds as DMA. Although most studies of arsenic metabolism have centered on arsenate and arsenite, other forms of arsenic are also metabolized in humans. Apostoli *et al.* (1997) reported on the metabolism of arsine gas (AsH_3) in an occupationally exposed worker. As species were analyzed in urine over a five-day post-exposure period by liquid chromatography and inductively coupled plasma mass spectroscopy. The As species most excreted were MMA, DMA, As^{III}, arsenobetaine (AsB), and to a lesser extent As^V. The data indicate a capability to oxidize As III to As V species probably via arsenite $As(OH)_3$. Arsenobetaine, an important form of arsenic in food, does not undergo subsequent biotransformation and is excreted via the urine.

Hopenhayn-Rich *et al.* (1993, 1996a,b) evaluated human arsenic metabolism in a series of studies. In a review of existing literature, they showed that the data did not support a methylation threshold hypothesis. The relative percentages of Asi, MMA, and DMA averaged approximately 15-20 percent, 10-15 percent, and 60-70 percent, respectively across different populations studied with no systematic increase in Asi percent with increasing exposure (Hopenhayn-Rich *et al.*, 1993). In a subsequent study in Nevada, individuals exposed to high As (1300 ppb) and low As (16 ppb) in drinking water were evaluated (Warner *et al.*, 1994), and in a larger study in Chile methylation patterns in 122 people with 600 ppb As in their water and 108 with 15 ppb in water were compared (Hopenhayn-Rich *et al.*, 1996a). Both studies found that methylation patterns did not vary significantly with exposure level, but revealed large inter-individual variability independent of exposure level. In a follow up study Hopenhayn-Rich *et al.* (1996b) returned to a subgroup of highly exposed subjects from a previously studied group in northern Chile who had been given water with low As levels (45 ppb) for two months. Seventy-three of these subjects were studied. The decrease in As exposure was associated with a small decrease in percent As in urine (from 17.8 percent to 14.6 percent) and in the MMA/DMA ratio (from 0.23 to 0.18). Other factors such as smoking, gender, age, years of residence, and ethnicity were associated mainly with the MMA/DMA ratio, especially smoking. However, the factors investigated accounted for only about 20 percent of the large inter-individual variability observed.

The possibility of genetic polymorphism in arsenic metabolism has been suggested by Vahter *et al.* (1995a), who studied native Andean women in northwestern Argentina who were exposed to a wide range of As concentrations in drinking water (2.5 to 200 µg As/L). The women exposed to the highest As concentration in water exhibited surprisingly low levels of MMA in their urine (2.3 percent). The range of MMA in typical human urine is 12-20 percent. Chiou *et al.* (1997a) studied the relationships among arsenic methylation capacity, body retention, and genetic polymorphisms of glutathione-*S*-transferase (GST) M1 and T1 in 115 human subjects. Percentages of As species in urine (mean ± SE) were: Asi, 11.8 ± 1.0; MMA, 26.9 ± 1.2; and DMA, 61.3 ± 1.4. Genetic polymorphisms of GST M1 and T1 were significantly associated with As methylation. Subjects with the null genotype of GST M1 had an increased percentage of Asi in urine, while those with the null genotype GST T1 had elevated DMA in their urine.

Del Razo *et al.* (1997) observed that 15 individuals bearing cutaneous signs of arsenicism had significantly longer times of exposure to Asi in drinking water and higher urinary concentrations of and proportions of MMA and MMA/Asi values and significantly lower DMA/MMA than 20 exposed individuals without cutaneous signs. Hsueh *et al.* (1998) studied urinary levels of As metabolites by age, sex, and previous exposure to Asi through artesian well water in 255 subjects in an arseniasis-hyperendemic area of Taiwan. A multivariate analysis indicated that urinary DMA was significantly inversely associated with age, with women exhibiting lower urinary As III, As V, MMA, and organic arsenic compared to men. The data suggest that women possess a more efficient As methylation capability than men, and that aging diminishes this.

Concha *et al.* (1998) studied arsenic metabolism in 39 women and 57 children in three villages in northern Argentina, two with moderately high As in the drinking water

(200 ppb) and one with low As (<1 ppb). Total As in urine was only slightly higher than the sum of Asi, MMA, and DMA, indicating that Asi was the main form of As ingested. The subjects excreted very little MMA. Women excreted 0.6 to 8.3 percent and children 0.9 to 12 percent, possibly indicating methylation polymorphism. The children had a significantly higher percentage of Asi in urine, ca. 50 percent (range 21 to 76 percent) versus 32 percent (range 6.5 to 53 percent) in the women. The percentage of Asi in the children was considerably higher than in previous studies of children (13 percent) and adults (15 to 25 percent). This could indicate a higher sensitivity to As-induced toxicity in children than in adults. It was also observed that as the total As in children's' urine increased, the percentage of Asi decreased and DMA increased, possibly indicating an induction of methylation capacity with increasing exposure.

Excretion

Most absorbed inorganic arsenic is excreted in the urine as inorganic arsenite or arsenate, MMA, DMA, and other unidentified organic forms. In humans, normally 60-80 percent of a single dose of inorganic arsenic is excreted within 5-7 days. The rate of excretion of inorganic arsenic is influenced by valence state. The rate of total arsenic excretion following exposure to As^{V} is generally much faster than following exposure to As^{III}. However, in the mouse the rate of excretion following exposure to As^{III} or As^{V} is very similar. This is mainly due to the efficient methylation of the arsenite (As^{III}), which compensates for the faster excretion of unmethylated As^{V} (Vahter and Marafante, 1983).

Because of high absorption in the gastrointestinal tract, very little arsenic is usually eliminated via the feces. Following parenteral administration, very low fecal arsenic levels are also observed. Arsenic is known to be secreted in the bile to some extent (Vahter, 1983). However, biliary excretion does not significantly contribute to elimination of arsenic from the body, presumably because of enterohepatic recirculation. Accumulation in hair, nails and skin can be regarded as a form of elimination. It has been estimated that the elimination through accumulation in hair is at most 0.6 percent of an ingested dose (World Health Organization, 1981). Exhalation of arsenic after exposure to inorganic arsenic has been studied in experimental animals (Vahter, 1983). These studies indicate that little if any arsenic is eliminated by this route. Chiou *et al.* (1997a) observed that the methylation capacity, indicated by the relative percentages of the As species in urine, was associated with body excretion of arsenic in humans. The As contents of hair and nails were found to be negatively associated with the percentage of MMA in urine and positively associated with the percentage of DMA in urine.

Several authors have studied the kinetics of As excretion in humans. Tam *et al.* (1979) administered ^{74}As arsenic acid (0.01 μg, ca. 6 μCi) to six adult males (age: 28-60; body weight: 64-84 kg) following an overnight fast. The urine was analyzed at 24 hr intervals for five days following As administration. In the first 24 hr period Asi excretion exceeded that of the methylated metabolites but thereafter the usual DMA > MMA > Asi pattern persisted, with DMA increasing in percentage of cumulative excretion at the later time points. A follow up study (Pomroy *et al.*, 1980) followed ^{74}As excretion for periods up to 103 days using a whole body counter, with measurement of excreta for the first seven days. Their results indicate that the excretion data were best represented by a three-component exponential function. The coefficients for the pooled data accounted for 65.7 percent of excretion with a half-life of 2.09 days, 30.4 percent with a half-life of 9.5 d, and 3.7 percent with a half-life of 38.4 d. A four-exponent function showed a better fit to one of the six subjects (half-lives: 0.017, 1.42, 7.70 and 44.1 d).

Buchet *et al.* (1981a,b) followed the urinary excretion of As metabolites after administration of 500 µg of sodium arsenite, MMA, or DMA to three to five male volunteers. Excretion was measured at 4, 8, 12, 24, 36, 48, 72, and 96 hr. Total As excreted in four days was 45.1 percent for arsenite, 78.3 percent for MMA, and 75.1 percent for DMA. The mean basal (pre-exposure) excretion of As amounted to 7.1 µg/day, comprised of Asi 18.3 percent, MMA 3.8 percent, and DMA 77.8 percent. After correction for daily basal excretion the mean percentages of metabolites following sodium arsenite administration (N = 3) were: Asi 25.0, MMA 21.3, and DMA 53.7 percent. Asi excretion exceeded that of the methylated metabolites during the first eight hours following ingestion, thereafter DMA predominated. After MMA ingestion (N = 4) the percentages of excreted metabolites after four days were: MMA 87.4 percent and DMA 12.6 percent. MMA was the most rapidly excreted metabolite with over 70 percent of the dose excreted within eight hours. Similarly, following DMA ingestion (N = 4) only DMA was excreted, with over 40 percent of the dose appearing in urine within eight hours.

Johnson and Farmer (1991) administered single oral doses of prawns containing ca. 540 µg As to three subjects, Vichy Celestins (VC) (220 µg As^V) to two subjects, and repeated daily doses VC (66 µg As^V) for ten days to a single subject. Six hr after consumption of the seafood 25 percent of the As dose had been eliminated, with nearly 50 percent eliminated after 20 hr. Urinary As elimination following a single oral intake of As^V was slower, with only 5.25 percent of the dose appearing in urine after six hr and 50 percent excreted in 54 to 70 hr after intake. Even after 166 hr, only 63.9 percent of Asi had been eliminated. A two component exponential model showed that almost 50 percent of the seafood As was excreted, with component half-lives of 6.9 to 11.0 hr and 75.7 hr (3.15 d), respectively. For As^V administration (N = 2), the two components had half-lives of 17.7 to 24.1 hr and 7.1 to 8.6 d, respectively.

Apostoli *et al.* (1997) evaluated the kinetics of As metabolites following an acute occupational inhalation exposure to arsine gas in a single male subject. Elimination of total As from blood was represented by a triphasic exponential model with half-lives of 27.6 hr, 59.4 hr, and 220.7 hr. Excretion of As metabolites in urine exhibited the following average half-lives: total As 68.4 hr, As^V 27.0 hr, MMA 56.3 hr, As^{III} 57.1 hr, DMA 71.8 hr, arsenobetaine 85.8 hr. Le *et al.* (1994) reported on the excretion of food arsenicals including arsenobetaine in crab and shrimp and arsenosugars in seaweed. With nine volunteers and commercial seaweed, both the urinary As excretion pattern and the excreted As species varied among individuals. After ingestion of 193 µg of arsenosugar As, peak excretion of As in urine occurred 10 to 60 hr after ingestion, with As excretion returning to background levels at ca 80-120 hr. It seems likely that the human gastrointestinal tract microflora play a role in the bioavailability of As in arsenosugars as with many other environmental glycosides of plant origin (Brown, 1988).

Mandal *et al.* (1998) studied 17 individuals (ages 1.5-70 yr) in West Bengal, India previously exposed for long periods to an average concentration of 200 µg As/L in drinking water but supplied with low arsenic water with < 2 µg As/L for drinking and cooking for two years. Arsenic was measured in urine, hair and nails over the two-year course of the study. Random fluctuations of arsenic concentrations in urine were seen with a declining trend during the first six months. Additional sources of incidental As exposure included consumption of edible herbs grown in contaminated water and occasional drinking of contaminated water. Arsenic concentration in nails and hair showed linear decreases with time, averaging -9.4 µg/kg-d and -2.3 µg/kg-d, respectively. Decreases in arsenic concentrations in urine averaged -0.55 µg As/L-d during the first half of the study, albeit with the fluctuations noted above. Initial average arsenic concentrations were about 310 µg/L urine, 8.5 mg/kg nail, and 7.0 mg/kg hair. Eight of the subjects (all over 28 yr of age) showed arsenical skin lesions, which did not resolve during the study period.

Kurttio *et al.* (1998) studied 47 individuals in Finland who were exposed to 17-980 µg As/L in drinking water for periods of 1-34 yr. Thirty-five current users of As contaminated water had a mean water concentration of 170 µg As/L, a mean exposure time of nine yr, and a cumulative mean arsenic dose of 472 mg/lifetime. A smaller group of 12 former users who had stopped using the As containing water 2-4 mo before urine sampling had values of 292 µg/L, 14 yr, and 828 mg/lifetime, respectively. Arsenic concentrations in hair of current users varied from 0.06 to 12.50 ppm (median 0.96 ppm) and for former users 0.09 to 21.2 ppm (median 5.32). For a control population the levels ranged from below detection to 0.18 ppm. Arsenic in hair was found to correlate well with total As in urine, arsenic concentration in drinking water, and daily As dose. An increase of 10 µg/L in the As concentration in drinking water and an increase in the daily dose of 10-20 µg As/d from drinking water corresponded to a 0.1 ppm increase in the hair arsenic ($r = 0.77$, $p < 0.001$).

Physiologically-Based Pharmacokinetic (PBPK) Models

Physiologically-based pharmacokinetic (PBPK) models employ data from various sources to mathematically simulate the uptake, distribution, metabolism and excretion of toxic chemicals in species of interest. Such models are used in risk assessment to estimate target tissue doses and to facilitate route-to-route and interspecies extrapolations. By contrast, pharmacodynamic (PD) models simulate biological responses to chemical exposures. A number of PBPK models for arsenic disposition and metabolism have previously been developed for experimental animals and humans (Menzel *et al.*, 1994; Mann *et al.*, 1994; Brown *et al.*, 1994; Brown and Collins, 1995; Mann *et al.*, 1996a,b). Although these models are based on somewhat different principles, they all seem to do a fair job in predicting the overall disposition of arsenic in animals and man. However, while the models often incorporate the latest ideas on the metabolism of inorganic arsenic with respect to oxidation state, methylated metabolites, and enzyme inhibition, they have yet to accommodate anything approaching a biological response or a pharmacodynamic (PD) capability. Such models could be modified to incorporate stochastic elements to natural variability in exposure and metabolism. In addition, various dose metrics for target tissues could be tied to biological response models according to the hypotheses that: (a) As or one of its metabolites exerts its carcinogenic action via a non-threshold genotoxic mechanism or; (b) the mechanism, while genotoxic, involves a threshold. A similar coupling of a physiological model and hypothetical biological response (DNA ligase activity: DNA damage) has been proposed for magnesium (Brown *et al.*, 1996). Additional work in this area would be useful in the overall context of arsenic risk assessment.

Physiological/Nutritional Role

Nutritional essentiality of arsenic in humans has been suggested by a number of experimental findings but has not been proven definitely (Anke, 1986, 1991; Anke *et al.*, 1976, 1978, 1985, and 1997). The nutritional role of arsenic has been investigated in goats, mini-pigs, rats, and chickens (Anke *et al.*, 1997; Uthus, 1992). It was demonstrated that arsenic-poor diets with < 35 µg/kg dry weight slowed growth in goats. Arsenic deficiency impairs the success of first service and the conception rate significantly. Arsenic-deficient animals had significantly more adsorbed fetuses than control animals, and the offspring had a considerably higher mortality rate during the second lactation. Deficient animals died suddenly. The mitochondria of the cardiac muscle of deficient goats showed ultrastructural changes. Arsenic is thought to play a role in methionine, glutathione, taurine, and/or polyamine metabolism due to its interaction with methyl groups. The arsenic requirement of goats, mini-pigs, rats, and chicks was estimated to be <50 µg/kg dry diet. No convincing data of human As deficiency syndromes were found. Nevertheless, an estimated "safe and adequate daily intake" for As in humans of 12-40 µg/d (6 µg/1000 kcal) has been proposed, based on animal studies (Uthus, 1994; Anke *et al.*, 1997).

Caution needs to be exercised in assuming an essential human nutritional role for As at intake levels of 12 µg/d. Most of the nutritional work was performed in animal species known to be more resistant to the acutely toxic effects of arsenic than are humans. For example, LD_{50} values in rats for inorganic arsenic are generally larger than estimated lethal doses in humans (ATSDR, 2000). Arsenic is a proven carcinogen in humans but is not readily carcinogenic in the animal species studied. Deficient diets used in the animal studies of <10 ng As/g or 15 µg/1.5 kg human diet would hardly be considered deficient in humans with normal estimated intakes of 12-45 µg As/d/1.5 kg diet. Thus animals would appear to need much more As and are much more tolerant of its toxicity.

The typical levels of As in drinking water (5 µg/L = 10 µg/d) are unlikely to result in As deficiency even in concert with an arsenic-free diet, and conversely, arsenic-free water would be unlikely to result in a deficiency syndrome with normal food sources, considering the low postulated requirement. If As does play a role in human metabolism and nutrition it is likely to do so at intake levels far lower than seen in animals, although OEHHA does not consider the evidence adequate to presume that there is a human dietary requirement for arsenic. Currently there is no demonstrated role of arsenic in mammalian metabolism. Some membrane transport proteins in bacteria bind As but alternatively bind antimony and play a role in resistance to the toxicity of these elements. A newly discovered, strictly anaerobic bacterium, *Chrysiogenes arsenatis*, was found to grow by reducing arsenate (As^{V}) to arsenite (As^{III}) and using acetate as electron donor and carbon source (Macy *et al.*, 1996). Arsenate could be replaced as electron acceptor by nitrite or nitrate but not by sulfate, thiosulfate, or iron oxide. This is perhaps the only example of As metabolism not related to toxicity resistance. Presumably there is an arsenate enzyme in this organism that remains to be isolated and characterized. Recently a human arsenite-stimulated ATPase (hASNA-I) was isolated from human embryo kidney 293 cells. Biochemical characterization of this protein indicates that it is of the same superfamily of ATPases represented by the *E. coli* ArsA transport protein (Kurdi-Haidar *et al.*, 1998). Its role in human metabolism is unknown.

TOXICOLOGY

Toxicological Effects in Animals and Plants

Acute Toxicity

The general toxic effects of arsenic in humans have been well characterized and are discussed in this document. Therefore, the following will contain only a brief summary of the effects observed in animals. Acute effects seen in animals after oral exposure are similar to effects seen in humans (U.S. EPA, 1984). Signs of acute poisoning in humans include intense abdominal pains, staggering, weakness, trembling, salivation, gastrointestinal effects such as vomiting and diarrhea, fast feeble pulse, prostration, hypothermia, collapse and death (Marcus and Rispin, 1988). Early symptoms in most human cases are those of severe gastritis or gastroenteritis, however, due to the vascular damage caused by absorbed arsenic, the first symptoms may not appear for several hours. A violent hemorrhagic gastroenteritis leads to loss of fluids and electrolytes, resulting in collapse, shock, and death (Gosselin *et al.*, 1984). Arsenite has been shown to induce oxidative DNA damage in human vascular smooth muscle cells *in vitro* (Lynn *et al.*, 2000). Since these effects were seen at low micromolar concentrations, it appears to be a plausible mechanism for vascular cell damage following acute *in vivo* exposures.

Oral LD_{50} values for various arsenic compounds range from 15 to 293 mg/kg in rats and from 10 to 150 mg/kg in other animals (U.S. EPA, 1984). No mortality occurred in rats given up to 30 ppm As as dry arsenic trioxide mixed in their feed or in Swiss mice given 10.4 mg As/kg and in C3H mice given 19.9 mg As/kg as As_2O_3 in aqueous solution by oral intubation (NRC, 1977). ATSDR (2000) lists oral LD_{50}s for inorganic As ranging from 15 to 175 mg/kg for rats and 26 to 39 mg/kg for mice. Most deaths occurred within one day of exposure. However, details on the causes of death were seldom reported.

An infant Rhesus monkey that died seven days after 3 mg/kg-d oral arsenate exhibited bronchopneumonia with extensive pulmonary hemorrhage, edema, and necrosis. Two other monkeys at the same dose level had no pulmonary lesions at a one-year sacrifice (Heywood and Sortwell, 1979). Acute oral exposure of rats to gallium arsenide at 1,040 mg/kg resulted in increased blood pressure and heart rate, whereas 520 mg/kg had no effect (Flora *et al*., 1997). Vomiting and gastrointestinal hemorrhage was observed in dogs after a single dose of 14 mg As/kg as roxarsone (ATSDR, 2000). Oligouria was observed after acute exposure and interstitial nephritis and tubular necrosis in rabbits after repeated doses of MMA (Jaghabir *et al*., 1989). Diarrhea and slight congestion of the intestines were observed in mice after single oral doses of 954 mg As/kg as DMA or 1,177 mg As/kg as MMA (Kaise *et al*., 1989).

Petrick *et al*. (2001) determined the LD_{50}s for i.p. administered monomethylarsonous acid (MMA^{III}) and arsenite in hamsters. Six animals were used for each dose with a total of 66 animals for MMA^{III} and 78 animals for As^{III}. Lethality was assessed during the 24 h period following arsenical administration. The LD_{50}s were 29.3 and 112.0 mg/kg, respectively. These data suggest that MMA^{III} is more acutely toxic than inorganic arsenite *in vivo* in hamsters. Previous studies have shown that MMA^{III} is more toxic than arsenite in cultured human cells *in vitro* (Petrick *et al*., 2000; Styblo *et al*., 2000).

Subchronic Toxicity

Subacute and chronic arsenic exposures generally affect many of the same organs or systems as those affected by acute arsenic exposure. The ones most affected by arsenic are those involved in absorption, accumulation, and/or excretion, i.e., the gastrointestinal tract, circulatory system, skin, liver, and kidney. However, other organs or systems that are particularly sensitive to the effects of arsenic, such as the nervous system, and those that are affected secondarily, such as the heart, are also affected (Squibb and Fowler, 1983).

Rats administered 2 to 10 mg As_2O_3 (1.5 to 7.6 mg As) per day by gavage for 40 days exhibited impaired avoidance conditioning, a behavioral index thought to reflect central nervous system functioning. No histopathological changes were observed in brain tissue (Osato, 1977). A variety of neurochemical effects, including an increase in the activity of lysosomal acid proteinase, was caused in rats by 0.77 mM sodium arsenite (As^{III}, 58 mg As/L) given to rats in drinking water for 11 days (Valkonen *et al.*, 1983). Administration of sodium arsenate to rats in drinking water (50 mg As/L) increased vascular response to b-adrenoreceptor stimulation and decreased response to angiotensin I. After 320 days exposure, however, no changes were noted in blood pressure, contractility of cardiac muscle, rate of contraction of the heart, or cardiovascular reactivity to various drugs (Carmingnani *et al.*, 1983).

In cats, electrocardiographic changes have been noted during several weeks of exposure to arsenate or arsenite in feed (0.5-1.5 ppm As in feed). Blood concentrations of As associated with electrocardiographic abnormalities were as low as 0.03 mg As/L (Massman and Opitz, 1954).

Hematological effects of arsenic in animals include decreased hemoglobin production, seen with arsenate and arsenite in rats and cats (Mahaffey and Fowler, 1977; Byron *et al.*, 1967; Massman and Opitz, 1954). Woods and Fowler (1977) have identified arsenic-induced disturbances of the heme biosynthetic pathway. Rozenshtein (1970) noted significant depression of the number of circulating sulfhydryl groups in whole blood in rats exposed to 3.7 or 46 μg As III/m^3 for two or three months. The data cited to support this observation represent monthly samples of 10 animals per group compared to pre-exposure means based on 20 animals per group. Because of the unusual accumulation of arsenic in rat red blood cells; however, this observation may have little relevance to other species.

Five or twenty daily three-hour exposures to airborne arsenic trioxide ($\geq$76 µg As/m^3) inhibited pulmonary bactericidal activity in mice (Aranyi *et al.*, 1985). Sodium arsenite inhibited the production of antibody-producing cells in mice at 0.5, 2.0, and 10.0 ppm in drinking water (Blakley *et al.*, 1980). Furthermore, most studies have shown arsenic to increase susceptibility to infections (Vos, 1977, Gainer and Pry, 1972). However, in one *in vitro* experiment low concentrations of arsenite ($10^{-6.5}$ to $10^{-5.5}$ M) stimulated the viral plaque-inhibiting action of mouse interferon (Gainer, 1972).

Histopathological changes in liver tissue accompany arsenic exposure. In a study of mice, arsenite in drinking water (50 mg As/L, and roughly 6 mg/kg-d) caused an acute reaction characterized by enlargement of some membrane surfaces and loss of glycogen (Mohelka *et al.* 1980). Three months of airborne exposure of female albino rats to 4.9 mg/m^3 arsenic trioxide (3.7 mg As/m^3) produced fatty degeneration of hepatic cells (Rozenshtein, 1970). Dose-dependent structural changes in rat liver from exposure to arsenic trioxide in drinking water, and impaired mitochondrial respiration in rats injected with arsenite have been reported (Ishinishi *et al.*, 1980; Ghatghazi *et al.*, 1980).

Genetic Toxicity

The earlier genetic toxicity data on arsenic are summarized in the Genetic Activity Profiles (GAP) database for short-term tests based on data of the U.S. EPA and the IARC monographs. The GAP97WIN program/data base is available online from U.S. EPA (www.epa.gov). For trivalent arsenic (As^{III}), the GERMCELL and IARC databases list 11 positive findings in 25 non-human animal, plant, or microbial test systems. These include chromosomal aberrations *in vitro* and *in vivo* (3), micronuclei induction in mice *in vivo* (1), SCEs in mammalian cells (2), and cell transformation *in vitro* (3). For pentavalent arsenic (As^V), the IARC database lists 6/13 positive findings: chromosome aberrations *in vitro* (3); SCEs *in vitro* (2); and cell transformation *in vitro* (1). In general, the lowest effective doses (LEDs) for As^{III} *in vitro* were in the 1-10 µM range, whereas for As^V the LEDs were usually 10-50 µM. The genotoxicity of arsenic in a variety of animal test systems is summarized in Table 3.

Jacobson-Kram and Montalbano (1985) and Basu *et al.* (2001) have published comprehensive reviews of arsenic genetic toxicity. Studies assessing the ability of arsenic to induce gene mutations have largely produced negative results. Several investigators (Rossman *et al.*, 1980; Lee *et al.*, 1985) have reported decreases in the spontaneous mutation frequency and enhanced colony formation ability in some bacterial strains following arsenic exposure under certain experimental conditions.

The results of two studies suggested positive mutagenic activity (Nishioka, 1975; Oberly *et al.*, 1982). However, the first study by Nishioka (1975) has been criticized for a number of reasons including misinterpretation of data and high cytotoxicity. Jacobson-Kram and Montalbano (1985) and Rossman *et al.* (1980) were unable to reproduce the results. In the study of Oberly *et al.* (1982), mutagenic activity was only observed in cultures of mouse L5178Y cells with less than 10 percent survival. Yamanaka *et al.* (1989) reported the mutagenicity of dimethylarsinic acid (DMA) in *E. coli* B tester strains. The mutagenicity for the WP2 and WP2uvrA strains was due to the dimethylarsine metabolite of DMA and required oxygen gas in the assay system.

In contrast to the largely negative mutagenicity results in microbial test systems, *in vitro* chromosomal aberration and sister chromatid exchange (SCE) studies in mammalian cell systems have consistently produced positive results. The mammalian cells used in many of the *in vitro* tests have included Syrian hamster embryo (SHE) and Chinese hamster ovary (CHO) cells.

Many of the *in vitro* studies demonstrated the trivalent form of arsenic to be 5 to 10 times more potent than the pentavalent form. The study by Nakamuro and Sayato (1981) evaluated the activity of six arsenic compounds. The following order of potency (from highest to lowest) was observed: $As_2O_3 > AsCl_3$, $NaAsO_2 > Na_2HAsO_4 > H_3AsO_4$, As_2O_5.

A study by Crossen (1983) indicated that arsenic is only clastogenic when present during the cell phase of DNA replication (i.e., the S-phase). When cells were treated only during the G-phase (i.e., prior to DNA replication), no increase in clastogenic activity was observed.

Wang and Huang (1994) observed that active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells, an X-ray sensitive Chinese hamster ovary cell line. XRS-5 cells were 6-fold lower in catalase activity than the parental CHO-K1 cells and catalase could reduce the frequency of arsenite-induced micronuclei, indicating a role for H_2O_2 in As clastogenicity. Alternatively, calcium and nitric oxide (NO) have also been shown to play a role in As clastogenicity (Liu and Huang, 1996, 1997; Lynn *et al.*, 1997). Gurr *et al.* (1998) demonstrated that 4-hr treatment of CHO-K1 cells with 5 µM arsenite caused a dose dependent increase in NO as well as calcium level. This increase was inhibited by NO synthase inhibitors and calcium chelators, but not by a catalase inhibitor. A four-hr treatment with arsenite above 10 µM also induced micronuclei in a dose dependent manner. The authors postulate that the disturbance of NO production may be involved in As-induced human disease including cancer.

Li and Rossman (1989a,b) demonstrated that arsenite treatment inhibits nuclear DNA ligase II activity in Chinese hamster V79 cells. Both arsenite (10µM, 3 hr) and N-methyl-N-nitrosourea (MNU) (4 mM, 15 min) inhibit total DNA ligase activity to 55 percent and 25 percent of control, respectively. However, three hr after MNU treatment DNA ligase was induced 2.5 fold versus the control value. Pre or post-treatment with arsenite inhibited inducible DNA ligase activity. Similar results were obtained in a DNA ligase II-specific assay of nuclear extracts, indicating that most of the total ligase inhibitory activity was due to DNA ligase II. Lee-Chen *et al.* (1994) observed inhibition by arsenite of rejoining of UV light and alkylation-induced DNA breaks in CHO- K1 cells. In addition to arsenite's inhibitory effects on DNA ligases, the findings also suggest a possible inhibition of poly (ADP-ribose) synthetase.

Table 3. Genetic Toxicity and Related Effects of Arsenic in Animal Systems*

				Reference
***In Vitro* Systems**				
Syrian hamster embryo cells	Na arsenite, Na arsenate	Gene mutation at the 6-thioguanine-ouabain-resistance loci	ND @ 10 µM, ND @ 100 µM	Lee *et al.*, 1985
	Na arsenite	Polyploids, Endoreduplication, Chromosome aberrations	3 µM, 3 µM, 6.2 µM	
	Na arsenate	Polyploids, Endoreduplication, Chromosome aberrations	32 µM, 64 µM, 64 µM	
Methotrexate resistant mouse 3T6 cells	Na arsenite Na arsenate	*dhfr* gene amplification	0.4 µM 2.0 µM	Lee *et al.*, 1988; Barrett & Lee, 1993
Hamster V79 cells	Arsenite	Inhibition of nuclear DNA ligase II	10 µM, 3 hr	Li & Rossman, 1989b
Hamster V79 cells	Arsenite, Arsenate	Tetraploid induction, mitotic arrest	ND @ 3.8 µM, ND @ 180 µM	Eguchi *et al.*, 1997
	MMA, DMA, TMAO	Mitotic arrest	3.6 mM, 7.2 mM, 7.4 mM	
	DMA, TMAO	Tetraploid induction	700 µM, 7.0 mM	
CHO cells	Arsenite	DNA endoreduplication, chromosome aberrations	1.0 µM, 0.1 µM	Kochhar *et al.*, 1996
	Arsenate	Chromosome aberrations	100 µM	
	Arsenite or arsenate	SCE induction	0.01 µM	
CHO-K1 cells	Arsenite	Inhibited rejoining of UV and alkylation damaged DNA, inhibition of poly (ADP-ribose) synthetase	40 µM, 4 hr	Lee-Chen *et al.*, 1994; Lynn *et al.*, 1997
CHO-K1 cells	Na arsenite	SCE induction, Micronuclei induction	20 µM, 6 hr, 5 µM, 6 hr	Fan *et al.*, 1996
CHO-K1 cells	Na arsenite	Stimulation of poly(ADP-ribose) polymerase	80 µM, 4 hr	Lynn *et al.*, 1998
CHO-K1 cells XRS-5 cells	Na arsenite	Micronuclei induction	80 µM, 4 hr, 10 µM, 4 hr	Wang & Huang, 1994

				Reference
CHO-K1 cells	Na arsenite	DNA degradation and apoptosis	40 µM, 4 hr	Wang *et al.*, 1996
CHO-K1 cells	Na arsenite	Micronuclei induction	80 µM, 4 hr; 10 µM, 4 hr	Liu & Huang, 1997; Gurr *et al.*, 1998
CHO-K1 – Human hybrid cells	Na arsenite 0.5-2.0 µM, 1 d, 5 d	A_L assay for intragenic and multilocus mutations	0.5 µM increased *S1*$^-$ mutants, 1 µM increased *HPRT*$^-$ mutants	Hei *et al.*, 1998
Mouse lymphoma L5178Y	Na arsenite, Na arsenate, MMA, DMA	Gene mutations at the TK +/- locus	1 µg/mL, 10 µg/mL, 2500 µg/mL, 10,000 µg/mL	Moore *et al.*, 1997
	Na arsenite, Na arsenate, MMA	chromosome aberrations	1.5 µg/mL, 10 µg/mL, 3000 µg/mL	
	Na arsenate, MMA	micronuclei	10 µg/mL, 4000 µg/mL	
	Na arsenite, Na arsenate	Polyploidy, endoreduplication	1.7 µg/mL, 11.5 µg/mL	
Chinese hamster V79 cells	Na arsenite, UV light	Co-mutagenicity at the *hprt* locus	10 µM, 3 hr + 5J/m^2 UVC, 55J/m^2 UVA; 10 µM, 24 hr + 200 J/m^2 UVB	Li & Rossman, 1991
Chinese hamster V79 cells	Arsenic trioxide	Micronuclei induction	1 µM	Gebel, 1998
Rat TRL 1215 liver epithelial cells	Na arsenite	DNA hypomethylation, Hyperexpressibility of MT gene	0.125µM, 18 wk	Zhao *et al.*, 1997
Rat H411E hepatoma cells	Na Arsenite	Altered *PEPCK* gene expression	0.33µM	Hamilton *et al.*, 1998
φX174 RFI DNA	Na Arsenate Na Arsenite Na MMA^V Na DMA^V MMA^{III} DMA^{III}	Nicking or degradation of DNA	None None None None 30 mM 150 µM	Mass *et al.*, 2001
***In Vivo* Systems**				
Mouse bone marrow cells	Arsenic trioxide	Chromosome aberrations	ND	Poma *et al.*, 1981b
Mouse bone marrow cells	DMA	Aneuploidy, Mitotic arrest	300 mg/kg i.p.	Kashiwada *et al.*, 1998
Chick embryo	Na arsenite	Altered *PEPCK* gene	100 µmol/kg	Hamilton *et al.*,

				Reference
liver		expression		1998
Mouse lung cells	DMA, Na	DNA strand breaks	1500 mg/kg p.o.	Yamanaka *et al.*, 1989

* ND = not detected; dhfr = dihydrofolate reductase; TMAO = trimethyl arsine oxide; TK = thymidine kinase; MT = metallothionein; HPRT = hypoxanthine guanine phosphoribosyl transferase; PEPCK = phosphoenolpyruvate carboxykinase.

Lynn *et al.* (1997) studied the effect of As on different stages in the DNA repair process in methyl methanesulphonate treated CHO-K1 cells and cell free extracts. The potency of the As inhibitory effect as deduced from concentration-response relations were: ligation of poly (rA)•oligo (dT) > ligation of poly (dA)•oligo (dT) ≈ DNA polymerization ≥ DNA repair synthesis > excision. Dithiothreitol could effectively remove As inhibition of both the ligation of poly (rA)•oligo (dT) and the activity of pyruvate dehydrogenase but had no effect on the As inhibition of poly (dA)•oligo (dT) ligation. Since both DNA ligase III and pyruvate dehydrogenase contain vicinal dithiols, the authors propose that As acts via interaction with vicinal dithiols in DNA ligase III.

Kochhar *et al.* (1996) studied the chromosomal alterations induced by arsenite or arsenate in cultured CHO cells. Arsenite treatment increased chromosome aberrations in a dose dependent manner between 10^{-7} and 10^{-5} M. At 10^{-6} and 10^{-5} M arsenite significant endoreduplication of chromosomes was observed. Other aberrations observed were chromosomes with more than one centromere, and chromatid type aberrations, mainly breaks. Arsenate showed similar effects without endoreduplication at higher dose levels (10^{-4}M). Both arsenite and arsenate induced significant increases in SCEs at 10^{-8} M and above.

The clastogenic effect of arsenic has been evaluated in mouse bone marrow cells and spermatogonia *in vivo* following the co-administration of ethyl methane sulfonate (EMS) (Poma *et al.*, 1981a) or TEPA with arsenic (Sram, 1976). No synergistic effect in chromosomal aberrations was observed (Poma *et al.*, 1981a). However, the study was only reported in an abstract form without experimental details, and only one dose combination of arsenic and EMS was examined. Sodium arsenite enhanced the clastogenic effect of TEPA in both bone marrow cells and spermatogonia at a concentration of 0.77 μM in drinking water (Sram, 1976).

Only one study assessing the clastogenic effects of arsenic *in vivo* has been reported (Poma *et al.*, 1981b). This was an evaluation of the frequency of chromosomal aberrations in mouse bone marrow cells and spermatogonia following i.p. administration of arsenic trioxide. In contrast to the consistently positive results observed in the *in vitro* cytogenetic studies described earlier, the results of this study showed no excess of chromosomal aberrations.

A more recent study by Kashiwada *et al.* (1998) investigated the cytogenetic effects of DMA on mouse bone marrow cells following a single i.p. administration of 300 mg/kg. DMA increased mitotic indices significantly at 16, 24, and 48 hr after injection, and prolonged the average generation time by 1.5 hr. This suggests that DMA may cause mitotic arrest *in vivo*. DMA significantly induced aneuploids. The aneuploids were 6 percent in the control saline treatment and 45 percent with DMA treatment ($p < 0.0001$). In the DMA treated group the hyperploids with 1 or 2 extra chromosomes comprised over 80 percent of all aneuploids.

Hamilton *et al.* (1998) used a 14-day chick embryo liver *in vivo* model to study the effect of chromium and arsenic on the expression of model-inducible gene: phosphoenolpyruvate carboxykinase (*PEPCK*). Arsenite significantly altered both basal and hormone-inducible expression at relatively nontoxic doses in the chick embryo *in vivo* (100 µmol/kg) and in rat hepatoma H411E cells in culture (0.33-1.0 µM). The authors suggest that As III acts principally through direct or indirect effects on specific transcription factors and other signaling pathways rather than on DNA per se.

Gebel (1998) observed a suppression of arsenic-induced micronuclei in V79 cells by trivalent antimony (Sb^{III}). Significantly elevated frequencies of micronuclei (MN) were obtained with 0.25 µM arsenic trioxide ($p < 0.05$, by Fisher's exact test). $SbCl_3$ caused significant increases in MN at concentrations of 10 µM and above. Combinations of As^{III} and Sb^{III} gave lower frequencies of MN than was expected by simple additivity. It is thought that Sb^{III} competes with As^{III} for sulfhydryl groups in DNA repair enzymes.

Mass *et al.* (2001) evaluated the genotoxic potential of trivalent As species in a DNA nicking assay. The effect of six arsenic species (Asi^{III}, Asi^{V}, MMA^{V}, DMA^{V}, MMA^{III}, and DMA^{III}) on the electrophoretic migration of ϕX174 RFI DNA was measured after two hr incubation at 37 °C (pH 7.4). Neither Asi^{III} (1 nM to 300 mM), Asi^{V} (1 µM to 1 M), MMA^{V}(1µM to 3M), nor DMA^{V}(0.1 to 300mM) was observed to nick or degrade ϕX174 DNA or alter its electrophoretic mobility. Only MMA^{III} and DMA^{III} were seen to affect the DNA. Either a complete degradation of DNA at higher concentrations or a nicking at lower concentrations was observed. MMA^{III} was effective at nicking DNA at 30 mM; however, at 150 µM DMA^{III}, nicking could be observed. The results appear to indicate that trivalent methylated As species can damage naked DNA without exogenous enzymatic or chemical activation.

Zhao *et al.* (1997) demonstrated an association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression in rat liver epithelial cell line. Rat liver cells were transformed by chronic exposure to low levels of sodium arsenite (0.125, 0.25, and 0.5 µM). Global DNA hypomethylation occurred concurrently with malignant transformation and in the presence of depressed levels of SAM. Arsenic-induced DNA hypomethylation was a positively correlated with dose and duration of exposure and remained after removal of arsenic. Hyperexpressibility of the metallothioneine (MT) gene, controlled by DNA methylation, was also detected in transformed cells. Acute As exposure or prolonged As exposure at nontransforming levels did not induce DNA hypomethylation. A positive linear relationship was observed between genomic DNA hypomethylation in transformed cells and subsequent tumor incidence when the cells were injected into athymic nude mice. DNA methyltransferase (MTase) expression was also evaluated. DNA MTase enzyme activity in arsenic transformed cells was depressed by up to 40 percent after 18 wk As exposure. DNA MTase was not significantly reduced by acute As exposure (24 hr). The authors propose chronic As-induced DNA hypomethylation as a likely mechanism of carcinogenicity for arsenic.

In overview of the results presented above and summarized in Table 3, it is important to note that the large majority of authors interpreted their results as indicating an indirect genotoxic effect of the arsenicals studied in their test systems. The most common explanation was the generation of reactive oxygen species (e.g., Wang *et al*., 1996; Hei *et al*. 1998; Wang and Huang, 1994; Yamanaka *et al*., 1989; Fan *et al*., 1996). Another indirect mechanism was the known ability of arsenic to inhibit DNA repair enzymes or to react with sulfhydryl groups in (nucleo)proteins leading to genome instability (e.g., Li and Rossman, 1991; Lee *et al.* 1985, 1988; Gebel, 1998; Liu and Huang, 1997; Lee-Chen *et al*., 1994; Zhao *et al*., 1997). Indirect effects mediated by transcription factor binding have also been indicated (Hamilton *et al*., 1998). Alternatively, some results are more indicative of a direct effect of trivalent arsenicals on DNA (Mass *et al*., 2001). Yamanaka *et al.* (2001) have proposed a methylarsenic peroxy radical ($CH_3AsOO\bullet$) derived from DMA^{III} as a putative DNA damaging agent.

However, Nesnow *et al*. (2002) have provided evidence that reactive oxygen species (ROS) are significantly involved in MMA^{III} and DMA^{III}-induced DNA damage in vitro. These authors used a supercoiled phage ϕX174 DNA assay and the ROS inhibitors Tiron, melatonin, and the vitamin E analogue Trolox. Each of these agents was found to significantly inhibit the DNA-nicking activity of both MMA^{III} and DMA^{III} at low micromolar concentrations. Also the spin trap agent 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was observed to be an effective inhibitor of DNA damage by these trivalent arsenicals. The authors also identified a DMPO-hydroxyl radical adduct formed in the presence of DMA^{III}.

Developmental and Reproductive Toxicity

Information on the developmental and reproductive toxicity of inorganic arsenic is available mainly from animal studies using arsenite and arsenate salts and arsenic trioxide. Data from a number of studies show that arsenic can produce developmental toxicity including malformation, growth retardation, and death in hamsters, mice, rats, and rabbits. A characteristic pattern of malformations is produced and developmental toxicity is dependent on dose, exposure route and the point in the gestation period when exposure occurs. See Golub *et al.* (1998), DeSesso *et al.* (1998), and OEHHA (1992a) for overviews of the numerous individual studies.

A variety of arsenic compounds has been assessed for developmental toxicity. These studies have shown that prenatal death, congenital malformations and inhibition of growth can result from exposure during organogenesis to arsenite, arsenate, dimethylarsinic acid, or methane arsonate. However, generally such effects are seen only at dose levels that also result in maternal toxicity. The fetal effects found after maternal exposure are influenced by a variety of factors: the chemical form of the arsenic, the route and timing of the exposure, and species susceptibility.

The potential for maternal toxicity and embryotoxicity and teratogenic activity of inorganic arsenic varies with its valence state. Arsenite (As^{III}) is more acutely toxic to both the mother and embryo or fetus than arsenate (As^{V}). For example, in mice, the dose resulting in maternal and/or fetal toxicity, is 40-45 mg/kg sodium arsenite, compared to 120 mg/kg sodium arsenate. Following intraperitoneal (i.p.) injection, the effective dose is 10-12 mg/kg sodium arsenite and 40 mg/kg sodium arsenate. The differences in toxicity between arsenite and arsenate could be due in part to the considerably longer retention time of arsenite compared to arsenate. Methylation of arsenic greatly reduces its toxicity for both the mother and offspring (Hood *et al.*, 1982; Willhite, 1981).

The rat appears to be an exception. Rogers *et al.* (1981) assessed the developmental toxicity of dimethylarsinic acid in rats and mice by gastric intubation on days 7 through 16 of gestation. Rats were much more sensitive to this form of arsenic than mice. In the rat, maternal toxicity (decreased weight gain and increased mortality) was evident at doses of 40 mg/kg or greater. At 40 mg/kg decreased fetal weight and increased number of litters containing fetuses with skeletal anomalies also occurred. Increased fetal mortality was seen at 50 mg/kg or greater. The incidence of irregular palatine rugae significantly increased at 30 mg/kg or greater. However, the significance of this abnormality is not known. A discriminatory and auxiliary masticatory function has been proposed for the palatine ridges. It has also been suggested that the rugae may play a role in food transport in the rat during eating (Rogers *et al.*, 1981).

Hood and co-workers have conducted a series of studies examining the influence of route of exposure (oral vs. i.p. injection) of arsenite and/or arsenate toxicity in mice (Hood and Harrison, 1982; Hood *et al.*, 1977, 1978). Comparison of oral versus i.p. dosing of sodium arsenite indicates that an oral dose of 40-45 mg/kg is comparable to an i.p. dose of 10-12 mg/kg in terms of inducing maternal mortality. Expressed in terms of elemental arsenic, the equivalent doses would be 23-26 mg As/kg and 6-7 mg As/kg, respectively. Toxic values for sodium arsenate are: oral - 120 mg/kg and i.p. - 40 to 45 mg/kg. Equivalent doses are 29 mg As/kg and 10-11 mg As/kg, respectively.

The equivalent doses of arsenite and arsenate stated above result in comparable maternal mortality. However, in general slightly more severe effects (i.e., higher prenatal deaths and higher incidence of malformations) are observed in the embryo or fetus following i.p. versus oral exposure (Hood, 1983). Although not as extensively studied, experimental evidence from hamsters administered sodium arsenite support the observations made in mice. Oral intubation of 25 mg/kg or an i.p. injection of five mg/kg (i.e., 14.4 mg As/kg and 2.9 mg As/kg, respectively) produced similar embryotoxic effects (Hood and Harrison, 1982). In the latter study, no teratogenic effects were noted at the doses employed.

Several pharmacokinetic factors could account in part for these differences in toxicity. A much greater amount of total arsenic, as well as higher peak levels, reaches the fetus following maternal i.p. injection than after oral exposure (Hood, 1983). The degree of methylation is also higher following oral treatment than after injection. Data on organic forms of arsenic are insufficient to determine whether route of exposure significantly influences toxicity.

The period of greatest susceptibility to teratogenic effects in mice, rats or hamsters is early organogenesis, i.e., gestation days 8 through 10 (Hood, 1972; Burk and Beaudoin, 1977; Ferm and Hanlon, 1985). Hood and coworkers have compared the effects of single and multiple dosing during this period (Hood *et al.*, 1977). A single oral dose of 120 mg/kg sodium arsenate given to mice on day 9, 10, or 11 caused prenatal toxicity, whereas oral dosing of 60 mg/kg on each of three consecutive periods (days 7-9, 10-12, or 13-15) produced no effects compared to the control group (Hood *et al.*, 1977, 1978).

Holson *et al.* (2000) evaluated prenatal development in rats orally administered arsenic trioxide from 14 days premating to gestational day (GD) 19. Groups of 25 Crl:CD (SD)BR female rats were given doses of 0, 1, 2.5, 5, or 10 mg/kg-d by aqueous gavage. No effects on dam survival were observed. Clinical signs were observed at 2.5, 5, and 10 mg/kg, namely excessive salivation. Food consumption was reduced in the 10 mg/kg-d group throughout the study. At 10 mg/kg-d both body weight and weight gain were decreased relative to controls late in gestation. Gestational body weights and weight gains were unaffected at the lower doses. Mean fetal body weight was significantly reduced in the 10 mg/kg-d group compared to controls (3.0 vs. 3.5, $P < 0.01$). Over 300 fetuses per treatment group (22-24 litters) were assessed for external, visceral and skeletal malformations and variations. A total of 12 fetuses in treatment and control groups had malformations. No external variations were seen and the only visceral variation was a small spleen from a fetus in the five mg/kg-d group. Statistically significant increases in the incidence of several skeletal variations were seen at the 10 mg/kg-d level. The latter included unossified sternebrae #5 and/or #6 (22 percent per litter versus 6.6 percent per litter among controls), slight or moderate sternebrae misalignment (1.1 percent versus 0.0 percent), and seventh cervical ribs (6.8 percent versus 1.2 percent). These variations were considered to be due to developmental growth retardation.

Nemec *et al.* (1998) evaluated the developmental toxicity of inorganic arsenic in mice and rabbits. CD-1 mice (25/dose group) and New Zealand White rabbits (20/dose group) were gavaged with aqueous arsenic acid (H_3AsO_4) doses of 0, 7.5, 24, or 48 mg/kg-d on gestation days (GD) six through 15 (mice) or 0, 0.19, 0.75, or 3.0 mg/kg-d on GD six through 18 (rabbits). The animals were examined at necropsy (GD 18, mice; GD 29, rabbits). Treatment related maternal toxicity including mortality (2/25) was observed only in the highest dose administered to mice. Effects on maternal weight gain were noted only on GD 6-9 ($p < 0.01$) and GD 15-18 ($p < 0.05$) of the mid dose and on GD 6-9 ($p < 0.05$) of the low dose. While overall maternal weight gains were statistically significantly reduced only at the top dose, there was an apparent negative trend in decreased GD18 body weights with increasing dose (56.2 g control, 54.9 g, 52.7 g, 46.7 g, respectively). While the authors identified a NOAEL for maternal toxicity of 7.5 mg/kg-d, the apparent negative trend noted above suggests that this may be a LOAEL of 7.5 mg/kg-d (4.0 mg As/kg-d).

Hood (1998) injected mice with 1,200 or 1,500 mg/kg-d methanearsonic acid (MMA) or with 800 or 1,200 mg/kg-d dimethylarsinic acid (DMA) on gestation days 8 through 14. MMA and DMA are the primary metabolites of inorganic arsenic in most animals and humans. Both arsenicals induced prenatal mortality and malformations in the developing offspring following maternal treatment on single gestation days. However, the doses employed were extremely high and “in the maternally toxic range.”

Machado *et al.* (1999) studied the interactions between genotype and arsenic exposure in SWV/Fnn or C57BL/6J mice injected i.p. with 10 mg/kg sodium arsenite on gestation days 6.5 through 9.0. A dose response was conducted on the C57BL/6J strain and the effect of the splotch mutation on neural tube development, introduced with the male C57BL/6J *Sp*/+, was assessed. A single i.p. dose of 20 mg/kg sodium arsenite was lethal to all dams treated. A 15 mg/kg dose was not maternally toxic but was 100 percent embryolethal, with 36 percent embryolethality occurring at 10 mg/kg. A five mg/kg arsenite dose resulted in no difference compared with controls. The introduction of the splotch allele (Sp/+) significantly increased neural tube defects and other specific malformations versus the wild type (+/+) (e.g., spina bifida aperta 8.8 percent vs. 0 percent; and exencephaly 46.8 percent vs. 11.8 percent, both $P < 0.05$ at 10 mg/kg arsenite, fetuses from 22 litters). Thus, mutation in a single gene can increase sensitivity to As-induced birth defects.

Stump *et al.* (1999) evaluated the effects of single i.p. or oral gavage doses of sodium arsenate (As^{III}) or arsenic trioxide (As^{V}) administered on GD nine to groups of 25 mated female rats. Intraperitoneal administration of inorganic arsenic (4.8 mg As^{III}/kg or 7.6 mg As^{V}/kg) on GD nine increased the incidence of fetal malformations, especially exencephaly, microphthalmia/anophthalmia, and other craniofacial defects. Oral administration of up to 22.7 mg/kg As^{III}/kg did not increase the incidence of fetal malformations. Only at doses that caused severe maternal toxicity, including lethality, did i.p. administration of As^{III} cause neural tube and ocular defects. Oral administration of higher doses of As^{III} caused some maternal deaths but no treatment-related fetal malformations. This study reported oral NOAELs for maternal effects of <3.8 mg As^{III}/kg and for developmental effects of 15.2 mg As^{III}/kg.

To date developmental studies have been conducted in five species: hamster, mouse, rat, rabbit and sheep. The mouse has been the most commonly utilized animal model to evaluate arsenite and arsenate following oral and parenteral injection and organic arsenic by oral administration. All three forms of arsenic have been assessed by injection in the hamster, but only arsenite has been evaluated following oral administration. Fewer studies have been conducted in the rat; arsenate by injection, organic arsenic by oral administration, and arsenic trioxide via inhalation or administered in feed have been investigated in this species. Rabbits received arsenic acid (As^{V}) by gavage and sheep received potassium arsenate by oral capsule.

The relative sensitivity of induction of teratogenic effects in commonly studied species appears to be rabbits/hamsters > mice > rats. In comparisons of mice and hamsters, the two most frequently studied species, hamsters appear to be more sensitive to the teratogenic effects of arsenic following a single dose (Golub *et al.*, 1998). With multiple dosing throughout embryogenesis, rabbits appeared more sensitive than mice (WIL Laboratories, 1988a,b). A small study in three pregnant sheep administered 0.5 mg arsenate/kg-d for 45, 140 or 147 days resulted in normal lambs (James *et al.*, 1966).

The pattern of malformations varies between species. The most common defects associated with inorganic arsenic exposure in mice are exencephaly, micrognathia and open eye (Hood, 1972). Bent, shortened or missing tails, and rib abnormalities are also observed. Malformations similar to those exhibited in mice are also seen in hamsters. Urogenital abnormalities including renal hypoplasia and agenesis, as well as defects in uterine, ovarian and testicular morphology have also been reported in the hamster (Ferm, 1972). However, in the rat a preponderance of skeletal defects with renal agenesis and anophthalmia and a few exencephalies are observed (Hood *et al.*, 1977).

The extremely limited data on developmental toxicity of organic forms of arsenic indicate that the rat is the most sensitive species. Oral dose levels of 30 mg/kg dimethylarsinic acid (16.3 mg As/kg) or more resulted in a significant increase in the incidence of irregular palatine rugae. Higher dose levels, 40 mg/kg or more (21.7 mg As/kg), were associated with increased maternal and fetal toxicity and a significant increase in skeletal anomalies. In the mouse, a dose of 200-400 mg/kg was needed to elicit maternal and fetal toxicity. Hood (1998) found that both MMA and DMA produced prenatal mortality and malformations in the developing offspring of pregnant mice dosed on single gestation days. However, the doses were extremely high (1200-1500 mg As/kg) and in the maternally toxic range.

Single-dose studies in mice indicate a steep dose-response relation for developmental effects. Hood (1972) found a four-fold greater incidence of malformations with 12 mg/kg compared with 10 mg/kg injections of sodium arsenite on day 10 of gestation. Baxley *et al.* (1981) observed a two-fold greater incidence of embryolethality with 45 mg/kg compared to 40 mg/kg administered by gavage on day 10. Ferm and Hanlon (1985) studied dose-response relations in hamsters. Five doses of arsenate (150, 175, 200, 225, and 250 mg/mL Na arsenate) and four durations of exposure (6, 7, 8, 9 d) were administered to groups of 5-11 animals during embryogenesis. The study employed implanted minipumps containing sodium arsenate to minimize maternal toxicity and to achieve a more uniform dose over the exposure period than could be achieved by i.p. injection. Dose-response relations were observed for all endpoints. Both dose level and duration were important in determining fetal loss and growth retardation. Dose level was more important in determining the occurrence of malformation than cumulative dose.

Hanlon and Ferm (1986) studied the active agent and effective internal dose. Arsenic levels in the maternal blood peaked two days after implantation of a minipump containing 0.642 M arsenate. Of the arsenic in plasma, 69 percent was arsenate, 7 percent was arsenite and 26 percent was methylated arsenic. Hanlon and Ferm estimated that a concentration of 4.3 μmol As/kg maternal blood "poses a minimal, but real, teratogenic threat in the hamster" and that at an estimated blood level of 8.4 μmol As/kg blood, 51 percent of surviving fetuses are malformed.

The oral dose of As that would produce the minimally teratogenic dose level in hamster blood (4.3 μ*M* for 24 hr) was estimated by OEHHA to be 2.8 mg/kg-d of arsenate (OEHHA, 1992b). This value was based on a physiologically based pharmacokinetic (PBPK) model of arsenic disposition in the hamster. Unknown model parameters were fitted using the data of Yamauchi and Yamamura (1985) and the model was constructed with Stella v. 2.1 software (High Performance Systems, Inc., Hanover, NH). This dose is comparable to the dose of 4 mg As/kg-d as arsenic acid (As^V) for malformation (exencephaly with facial cleft) by gavage administration to pregnant mice (WIL Laboratories, 1988a). In rabbits a dose of two mg As/kg-d as arsenic acid (As^V) produced developmental toxicity by gavage administration (WIL Laboratories, 1988b), albeit with considerable maternal mortality at that dose.

Based on these data the hamster conceptus appears to be the most sensitive species to the developmental toxicity of arsenic. The minimum effective fetotoxic dose in hamsters is about 2.8 mg/kg-d based on the PBPK analysis of the study by Hanlon and Ferm (1986).

Animal studies have not shown an effect of arsenic on fertility in males or females. Dominant lethal mutation (DLM) studies in male mice and rats have been summarized by Golub *et al.* (1998). Orally administered sodium arsenite has been evaluated for its ability to induce DLMs (Sram and Bencko, 1974; Gencik *et al.*, 1977; Deknudt *et al.*, 1986), but no increases in dominant lethality were seen. However, when sodium arsenite was administered in conjunction with triethylene phosphoramide (TEPA), a known mutagen, the incidence of dominant lethality attributable to TEPA was significantly enhanced (Sram and Bencko, 1974). The sodium arsenite exposure levels used in this study were 10 and 100 mg As/L of drinking water. The lower dose did result in a slight synergistic elevation in DLM, although a significant increase was only observed at the 100 mg/L level.

Omura *et al.* (1996) found no effect of 1.3 mg/kg arsenic trioxide, administered intratracheally, on testes weight, epididymal weight, sperm count or sperm morphology.

A mouse multigeneration study of arsenic acid administered in feed at 0.53, 2.65, and 13.25 mg As/kg-d (Hazleton Laboratories, 1990) indicated no apparent effect in either generation on the male fertility index. In the high dose group, litter size was lower than controls for both generations (8 vs. 11 pups) being significant in the F_2 generation. The viability and weaning index were also significantly affected in the F_0 dams, and the weaning index was significantly affected in the second generation. These results appear to indicate an effect on the viability of the conceptus and pups. Reproductive indices (mating, female fertility, gestation indices) were not affected even at doses that produced systemic toxicity.

The only other inorganic form of arsenic investigated for its potential reproductive toxicity is arsenic trioxide (Bencko *et al.*, 1968). In this study on hairless mice, histopathological effects on the testes were only seen at levels that caused overt general toxicity.

The only organic form of arsenic that has been evaluated for male reproductive toxicity is monosodium methanearsonate (MSMA) (Prukop and Savage, 1986). Male ICR Swiss mice were dosed orally every other day (i.e., 3 times per week) with either water or 119 mg/kg MSMA for a period of 19 days (9 doses). The average daily dose would be 56.4 mg MSMA/kg-d (i.e., 26 mg As/kg-d). Males were placed with untreated females during the exposure period. The pregnancy rate among females mated with treated males was significantly lower than among those mated with control males (50 percent versus 90 percent). No differences in litter size, litter weight, or frequency of stillbirths were seen.

Based on these animal studies it would appear unlikely that environmental levels of arsenic exposure would be sufficient to cause any developmental or reproductive effects in exposed humans. This conclusion has been drawn in two recent articles on arsenic developmental toxicity (Jacobsen *et al*., 1999; Holson *et al*., 2000). However, a few epidemiological studies have indicated possible causal associations between arsenic exposures and adverse effects such as fetal and infant mortality, neuropsychological development and IQ (see Effects in Humans below).

Immunotoxicity

Single inhalation exposures of mice to arsenic trioxide (0.94 mg As/m^3) led to increased susceptibility to respiratory bacterial pathogens, apparently via injury to alveolar macrophages (Aranyi *et al.*, 1985). Sikorski *et al.* (1989) observed a decreased humoral response to antigens and decreases in several complement proteins in mice given 5.7 mg As/kg sodium arsenite intratracheally. No evidence of immunosuppression was detected in mice exposed orally to arsenate at levels up to 100 ppm (20 mg As/kg-d) (Kerkvliet *et al.*, 1980). Sakurai *et al.* (1998) observed that inorganic arsenicals, arsenite and arsenate, were strongly toxic to mouse peritoneal or alveolar macrophages *in vitro* with IC_{50} values of 5 μM and 650 μM, respectively. These inorganic arsenicals caused necrotic death (80 percent) with partially apoptotic cell death (20 percent). They also induced a marked release of inflammatory cytokine, tumor necrosis factor α (TNFα), at cytotoxic doses. In contrast, the cytotoxic effects of methylated arsenic compounds were lower that those of the inorganic arsenicals. The IC_{50} value of DMA was about 5 mM, and MMA and TMAO had no toxicity at concentrations of 10 mM. In addition, these methylated compounds suppressed the TNFα release from macrophages. DMA induced mainly apoptotic cell death but appeared to operate by a different mode of action than the inorganic arsenicals. In a separate study Sakurai *et al.* (1997) observed weak but significant cytotoxicity for the arsenosugar (*R*)-(2', 3'-dihydroxypropyl)-5-deoxy-5-dimethylarsinoyl-β-D-riboside, a seaweed component. This compound had an IC_{50} value of 8 mM for alveolar macrophages.

Lantz *et al.* (1994) observed adverse effects of inorganic arsenic exposure on alveolar macrophage function in rats. One day after intratracheal instillation of 1 mg As/mL (either sodium arsenate or sodium arsenite) to male Sprague-Dawley rats, lavaged pulmonary alveolar macrophages (PAM) showed a significant increase in superoxide and decreases in basal and lipopolysaccharide (LPS)-induced release of TNF-α. Lavaged PAM from control and test animals was exposed *in vitro* to concentrations of the arsenicals from 0.1 to 30 μg As/mL. Significant dose-dependent inhibition of superoxide production was evident only after 24 hr exposure. Arsenite was effective at concentrations as low as 0.1 μg As/mL compared to 1.0 μg As/mL for arsenate. Suppression of LPS-induced release of TNF-α also occurred at lower concentrations of As III than of As V. Arsenate caused inhibition of LPS-induced PGE_2 production above 1.0 μg As/mL whereas arsenite had no effect on PGE_2 production. Overall, As-induced alterations of PAM function may compromise host defense against infection and alter immune surveillance.

It is uncertain what effect, if any, arsenic-induced immunotoxicity may have on other arsenic endpoints, specifically cancer.

Neurotoxicity

Neurological effects were not reported in chronic oral studies in dogs or monkeys exposed to arsenate or arsenite (Byron *et al.*, 1967; Heywood and Sortwell, 1979). The NOAEL in the two yr dog study was 3.1 mg As/kg-d and in the one yr monkey study, 2.8 mg As/kg-d. Some organic arsenicals such as phenyl arsenates may be neurotoxic at high doses. In pigs subchronic oral exposure to roxarsone (0.87-5.8 mg As/kg-d for one month) caused muscle tremors, partial paralysis, and seizures (Edmonds and Baker, 1986; Rice *et al.*, 1985). A time dependent degeneration of myelin and axons in the spinal cord was also observed histologically (Kennedy *et al.*, 1986). These signs were not seen in rats exposed to roxarsone but rather hyperexcitability, ataxia, and trembling at the highest dose of 11.4 mg As/kg-d were observed (NTP, 1989).

Meija *et al.* (1997) studied the effects of lead-arsenic combined exposure on central monoaminergic systems in the mouse brain. Lead acetate (116 mg/kg-d), sodium arsenite (11 or 13.8 mg/kg-d), a lead-arsenic mixture (116/13.8), or vehicle controls were given to groups of 16-20 male BALB/c mice by gastric intubation during 14 days. Regional brain concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3 acetic acid (5-HIAA), As, and Pb were measured. Arsenic alone caused regional increases in DOPAC, DA, 5-HT or their metabolites and a decrease in NE. Arsenic combined with lead provoked significant changes in all three monoamines and their metabolites similar to As alone. The mixture also provoked a 38 percent decrease of NE in the hippocampus and increases of 5-HT in midbrain and frontal cortex (100 and 90 percent, respectively) over control values, alterations that were not elicited by either metal alone. The doses in this study were quite high, close to the LD_{10} for arsenite. Nevertheless, this study demonstrates the interaction of two common environmental contaminants and the need for additional testing of chemical mixtures.

Delgado *et al.* (2000) evaluated the effects of arsenite in drinking water on central monoamines and plasma levels of adrenocorticotropic hormone (ACTH) in mice. Groups of 12 male BALB/c mice per group received water containing 0, 20, 40, 60, or 100 ppm As as sodium arsenite. The average intakes were 0, 4, 8, 12, or 20 mg As/kg-d. Nine weeks exposure to As-containing drinking water did not result in statistically significant body weight loss or apparent signs of toxicity. All treatment groups had significantly higher brain As levels compared with controls ($P < 0.001$). Plasma ACTH level increased significantly only in the 20 ppm dose group (91.2 vs. 343 pg/mL, $P < 0.05$). ACTH plasma levels were significantly correlated with NE concentrations in the medulla and pons, but not with hypothalamic NE levels. The results show that chronic arsenic exposure produces changes in central monoamines that are not associated in a dose-dependent manner with changes in plasma ACTH. The authors note that the basis of arsenic effects on the central neurochemistry is not well defined and they speculate that the sulfhydryl-binding and reactive oxygen generating properties of As may be involved.

Chattopadhyay *et al.* (2002) observed arsenic-induced changes in growth development and apoptosis in neonatal and adult brain cells in vivo and in tissue culture. Sodium arsenite was administered in drinking water at 0, 0.03, 0.3, and 3.0 ppm for 20 days to pregnant rats (5 animals/dose group). The high dose level of arsenite led to a loss of gestation by 20 percent and to neonatal death of 25 percent. Both adults (postgestational) and neonatal rats were evaluated for spontaneous behavior and both groups exhibited a dose-dependent reduction of activity at the highest dose of 50 and 70 percent, respectively. Postgestational and neonatal rat brain explants were cultured in medium with and without 0.3 ppm arsenite. The control explants showed signs of viability, outgrowth of cells, development of neuronal processes and establishment of confluence and networking. By contrast the treated explants showed reduced growth, loss of ground matrix, and inhibition of neural networking. The viability of cells was measured over 18 days in culture using Trypan Blue exclusion. The viability was defined as the ratio of unstained cells to the total number of cells times 100. The control postgestational and neonatal cell viability declined from 80 to 55 percent and from 90 to 60 percent, respectively. The arsenite exposed cells exhibited greater overall reductions in viability over the 18 days of 80 to 50 percent and 70 to 50 percent, respectively.

A parallel experiment in human fetal brain explants with the same in vitro exposure exhibited a reduction in viability of 90 to 65 percent for controls and 90 to 55 percent for arsenite-treated cells. Cells in culture were analyzed for release of NO and reactive oxygen species (ROS). Both human fetal and neonatal rat brain cells were observed to release significantly more NO and ROS when maintained in culture with 0.3 ppm arsenite. Both human and rat cells cultured in 0.3 ppm arsenite also showed decreased DNA and protein synthesis by incorporation of ^{3}H-thymidine and ^{14}C-leucine versus controls. Morphometric analyses indicated that the human fetal brain explants exposed to arsenic showed apoptotic changes in both isolated and associated cells. This was characterized by inhibition of growth, loss of ground matrix, frothing of cytoplasm, vacuolation, nuclear condensation, fragmentation, and final loss of the cell. The results indicate that arsenic toxicity may induce damage to brain cells prior to more visible clinical signs. Also the experiments carried out with the rat neonatal brain where the tissue was exposed to arsenic through the entire embryonic development exhibited the potential of growth and development with signs of oxidative stress, loss of protein content in synaptosomes and apoptosis.

None of the animal neurotoxicity studies described here appears suitable for quantitative risk assessment.

Biochemical and Cellular Toxicity

Ochi (1997) studied the mechanisms associated with As-induced increases in glutathione (GSH) levels in cultured Chinese hamster V79 cells. Arsenite at a concentration of 5 μM caused a marked increase of GSH at eight hr after addition. The GSH increase was associated with an increase in cystine uptake into the cells, but not with increased γ-glutamylcysteine synthetase (γ-GCS) activity. DMA (0.2-5 mM) also caused an increase in the GSH level in a time- and concentration-dependent manner. This was accompanied by an increase in γ-GCS activity and cystine uptake. DMA caused a reduction in the rate of utilization of cysteine for protein synthesis while enhancing its utilization for GSH synthesis. MMA was not effective in causing an increase in GSH level.

Repetto *et al.* (1994) investigated the effects of inorganic arsenic on mouse neuroblastoma cells *in vitro*. Actively dividing Neuro-2a cell cultures were exposed for 24 hr to varying concentrations of arsenite or arsenate. Arsenite exposure resulted in a marked dose-dependent inhibition of cell proliferation, determined by total protein content, with an EC_{50} value of 10 µM. Inhibition of relative neutral red uptake was not seen up to 77 µM (EC_{50} =28 µM) and cytoplasmic membrane permeability to lactate dehydrogenase (LDH) was increased only at high concentrations, EC_{50} = 54 µM. However, hexosaminidase (HEX) secretion was a sensitive marker for As exposure (EC_{50} = 0.09 µM). Metabolic function was impaired, with inhibition of LDH activity (up to 65 percent) at 0.8 µM (EC_{50} = 0.4 µM). Succinate dehydrogenase (SDH) activity inhibition did not occur at levels less than 8 µM (EC_{50} = 46 µM) and HEX activity was nearly completely inhibited at 0.8 µM (EC_{50} = 0.25 µM). Acetylcholinesterase (AChE) was also inhibited in a concentration-dependent manner at 1 µM As^{III} and above, EC50 = 18 µM. Arsenate was less toxic than arsenite and inhibition of cell proliferation occurred at higher concentrations (EC_{50} = 54 µM), albeit with a similar dose-response slope. Relative neutral red uptake, LDH leakage, and HEX release were all less affected, with EC_{50} values of 0.17, 2.5, and 2.7 mM As^{V}, respectively. LDH activity was significantly inhibited by As^{V}, EC_{50} = 1.7 µM. However, both HEX and SDH activities were increased by As^{V}. AChE was inhibited at high concentration, EC_{50} = 323 µM.

Ochi *et al.* (1998) evaluated the effects of arsenite and DMA on cell morphology, cytoskeletal organization, and DNA synthesis in cultured Chinese hamster V79 cell *in vitro*. DMA (2 mM) caused mitotic arrest (43 percent increase in mitotic index) and induction of multinucleated cells with a delay of 12 hr relative to mitotic arrest. Arsenite (5 µM) was less effective than DMA in causing mitotic arrest (ca.15 percent increase in mitotic index) and in inducing multinucleated cells. The mitotic arrest caused by DMA was accompanied by disruption of the microtubule network. However, neither DMA nor arsenite caused disorganization of actin stress fibers, even at concentrations that caused marked growth retardation (up to 18-25 percent versus control cells). Earlier work in Swiss 3T3 cells indicated that low levels of arsenite (2.5 µM) caused loss of actin filaments and only higher concentrations (20 µM) caused loss of microtubules (Li and Chou, 1992). Exposure of cells to arsenite for six hr caused concentration-dependent inhibition of DNA synthesis (2-10 µM) (measured by uptake of radioactive thymidine by the cells). DMA exposure for six hr did not affect DNA synthesis. When incubations were extended to 18 hr with these compounds, the effect of As^{III} on DNA synthesis was mitigated at low concentrations (2-5 µM), whereas DMA now caused concentration dependent inhibition of DNA synthesis (2-10 mM).

When large concentration differences are reported for biological effects caused by inorganic arsenic species vs. methylated species, often 2-3 orders of magnitude (see also Tables 4 and 5 below), it is possible that the methylated species effects could result from inorganic arsenic impurities on the order of 0.1 to 1 percent. Alternatively, the methylated species may give lower values for kinetic reasons. For example, the MMA^{V} reductase has a high m*M* Km value and conversion to the more toxic MMA^{III} will only occur at comparatively high concentrations (Zakharyan *et al.*, 2001).

Shimizu *et al.* (1998) examined the relationship among GSH, metallothioneine (MT) gene expression, and arsenic-induced toxicity or c-myc expression in cultured rat myoblast (L6) cells. *In vitro* exposure of L6 cells to L-buthionine sulfoximine (BSO) (1 to 25 μM) resulted in dose-dependent decreases in GSH. GSH depletion sensitized cells to both arsenite and arsenate. Zinc pretreatment, at levels that highly activated MT expression, had no effect on arsenite-induced cytotoxicity. Arsenite (1 μM) alone modestly increased c-*myc* expression from one to four hours after treatment to a maximum of 2-fold over the control. After GSH depletion cells responded to arsenite exposure with larger increases in c-*myc* transcription (3.2-fold over control). The authors concluded that cellular levels of GSH, but not MT gene expression, play an important role in resistance to As toxicity and aberrant gene activation. Depletion of cellular GSH enhances arsenic-induced proto-oncogene activation, possibly contributing to subsequent cell transformation.

Styblo *et al.* (2000) evaluated the comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. The compounds tested were sodium arsenite (As^{III}) and arsenate (As^{V}), MMA^{V}, DMA^{V}, methylarsine oxide ($MAs^{III}O$) and iododimethylarsine ($DMAs^{III}I$), and the glutathione complex $DMAs^{III}GS$. The cytotoxicities of the arsenicals were examined in primary cultures of rat hepatocytes exposed to 0, 0.4, 1, 4, 10, or 20 μM for 24 hr. Pentavalent arsenicals (As^{V}, MMA^{V}, DMA^{V}) were not cytotoxic at concentrations up to 20 μM. However, trivalent arsenicals decreased cell viability in a concentration-dependent manner. Exposure to 10 μM As^{III} reduced the rate of MMT (thiazolyl blue) conversion to formazan by mitochondrial dehydrogenases by 40 percent. Exposure of cells to four μM $MAs^{III}O$ or 10 μM $DMAs^{III}I$ resulted in almost complete inhibition of conversion. Dramatic changes in cell morphology were also observed, including rounding and shrinking of cells and granulation of the cytoplasm. $DMAs^{III}GS$ was less cytotoxic than $DMAs^{III}I$. No toxic effects were seen in cells treated with up to 20 μM KI.

Kaltreider *et al.* (2001) evaluated the effects of arsenite on the biochemical function of the glucocorticoid receptor (GR) in hormone-responsive H4IIE rat hepatoma cells. Non-cytotoxic arsenite treatments (0.3-3.3 μM) significantly decreased dexamethasone-induced expression of transiently transfected luciferase constructs containing either an intact hormone-responsive promoter from the mammalian phosphoenolpyruvate carboxykinase (PEPCK) gene or two tandem glucocorticoid response elements (GRE). Arsenite pretreatment did not block normal dexamethasone-induced nuclear translocation of GR. The results suggest that arsenite can interact directly with GR complexes and selectively inhibit GR-mediated transcription, which is associated with altered nuclear function. Glucocorticoids induce a number of cellular and physiological effects that are mediated mainly through their interaction with the cytosolic steroid receptor GR. GR is a member of the nuclear superfamily, it mediates glucose homeostasis, immune modulation, cellular growth and differentiation and other responses in a variety of tissues. GR is normally sequestered in a preactive state in the cytosol, bound in a complex that includes multiple heat shock proteins. Upon steroid binding, GR conformation is altered leading to the translocation of the ligand-bound GR to the nucleus in a form that can interact with DNA. Once in the nucleus, GR binds to its DNA recognition element, GRE. As-induced alterations in GR function may play a role in the mechanism of arsenic carcinogenesis since GR mediates the suppression of tumor promotion in skin and lung by suppressing cell growth and inducing differentiation. Since the mechanism by which arsenite inhibits GR-dependent transcription appears to involve nuclear events, it may represent a new class of endocrine disruptors that alter receptor function rather than compete for hormone binding sites. It is unknown whether arsenic has similar effects on other members of the steroid receptor family, such as the estrogen and progesterone receptors.

While few of the *in vitro* studies above in animal cells have been conducted in human counterparts, the results of Styblo *et al*. (2000) in rat and human hepatocytes were similar (see Effects in Humans below). This suggests that experimental *in vitro* studies in animal systems may be useful in elucidating mechanisms of As toxicity that are relevant to human toxicity and risk assessment.

Chronic Toxicity

Most of the animal studies of chronic duration have focused on the cancer endpoint, and are discussed in the next section. Byron *et al*. (1967) studied the chronic effects of inorganic arsenic in two-year studies in rats and dogs.

Osborne-Mendel rats, 25 per sex per dose group, were fed sodium arsenite at 0, 15.63, 31.25, 62.5, 125, and 250 ppm As or sodium arsenate at 0, 31.25, 62.5, 125, 250, and 400 ppm As, both in commercial diet. Increased mortality was observed at one year at the high dose of both compounds: arsenite - seven percent vs. two percent in control; arsenate eight percent vs. zero percent in controls. At the highest dose levels with either compound (250 and 400 ppm) body weights were depressed throughout the experiment compared to controls. At doses of 31.25 ppm and above, body weight gain appeared to be depressed in females with either As treatment. With males body weight gain was depressed at ≥62.5 ppm arsenite and at ≥125 ppm arsenate. The highest dose of sodium arsenite showed a slight decrease in hemoglobin at three and 11 months and in hematocrit at 11 months in females only. No other blood effects were seen at lower doses. Sodium arsenate at the highest dose showed a slight elevation in leukocyte count in females over two years and in males in the first year. No differences in organ weights were seen. At the highest doses of both compounds there was an enlargement of the common bile ducts. The total number enlarged were: arsenate 400 ppm, 42; arsenate 250 ppm, 25; arsenite 250 ppm, 45. The enlargements were graded on a scale from one to greater than seven mm duct diameter, with one mm considered the normal duct diameter. The chief microscopic lesion was a thickening of the wall of the enlarged common bile duct to about one mm vs. normal thickness of 0.1 mm. This thickening was due to slight to moderate fibrosis, occasionally including infiltration with inflammatory cells. Based on body weight gain depression in this study a chronic LOAEL for arsenate was 31.25 ppm (1.5 mg As^{V}/kg-d) and a chronic NOAEL for arsenite was 31.25 ppm (1.6 mg As^{III}/kg-d).

Beagle dogs, three per sex per dose group, were fed sodium arsenite or sodium arsenate at 0, 5, 25, 50, or 125 ppm in the diet for two years. At the highest sodium arsenite dose level four dogs died after 3-9 months, one after 19 months, and the remaining dog was found moribund and was sacrificed at eight months. All the high dose dogs showed weight loss of 44 to 61 percent. Anorexia and listlessness were the only clinical signs noted. A slight to moderate anemia was observed in the high dose arsenite dogs. The surviving treated dogs (≤50 ppm) appeared indistinguishable from the controls. No systemic treatment-related effects were observed for sodium arsenate treated dogs except for body weight depression and a mild anemia at the high dose level. One female death at the high dose at 13.5 months was considered treatment related. Based on body weight depression and excess mortality in this study, a chronic NOAEL for arsenate was 50 ppm (1.25 mg As^{V}/kg) and for arsenite was 50 ppm (1.25 mg As^{III}/kg-d).

Schroeder and Balassa (1967) observed increased mortality in CD mice (54/sex/dose group) administered five ppm arsenite in drinking water for 18 months. A companion study in Long-Evans rats (50/sex/dose group, Schroeder *et al*., 1968) also at five ppm arsenite in drinking water until natural death (52 months), showed no adverse effects. Extensive arsenic accumulation was observed in all tissues, particularly aorta, red blood cells, liver, lung, and spleen.

The studies of Byron *et al*. (1967) in rats and dogs might be suitable for quantitative risk assessment.

Carcinogenicity

The human carcinogenicity of arsenic has been established by epidemiological evidence. However, bioassays in animals have not yet convincingly demonstrated arsenic carcinogenicity, although effects have been noted in some studies. A variety of arsenic compounds has been examined for carcinogenic activity: arsenic trioxide (As_2O_3), potassium arsenite ($KAsO_2$), sodium arsenite ($NaAsO_2$), sodium arsenate ($Na_2HAsO_4 \bullet 7H_2O$), and lead arsenate ($PbHAsO_4$). Two organic forms of arsenic have also been assessed: arsanilic acid ($C_6H_4NH_2AsO(OH_2)$) and dimethylarsinic acid ($C_2H_6AsO_2H$). Several of the arsenic compounds listed above have also been assessed in combination with various initiating and/or promoting agents.

Arsenic trioxide (As_2O_3) carcinogenicity has been evaluated in rats and mice (Hueper and Payne, 1962; Baroni *et al*., 1963; Knoth, 1966; Rudnai and Borzsonyi, 1981). No increase in tumor incidence was seen in two of the four studies. This observation was also true whether arsenic trioxide was given orally or applied dermally in combination with an initiator or promoter (i.e., DMBA, urethane or croton oil). The third study (Knoth, 1966) did report an increase in tumor incidence in treated animals as reviewed by IARC (1980) and U.S. EPA (1984). Thirty mice were given orally one drop of a drug (Psor-Intern or Fowler's solution) containing arsenic trioxide once a week for 5 months. The total dose (as calculated by the author) was 7 mg As_2O_3 per animal. A higher incidence of adenomas of the skin, lung, peritoneum, and lymph nodes occurred at 14 months when compared to concurrent controls. No tumors were found in 15 control mice. The description of the study design, analysis, and results were very brief and incomplete, precluding critical assessment of this study.

In the fourth study (Rudnai and Borzsonyi, 1981), arsenic trioxide was administered by subcutaneous (s.c.) injection to pregnant mice and then postnatally to their offspring (as reviewed by U.S. EPA, 1984). Offspring thus exposed were reported to have significantly elevated lung tumor incidence rates at 1 year of age. The study description was very limited; for example, the authors did not report whether the tumors observed were malignant. Moreover, data generated after administration by s.c. injection may not be relevant to humans exposed orally or by inhalation.

The carcinogenic potential of potassium arsenite ($KAsO_2$) has been examined by only one investigator (Boutwell, 1963). Potassium arsenite was administered orally in conjunction with dermal exposure to an initiator, DMBA, and a promoter, croton oil. Significant body weight depression occurred, but the results did not indicate any carcinogenic response. In another experiment, potassium arsenite was applied directly to the skin in conjunction with the initiator and/or promoter. However, no significant alteration in tumor incidence was seen. It should be noted that the mouse strain utilized in this study was skin tumor susceptible (STS).

Sodium arsenite ($NaAsO_2$) has been the most extensively studied arsenic compound. Its carcinogenicity has been evaluated in rats, mice and dogs (Byron *et al.*, 1967; Kanisawa and Schroeder, 1967, 1969; Schrauzer and Ishmael, 1974; Schrauzer *et al.*, 1978; Shirachi *et al.*, 1983; Blakeley, 1987b). Sodium arsenite did not exhibit carcinogenic activity when given in drinking water or in the diet at exposure levels ranging from 2 to 250 ppm arsenic. The tumor incidence actually decreased compared to controls in mice exposed to 5 to 10 ppm arsenic (Kanisawa and Schroeder, 1967; Schrauzer and Ishmael, 1974). Weight depression did occur at these levels (Kanisawa and Schroeder, 1967). One of the mouse strains employed, C3H/St, exhibits a very high spontaneous mammary tumor incidence. When sodium arsenite was administered to this strain in drinking water at the 10 ppm arsenic level, the spontaneous tumor rate decreased. However, the growth rate of those tumors that did develop was much higher than in controls (Schrauzer and Ishmael, 1974). Tumor growth rate was monitored as change in tumor volume over time. Whether growth was the result of hypertrophy or a hyperplastic response is not known. The tumors also had a greater tendency to metastasize. At a lower concentration of sodium arsenite (2 ppm arsenic) the tumor incidence was no different to that of the concurrent controls (Schrauzer *et al.*, 1978). The length of time to tumor onset, however, was apparently doubled. The tumor growth stimulation was also evident at this concentration.

The carcinogenic activity of sodium arsenite administered in combination with other known carcinogenic agents has been investigated (Shirachi *et al.*, 1983; Blakeley, 1987). When a high level of arsenic (160 ppm in drinking water) as sodium arsenite was given to rats in conjunction with an i.p. injection of diethylnitrosamine (DENA), a significant increase in kidney tumors was observed compared to DENA alone (Shirachi *et al.*, 1983). No tumors were observed in the control or arsenic-alone groups. All animals utilized in this study were partially hepatectomized. Arsenic treated animals consumed 40 percent less water than controls. How these factors may have affected the incidence of tumor formation is unknown.

In another study, which utilized the carcinogen urethane as the initiating agent, exposure to sodium arsenite in drinking water resulted in a dose-related decrease in tumor incidence and tumor size (Blakeley, 1987). Similar experimental results have been produced with sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$). When administered orally, sodium arsenate did not exhibit carcinogenic activity (Byron *et al.*, 1967; Kroes *et al.*, 1974; Blakeley, 1987). When given orally concurrently with injected urethane, sodium arsenate, like sodium arsenite, had a protective effect in that the tumor incidence and tumor size was significantly reduced (Blakeley, 1987).

Sodium arsenate carcinogenicity has also been evaluated after administration by injection (Oswald and Goerttler, 1971) as reviewed by U.S. EPA (1984) and IARC (1980). Pregnant mice were injected subcutaneously throughout pregnancy (total of 20 injections) with 0.5 mg As/kg as a 0.005 percent aqueous solution of sodium arsenate. Forty-six percent of the treated mothers developed leukemia or lymphomas whereas the incidence in the control animals was zero. Subgroups of the offspring from treated mothers were treated with 0 or 0.5 mg As/kg by s.c. or i.v. injection once a week for 20 weeks. An increased incidence of lymphocytic leukemia or lymphomas was observed in all offspring from treated mothers. The highest incidence of leukemia or lymphomas occurred in those offspring that were administered arsenic intravenously. The results of this study are difficult to interpret since some of the animals from the various treatment groups were still alive, and therefore, had not been evaluated at the time results were published (Oswald and Goertler, 1971) as reviewed by U.S. EPA (1984) and IARC (1980). In addition, the parenteral route of administration may not be applicable to human environmental exposure to arsenic in drinking water.

The only other inorganic form of arsenic evaluated for carcinogenic activity is lead arsenate ($PbHAsO_4$) (Kroes *et al.*, 1974). Rats were fed 0, 463, or 1,850 ppm lead arsenate in the diet for 27 months. Food intake, body weight, and survival were significantly affected at the 1,850 ppm level. No significant alteration in benign or malignant tumor incidence was seen.

A recent report by Waalkes *et al*. (2003) describes a transplacental carcinogenicity assay of inorganic arsenic. Groups of 10 pregnant C3H mice received drinking water containing 0, 42.5 and 85 ppm arsenite *ad libitum* from gestation day 8 to 18. The offspring were weaned and put into gender-based groups (N = 25) according to maternal exposure. Male survival and body weights were affected by arsenic exposure and the study was limited to 74-weeks. Female mice were less affected and the study was carried out for the full 90-week period. The offspring received no additional arsenic treatment. At study termination male offspring showed a marked dose-dependent increase in hepatocellular carcinoma (control, 12 percent; 42.5 ppm, 38 percent; 85 ppm, 61 percent; trend P = 0.0006) and in liver tumor multiplicity (tumors/liver, 5.6-fold over control at 85 ppm; trend P < 0.0001). A dose-dependent increase in adrenal tumor incidence and multiplicity (2.2-fold) was also seen (tumors: control, 38 percent; 42.5 ppm, 67 percent; 85 ppm, 91 percent; trend P = 0.001). In female offspring, dose-dependent increases occurred in ovarian tumors (control, 8 percent; 42.5 ppm, 26 percent; 85 ppm, 38 percent; trend P = 0.015) and in uterine proliferative lesions (hyperplasia + tumors; control, 16 percent, 42.5, 56 percent; 85 ppm, 62 percent; trend P = 0.001). Lung carcinoma was seen in female offspring (control, 0 percent; 42.5 ppm, 4 percent; 85 ppm, 21 percent; trend P = 0.0086). Oviduct proliferative lesions were seen in female offspring (4, 13, 29 percent, respectively; trend P = 0.0145).

This study shows that inorganic arsenic exposure of pregnant mice during the later stage of gestation induces a variety of tumors in the resulting offspring. The tumors, including aggressive epithelial malignancies of liver and lung, occurred in a dose dependent manner without any treatment other than prenatal inorganic arsenic.

Rossman *et al*. (2002) have reported a UV radiation (UVR)-arsenite model in hairless mice. Two groups of 15 hairless but immunocompetent female Skh1 mice were given 0 or 10 mg/L sodium arsenite (5.8 ppm arsenite) in drinking water and irradiated with a low (nonerythemic) 1.7 kJ/m^2 solar UVR dose three times per week. After 26 weeks the irradiated mice given arsenite had a 2.4-fold increase in skin tumors compared to the irradiated control mice (127 vs. 53 tumors, respectively, $P < 0.01$ by Fisher's exact test). The tumors were mostly squamous cell carcinomas but those in the arsenite treated mice were larger and more invasive than seen in the controls (50 percent vs. 26 percent, respectively). The tumors appeared only in mice that received UVR, and only on the exposed areas (backs) of the animals. Times to first tumor ranged from about 55 to 130 days for the arsenite treated mice vs. 85 to 175 days for the irradiated controls. These results are interesting but need to be repeated with multiple doses to properly assess tumor incidence-dose and time to tumor-dose responses

The carcinogenic activity of two forms of organic arsenic has been investigated (Boutwell, 1963; Innes *et al.*, 1969). Arsanilic acid ($C_6H_8NH_2AsO(ON)_2$) has only been assessed in combination with DMBA and croton oil (Boutwell, 1963). STS mice were utilized in this study. The incidence of papillomas and carcinomas was not significantly different regardless of whether or not the exposure regimen included arsanilic acid. The dimethylarsinic acid form ($C_2H_6AsO_2H$) of arsenic has also been assessed (Innes *et al.*, 1969). Mice were exposed from 7 days to 18 months of age. No significant carcinogenic activity was observed.

Yamamoto *et al.* (1995) observed that dimethylarsinic acid (DMA) significantly enhanced the tumor induction in the urinary bladder, kidney, liver, and thyroid in rats pretreated with five carcinogens. Twenty male F344/DuCrj rats were used per group. Pretreatment with diethylnitrosamine (DEN), N-methyl-N-nitrosourea (MNU), N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), 1,2-dimethylhydrazine (DMH), and N-bis (2-hydroxypropyl) nitrosamine (DHPN) was conducted during the first four weeks of the study. This was followed by no further treatment (control group), DMA administration at 50, 100, 200, or 400 ppm in drinking water per group on weeks 6 through 30, or DMA administration without pretreatment at 100 or 400 ppm in drinking water on weeks 6 through 30. At 400 ppm the increases in tumor incidences at week 30 were: bladder, 80 percent; kidney, 65 percent; liver, 65 percent, and thyroid, 45 percent. Urinary bladder carcinogenesis was strongly enhanced by DMA even at the lowest dose level of 50 ppm. Tumor inductions in the kidney and thyroid gland were moderately enhanced by DMA in a dose dependent manner. Strong enhancement of the liver tumor induction was seen at 400 ppm. This study indicates that DMA may act as a carcinogen or promoter for urinary bladder, kidney, liver, and thyroid gland. However, due to the complexity of the protocol and high doses employed, the study results are difficult to interpret.

Wanibuchi *et al*. (1996) observed an increased incidence of urinary bladder tumors in rats at DMA doses of 25 mg/L and higher following pretreatment with (BBN). The rats were administered DMA in drinking water at 0, 2, 10, 25, 50, and 100 mg/L for 32 weeks. Slight effects were seen at 10 mg/L but no effects were seen at 2 mg/L.

Li *et al* (1999) administered DMA at 100 mg/L in drinking water for 32 weeks following four weeks pretreatment with BBN and observed a similar incidence of bladder tumors in NCI-Black Reiter male rats and in F344 male rats. The NCI-Black-Reiter strain does not produce or excrete $alpha_{2u}$-globulin in the urine. The results suggest that this protein is not involved in DMA-induced bladder cancer in rats.

Wei *et al*. (1999, 2002) conducted a two-year bioassay with DMA in F344 rats. Four groups of male rats (N = 36) were administered 0, 12.5, 50, or 200 ppm DMA, respectively, in drinking water for 104 weeks. From 97 to 104 weeks urinary bladder tumors were found in 0/36, 0/33, 8/31, and 12/31 animals, respectively. Preneoplastic lesions, papillary or nodular hyperplasias, were seen in 12/31 and 14/31 of the mid and high dose animals, respectively. DMA and TMAO were the predominant metabolites detected in urine, with small amounts of MMA^{III} and tetramethylarsonium (TeMa). Significantly increased BrdU labeling indices were seen in apparently normal bladder epithelium at the mid and high doses. Mutation analysis of DMA-induced rat urinary bladder tumors showed a low rate of H-ras mutations (2/20). No alterations were seen in p53, K-ras, or beta-catenin genes. Only one transitional cell carcinoma (TCC, 6 percent) exhibited an increase of p53 by immunohistochemistry. In 16/18 TCCs and ¾ of the papillomas decreased p27 was seen. Cyclin D1 overexpression was seen in 26/47 of the hyperplasias, ¾ of the papillomas, and 10/18 of the TCCs. Increased COX-2 expression, a marker of oxidative stress, was seen in 17/18 TCCs, 4/4 papillomas, and 39/47 hyperplasias. In a parallel experiment 8-OHdG formation in rat urinary bladder was significantly increased after treatment with 200 ppm DMA in drinking water for two weeks compared with controls. The authors concluded that DMA is carcinogenic for the rat urinary bladder. The data also indicated that multiple genes were involved in stages of DMA-induced tumor development.

Morikawa *et al*. (2000) have reported DMA promotion of skin carcinogenesis in *Keratin* (K6)/ODC transgenic female mice following initiation with 7,12-dimethylbenz[a]anthracene. DMA alone had no effect in the mice. Mouse strains more commonly used for skin cancer experiments were also negative (Huff *et al*., 2000).

None of the studies described appears suitable for quantitative risk assessment.

Animal carcinogenicity summary

In general, inorganic or organic arsenic failed to exhibit significant carcinogenic activity when given orally to rodents. The only exception is the study by Knoth (1966). However, the description of the study design, analysis and results were incomplete, precluding critical assessment of this study. When arsenic in the form of sodium arsenate or arsenic trioxide was given by s.c. injection, significant carcinogenic activity has been demonstrated. Although the applicability of these studies to human environmental exposure may be questionable, the production of leukemia or lymphomas in mice after parenterally administered arsenic cannot be discounted outright. Ishinishi *et al.* (1983)

and Pershagen *et al.* (1984a,b) have demonstrated increased incidences of lung tumors in hamsters given arsenic trioxide by intratracheal instillation. However, the tumor incidences were low (e.g., 3/47) (these older animal studies are discussed in an earlier report, OEHHA, 1992a). Huff *et al.* (2000) have noted ten other human carcinogens with limited or no evidence of carcinogenicity in animal bioassays. These authors conclude that "while the collective evidence on the carcinogenicity of inorganic arsenic appears quite close to being considered sufficient evidence in experimental animals (Chan and Huff, 1997; IARC, 1980,1987), an adequate and definitive long-term experiment on arsenic (and in particular arsenic trioxide) has not yet been done."

Several studies have evaluated the effects of arsenic exposure in combination with initiating and/or promoting agents. The effects of arsenic have also been examined in mouse strains that are susceptible to or have a high background incidence of spontaneous tumors. The results from these investigations in rodents indicated no clear initiating or promoting activity in those strains. In fact, a protective effect has been observed in some studies. When arsenate was given in conjunction with urethane, a decrease in the number of tumors and in tumor size was reported (Blakely, 1987). However, arsenic in drinking water enhanced the kidney cancer response to DENA in one study (Shirachi *et al.*, 1983). In C3H/St mice, a strain that has a high spontaneous incidence of mammary tumors, sodium arsenite appears to inhibit the development and growth of precancerous cell populations (i.e., decrease or delayed tumor development). However, once tumors developed, the growth rate was faster in the arsenic-treated animals.

The studies of DMA-induced carcinogenicity following carcinogen pretreatment are difficult to interpret with respect to human risk largely due to the high doses required to produce an effect. Also, there are significant differences in metabolism of arsenic in the rat versus the human. Rats store arsenic in red blood cells, unlike humans, and the extent of methylation and dimethylation vary (Cohen *et al*., 2001). The findings of Wei *et al*. (1999) of direct carcinogenicity of DMA in rat urinary bladder appear to confirm the pretreatment studies.

The observation of transplacental carcinogenicity of inorganic arsenic in mice (Waalkes *et al.*, 2003) is particularly noteworthy. In this study, exposures of 42.5 and 85 ppm in drinking water to pregnant mice for 10 days during the later stage of gestation resulted in high yields of aggressive tumors in the offspring without additional arsenic treatment. By contrast, DMA exposure of rats noted above involved 23.5 to 93.6 ppm as arsenic in drinking water for 104 weeks and produced only urinary bladder tumors, which appeared only after 97 weeks.

The Waalkes *et al*. study indicates that the gestational period is one of high sensitivity to the carcinogenic effects of arsenic. Also it indicates that inorganic arsenic can be a complete carcinogen since its effects were seen long after exposure, did not require continued exposure, and were not reversible upon cessation of exposure as would be expected with a tumor promoter. The authors note that inorganic arsenic may act as a tumor progressor, affecting some pool of minimally neoplastic cells in the fetal target tissues. The findings of Rossman *et al*. (2001) that continuous exposure to inorganic arsenic in drinking water enhanced the aggressiveness of skin tumors in mice resulting from ultraviolet radiation tends to support the role of As as a tumor progressor.

Toxicological Effects in Humans

Acute Toxicity

The fatal dose of arsenic trioxide for humans is estimated to be between 70 and 180 mg (Vallee *et al.*, 1960), although 120 mg appears to be the most commonly quoted minimum lethal dose. Some sources have quoted a lethal dose as low as 10 mg while others have reported recovery from as much as 230 grains (15 grams) (Buchanan, 1962). On a unit body weight basis, the trivalent form of arsenic appears to be about four times as toxic as the pentavalent form.

Victims of lethal oral arsenic poisoning generally followed one of two clinical patterns. "Acute massive intoxication" occurs when the victim takes a large dose of arsenic on an empty stomach, and may be fatal within a few hours as a consequence of cardiac failure (Jenkins, 1966). In the more typical cases involving the ingestion of a lesser amount of arsenic, the first sign of poisoning occurred from half an hour to several hours after the ingestion. Initially there is throat constriction, a metallic taste in the mouth and a garlicky odor in the breath, followed by acute gastrointestinal effects, including severe abdominal pain, vomiting, and diarrhea, sometimes with muscular cramps and headache. Finally, 24 hours to several days after the initial exposure, there is a general vascular collapse leading to shock, coma, and death.

Patients who survived acute symptomatic arsenic ingestion (either because they took a sub-lethal dose, or because they received quick treatment for a lethal dose) showed a range of effects. The most common were gastrointestinal and cardiac disturbances, muscle cramps and facial edema (World Health Organization, 1981).

Subchronic Toxicity

At relatively low acute intake levels arsenic provokes mild gastrointestinal effects. Feinglass (1973) reported the acute gastrointestinal effects of acute and subacute exposure to well water contaminated with 11,800 to 21,000 ppb of arsenic. Victims drinking between 10 and 85 cupfuls of such water over a 10-week period experienced gastrointestinal effects (nausea or vomiting, dryness or burning of the mouth and throat, abdominal pain, and diarrhea). One of the most common long-term indicators of acute arsenic exposure is Mees' lines: ridges that appear on the fingernails six to eight weeks after the exposure (Jenkins, 1966). General desquamation of the skin has also been seen several weeks after exposure (Zaloga *et al.*, 1985).

Prasad and Rossi (1995) report a case of kidney toxicity associated with presumed arsenic poisoning. The patient had a 24-hour urinary arsenic of 91 μg/L. Percutaneous kidney biopsy showed tubular cell atrophy and extensive fibrosis on the interstitium. The findings were interpreted as ongoing chronic interstitial nephritis. The source of arsenic exposure was not confirmed, although cessation of organic health food consumption led to a urinary arsenic concentration of only 6.5 μg/L in three months.

Genetic Toxicity

In vivo studies

Chromosomal aberrations and SCE levels have been evaluated in humans with a history of arsenic exposure. Lerda (1994) studied human subjects exposed to drinking water containing 0.13 mg/L (130 ppb) arsenic for a period of at least 20 years. A control group of 155 people was exposed to less than 20 ppb arsenic in drinking water for more than 20 yr. The exposed group had a significantly elevated blood lymphocyte SCE response of 10.46 mean SCE/cell ± 1.02 SD, versus 7.49 ± 0.97 for the control group (Student's t-test, $p < 0.001$). The urinary arsenic was also significantly higher in the exposed group, 0.16 mg/L versus 0.07 mg/L ($p < 0.001$).

Eighteen human subjects in Nevada who were chronically exposed to arsenic in their drinking water (1.3 mg/L, 1312 ppb) exhibited a 1.8-fold increase (90 percent CI 1.06-2.99) in the frequency of micronucleated bladder cells (Warner *et al.*, 1994). The matched control group had an average drinking water arsenic level of 16 ppb. The frequency of micronucleated bladder cells was positively associated with the urinary concentration of inorganic arsenic and its methylated metabolites. There was no increase in micronucleated buccal cells associated with arsenic intake. Biggs *et al.* (1997) studied the occurrence of urinary bladder cell micronuclei in two populations of human subjects in Northern Chile with low or high arsenic in their drinking water supplies. Urinary arsenic was measured and found to average 582 µg/L (range 61-1893) in the high exposure group (N=124) vs. 59 µg/L (range 4-266) in the low exposure group (N=108). The groups were divided into quintiles based on urinary arsenic excretion, i.e., <54, 54-137, 138-415, 416-729, >729 µg/L. Each exposure quintile showed an increase in micronucleated cells (MNC) except the highest, i.e., 1.61, 3.39, 3.69, 4.77, and 1.52 MNC/1000 cells. Urinary arsenic was speciated to inorganic arsenic Asi, MMA, and DMA. The strongest association was between the sum of species and the prevalence of bladder cell micronuclei.

Dulout *et al.* (1996) evaluated chromosomal aberrations in peripheral blood lymphocytes from 22 Andean women and children from Argentina exposed to arsenic in drinking water at about 0.2 mg/L. The genotoxicity endpoints studied were micronuclei in binucleated cells (MN), SCEs, and fluorescence in situ hybridization (FISH) with chromosome specific DNA libraries. When compared to a control population exposed to very low arsenic in drinking water, the exposed group showed highly significant increases in the frequencies of micronuclei and of trisomy in lymphocytes. There were no notable effects on SCEs, specific translocations, or on cell cycle progression. A portion of the micronuclei appears to originate from whole chromosome loss. The exposed children (N = 10) exhibited 35 ± 4.6 SEM MN/1000 cells and exposed women (N = 12) 41 ± 4.9 SEM MN/1000 cells. A total of 22 control children and women exhibited 6.9 ± 1.7 SEM, indicating a highly statistically significant difference, $p < 0.001$.

Gonsebatt *et al.* (1997) evaluated two populations in Mexico for cytogenetic effects in blood lymphocytes associated with arsenic exposure via drinking water. The groups of 30-35 residents were exposed to either 30 µg/L (range 7-62) or 408 µg/L (range 396-435) arsenic. Approximately 1/3 of each group was comprised of smokers. The incidence of chromosome aberrations was significantly higher in the high exposure group: 7.12 ± 1.00 SEM percent vs. 2.96 ± 0.54 SEM percent ($p < 0.05$ by t-test). Exposed individuals showed a significant increase in the frequency of chromatid and isochromatid deletions in lymphocytes and of MN in oral and urinary epithelial cells. Males were more affected than females and a higher number of micronucleated oral cells were found among those individuals with skin lesions. The observed types of genetic damage provide additional evidence that arsenic is a clastogenic and aneugenic genotoxicant.

Maki-Paakkanen *et al.* (1998) described an association between structural chromosome aberrations (CAs) in peripheral blood lymphocytes and arsenic exposure via drinking water wells in 42 individuals in Finland. The median As concentration in well water was 410 µg/L, in urine total As was 180 µg/L, and in hair 1.3 µg/g. Eight control individuals were also analyzed who consumed water with low As (< 1.0 µg/L). Increased As exposure indicated by increased concentrations of As species (Asi, MMA, DMA) in urine and cumulative arsenic dose (kg/lifetime) in crude and adjusted linear regression models was associated with increased frequency of CAs. An increased MMA/As total and decreased DMA/As total ratios were associated with increased CAs when all aberration types were considered. Current users of As contaminated water showed stronger associations than all participants in the study. The genotoxicity of arsenic in a number of human *in vivo* studies is summarized in Table 4.

Table 4. Genetic Toxicity of Arsenic in Human Studies *In Vivo*

				Reference
Human Subjects, 18 exposed, 18 control Nevada, U.S.	Arsenic in drinking water, 1.3 mg As/L water, 4 yr, 16 µg As/L water, 5 yr	Micronuclei (MN) induced in exfoliated bladder cells	2.79 ± 0.73 SE/1000 cells vs. 1.57 ± 0.28 SE/1000 cells, $p = 0.09$	Warner *et al.*, 1994
		Centromere-specific micronucleus probe, FISH	MN frequency, percent 0.091 vs. 0.055, $p = 0.07$	Moore *et al.*, 1997b
Human subjects, 70 exposed, 55 control, Chile	Arsenic in drinking water 600 µg As/L 19.3 yr; 15 µg As/L 28.3 yr	Micronuclei in bladder cells,	3.2 MN/1000 cells vs. 2.6 MN/1000 cells $p < 0.001$	Moore *et al.*, 1997a
	Urinary As quintiles: < 53.8 µg/L 53.9-137.3 µg/L 137.4-414.6 µg/L	Centromere-specific MN probe	MN^-/1000 cells 0.26 1.22 1.94	

				Reference
	414.7-728.9 µg/L >728.9 µg/L		1.36 0.25	
Male human subjects, 34 exposed, stop exposure for 8 wk Chile	Arsenic in drinking water 600 µg As/L to 45 µg As/L; Urinary As: 742 µg As/L to 225 µg As/L	Micronuclei induction in bladder cells	MN/1000 cells 2.63 vs. 1.79 MN/1000 cells, $p < 0.05$ 3.54 vs. 1.47 , $p = 0.002$ for subcytotoxic subgroup	Moore *et al.*, 1997c
Human subjects 124 exposed 108 controls Chile	Arsenic in drinking water ≤ 670 µg As/L 15 µg As/L water Urinary As quintiles: <53.8 µg/L 53.9-137.3 µg/L 137.4-414.6 µg/L 414.7-728.9 µg/L >728.9 µg/L	Micronuclei in bladder cells	MN/1000 cells 1.61 3.39, $p = 0.001$ 3.69, $p < 0.001$ 4.77, $p < 0.001$ 1.52, $p = 0.5$	Biggs *et al.*, 1997
Human subjects 35 exposed 34 controls Mexico	Arsenic in drinking water 408.2 µg As/L 29.9 µg As/L	Chromosome aberrations in cultured blood lymphocytes	Chromosome aberrations, percent 7.12 ± 1.0 SE vs. 2.96 ± 0.54 SE	Gonsebatt *et al.*, 1997
	740 µg As/L urine 34 µg As/L urine	Micronuclei in urothelial cells	MN/1000 urothelial cells 2.22 ± 0.99 vs. 0.48 ± 0.1, $p < 0.05$	
		Micronuclei in oral mucosal cells	MN/1000 cells 2.21 ± 0.47 vs. 0.56 ± 0.13, $p < 0.05$	
Human subjects 282 exposed 155 control Argentina	Arsenic in drinking water 130 µg As/L 20 µg As/L	SCE in blood lymphocytes	SCE/cell 10.46 ± 1.02 vs. 7.49 ± 0.97, $p < 0.001$	Lerda, 1994
Human subjects 22 exposed 22 controls Argentina	Arsenic in drinking water 200-500 µg As/L very low Urinary As Exposed women	Micronuclei in peripheral blood lymphocytes; SCEs in peripheral	MN/ 1000 cells 38 ± 3.2 vs. 6.9 ± 1.7, $p < 0.001$; No difference in SCEs	Dulout *et al.*, 1996

				Reference
	260 vs. 8.4 µg As/L in controls Exposed children 310 vs.13 µg As/L in controls	blood lymphocytes; Chromosome aberrations by FISH	No translocations but signif. increase in aneuploidy (0.21 percent trisomy in exposed group vs. 0 percent in controls)	

In vitro studies

The GAP data base for As III lists 7/8 positive tests in human systems: Chromosome aberrations (3); micronuclei induction *in vitro* (1); SCEs in lymphocytes *in vitro* and *in vivo* (2); and DNA strand breaks *in vitro* (1). The lowest effective concentrations for *in vitro* tests ranged from three nM (chromosome aberrations in fibroblasts) to 1 mM for DNA strand breaks. For pentavalent arsenic, only three tests are listed. A positive response was observed with chromosome aberrations in human lymphocytes *in vitro*, LED = 2.7 µM. A weak response was seen with SCEs in human lymphocytes. The highest ineffective dose was 1 mM for UDS in human fibroblasts *in vitro* (U.S. EPA, 1997b).

Jha *et al.* (1992) studied the effects of sodium arsenite alone or in combination with X-rays on human peripheral blood lymphocytes *in vitro*. Sodium arsenite was found to: inhibit cell cycle progression of phytohemagglutinin (PHA)-responsive lymphocytes; induce chromatid-type aberrations and SCEs in a positive dose related manner; and potentiate X-ray and UV-induced chromosomal damage. The authors suggest that arsenite acts by inhibiting DNA ligase activity.

Wiencke and Yager (1992) found that normal human lymphocytes from three subjects treated *in vitro* with 1-2 µM arsenite had significantly increased SCEs ($p < 0.05$; Student's t-test). Lymphocytes treated with diepoxybutane (DEB, 6 µM) alone showed significantly increased SCEs and when exposed to both arsenite and DEB showed highly significant increases in chromosomal aberrations (chromatid deletions 4-8-fold and chromatid exchanges 7-40-fold). The authors suggest an effect of arsenite on repair of DEB-induced DNA damage that leads to chromosomal aberrations but not SCEs.

Dong and Luo (1994) examined the effects of sodium arsenite and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) on human fetal lung fibroblasts *in vitro*. Arsenic at concentrations of 1-10 µM increased unscheduled DNA synthesis (UDS). UDS induced by 34 µM MNNG in combination with arsenic was significantly increased at 3 µM As but not at 0.1, 0.5, 1.0, or 5 µM As. Three µM As seems to be an optimal concentration for DNA-protein cross-link induction.

Vega *et al.* (1995) observed a dose dependent increase in hyperploid cells in human lymphocyte cultures treated with arsenite *in vitro*. Arsenite induced hypoploidy, hyperploidy and polyploidy in first and second division cells in cultures from all four human donors. A positive dose response for frequency of aneuploid first division cells was described by the linear regression ($y = 2.91x + 43.78$, $r = 0.75$, $p = 0.0001$) and for second division cells by ($y = 3.86x + 61.11$, $r = 0.82$, $p = 0.0001$). In both cases, y is the percentage of heteroploid cells and x is the log μmolar concentration of arsenite ion. Significant effects were observer over the concentration range of 10^{-2} to 10^{-10} *M* arsenite. Sodium arsenite was also found to cause mitotic arrest in a dose dependent manner over the same range of doses. The authors postulate that arsenite acts via binding to sulfhydryl groups on tubulin to prevent polymerization and induce mitotic arrest. Arsenite was more effective than colcemid at inducing aneuploidy but less effective in causing mitotic arrest at equal concentrations.

Rossman *et al.* (1997) observed that a human keratinocyte cell line (AG06) lacked inducible tolerance to arsenite that was observed in Chinese hamster V79 cells. Similar results were obtained with HeLa (cervical carcinoma) cells, HTB139 human meduloblastoma cells, CRL1295 diploid human fibroblasts, and 2008 ovarian carcinoma cells. The human cell lines head ID_{50} values between 0.2-2.0 μM vs. 12.5 μM for the V79 wild type, 35.0 μM for an As resistant V79 variant, and 25.0 μM for CHO cells. The human keratinocytes were the most sensitive of the cell lines tested (ID_{50} = 0.2 μM).

In an unusual study of arsenic mutagenesis using the plasmid shuttle vector pZ189 propagated in DNA repair proficient human fibroblasts, it was observed that arsenite is mutagenic (Wiencke *et al.*, 1997). The base substitutions observed involved A:T→ T:A transversions. The concentrations employed were 1.0, 2.5, and 5.0 μM in the normal GM637 human fibroblast cell line. The induced mutant frequencies were 0, 0.7 and 6.0 x 10^{-4}, respectively. A cooperative effect with UV-irradiation (320 J/m^2) was also observed with observed/expected ratios of 4.9, 1.5, 1.5, respectively, ($p < 0.01$).

Oya-Ohta *et al.* (1996) tested a number of inorganic and organic arsenicals for the ability to induce chromosomal aberrations in cultures human fibroblasts. All of the arsenicals tested showed clastogenic activity. The rank order of clastogenicity was arsenite > arsenate > dimethylarsinic acid (DMA) > methylarsonic acid (MMA) > trimethylarsine oxide (TMAO). DMA was a potent clastogen and produced chromosome pulverizations when present at concentrations above 7 x 10^{-3} *M*. Arsenosugar, arsenocholine, arsenobetaine and tetramethylarsonium iodide were less effective clastogens. Arsenite caused significant aberrations at exposures at or above 3.8x10^{-6} *M* for 24 hr and arsenate at or above concentrations of 1x10^{-5} *M* for 24 hr. The authors observed that cellular GSH protected against the clastogenic effects of arsenite, arsenate, and MMA while apparently stimulating the clastogenic activity of DMA.

Rasmussen and Menzel (1997) evaluated arsenic induced SCEs in human lymphocytes and lymphoblastoid cell lines. SCEs were increased in primary lymphoblast cultures in a dose dependent manner over the 10^{-7} to 10^{-5} *M* arsenite concentration range. Arsenate and DMA were found not to increase SCEs significantly over the same concentration range. Comparison of SCE frequency in primary lymphocyte cultures among 14 individuals showed variation in sensitivity to arsenite, with some showing no significant effect while others a 2-3-fold increase in SCEs.

Schaumloffel and Gevel (1998) studied the comparative genotoxicity of As^{III} and Sb^{III} in human peripheral lymphocyte cultures. Trivalent As was five times more cytotoxic and 10 times more potent in induction of MN than was antimony. Both gave linear dose responses over the exposure concentration range of 0.1 to 2.0 μ*M*. Combined effects of As^{III} and Sb^{III} indicated simple additivity. In the single cell gel test with human lymphocytes, a significant induction of DNA damage was seen with 0.01 μ*M* As^{III} and 5 μ*M* Sb^{III}.

Mass and Wang (1997) studied the effect of arsenic on DNA methylation of the tumor suppressor gene p53 in human lung cells. Since As is metabolized by methyl transferase (MTase), the authors suspected that arsenic might interfere with MTase/S-adenosylmethionine (SAM)-dependent methylation of DNA. Exposure of human lung adenocarcinoma A549 cells to sodium arsenite (0.08-2.0 μ*M*) or sodium arsenate (30-300 μ*M*) but not DMA (2-2000 μ*M*) resulted in significant dose-dependent hypermethylation of the cytosine in a fragment of the p53 promoter. For arsenite doses of 0, 0.08, 0.4, and 2.0 μ*M*, the number of 5-methyl cytosines/clone were: 0.21, 0.42, 0.65, and 1.4, respectively. The authors postulate a model for arsenic carcinogenesis based on perturbations of DNA methylation.

Salazar *et al.* (1997) demonstrated arsenite induced increases in p53 suppressor gene expression in human cell lines: in Jurkat cells at 1 μM; and at 10 μM in HeLa cells and a lymphoblast cell line transformed with Epstein-Barr virus (LCL-EBV).

Yamanaka *et al.* (1997) studied the effect of methylation on arsenic-induced genotoxicity in human alveolar epithelial type II (L-132) cells in culture. Arsenite, MMA, and DMA were evaluated. DMA at 5-100 μM caused DNA single strand breaks resulting from inhibition of repair polymerization. Arsenite and MMA did not exhibit similar activity even at 100 μM. When 100 μM MMA was combined with 10 mM SAM, a methyl group donor, DNA repair synthesis was induced along with increased amounts of DMA. The authors conclude that in this system methylation of inorganic arsenic to dimethylarsinic acid represents a genotoxication rather than a detoxication process.

Rossman and Wolosin (1992) evaluated the ability of five carcinogens to induce gene amplification of the SV40 and *dhfr* sequences in SV40-transformed human keratinocytes. UV, X-rays, MNNG, and mitomycin C amplified SV40 2-8 fold and *dhfr* 1.5- to 3-fold. Arsenite did not amplify SV40 but was the best inducer of *dhfr* amplification, 3.2–fold. The authors interpret the differences as being the result of lower DNA damaging ability of As compared to the other carcinogens.

Mass *et al.* (2001) evaluated the genetic toxicity of methylated trivalent arsenic species in human peripheral lymphocytes and against ϕX174 RF1 DNA. Methyloxoarsine (MAs^{III}) and iododimethylarsine ($DMAs^{III}$) were assessed using a DNA nicking assay and a single-cell gel (SCG, "comet") assay. Both compounds were able to nick and/or completely degrade ϕX174 *in vitro* during two hr incubations. $DMAs^{III}$ exhibited activity at concentrations as low as 150 μM. Similar exposures to sodium arsenite, sodium arsenate, and the pentavalent arsenicals MMA and DMA did not nick ϕX174 DNA. In the SCG assay, the methylated trivalent arsenicals were more potent than the other arsenic species tested. The relative potencies based on the slopes of migration of DNA in the assay (μm/μM) were: $MAs^{V} = DMAs^{V} < 1$; $As^{III} = 1$; $As^{V} = 1.4$; $MAs^{III} = 77$; $DMAs^{III} = 386$. These results suggest key roles for the trivalent arsenical metabolites monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) as directly acting genotoxicants in human arsenic toxicity. Both of these metabolites have been detected in urine from individuals ingesting As-contaminated drinking water (Le *et al.*, 2000a,b; Aposhian *et al.*, 2000a,b).

The recent observations of Mure *et al*. (2003) however, would indicate caution in regard to the genotoxicity of MMA^{III}. They found that 0.025 to 0.1 μM arsenite transformed human osteosarcomas TE85 (HOS) cells to anchorage independence after eight weeks of exposure. Other carcinogens only required days of exposure. Arsenite also caused delayed increases in mutagenicity to 6-thioguanine resistance at $\leq$ 0.1μM after almost 20 generations of continuous exposure. Arsenite also induced gene amplification of the dihydrofolate deductase gene at 0.0125 to 0.1 μM. When these assays were repeated with MMA^{III} no significant transformation or mutagenesis were seen, suggesting that arsenite rather that its metabolites was the genotoxicant, at least in this system. The authors speculate that long-term exposure to low concentrations of arsenite may affect signaling pathways resulting in progressive genomic instability.

The following general conclusions may be drawn from the genetic toxicity studies summarized in the text and in Tables 4 and 5:

- Arsenic is a well-established genotoxicant in mammalian cells;
- Arsenic causes gene mutations in some systems but these are likely lethal in most and hence poorly recoverable;
- Arsenic does not appear to directly damage DNA except possibly at highly cytotoxic levels;
- Arsenic induces chromosomal aberrations (including micronuclei and aneuploidy) and SCEs;
- Arsenic enhances oxidative stress and influences the production of NO;
- Arsenic affects the methylation of DNA in tumor suppressor genes;
- Arsenic causes gene amplification;
- Arsenic inhibits DNA synthesis and repair;
- Arsenic acts as a co-mutagen;
- Methylated and dimethylated arsenic, while more readily excreted *in vivo*, also exhibit genotoxicity, albeit at higher exposure levels;
- Arsenic causes mitotic arrest, possibly by reaction with tubulin.

A number of possible mechanisms of arsenic's interference with DNA synthesis and/or repair have been proposed, including inhibition of DNA ligases or polymerases, effects on accessory proteins (not yet demonstrated), or through effects on p53 expression (U.S. EPA, 1997a).

Jacobson-Kram and Montalbano (1985) suggested a possible reason for the discrepancy between gene mutation and chromosomal aberration studies. The protocols for gene mutation assays generally involve relatively short cellular incubation periods (2-3 hr), while chromosomal aberration protocols involve much longer incubation times (12-48 hr). Since arsenic appears to exert its effect only during the DNA replication phase, the incubation period in gene mutation studies may be too short. Alternatively, as suggested by Hei *et al.* (1998), arsenic may induce largely multilocus deletions that are incompatible with cell survival. That is, many of the types of mutations induced by As are poorly recovered due to lethality.

Lee *et al.* (1988) have demonstrated that arsenic is capable of inducing gene amplification. Amplification of the dihydrofolate reductase (*dhfr*) gene was measured in mouse 3T6 cells by selecting cells that form colonies in the presence of methotrexate (MTX). Treatment of mouse 3T6 cells with sodium arsenite (0.2 - 6.2 µM) or sodium arsenate (1-32 µM) induced dose-dependent increases in the number of MTX colonies. Approximately 50 percent of the MTX clones induced had amplified copy numbers (2 to 11-fold) of the *dhfr* gene. Sodium arsenite was active at a lower concentration than sodium arsenate.

The mechanism by which arsenic induces gene amplification remains unknown. However, these authors have proposed that since amplification of oncogenes is observed in many human tumors, the ability of arsenic to induce gene amplification may be related to its carcinogenic effects. The genotoxicity of arsenic in human *in vitro* systems is summarized in Table 5.

Relatively few studies in Table 5 have included MMA and/or DMA and these have generally indicated either a lack of activity or activity at much higher effective concentrations than inorganic arsenic species. These results appear consistent with the in vivo initiation/promotion studies with DMA discussed above which also required relatively high doses to achieve effects in rats (see Toxicological Effects in Animals: Carcinogenicity). The one exception appears to be the study of Mass *et al*. (2001), which used trivalent and pentavalent arsenic species to assess DNA damage in human peripheral lymphocytes in vitro. By comparing the slopes of the regression lines relating the arsenical concentrations to the lengths of the induced tail moments in µm in the Comet Assay (i.e., µm/µ*M*), the authors were able to rank the "potencies" of the arsenicals as follows: $DMA^{III}>MMA^{III}>>As^{III} \sim As^{V}>MMA^{V} \sim DMA^{V}$. No exogenous enzymatic activation was required for activity and the trivalent arsenicals were considered to be direct-acting genotoxicants. This study indicates that inorganic arsenic could be metabolized to highly reactive genotoxic trivalent methylated arsenic species. This work needs to be confirmed and extended to other tissues and model systems. It is also important to note, as the authors do, that MMA^{III} and DMA^{III} are not the only genotoxic species of arsenic that could exist.

Table 5. Arsenic Genotoxicity and Related Effects in Human Systems *In Vitro*

				Reference
Peripheral Blood Lymphocytes	1-5- µM Na arsenite, 48 hr	Chromosomal aberrations and chromatid breaks SCEs	1 µM As 10-15 percent vs. 4 percent 1 µM As	Jha *et al.*, 1992

				Reference
			7/cell vs. 4/cell	
Human fibroblasts	5 µM, 24 hr	Micronuclei (MN)	45 MN/1000 cells vs. 10 MN/1000 cells in control	
Peripheral Blood Lymphocytes	0.5-2.0 µM Na arsenite	Chromosomal aberrations SCEs	No activity without combination with DEB 6 µM 1.0-1.5 µM = 11.7-13.6 SCEs/cell vs. 8.1-9.8 SCEs/cell in controls (N = 3)	Wiencke & Yager, 1992
Peripheral Blood Lymphocytes	1-10^{-10}µM Na arsenite, 24 hr	Aneugenicity Mitotic arrest	10^{-10} µM % heteroploid cells (y) linear with $\log_{10}$ dose (x): $y = 2.91x + 43.78$, $r^2 = 0.57$ 10^{-10} µM in 4/5 subjects, $p < 0.001$	Vega *et al.*, 1995
Peripheral Blood Lymphocytes	Arsenic trioxide 0.1-5 µM Na arsenate 0.1-10 µM	SCEs Chromosome aberrations	0.5 µM, $p < 0.001$ No effect on SCEs, toxic at 10 µM 10^{-2} µM, mean = 53 CA/cell	Gebel *et al.*, 1997
Peripheral Blood Lymphocytes	Na arsenite 0.5-5 µM, 24 hr	Micronuclei induction	0.5 µM gives 16 vs. 7 MN/1000 cells in control, $p < 0.05$	Schaumloffel & Gebel, 1998
Fetal Lung Fibroblasts (2B5 cells)	1-10 µM Na arsenite	DNA Damage	1.0 µM As for unscheduled DNA synthesis & inhibition of replicative DNA synthesis	Dong & Luo, 1994
			3.0 µM As optimum for DNA-protein cross-links	Dong & Luo, 1993
EBV Burkitt's lymphoma cells	5, 20,100 µM Na , 4 hr	DNA-protein cross-links	Significant cross-links found only with high cytotoxicity	Costa *et al.*, 1997
Peripheral blood lymphocytes	Na arsenite, 0.5-5 µM, 40 hr	SCEs	0.5 µM, linear dose response	Rasmussen & Menzel, 1997
Lympho-blastoid cells	Na arsenite 0.5-10 µM Na arsenate & DMA 0.5-10 µM	SCEs	1.0 µM Negative at 10 µM	
Human	1-5 µM	Gene mutation	Increase in A:T→T:A	Wiencke *et*

				Reference
fibroblasts, pZ189 plasmid shuttle vector			and G:C → C:A transversions and base substitutions at 5 μM	*al.*, 1997
HeLa cells, EBV transformed lymphoblasts	1-10 μM arsenic, 24 hr	Expression of p53 tumor suppressor gene	Increased expression at 10 μM As	Salazar *et al.*, 1997
Jurkat cells	1-10μM arsenic		Increased expression at 1 μM As	
Keratinocytes	0.28-28μM Na arsenite	Interleukin-8 gene expression	28 μM As stimulated IL-8: 693 vs. 141 pg/mL/10^5 cells in control	Yen *et al.*, 1996
HeLa cells	50 μM arsenite 50 μM arsenate	AP-1 transcriptional activity, Jun kinases (JNK)	Stimulation of AP-1 activity and JNK inhibition No activity found	Cavigelli *et al.*, 1996
Cultured Fibroblasts	1-10 μM Na arsenite 16-321 μM Na arsenate 1.4-21.4 mM MMA 0.7-21.7 mM DMA 3.7-14.7 mM TMAO	Chromosomal aberrations and abnormal cells	percent Abnormal cells (in 24 hr) 13; 3.8 μM, $p < 0.01$ 16; 16 μM, $p <0.01$ 19; 1.4 mM, $p < 0.01$ 28; 0.7 mM, $p < 0.01$ 26; 3.7 mM. $p < 0.01$	Oya-Ohta *et al.*, 1996
Alveolar Type II (L-132) cells	10 mM DMA, 10 hr	DNA damage	10 mM DMA single strand DNA breaks, replicative DNA synthesis suppressed, shortened DNA chain length	Tezuka *et al.*, 1993
Alveolar Type II (L-132) cells	Dimethyl arsine from 200 µmol DMA	DNA damage	DNA single strand breaks caused by dimethyl arsenic-peroxyl radical	Yamanaka *et al.*, 1990
Alveolar Type II (L-132) cells	100 μM MMA or arsenite, 6 hr 5-100 μM DMA, 6 hr	DNA damage	No DNA strand breaks at 100 μM DNA breaks at 5 μM	Yamanaka *et al.*, 1997
Keratinocyte SCC-9, SIK, and hEp cells in culture	Na arsenate 0.3-10 μM; Na arsenite 0.1-3 μM	Gene expression	Suppression of 4 markers of suprabasal differentiation, arsenite $EC_{50} \cong 1$μM and	Kachinskas *et al.*, 1997; Jessen *et al.*, 2001

				Reference
			arsenate 2 µM	
Primary keratinocyte cultures	Na arsenite 0.5-50 µM	Altered β-adrenergic receptor density (Bmax) and affinity (Kd)	0.5 µM reduces Bmax: 69.3fmol/mg vs. 97.6 fmol/mg in control, $p < 0.05$; no effect on Kd	Chang *et al.*, 1998
Epidermal keratinocytes	Na arsenite 1-4 µM	Growth factor secretion Transforming growth factor-α	0.5 µM increases 1.0 µM increasesα	Germolec *et al.*, 1996, 1997
Adenocarcinoma A549 cell line	Na arsenite 0.08-2 µM, 7 d Na arsenate 30-300 µM, 7 d DMA 2-2000 µM, 7 d	DNA methylation of p53 promoter	0.4 µM increased 5-MeC/clone, $p < 0.05$, linear dose response 30 µM increased 5-MeC/clone No increased methylation	Mass & Wang, 1997
SV40-transformed keratinocytes	Na arsenite 5-20 µM, 16 hr	Gene amplification of SV40 and *dhfr* sequences	6 µM As caused 3-fold amplification of *dhfr* (MTX^r) with no amplification of SV40	Rossman & Wolosin, 1992
Human peripheral lymphocytes	Na arsenate Na arsenite Na MMA^V Na DMA^V MMA^{III} DMA^{III}	Single-cell gel "Comet" assay Slopes of µm/µM converted to relative potency	1 (relative potency) 1.4 <1 <1 77 386	Mass *et al.*, 2001

Arsenic in chemotherapy

Arsenic compounds have been used to treat patients with acute leukemia and chronic myelogenous leukemia (CML) since the early 1930s (Forkner *et al.*, 1931). Recently arsenic trioxide (As_2O_3) has demonstrated a specific beneficial effect in the treatment of acute promyelocytic leukemia (APL) (Chen *et al.*, 1996b). In addition to inducing complete remission in the large majority of patients, arsenic trioxide could also trigger apoptosis of APL cell lines at higher concentrations and induce partial differentiation at lower concentrations. Zhang *et al.* (1998) studied the *in vitro* effects of arsenic trioxide on seven lymphoid lineage cell lines. They demonstrated that arsenic trioxide inhibited the proliferation of myeloid and lymphoid cultured cell lines. Apoptosis was induced at 1 µM arsenic trioxide in cell lines such as NB4, NKM-1, and NOP-1 but not in Raji, Daudi and HL-60 cells. The induction of apoptosis was associated with the down-regulation of bcl-2 protein.

Akao *et al.* (1998) showed that 1 µM arsenic trioxide for 24-48 hr inhibited cell growth of four B-cell leukemia cell lines. In two of these, KOCL-44 and LyH7, apoptosis was identified by morphological and nucleosomal DNA fragmentation studies. Three of four B-cell lines that were growth inhibited were acute infantile leukemia with t(11:19)(q23:p13) translocations. The arsenic-induced apoptosis in KOCL-44 and LyH7 cells was linked to activation of caspase 1-like and caspase 3-like proteases.

Chen *et al.* (1998) propose a mechanism of arsenite-induced apoptosis involving the following sequential steps: (1) initial activation of flavoprotein-containing superoxide-production enzyme such as NADPH-oxidase and an increase in cellular superoxide levels; (2) conversion of superoxide to hydrogen peroxide; (3) release of cytochrome c (from mitochondria) to the cytosol, activation of CPP32 protease, and PARP (a DNA repair enzyme) degradation. Action of arsenite on Bcl-2 gene expression (Bcl-2 protein can attenuate As-induced apoptosis) also seems to occur between steps (1) and (3) above.

Developmental and Reproductive Toxicity

In an ecological study of a Hungarian population (N = 25,648), spontaneous abortion and stillbirth were examined for elevated arsenic exposure via drinking water (Borzsonyi *et al.*, 1992). Data were collected over an 8-yr period and compared with a population in a neighboring area with low arsenic. The arsenic exposed population demonstrated increased incidence of hyperpigmentation and hyperkeratosis. There was some indication of an association of arsenic exposure with spontaneous abortion (RR = 1.36, 95 percent CI 1.1-1.6) and a stronger association with stillbirth (RR = 2.70, 95 percent CI 1.15-6.35); both effects were statistically significant.

Two case-control studies evaluated arsenic exposure via drinking water and adverse reproductive effects.

Zierler *et al.* (1988) studied the association of cardiac defects and in utero exposure to nine metals in 270 cases of children with congenital heart disease and 650 control children. An increased frequency of coarctation of the aorta (prevalence odds ratio = 3.4, 95 percent CI 1.3-8.9) was observed among children born to mothers residing in areas with detectable levels of arsenic in the public drinking water supply during the first trimester of pregnancy. No association of arsenic exposure was found with three other cardiac defects studied. Due to study design limitations it is not possible to conclude that arsenic exposure caused the increased frequency of the cardiac lesion.

In a case-control study, Aschengrau *et al.* (1989) evaluated exposure to arsenic in 286 women with evidence of spontaneous abortion (SA) and compared them to 1391 control women. For the metal analyses, the interval from the date of a matched water sample to the date of conception ranged from five days to 3.7 yr, with a median value of 2.1 yr (1.6 yr for cases and 2.2 yr for controls). The crude odds ratio of exposure to inorganic arsenic in drinking water was 1.3 (95 percent CI 1.0-1.6). Exposure to water containing higher arsenic levels was more strongly associated with spontaneous abortion. After adjustment for multiple cofounders using a multiple logistic regression model, only exposure to higher levels of arsenic was found to be associated with SA, although the magnitude was not statistically significant (exposure odds ratio = 1.5, 95 percent CI = 0.4-4.7). As the authors note, this study has shortcomings in the measurement, recording and classification of exposure. Additional inhalation exposure and acute exposure studies were reviewed by Golub *et al.* (1998).

Tabacova *et al.* (1994) determined urinary arsenic, cadmium and lead in 50 women in a heavily industrialized region of Bulgaria. No differences were found in urinary As subgroups that experienced pregnancy complications (toxemia, anemia, and threatened abortion).

Potential paternal effects of arsenic exposure were studied by Beckman (1978). When both parents were employed at a smelter emitting As, the spontaneous abortion rate was higher than if only the mother was employed there. A higher spontaneous abortion rate was observed for parity >2 (n = 117) but not for parity 1 or 2 (Nordstrom *et al.*, 1979a).

Shalat *et al.* (1996) reviewed the literature concerning the role of arsenic exposure in the causation of human neural tube defects (NTDs). The prevalence of NTDs varies widely in different geographical areas for fewer than four per 10,000 live births (France) to over 20 per 10,000 live births (Mexico, N. Ireland). Incidence of NTDs is usually higher in female than in male infants. The association between human prenatal As exposure and congenital malformations including NTDs has not yet been fully resolved. However, given the data on arsenic's teratogenic potential in multiple animal species, it seems likely that humans would also be susceptible to such adverse effects.

Hopenhayn-Rich *et al.* (2000) conducted an ecologic retrospective study of chronic arsenic exposure and risk of infant mortality in two areas of Chile: Antofagasta, with a documented history of As contaminated drinking water, and Valparaiso, a comparable low-exposure city. Between 1950 and 1996 infant and late fetal mortality rates declined markedly in Chile as in other Latin American countries. Antofagasta experienced an 86 percent decline in the late fetal mortality rate, an 81 percent decline in neonatal mortality rate, and a 92 percent decline in the post neonatal mortality rate. The declines in infant mortality rates in Valparaiso were 64, 77, and 92 percent, respectively. Despite the overall decline, rates for all mortality outcomes increased in Antofagasta during 1958-1961 and declined thereafter. The increases and declines overall coincide with the period of higher arsenic levels in the drinking water (860 µg As/L in Antofagasta). Results of a Poisson regression analysis of the rates of late fetal, neonatal and postneonatal mortality showed elevated relative risks for high arsenic exposure in association with each of the three mortality outcomes. The association between arsenic exposure and late fetal mortality was the strongest (RR = 1.72, 95 percent CI 1.54-1.93). Neonatal mortality (RR = 1.53, 95 percent CI 1.40-1.66) and postneonatal mortality (RR = 1.26, 95 percent CI 1.18-1.34) were also elevated. These findings provide suggestive evidence for arsenic-related human developmental toxicity.

Ihrig *et al.* (1998) conducted a hospital-based case-control study of stillbirths and environmental arsenic exposure using an atmospheric dispersion model linked to a geographical information system. They collected data on 119 cases and 267 controls in a central Texas area including a facility with 60-year history of arsenic-based agricultural product manufacture. Four exposure groups were categorized (0, < 10 ng/m^3; 10-100 ng/m^3; and > 100 ng/m^3). For the period 1983-93 they fit a conditional logistic regression model including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. Effects were only seen in the Hispanic group, with the medium exposure group having a prevalence odds ratio and 95 percent confidence interval of 1.9 (0.5-6.6) and the high exposure group 8.4 (1.4-50.1). The authors postulate a possible influence of genetic polymorphism affecting folate metabolism in Hispanic populations, possibly leading to increased neural tube defects and stillbirths. This study is limited by small numbers; for example, there were only seven cases in the high exposure group and five of these were Hispanic.

Calderon *et al.* (2001) conducted a cross-sectional study to examine the effects of chronic exposure to lead (Pb), arsenic (As), and nutrition on the neuropsychological development of children. Two populations of children (N = 41, 39) with differing As exposure levels (63 vs. 40 μg/g) but similar Pb exposures (8.9 vs. 9.7 μg Pb/dL blood, respectively) were compared using the Wechsler Intelligence Scale for Children (WISC) Revised Version for Mexico. After controlling for significant potential confounders, verbal IQ was observed to decrease with increasing urinary arsenic concentration ($P < 0.01$). Language, verbal comprehension, and long-term memory also appeared to be adversely affected by increasing arsenic exposure. Blood lead was significantly associated with a decrease in attention (Sequential Factor). However, since blood lead is an imprecise measure of lead burden there could be some residual confounding in this study.

The relationship between arsenic exposure via drinking water and neurological development as indicated by IQ was assessed in Thailand (Siripitayakunkit *et al.*, 1999). A total of 529 children aged six to nine were studied using a cross-sectional design. The children were randomly selected from 15 schools. The male:female ratio was 1.08. Arsenic levels in hair were used to assess exposure and the Wechsler Intelligence Scale Test for children was used to assess IQ. The mean hair arsenic was 3.52 μg/g (SD = 3.58) and the median hair arsenic was 2.42 μg/g (range = 0.48 to 26.94 μg/g). Fifty-five percent of the children had As levels between 1.01 and 3 μg/g. Only 44 (8.3 percent) had normal arsenic levels in hair (≤1 μg/g). The mean IQ of the study was 90.44 (range 54 to 123). Most of the IQs were classified as average (45.7 percent) or dull normal (31.6 percent). Approximately 14 percent and 3 percent of the children were in the borderline and mental defective groups, respectively. The percentage of children in the average IQ group decreased significantly from 57 percent to 40 percent with increasing arsenic exposure. The percentage in the lower IQ group increased with increasing As (23 percent to 38 percent) and in the low IQ group (0 percent to 6 percent). In a comparison of IQ between children with As hair levels ≤ 2 ppm or >2 ppm, arsenic was found to explain 14 percent of the variance in IQ after controlling for father's occupation, mother's intelligence score, and family income. Although the cross-sectional study design does not allow for establishment of the time precedence of exposure to arsenic, the investigators stated that the subjects of the study were born in a period of chronic arsenic poisoning and that this cohort has been continuously exposed since birth due to their non-mobility. The study suffers from small numbers of children exposed to low arsenic (hair arsenic ≤ 1 ppm). The data are summarized in Table 6.

In the historical cohort study of Ahmad *et al*. (2001), the incidences of three adverse pregnancy outcomes (spontaneous abortion, stillbirth, preterm birth) were determined by interviews using a questionnaire and checklist. Respondents (N = 96 per group) were randomly selected from the exposed population and controls were matched for age, education, and socioeconomic status. Statistical comparisons were made between a low exposure community (drinking water concentration <0.02 mg As/L) and a high exposure community (drinking water concentration >0.05 mg As/L in 85 percent of wells). Subgroups within the high exposure community with briefer and longer exposures (5-15 years or ≥ 15 years) were also compared. Comparisons were statistically significant for spontaneous abortion (P = 0.008), stillbirth (P = 0.046), and preterm birth (P = 0.018) between exposed and nonexposed groups as well as between shorter and longer exposure groups.

Yang *et al*. (2003) conducted a study of pregnancy outcome in an arseniasis-endemic area of northeastern Taiwan. The arsenic exposed area had drinking water arsenic concentrations ranging from undetectable (<0.15 ppb) to 3590 ppb. The study compared data on 3872 singleton live births in the arsenic exposed area (AE) with 14,387 births in the non-arsenic exposed area (NAE) collected in 1983 thru 1997. The results indicate that, after adjusting for potential confounders, arsenic exposure via drinking water was associated, but not statistically significantly, with risk of preterm delivery, odds ratio = 1.10 (95 percent CI 0.91- 1.33). The estimated reduction of birth weight was 29.05 g (95 percent CI 13.55-44.55). This reduction in birth weight was statistically significant (P = 0.001). The authors conclude that increased arsenic exposure via drinking water results in a greater risk of reduction of infant birth weight.

In general, the studies on developmental and reproductive toxicity do not appear suitable for quantitative risk assessment of arsenic in drinking water. An exception is possibly the latter study by Siripitayakunkit *et al*. (1999).

Table 6. IQ versus Hair Arsenic for Selected Children Aged Six to Nine in Thailand (Siripitayakunkit *et al*., 1999)

				> 5.0 µg As/g hair
≤ 69	0	2.1	2.9	6.3
70-79	11.4	12.3	16.0	11.6
80-89	22.7	27.4	33.2	37.9
90-109	56.8	50.7	43.0	40.0
110-119	9.1	6.8	4.9	4.2
120-129	0	0.7	0	0
Total	100	100	100	100
N	44	146	244	95
Mean	96.0	93.3	90.7	89.8

Immunotoxicity

Bencko *et al.* (1988) found no abnormalities in serum concentrations of immunoglobulins in workers exposed to arsenic in a coal-burning power plant. However, the levels of arsenic were not measured. Gonseblatt *et al.* (1992) studied the response to phytohemagglutinin (PHA) stimulation of peripheral blood lymphocytes from healthy human volunteers incubated with arsenate or arsenite at concentrations of 10^{-7} M, 10^{-8} M, or 10^{-9} M. Delays in cell-cycle kinetics were seen at all concentrations of both arsenicals in a dose-dependent pattern. Gonseblatt *et al.* (1994) compared lymphocyte-replicating ability in 33 subjects consuming drinking water with a mean arsenic concentration of 412 µg/L and in 30 control subjects consuming water with a mean concentration of 37 µg/L. First-morning-void urine from the two groups had As concentrations of 758 µg/L and 37 µg/L, respectively. The cell cycle progression of lymphocytes from S phase to M phase following PHA incubation was decreased in the As-exposed subjects, suggesting an impairment of immune response.

Samet *et al.* (1998) studied the activation of mitogen-activated protein kinases (MAPKs) in human bronchial epithelial cells exposed to various metals *in vitro* including arsenic. Treatment of BEAS cells for 15 min with 500 µM sodium arsenite resulted in a differential activation of kinases of varying molecular masses corresponding to extracellular receptor kinase (ERK), c-Jun NH2-terminal kinase (JNK), and P38 kinase. The transcription factors c-Jun and ATF-2, substrates of JNK and P38, respectively, were markedly phosphorylated in BEAS cells treated with As III. The same exposure to As that activated MAPKs also induced a subsequent increase in interleukin (IL-8) protein expression in BEAS cells. The authors speculate that the activation of the distinct MAPKs ERK, JNK, and P38 in metal-exposed human bronchial epithelial cells may result in cellular responses such as growth proliferation, apoptosis, and modulated inflammatory protein expression. The expression of the cytokines IL-6, IL-8, and tumor necrosis factor-α (TNF-α) is regulated through signaling pathways that involve MAPKs and the activation of the transcription factors ATF-2 and c-Jun.

Neurotoxicity

Peripheral neuropathies, beginning with loss of sensation and developing into paralysis and muscle atrophy, frequently develop in patients 10 days to three weeks after acute exposure to arsenic (Hay and McCormack, 1987; Chuttani *et al.*, 1967). These cases are frequently diagnosed as Guillain-Barre syndrome (Donofrio *et al.*, 1987). Adults who have severe gastrointestinal reaction to arsenic rarely escape this complication (Jenkins, 1966). Six to 12 months after arsenic ingestion these signs of poisoning may gradually disappear. The less severe cases often show complete recovery, while the most severe cases are permanently disabled. One woman was permanently bedridden with paresthesia and weakness in all four limbs after swallowing 3 oz of rat poison containing 3.5 percent of arsenic trioxide (Jenkins, 1966).

Encephalopathy, usually reversible, is also encountered in victims of arsenic poisoning. One man swallowing one gram of sodium arsenite suffered from both peripheral neuropathy and encephalopathy. However, unlike most encephalopathy cases reported in the literature, the patient did not recover (Fincher and Koerker, 1987).

Harrington *et al.* (1978) investigated a group of 232 people living in 59 households in Ester Dome, Alaska, an area that had been shown to have a high arsenic concentration in the well water. Each participant was asked to complete a questionnaire regarding residential, occupational, dietary, and water consumption history, and whether they were currently suffering from certain symptoms. Each participant was also given a brief dermatologic and neurologic examination. A venous blood sample was taken from each participant and a sample of tapwater from each house was analyzed for arsenic content. Samples of urine, hair and toenails from each participant were also taken for arsenic analysis.

The 59 households had a mean well-water arsenic concentration of 224 μg/L (range 1.0 - 2450 μg/L). Both trivalent and pentavalent arsenic were present in both well water and urine samples. The 211 subjects were divided into four groups based on their arsenic exposure through well-water consumption. However, there were no differences between any of the four groups in the prevalence of signs or symptoms based on the questionnaire data, the physical examination, or the blood counts.

Kreiss *et al.* (1983) conducted a neurological evaluation of 147 residents of Ester Dome, Alaska; who were less than 60 years of age and who had lived in their residences for at least 2 years. The mean age was 36.3 years and on average, they had lived there 74 months. Well-water arsenic concentrations ranged from 1 to 4781 μg/L, with a mean of 347.3 μg/L. Residents were divided into three groups based on their arsenic exposure.

All subjects were given neurologic examination and nerve conduction tests by neurologists. Although some of the subjects had signs or symptoms suggestive of sensory peripheral neuropathy or one or more abnormal nerve conduction velocities, there was no dose-response relationship. The authors concluded that they had found no evidence of either clinical or subclinical neuropathy in this Alaskan community.

Southwick *et al.* (1983) investigated the effects of exposure to more than 150 μ/L of arsenic in well water on the health of the inhabitants of the towns of Hinckley and Deseret, Utah. Inhabitants of the nearby town of Delta (average As level 17 μg/L) were used as controls. One hundred and forty five exposed subjects and 105 controls were given a dermatological examination for signs of arsenic toxicity. A neurological examination, including nerve conduction velocity tests, was conducted on the 83 exposed and 67 control subjects who were 47 years of age or under. Any signs of peripheral vascular disorder were noted. No significantly increased prevalence of any of the potential signs of arsenic toxicity was found in the exposed group compared to the controls. However, for each health indicator studied, the exposed group showed a slightly higher percentage of abnormalities.

Studies in Japan and Czechoslovakia have reported hearing loss in children (studies described in Tabacova, 1986). In Japan, 12,000 infants were accidentally poisoned with dry milk contaminated with inorganic arsenic. Doses were estimated to be about 3.5 mg/d for 30 d. Anemia, kidney and liver damage were seen, and there were 130 deaths (Hamamoto, 1955; Nakagawa and Ibuchi, 1970). Disturbances of CNS functions were reported in survivors 15 yr after exposure, including severe hearing loss in 18 percent of 415 children studied, and electroencephalographic abnormalities (Yamashita *et al.*, 1972; Ohira and Aoyama, 1972). Pathological eye effects were also seen, including a case of bilateral optic atrophy. Moderate hearing losses apparently due to inner ear damage were reported in children 10 yr of age living near a coal-fired plant emitting large quantities of As (Bencko *et al.*, 1977).

Franzblau and Lilis (1989) reported acute arsenic poisoning resulting from contaminated well water consumption. A married couple moved into a new home and soon experienced a variety of problems including acute gastrointestinal symptoms, central and peripheral neurotoxicity, bone marrow suppression, hepatic toxicity, and mild mucous membrane and slight cutaneous changes. Initial urinary arsenic exceeded 2200 μg/L in the female and 1300 μg/L in the male. In the female, concentrations of arsenic in hair ranged from 0.5 to 6.3 μg/g. Six months after exposure this patient continued to complain of numbness, tingling, and hypesthesia in the lower extremities. In the male, analysis of hair samples revealed As concentrations ranging from 32 to 52 μg/g. His central neurologic symptoms including confusion, disorientation, mental sluggishness and visual changes diminished during chelation therapy but trembling of the extremities persisted for a number of weeks. Subsequent analysis of the well water revealed total As concentrations of 9,000 to 11,000 μg/L. A single speciation gave 6,800 μg/L as arsenates (AsV) and 2,400 μg/L as arsenites (AsIII). The well water was initially tested only for microbiological contaminants despite being only 90 m from an abandoned iron mine.

Greenberg (1996) reported a rare case of acute demyelinating polyneuropathy resulting from arsenic ingestion. The acute neuropathy initially characterized by acute nausea, vomiting, frontal headache, dry cough, and swollen face and eyes, was initially misdiagnosed as Guillain-Barre syndrome (GBS), with electrophysiological and spinal fluid examinations supportive of GBS. The 24-hr urine showed 3176 μg As/L (normal < 80) and after one week was 850 μg/L, resulting in a rediagnosis of acute arsenic poisoning. Within one week the patient developed a ventricular arrhythmia and was found to have pericardial effusion, bilateral pleural effusions, and pancytopenia. Sixteen days after initial onset of symptoms there was severe burning pain, numbness, and swelling in the hands and feet; marked impairment of in grip strength; and difficulty walking, with pain and inability to feel the floor with the feet. Electrodiagnostic studies showed an acquired demyelinating neuropathy.

While the studies described above demonstrate the neurotoxic potential of arsenic, none appears suitable for the quantitative risk assessment of arsenic in drinking water.

Hematotoxicity

A number of arsenic compounds are toxic to blood cells. Exposure to arsenic can result in anemia and leukopenia, which may be because arsenic can cause bone marrow suppression. Acute exposures can produce decreased hematocrit and intravascular hemolysis.

Arsine gas (AsH_3) is a severe hemolytic toxicant that can be acutely fatal (Fowler and Weissberg, 1974). The sequence of toxic events in arsine-induced hemolysis was studied in human erythrocytes *in vitro* by Winski *et al.* (1997). The earliest indicators of damage were changes in sodium and potassium levels. Within five minutes of beginning incubation with 1 mM AsH_3 the cell lost volume control, indicated by leakage of K^+, influx of Na^+, and increases of hematocrit. Arsine did not significantly alter ATP levels or inhibit ATPases. The changes noted were followed by profound disturbances of the plasma ultrastructure. These events preceded hemolysis, which was not significant until

30 min. On contact with arsine, methemoglobin was rapidly formed but reached only 2-3 percent of total cellular hemoglobin. The authors conclude that the *in vivo* hemolysis of human erythrocytes by arsine does not occur via an oxidative mechanism involving hemoglobin.

Winski and Carter (1998) evaluated arsenate toxicity in human erythrocytes and its possible role in vascular disease. Human erythrocytes were incubated *in vitro* with sodium arsenate (As^{V}) or sodium arsenite (As^{III}), and assessed for damage. After five hours incubation with 10 mM As^{V} or As^{III}, significant cell death (hemolysis) only occurred in the As^{V} treated cells. Morphologic changes were observed by scanning electron- and light microscopy. As^{V} induced a concentration dependent discocyte-echinocyte transformation extending to the formation of sphero-echinocytes. Significant echinocyte formation was seen at the lowest concentration of arsenate employed, 0.1 mM. Sphero-echinocytes were significantly increased at 5 mM and higher. Damaged cells exhibited depletion in cellular ATP, which became statistically significant at five hr exposure to 0.01 mM arsenate. Treatment with 0.001 mM arsenate also showed a depletion in ATP and overall there was a clear dose-response (percent of control ATP level vs. log As^{V} concentration). As^{V} was at least 1000 times more toxic than As^{III} based on ATP depletion. The consequences of ATP depletion for the red cell may be severe. ATP is used to maintain membrane shape, deformability, and osmotic stability. Depletion of ATP has been reported to decrease filterability and deformability and to increase blood viscosity (LaCelle, 1970; Rendell *et al.*, 1992;Winski and Carter, 1998). Such changes may contribute to microvascular occlusion, local tissue ischemia, and consequent tissue damage (Weed *et al.*, 1969; Somer and Meiselman, 1993). The occlusive nature of arsenic-induced circulatory disorders suggests that ATP depletion in red cells may play a role in the disease mechanism. Ma *et al.* (1997) reported that patients with arsenicism from Inner Mongolia, China, had circulating erythrocytes with abnormal shapes and damaged cellular membranes.

Meltzer *et al.* (1994) reported that 11 subjects consuming fish diets for six weeks which contained 436-1795 µg As/d, 71-177 µg Se/d, and 9.8-24 µg Hg/d versus 37-170 µg As/d, 33-115 µg Se/d, and 1.2-8 µg Hg/d in 10 control subjects, had dose-dependent increases in cutaneous bleeding times. The dietary As load strongly correlated with both bleeding times and changes in bleeding times ($r = 0.48$, $p < 0.01$ and $r = 0.54$, $p < 0.002$, respectively). Dietary Hg showed a strong negative correlation with HDL-cholesterol ($r = - 0.76$, $p < 0.01$). Selenium had only a modest effect on bleeding time. A multiple regression equation with bleeding time as the independent variable and the covariates of platelet 18:2 fatty acid (P182), dietary arsenic (As), blood Hg (BHg), dietary Hg (DHg), platelet 16:0 fatty acid (P160), and a constant (C), gave an excellent fit, $r = 0.80$, $0.05 < p < 0.005$. The authors caution against over interpretation of these results since they are based on relatively few subjects. Insofar as the noted effects on bleeding time could be considered beneficial effects of fish consumption, it is surprising that arsenic is apparently playing a positive role in this context.

Wu *et al*. (2001) studied the effect of arsenic exposure on reactive oxidants and antioxidant capacity in human blood. Sixty-four subjects aged 42 to 75 years were recruited from an area of northeastern Taiwan where the arsenic concentration in well water varies from 0 to ≥ 3000 µg/L. A chemiluminescence method was used to measure superoxide as "reactive oxidant" and an azinodiethylbenzthiazoline sulfate method to determine antioxidant capacity in blood plasma of study subjects. The arsenic concentration in whole blood of the subjects ranged from 0 to 46.5 µg/L and exhibited a positive association with plasma reactive oxidants (R = 0.41, P = 0.001). A corresponding negative association was seen between arsenic blood concentration and plasma antioxidant activity (R = - 0.30, P = 0.014). The results suggest a causal relation between arsenic ingestion via contaminated well water and increased reactive oxidants in blood. Persistent oxidative stress in peripheral blood may be a mechanism supporting carcinogenesis and atherogenesis observed in chronic arsenic exposure.

Pi *et al.* (2002) studied 33 individuals from Wuyuan, Inner Mongolia, China who had been drinking well water with high concentrations of arsenic (mean value = 0.41 mg/L) for about 18 years. Ten nearby residents exposed to lower arsenic well water concentration (mean value = 0.02 mg/L) served as a control group. The mean concentrations of Asi, MMA, and DMA in the blood of the exposed group were 8.2, 20.7, and 13.2 µg/L versus 2.7, 2.1, and 2.6 µg/L in the control group, respectively. Although no increase over control was noted in serum superoxide dismutase activity, a significant increase in the mean level of lipid peroxides was seen (8.8 vs. 7.1 µM, $P < 0.05$). Also, the exposed group showed a significant decrease in whole blood nonprotein sulfhydryl levels (4.3 vs. 7.5 µmol/g Hb, $P < 0.01$). The results support a link between ingested arsenic via contaminated well water and the induction of oxidative stress.

Vascular Disease

Vascular diseases have long been noted to be associated with chronic arsenic exposures among German vineyard workers (Grobe, 1976) and inhabitants of Antofagasta, Chile (Borgono *et al.*, 1977). Peripheral vascular diseases have been reported to be associated with the occurrence of arsenic in well waters in Taiwan (Chen and Wu, 1962; Chi and Blackwell, 1968; Tseng, 1977; Chen *et al.*, 1988a). In a review of the literature that included 177 citations, Engel *et al.* (1994) concluded that there was good epidemiological evidence indicating that chronic arsenic consumption at high levels is a cause of severe vascular disease with resulting gangrene and limb amputations. These authors also concluded that it was plausible, though epidemiologic evidence was limited (at that time), that arsenic might cause increases in vascular mortality beyond that found in patients with severe peripheral vascular disease.

Wu *et al.* (1989) found significant trends of mortality rates from peripheral vascular diseases and cardiovascular diseases with concentrations of arsenic in well water. However, no significant association was observed for cerebrovascular accidents. Engel and Smith (1994) evaluated arsenic in drinking water and mortality from vascular disease in 30 U.S. counties from 1968 to 1984. Mean As levels in drinking water ranged from 5.4 to 91.5 µg/L. Standardized mortality ratios (SMRs) for diseases of arteries, arterioles, and capillaries (DAAC) for counties exceeding 20 µg/L were 1.9 (90 percent C.I. = 1.7-2.1) for females and 1.6 (90 percent C.I. = 1.5-1.8) for males. SMRs for three subgroups of DAAC including arteriosclerosis and aortic aneurysm were also elevated, as were congenital abnormalities of the heart and circulatory system.

Tseng *et al.* (1996) studied the dose relationship between peripheral vascular disease (PVD) and ingested inorganic arsenic in blackfoot disease endemic villages in Taiwan. A total of 582 adults (263 men and 319 women) underwent Doppler ultrasound measurement of systolic pressures on bilateral ankle and brachial arteries and estimation of long-term arsenic exposure. The diagnosis of PVD was based on an ankle-brachial index of < 0.9 on either side. Multiple logistic regression analysis was used to assess the association between PVD and As exposure. A dose-response was observed between the prevalence of PVD and long-term As exposure. The odds ratios (95 percent confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14), and 4.28 (1.26-14.54) for those who had cumulative As exposures of 0.1 to 19.9 and ≥ 20 (mg/L) x yr, respectively. A follow up study (Tseng *et al.*, 1997) indicated that PVD was correlated with ingested As and not with abnormal lipid profiles. The lipid profiles studied were total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), apolipoprotein AI, and apolipoprotein B. Other lipids such as modified LDL, subclasses of LDL and HDL, and other lipoproteins such as lipoprotein(a), which may track as better indicators of atherosclerosis, were not included. In addition, the roles of platelet aggregation and coagulation profiles were not studied.

Chen *et al.* (1996a) evaluated the dose-response relationship between ischemic heart disease (ISHD) mortality and long-term arsenic exposure. Mortality rates from ISHD among residents in 60 villages in an area of Taiwan with endemic arseniasis from 1973 through 1986 were analyzed for association with As concentrations in drinking water. Based on 1,355,915 person-years and 217 ISHD deaths, the cumulative ISHD mortalities from birth to age 79 yr were 3.4 percent, 3.5 percent, 4.7 percent, and 6.6 percent for the median As concentrations of < 0.1, 0.1-0.34, 0.35-0.59, and ≥ 0.6 mg/L, respectively. Multivariate-adjusted relative risks (RRs (95 percent C.I)) associated with cumulative arsenic exposure from well water were 2.46 (9.53-11.36), 3.97 (1.01-15.59), and 6.47 (1.88-22.24) for 0.1-9.9, 10.0-19.9, and 20+ (mg/L)-yr, respectively, compared with those without As exposure.

Chiou *et al.* (1997b) evaluated the dose-response relationship between prevalence of cerebrovascular disease and ingested arsenic among residents of the Lanyang Basin in northeast Taiwan. A total of 8102 adults from 3901 households were recruited for the study. Arsenic in well water of each household was determined by hydride generation and atomic absorption spectrometry. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95 percent confidence intervals for various risk factors of cerebrovascular disease. A significant dose-response relationship was observed between As concentration in well water and prevalence on cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The dose-response was even more prominent for cerebral infarction, with multivariate-adjusted odds ratios (95 percent C.I.) of 1.0, 3.4 (1.6-7.3), 4.5 (2.0-9.9), and 6.9 (3.0-16), respectively, for those who consumed well water with As concentration of 0, 0.1-50.0, 50.1-299.9, and >300 μg/L. For cumulative arsenic exposures of <0.1, 0.1-4.9, and ≥5.0 (mg/L)yr the odds ratios were 1.00, 2.26, and 2.69 for cerebrovascular disease, and 1.00, 2.66, and 3.39 for cerebral infarction, respectively.

All of the values above for As exposed groups were significantly greater than unexposed at $p < 0.05$ or less.

Wang *et al*. (2002) studied the association between long-term arsenic exposure and carotid atherosclerosis (CA) in 463 residents of an arseniasis endemic area in Taiwan. The extent of CA was measured by duplex ultrasonography. The presence of plaque and/or the increase in the intimal-medial thickness were used to assess the progression of CA. Diabetes mellitus was assessed by oral glucose tolerance test and hypertension by mercury sphygmomanometers. Information regarding the consumption of high-arsenic artesian well water, cigarette smoking, and alcohol consumption was obtained by questionnaire interviews. Logistic regression was used to estimate the odds ratio and its 95 percent C.I. of CA for various risk factors. Three indices of long-term exposure to ingested arsenic, the duration of consuming artesian well water, the average concentration of arsenic in the well water, and the cumulative arsenic exposure, were all significantly associated with the prevalence of CA in a dose response relationship. This relationship remained significant after adjustment for age, sex, hypertension, smoking, diabetes mellitus, alcohol consumption, waist-to-hip ratio, total serum cholesterol, and LDL cholesterol. The multivariate-adjusted odds ratio was 3.1 (95 percent C.I. 1.3-7.4) for those who had cumulative arsenic exposure of ≥ 20 (mg/L)yr compared with those without exposure to arsenic from artesian well water. The authors conclude that chronic arsenic exposure is an independent risk factor for atherosclerosis and that CA is a novel biomarker for arseniasis. Although the effects in this study were subclinical, since they were observed before the development of events such as acute myocardial infarction and stroke, but still late in the atherosclerotic process, they may be considered "adverse" effects due the serious potentially fatal outcomes to which they may lead. Thus the value noted above may indicate a chronic LOAEL for carotid atherosclerosis resulting from arsenic exposure via drinking water.

Chen *et al.* (1995) also investigated the association between long-term exposure to inorganic arsenic and the prevalence of hypertension. A total of 382 men and 516 women were studied in villages where arseniasis was hyperendemic. Hypertension was defined as a systolic blood pressure of 160 mm Hg or greater, or a history of hypertension treated with antihypertensive drugs. The long-term arsenic exposure was calculated from the history of artesian well water consumption obtained through subject questionnaires and the measured arsenic concentration in well water. Residents in villages where long-term arseniasis was endemic had a 1.5-fold increase in age- and sex-adjusted prevalence of hypertension compared with residents in nonendemic areas. Duration of well water consumption, average As water concentration, and cumulative As exposure were all significantly associated with hypertension. For the cumulative As exposure in (mg/L)yr the percent prevalence values were: 0, 5.0; 0.1-6.3, 4.9; 6.4-10.8, 12.8; 10.9-14.7, 22.1; 14.8-18.5, 26.5; > 18.5 (mg/L)yr, 29.2 percent.

As part of a study of arsenic exposure via drinking water and mortality outcome in Millard County, Utah, Lewis *et al.* (1999) found a statistically significant association with mortality from hypertensive heart disease. Median drinking water concentration of arsenic ranged from 14 to 166 µg/L for the 946 subjects in the study. The standard mortality ratios (SMR) without regard to specific exposure levels were (SMR = 2.20, 95 percent C.I. 1.36-3.36) for males and (SMR = 1.73, 95 percent C.I. 1.11-2.58) for females. When analyzed by cumulative exposure groups of low (< 1.0 (mg/L)yr), medium (1.0-4.9 (mg/L)yr), and high (≥ 5.0 (mg/L)yr) there was no apparent dose response. However, the cumulative dose estimates in this study are lower than in the Chen *et al.* (1995) discussed above, so the results of the two studies are not inconsistent.

Rahman *et al*. (1999) conducted a study of hypertension among subjects with and those without exposure to arsenic via drinking water in Bangladesh. Wells with and without arsenic were identified and 1595 subjects who apparently used the wells and were ≥ 30 years of age were interviewed. Of the 1595 subjects, 1481 had a history of well water consumption and 114 did not. Time weighted mean arsenic levels in mg/L and (mg/L)yr were estimated for each subject. Exposure categories were derived as <0.5 mg/L, 0.5 to 1.0 mg/L, and > 1.0 mg/L and cumulative exposures as <1.0 (mg/L)yr, 1.0 to 5.0 (mg/L)yr, >5.0 to 10 (mg/L)yr, and > 10 (mg/L)yr. Hypertension was defined as a systolic blood pressure ≥140 mm Hg. Using "unexposed" subjects as the reference, the prevalence ratios (95 percent C.I.) for hypertension adjusted for age, sex, and body mass index (BMI) were 1.2 (0.6-2.3), 2.2 (1.1-4.3), 2.5 (1.2-4.9) and 0.8 (0.3-1.7), 1.5 (0.7-2.9), 2.2 (1.1-4.4), and 3.0 (1.5-5.8) for the metrics of mg/L and (mg/L)yr, respectively. Both metrics showed significant dose response trends (P << 0.001) for crude and adjusted data sets.

In general, studies among workers exposed to arsenic, principally via inhalation, have shown weak or no associations with the incidence of vascular disease. Hertz-Picciotto *et al.* (2000) examined circulatory disease mortality data among 2,802 smelter workers in Tacoma, Washington. Six cumulative exposure levels from <750 to ≥20,000 μg As/m^3-year were analyzed. For total circulatory disease, the baseline analysis showed no significant effect at any exposure level. Adjustments for healthy worker survivor effects (HWSE) including work status and 10 to 20-year lags led to some significant rate ratios e.g., RR = 1.6 (95 percent C.I. 1.2-2.1) for 8,000-19,999 μg As/m^3-yr with a 20-year lag. Restricting the analysis to cardiovascular disease increased the rate ratios in general, the highest value being 2.0 (95 percent C.I. 1.4-3.0) for 8,000-19,999 μg As/m^3-yr with a 10-year lag and work status adjustments. These authors concluded that HWSE obscures an effect of arsenic on circulatory disease in occupationally exposed individuals.

A number of the studies on vascular effects appear suitable for quantitative risk assessment including cerebrovascular disease (Chiou *et al*., 1997b), ischemic heart disease (Chen *et al*., 1996a), peripheral vascular disease (Tseng *et al*., 1996) and hypertension (Chen *et al*., 1995; Rahman *et al*., 1999).

Diabetes Mellitus

Chronic exposure to arsenic has been associated with late-onset or Type 2 diabetes or diabetes mellitus in several studies.

In a study related to those above, Lai *et al.* (1994) studied inorganic arsenic ingestion and the prevalence of diabetes mellitus. A total of 891 adult residents of villages in southern Taiwan where arseniasis is hyperendemic were included in the study. Diabetes status was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. Cumulative arsenic exposure in ppm-yr was determined from the detailed history of drinking artesian well water. There was a dose-response relation between cumulative arsenic exposure and prevalence of diabetes mellitus. The relation remained significant after adjustment for age, sex, body mass index, and activity level at work, by a multiple logistic regression analysis giving multivariate-adjusted odds ratios of 6.61 and 10.05, respectively, for exposures of 0.1-15 ppm-yr and > 15.0 ppm-yr versus an unexposed group.

In an effort to confirm this association between diabetes mellitus and arsenic observed for drinking water in Taiwan, Rahman and Axelson (1995) reviewed 1978 case-control data from a Swedish copper smelter. Twelve cases of diabetes mellitus (death certificate) were compared with 31 controls without cancer, cardiovascular and cerebrovascular disease. The odds ratios for diabetes mellitus with increasing arsenic exposure categories were 1.0 (reference level), 2.0, 4.2, and 7.0 with the 95 percent C.I. including unity. The trend was weakly significant, $p = 0.03$. Albeit with limited numbers, the study provides some support for a role of arsenic exposure in the development of diabetes mellitus.

Rahman *et al*. (1998) assessed arsenic exposure as a risk factor for diabetes mellitus in western Bangladesh. The survey conducted in 1996 included 163 subjects with keratosis taken as exposed to arsenic and 854 unexposed individuals. Diabetes mellitus was determined by history of symptoms, previously diagnosed diabetes, glucosuria, and blood sugar level after glucose intake. Three time-weighted average exposure levels were derived: < 0.5 mg/L; 0.5 to 1.0 mg/L; and > 1.0 mg/L. For the unexposed and the three exposure levels the adjusted prevalence ratios (95 percent C.I.) were 1.0, 2.6 (1.2-5.7), 3.9 (1.5-8.2), 8.0 (2.7-28.4), respectively. The Chi squared test for trend was very significant ($P<0.001$). Although this study is somewhat weaker than the earlier study of Lai *et al*. (1994) in having smaller numbers and lack of comprehensive long-term well water analysis for arsenic, it does corroborate the earlier Taiwanese study.

Tseng *et al*. (2000) followed up the study of Lai *et al*. (1994) with a prospective cohort study. A total of 446 nondiabetic residents of arseniasis-endemic villages in Taiwan were followed biannually by oral glucose tolerance test. Diabetes was defined as a fasting plasma glucose level ≥ 7.8 mmol/L and/or a two hour post-load glucose level of ≥ 11.1 mmol/L. During the follow-up period of 1500 person-years, 41 cases developed diabetes with an overall incidence of 27.4/1000 P-yr. The incidence of diabetes correlated with age, BMI, and cumulative arsenic exposure (CAE). The multivariate adjusted risks were 1.6, 2.3, and 2.1 for greater versus less than 55 years, 25 kg/m^2, and 17 (mg As/L)yr, respectively. The incidence rates (per 1,000 P-yr) were 18.9 for CAE < 17 (mg/L)yr and 47.6 for CAE ≥ 17 (mg/L)yr. The crude relative risk (95 percent C.I.) was 2.5 (1.4-4.7) and the adjusted relative risk was 2.1 (1.1-4.2) for higher vs. lower CAE. The results support earlier finding of a dose-dependent association between long-term arsenic exposure and diabetes mellitus, based on prevalence of diabetes mellitus.

Wang *et al*. (2002) evaluated the prevalence of non-insulin dependent diabetes mellitus and related vascular diseases in southwestern arseniasis-endemic and non-endemic areas of Taiwan. The National Health Insurance Database in 1999-2000 was used to derive the prevalences by age and gender. A total of 66,667 residents of arseniasis-endemic and 639,667 in non-endemic areas, aged 25 years and older, were included in the study. The prevalence of diabetes age-gender adjusted to the general population in Taiwan was 7.5 percent (95 percent C.I., 7.4-7.7 percent) in the arseniasis area and 3.5 percent

(3.5-3.6 percent) in the non-arseniasis area. Among both diabetics and non-diabetics, higher prevalences of microvascular and macrovascular diseases were seen in the arseniasis areas versus the non-endemic areas. For microvascular disease the age-gender adjusted prevalence was 20 percent vs. six percent in the endemic and non-endemic areas, respectively for diabetics, and 8.6 percent vs. one percent for non-diabetics. The corresponding prevalences for macrovascular disease were 25.3 percent vs. 13.7 percent for diabetics and 12.3 percent and 5.5 percent for non-diabetics. The authors concluded that ingested arsenic had a greater contribution than diabetes on the development of microvascular diseases. The also suggested that risk assessment of arsenic exposure for diabetes and related vascular diseases should be integrated with cancer risk assessment.

It should be noted that studies of mortality rather than morbidity are likely to underestimate the true burden of living with chronic vascular disease or diabetes mellitus.

The studies of Lai *et al*. (1994) and Rahman *et al*. (1998) on diabetes mellitus and arsenic in drinking water appear to be suitable for quantitative risk assessment. Tseng *et al*. (2002) in reviewing six epidemiologic studies of arsenic and diabetes mellitus noted that while consistent associations were seen, weak study designs and the use of glucosuria or diabetes death as diagnostic criteria, and lack of adjustment for possible confounders were limitations that reduced the strength of the evidence.

Respiratory Disease

Non-malignant pulmonary effects have also been associated with ingestion of inorganic arsenic. Studies in Chile lend support to this association. In 1970, Rosenberg conducted autopsies on five children manifesting characteristic signs of chronic arsenic poisoning, including hyperpigmentation and/or keratoses, who died between 1968 and 1969 in Antofagasta. Lung tissue was examined in four of the five children, with abnormalities found in each and two having pulmonary interstitial fibrosis (Rosenberg, 1974). A 1976 cross-sectional survey in Antofagasta examined 144 schoolchildren with arsenic-induced skin lesions, and reported that bronchopulmonary disease occurred 2.5 times more often in these children (15.9 percent) compared with children with normal skin (6.9 percent) (Borgono *et al.*, 1977). In survey data collected between 1968 and 1972 in Antofagasta, Chile, Zaldivar reported that the prevalence of cough and/or dyspnea among 398 children correlated with mean drinking water arsenic concentrations (Zaldivar and Ghai, 1980). In addition, they found the prevalence of bronchiectasis was 23 times higher and recurrent bronchopneumonia was 3.44 times higher in children with chronic arsenical dermatosis than in the general population of Chilean children (Zaldivar, 1980). Finally, over a three-year period following installation of a water arsenic treatment plant in Antofagasta, the prevalence of cough and/or dyspnea dropped from 38 percent to 7 percent ($p < 0.001$), a rate similar to that found in a non-exposed region of Chile.

In a recent study performed in Chile, even though COPD mortality was not increased overall, SMRs for the years 1989-1993 were increased for both men and women aged 30-39 years (combining men and women, 10 deaths observed, 0.9 expected, SMR=11.1, 95 percent C.I. 5.3-20.4, $p<0.001$) (Smith *et al.*, 1998). Those in this age group were likely to have had their highest exposure to inorganic arsenic as children in the period 1955-1970, when arsenic levels in water in this region, particularly in the city of

Antofagasta, were at their peak. The potential impact of childhood exposure on the lung is also supported by the fact that in both men and women the highest lung cancer SMRs were for young adults with potential childhood exposure, with the lung cancer SMR being as high as 11.7 among men in the age range 30-39. There were 14 deaths in this group versus 1.2 expected, SMR = 11.7, (95 percent C.I. 6.3-19.6, p<0.001).

Studies in West Bengal, India, also contribute information on ingested arsenic and non-malignant respiratory effects. Symptoms of cough were reported by 89 of 156 patients with arsenic-associated skin lesions and 17 of these patients showed evidence of restrictive disease on pulmonary function testing (Mazumder *et al.*, 1998). To further investigate the relationship of non-malignant respiratory disease with ingested arsenic, we have analyzed data from a cross-sectional survey of 7,683 participants who were clinically examined and interviewed, and the arsenic content in their current primary drinking water source was measured (Mazumder *et al.*, 2000). Because there were few smokers, analyses were confined to nonsmokers (N = 6,864 participants). Study subjects included those who had arsenic-associated skin lesions such as hyperpigmentation and hyperkeratosis, and who were also highly exposed at the time of the survey (arsenic water concentration ≥500 µg/L). Individuals with normal skin and low arsenic water concentration (<50 µg/L) were used as the referent group. Reported shortness of breath was 23-fold greater among the exposed women with skin lesions than in the referent group (age-adjusted prevalence odds ratio, POR = 23.3, 95 percent C.I. 5.8-92.8). The prevalence of cough and crepitations were also dramatically elevated among participants with skin lesions (see Table 7). These results warrant further investigation concerning non-malignant respiratory effects of inorganic arsenic. The findings from this large India survey raise the possibility that respiratory effects are largely present only in people with skin lesions, but this needs to be confirmed in a more focused study.

Table 7. Prevalence Odds Ratio for Participants with and without Skin Lesions Exposed to >500 µg/L (with 95 Percent Confidence Interval)

	MALE	FEMALE
With Skin Lesions		
Cough	5.0 (2.6-9.9)	7.8 (3.1-19.5)
Crepitations	6.9 (3.1 –15.0)	9.6 (4.0-22.9)
Shortness of Breath	3.7 (1.3-10.6)	23.2 (5.8-92.8)
Without Skin Lesions		
Cough	0.9 (0.5-1.7)	1.8 (1.0-3.4)
Crepitations	1.2 (0.5-2.6)	1.6 (0.8-3.2)
Shortness of Breath	1.5 (0.6-3.7)	5.2 (1.9-14.8)

While respiratory effects of arsenic exposure appear to be significant, the data available do not appear suitable for quantitative risk assessment.

Biochemical and Cellular Toxicity

Styblo *et al.* (2000) investigated the effects of trivalent and pentavalent arsenicals in human hepatocytes incubated for 24 hr with 0.4 to 20 μM test agents. Exposure to 4 to 20 μM As^{III} or As^{V} had similar effects on cell viability as measured by decreasing rates of MTT (thiazolyl blue) conversion (20 to 25 percent) without apparent changes in cell morphology. By contrast, exposure to 1 to 10 μM $MAs^{III}O$ caused a dose-dependent toxicity and morphological changes in the cells similar to those seen in rat hepatocytes (see above). The estimated IC_{50} for $MAs^{III}O$ was 5.5 μM. Treatment with the glutathione complex $DMAs^{III}GS$ had no significant effect on MTT conversion or cell morphology. Pentavalent arsenicals were not cytotoxic at concentrations up to 20 μM.

The effects of tri- and pentavalent arsenicals were also investigated in human epidermal keratinocytes (Styblo *et al.*, 2000). Increased rates of MTT conversion were observed in cells exposed to 0.1 to 0.4 μM As^{III} or to 0.1 μM $DMA^{III}GS$, indicating increased cell proliferation or activation of enzymes involved in MTT metabolism. Treatment with higher concentrations of trivalent arsenicals decreased the rate of MTT conversion in a dose-dependent manner: e.g., As^{III} 60 percent of control at 20 μM; $MAs^{III}O$ 10 percent of control at 10 μM; and $DMAs^{III}GS$ 40 percent of control at 10 μM. The estimated IC_{50} values for $MAs^{III}O$ and $DMAs^{III}GS$ were 2.6 and 8.5 μM, respectively. Cellular morphology was also affected at these concentrations and higher. $MAs^{III}O$ was the most cytotoxic of the agents tested. As with the hepatocyte assays, pentavalent arsenicals were not cytotoxic at concentrations up to 20 μM.

Similar experiments were conducted with human bronchial cells and Urotsa cells, a SV-40-transformed epithelial cell line derived from normal human urinary bladder. The bronchial cells were sensitive to the toxic effects of all the trivalent arsenicals tested with estimated IC_{50} values of: As^{III} 3.2 μM, $MAs^{III}O$ 2.7 μM, and $DMAs^{III}GS$ 6.8 μM. Pentavalent arsenicals were not cytotoxic up to 20 μM. Urotsa cells showed the highest sensitivity to $MAs^{III}O$. The estimated IC_{50} values were: As^{III} 17.8 μM, $MAs^{III}O$ 0.8 μM, and $DMAs^{III}GS$ 14.2 μM. Trivalent methylated arsenicals appeared significantly more toxic for normal human hepatocytes, epidermal keratinocytes, bronchial epithelial cells, and urinary bladder cells than pentavalent As species. These and other data indicate that methylation of inorganic arsenic may not connote detoxication in all cases and may generate methylated metabolites of comparable or higher toxicity than the parent inorganic arsenic species. Furthermore, the cytotoxicity shown by trivalent arsenicals was apparently independent of the target cell's methylation capacity (Styblo *et al.*, 2000).

Jessen *et al*. (2001) studied the response of human keratinocytes to arsenic suppression of their differentiation. Four representative differentiation marker mRNAs (involucrin, INV; keratinocyte transglutaminase, TGM1; small proline-rich protein 1, SPRR1; and filaggrin, FIL) were suppressed by arsenite and arsenate in normal and malignant keratinocytes in vitro. SIK, SCC-9, and hEp cells were grown in concentrations of 0.3,1, 3, and 10 μM arsenate and 0.1, 0.3, 1, and 3 μM arsenite. Relative mRRNA values were measured from Northern blots of triplicate cultures and graphed to permit determination of concentrations giving half-maximal suppression (EC_{50}). EC_{50} values for arsenate suppression ranged from 0.5 to 6 μM both for FIL. For arsenite suppression, EC_{50}s ranged from 0.3 for SPRR1 to 1.4µM for FIL. In general, the SIK and SCC9 cell lines were more sensitive to the effects of arsenic. The suppression was almost completely reversed nine days after removal of arsenate from the culture medium. In the case of to INV gene, suppression was mediated primarily by two AP1 response elements in the gene promoter. Both glucocorticoid and serum stimulation of differentiation occurred to a similar extent in the presence and absence of arsenic, indicating that neither was a target of arsenic action. Alternatively, 12-*O*-tetradecanoylphorbol-13-acetate prevented the suppression of keratinocyte transglutaminase, suggesting action upstream from protein C kinase. In general, these results contrast those of Kaltreider *et al*. (2001) noted above for rat H4IIE hepatoma cells and indicate significant cell type and/or species dependence of arsenic effects on nuclear receptors. For example, Hong *et al*. (2001) have identified the SMRT corepresor as a potentially important target of arsenic action operating in normal and neoplastic cells.

Skin Effects

Tseng *et al.* (1968) examined 40,421 inhabitants of 37 villages in an area on the Southwest coast of Taiwan where artesian well water with a high arsenic concentration (mostly 0.4-0.6 ppm, but ranging from 0.01 to 1.82 ppm) had been used for more than 45 years. The examination paid particular attention to skin lesions, peripheral vascular disorders, and cancers. Well water samples were collected from most of the villages where such water was still being used and villages were arbitrarily designated into "low," "mid," and "high" groups according to their well-water arsenic concentration (<0.3, 0.3-0.6 and >0.6 ppm, respectively). Overall, there were 7,418 cases of hyperpigmentation, 2,868 of keratosis, 428 of skin cancer, and 360 of Blackfoot disease. By contrast, in a control population of 7,500 persons (two-thirds of whom had non-detectable arsenic in their water supply and the remaining third 0.001 - 0.017 ppm), which was surveyed in the same manner, no case of any of the above disorders was found.

In the exposed population, dose-response relationships were found for skin cancer and Blackfoot disease (Tseng, 1977), with elevated levels of both diseases, relative to the control population, in all three exposure groups. (Comparable dose-response data were not presented for hyperpigmentation and keratosis.) Prevalence rates of both diseases were also found to increase with age and generally, to be higher for males than for females. The overall male to female ratios for skin cancer and Blackfoot disease were 2.9 and 1.3, respectively.

As mentioned previously there is some dispute as to whether the arsenic is itself responsible for the Blackfoot disease (Yu *et al.*, 1984; Yu, 1984). However, in this study the rate of Blackfoot disease occurring with one or more skin cancer, keratosis or hyperpigmentation in the same individual was greater than would have been expected by chance. This lends support to the view that the four conditions are likely to have a common cause.

Yue-Zhen *et al.* (1985) reported a study of people who had been drinking water from a well at an oil extracting plant in the Kuitun Area, Xinjiang Uighur Autonomous Region, China. The well contained 0.6 mg/L arsenic, in addition to 3.45 mg/L fluoride. Of the 359 exposed persons, 336 (93.6 percent) were examined and 44.6 percent (150/336) were found to have skin lesions characteristic of chronic arsenicism. The shortest period between initiation of arsenic exposure and diagnosis of skin lesions was six months and the longest 12 years. Generally, depigmentation was the first symptom to appear, followed by keratosis. The authors assumed that the high fluoride content of the water contributed to the high morbidity rate (compared with the Taiwanese experience) but no evidence was presented to support this view.

Cebrian *et al.* (1983) compared two towns in the north of Mexico, one of which (El Salvador De Arriba) had an arsenic level in its water supply of 0.41 mg/L (range 0.16 - 0.59) and the other (San Jose del Vinedo) a level of 0.005 mg/L. The authors estimated that 70 percent of the arsenic was arsenate and the remainder arsenite. In each town, every third house was selected for survey and every member of each family present was examined. In the exposed town, this amounted to 57 households with 296 individuals and in the control town, 68 households housing 318 individuals. Both samples were comparable in terms of socioeconomic status and in terms of age and sex distribution, except for a higher proportion of over-60 individuals in the control town.

The prevalence of cutaneous signs of chronic arsenic poisoning in the exposed population was 21.6 percent compared to 2.2 percent in the controls (Cebrian *et al.*, 1983). The prevalence of skin pigmentation changes of the affected individuals increased with age until age 50 and until age 40 for the other signs, after which there were non-significant decreases. The most common cutaneous sign among those affected in the exposed population was hypopigmentation (81 percent), followed by hyperpigmentation (56 percent), palmoplantar keratosis (52 percent), papular keratosis (23 percent), and ulcerative lesions (6 percent). The clinical presentation of ulcerative lesions was consistent with their being either epidermoid or basal cell carcinomas. However, they were not recorded as such because of the reluctance of people to give samples for histopathologic confirmation.

Minimum doses and shortest times of exposure after which lesions were detected were estimated as follows: hypopigmentation (2 g and 8 years), hyperpigmentation and palmoplantar keratosis (3 g and 12 years), papular keratosis (8 g and 25 years) and ulcerative lesions (12 g and 38 years).

Borgono and Greiber (1972) examined the effects of arsenic on the inhabitants of the city of Antofagasta, Chile, that from 1958 to 1970 (when a treatment plant was installed) had a water supply with an arsenic content of 0.8 ppm. In their 1969 investigation it was shown that, relative to a sample of 98 inhabitants (hospital patients) of the city of Iquique (water-supply arsenic level not given), a group of 180 Antofagasta hospital patients had a high rate of a number of clinical manifestations. These included bronchopulmonary disease, hyperkeratosis, chronic cough, and various cardiovascular problems, particularly Raynaud's syndrome and acrocyanosis. When the Antofagasta patients were divided into those with skin pigmentation and those with normal skin, the pigmented group had even higher rates of these conditions. There is the possibility of a selection bias operating here as the criteria for the selection of the members of these patient groups were not stated.

Borgono *et al.* (1977) have reported an investigation conducted in 1976 of 645 Antofagasta school children, 306 under 6 years and 339 over that age, who were examined for skin lesions. The purpose was to determine whether the installation of the treatment plant had made a difference to the prevalence of the symptoms of chronic arsenicism (the reduced arsenic level of the water supply is not given). No skin lesions were found in children up to eight years of age. However, it is not clear whether this was an effect of the reduced arsenic content of the water supply or simply that a longer exposure was necessary to produce clinical effects.

Hindmarsh *et al.* (1977) examined 92 of a group of 110 people using the 29 wells in Waverly, Nova Scotia, which had arsenic levels exceeding the Canadian maximum permissible limit of 0.05 ppm. A control group of 21 people exposed to well waters containing 0.05 ppm of arsenic or less was also examined. Clinical examinations were performed and medical histories were taken by physicians blind to the arsenic exposure status of the subjects. Patients were considered abnormal if two features of either the medical history or the physical examination were abnormal. All the people undergoing medical examinations were invited to have an electromyographic examination. Thirty-three of those using high arsenic wells accepted, as did 12 of the controls. Dividing the subjects into three exposure groups according to their well-water arsenic concentration, ordered 2 x c contingency testing showed a positive relationship ($p = 0.0026$) between the frequency of positive clinical findings and drinking water arsenic concentration. Of the people who underwent electromyographic examination, 50 percent (7/14) of those who were exposed to >0.1 ppm showed abnormalities. The corresponding figures for those exposed to between 0.05 and 0.1 ppm and to <0.05 ppm were 17 percent (3/18) and 0 percent (0/12), respectively. Of the three people in the middle exposure group who demonstrated electromyographic abnormalities, two were exposed to 0.09 ppm and one to 0.06 ppm.

Valentine *et al.* (1985) compared residents of four U.S. communities with water-supply arsenic levels ranging from 51 to 393 μg/L with two control communities with arsenic levels below 50 μg/L. All residents used tap water and had resided in their community for at least one year prior to data collection. Based on health histories obtained by questionnaire, there were no differences in symptom reporting for neurological, circulatory, or skin disorders.

Goldsmith *et al.* (1972) studied 171 individuals living in 76 households in Lassen County, California, which has high well-water arsenic levels in some areas. From each individual a water sample, a hair sample and responses to a demographic and health questionnaire were sought. Both samples and the completed questionnaire were obtained for 98 individuals. The range of well-water arsenic levels found was from 100 to 1,400 μg/L and a significant association was found between well water and hair arsenic levels. However, no significant associations were obtained for any of the reported symptoms or illnesses when subjects were dichotomized into high and low exposure groups based on hair arsenic content.

Mazumder *et al.* (1998) investigated arsenic-associated skin lesions of keratosis and hyperpigmentation in 7,683 exposed subjects in West Bengal, India. While water arsenic concentrations ranged up to 3,400 μg/L, over 80 percent of the subjects were consuming water with < 500 μg/L. The age-adjusted prevalence of keratosis was strongly related to water As concentration, rising from zero in the lowest exposure level (< 50 μg/L) to 8.3 percent for females drinking water containing >800 μg As/L, and 0.2 to 10.7 percent in males, respectively. A similar dose-response was observed for hyperpigmentation, 0.3 to 11.5 percent for females, and 0.4 to 22.7 percent for males. Overall, males had 2-3 times the prevalence of both keratosis and hyperpigmentation than females apparently ingesting the same doses of arsenic per body weight. Subjects that were more than 20 percent below standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratoses, suggesting that malnutrition may play a role in increasing susceptibility.

Habibul *et al.* (2000) studied associations between drinking water arsenic and urinary arsenic levels and the occurrence of skin lesions in Bangladesh. The survey included 167 residents of three contiguous villages in Bangladesh of which 27 (16.2 percent) had keratosis, 34 (20.4 percent) had melanosis, and 36 (21.6 percent) had either keratosis and/or melanosis. Subjects with skin lesions were more likely to have a higher level of arsenic in their drinking water or urine (with or without creatinine adjustment). Also subjects with skin lesions were more likely to have a higher cumulative arsenic index (i.e., yearly water consumption x arsenic concentration in water x years of well use). Significantly, a sizeable proportion of subjects with skin lesions was seen at the lowest As levels: 13/36 (36.1 percent) drank water with <50μg As/L; and 5/36 (13.9 percent) with <10 μg As/L. Overall there was more than a three-fold elevated risk of skin lesions for those subjects who had the highest levels of urinary arsenic. Adjustment for urinary creatinine did not markedly alter this finding. The risk was also higher, but not significantly so, when arsenic was measured from current water concentration or the cumulative arsenic index.

The studies of Tseng (1977), Tseng *et al*. (1968), and Mazumder *et al*. (1998) on arsenic-induced skin effects, especially skin keratosis, appear to be suitable for quantitative risk assessment.

Overview of Noncancer Epidemiology

It is apparent from the foregoing study descriptions that exposure to arsenic via drinking water is associated with a number of serious health effects, often in a dose-related manner. Tsai *et al*. (1999) compared mortality due to all causes in areas of Taiwan with high levels of arsenic in drinking water. Standardized mortality ratios (SMRs) for noncancer and cancer diseases, by sex, during the period 1971 to 1994 were calculated both with local and national reference groups. Arsenic levels in the study group drinking water ranged from 0.25 to 1.14 ppm (median = 0.78 ppm). The local study area reported 11,193 male and 8,874 female deaths compared to 113,576 and 80,350 in the local reference, and 1,290,606 and 836,203 in the national reference groups, respectively.

For males with the local reference, significant SMRs (95 percent C.I.) were seen for diabetes mellitus 1.35 (1.16-1.55), ischemic heart disease 1.75 (1.59-1.92), cerebrovascular disease 1.14 (1.08-1.21), vascular disease 3.56 (2.91-4.30), bronchitis 1.48 (1.25-1.73), asthma 1.18 (1.08-1.31), liver cirrhosis 1.17 (1.02-1.34), and nephritis 1.16 (1.01-1.39). Comparisons with the national reference group gave significant SMRs for ischemic heart disease 1.50 (1.36-1.64), heart disease 1.17 (1.08-1.28), cerebrovascular disease 1.09 (1.03-1.15), vascular disease 3.09 (2.53-3.73), bronchitis 1.87 (1.59-2.18), liver cirrhosis 1.17 (1.08-1.28), and nephritis 1.23 (1.07-1.41).

For females with the local reference, significant SMRs (95 percent C.I.) were seen for diabetes mellitus 1.55 (1.39-1.72), hypertension 1.20 (1.06-1.37), ischemic heart disease 1.44 (1.27-1.61), cerebrovascular disease 1.24 (1.18-1.31), vascular disease 2.30 (1.78-2.93), bronchitis 1.53 (1.30-1.80), and nephritis 1.16 (1.01-1.39). Similar values were seen with the national reference group except hypertension and nephritis were no longer significant.

For comparison, SMRs for all malignant cancers in males were 2.19 (2.11-2.28) for local and 1.94 (1.87-2.01) for national reference. Specific cancers were seen in the digestive, respiratory, genitourinary, and lymphatic systems. Significant male SMRs for both local and national reference groups included intestine 2.10 (1.20-1.83, local), lung 2.46 (1.77-3.34, local), skin 5.97 (4.62-7.60, national), prostate 2.52 (1.86-3.34, local), urinary bladder 10.50 (9.37-11.73, national), kidney 6.80 (5.49-8.32, national), and lymphoma 1.63 (1.23-2.11, local). The higher of the two reference values is given in each case. For females, SMRs for all malignant cancers were 2.40 (2.30-2.51) and 2.05 (1.96-2.14), for local and national, respectively. Significant individual cancer SMRs with both reference groups included pharyngeal 2.36 (1.13-4.34, local), rectum 1.87 (1.64-2.14, national), lung 4.13 (3.77-4.52, local), skin 6.81 (5.29-8.63, national), kidney 10.49 (8.75-12.47), bladder 17.65 (5.70-19.79), and lymphoma 1.70 (1.18-2.37).

This study compares mortality; however, since not all diseases are fatal the figures tend to underestimate the risks of serious adverse health effects. Also it is important to note that the noncancer SMRs, while lower than the most serious specific cancer endpoints, are not much lower than overall SMRs due to malignant cancers possibly caused by chronic arsenic exposure. Hence, the noncancer endpoints discussed in this report need to be taken as seriously as the cancer endpoints. Fortunately there appear to be suitable data available for the quantitative risk assessment of several significant noncancer disease endpoints, including cerebrovascular and cardiovascular disease, hypertension, diabetes mellitus, and skin keratosis.

Carcinogenicity

Inorganic arsenic was one of the first chemicals for which there was evidence of a carcinogenic effect. As early as 1879, it was suggested that high rates of lung cancer in German miners may have been caused by inhaled arsenic (Neubauer, 1947). A few years later, Hutchinson (1887, 1888) reported skin cancer in patients who had taken arsenical medications. Numerous epidemiologic studies have since confirmed that ingested arsenic can cause skin cancer and inhaled arsenic lung cancer (IARC, 1980; 1987). Until recently, the evidence that ingestion of arsenic is a cause of various cancers other than skin cancer came mainly from studies in Taiwan (Chen *et al.*, 1985, 1988a,b; Wu *et al.*, 1989) and to a lesser extent from two studies in Japan (Tsuda *et al.*, 1990). A review published in 1992 concluded that these studies strongly suggested that ingested inorganic arsenic causes cancer of the bladder, kidney, lung and liver, and possibly other sites, but that confirmatory studies were needed (Bates *et al.*, 1992). Since then several studies have provided strong additional evidence that arsenic ingestion causes internal cancers, in particular cancers of the bladder and lung (Hopenhayn-Rich *et al.*, 1996a; Smith *et al.*, 1998; Chiou *et al.*, 1995, 2001; Guo *et al.*, 1997; Tsuda *et al.*, 1995).

Skin

There is sufficient evidence from epidemiological studies to demonstrate a causal association between exposure to arsenic and human skin cancer (U.S. EPA, 1988; ATSDR, 1997). Numerous studies have examined the effects of chronic ingestion of arsenic in drinking water, in other arsenic-contaminated beverages, and in medicines. Characteristic skin manifestations include hyperpigmentation (melanosis), hyperkeratosis, and carcinomas.

Hyperpigmentation is not generally considered to be a pre-malignant condition, although it may serve as a marker for hazardous exposure to arsenic. There is, however, some debate about the malignant potential of arsenic-induced hyperkeratoses. Hyperkeratotic lesions tend to occur after long-term ingestion of arsenic and characteristically, are found on the palms and on the soles of the feet (Yeh *et al.*, 1968). Yeh (1973) divided these arsenic-induced hyperkeratotic lesions into two types. Type A was not considered to be premalignant, while Type B contained cells with marked atypia, which could be considered premalignant. This distinction is of some importance in the attempt to quantify the carcinogenic potential of arsenic. The present consensus in the scientific literature seems to be that hyperkeratotic lesions, especially those which appear as small corn-like elevations, have the potential to progress to squamous cell carcinomas, though most remain benign for decades.

Skin cancers resulting from chronic exposure to arsenic include in-situ cell carcinomas (Bowen's disease), invasive squamous cell carcinomas, and multiple basal cell carcinomas. One case report (Shneidman and Belizaire, 1986) describes the development of dermatofibrosarcoma protuberans in a woman with a history of chronic arsenic ingestion and concurrent diagnosis of keratosis, Bowen's disease, and basal cell carcinoma. Squamous cell carcinomas often arise from hyperkeratotic lesions or from sites of in-situ carcinoma (Bowen's disease), while basal cell carcinomas more frequently arise from normal tissue. Many reports have confirmed that chronic arsenic ingestion, especially at relatively high dose levels, often results in the appearance of multiple skin cancers (Sommers and McManus, 1953). Fifteen of 27 patients with multiple skin cancers were exposed to arsenic through treatment with an arsenical medication (Sommers and McManus, 1953). Fourteen of these had been treated with Fowler's solution (1 percent arsenic trioxide; 7.6 g/L As), 12 for psoriasis, and 2 for epilepsy. One had been treated with injected arsenicals for syphilis. While no exact dosages were reported, it was noted that some of these patients had taken Fowler's solution for only a few months. The latent period for arsenical carcinogenesis in this study ranged from 13-50 years, with an average of 24 years.

A retrospective study of patients treated with a 1:1 dilution of Fowler's solution (3.8 g/L As) found a dose-response relationship between the amount of ingested arsenic and the occurrence of hyperkeratosis and skin cancer (Fiertz, 1965). Patient records identified 1,450 people who had been treated with arsenic 6 to 26 years previously. Of these, only 262 participated in the study. Considering the low response rate (18 percent), selection bias is very likely a factor in this study. Another drawback is the lack of a control group. All of the participants were less than 65 years old, and most had received the Fowler's solution for treatment of skin disease (psoriasis 24 percent, neurodermatitis 23 percent, chronic eczema 27 percent, other 25 percent).

Of the participating patients, 40.4 percent were found to have hyperkeratotic lesions, mostly on the palms and soles. The prevalence of hyperkeratosis increased with arsenic dose, being greater than 50 percent among patients who had received more than 400 mL of Fowler's solution (containing a total exposure of 3 g or more of As). Eight percent of the 262 patients had developed skin cancers. The prevalence of skin cancer among patients who had ingested between 200 and 800 mL of Fowler's solution (1.5 g and 6.1 g As, respectively) was between 5 and 10 percent, increasing to > 20 percent in the group estimated to have received more than 1,000 mL of Fowler's solution (7.6 g As). Ten of the 21 cancer patients had multiple basal cell carcinomas, with squamous cell carcinoma, single basal cell carcinoma, and Bowen's disease occurring less frequently. Sixteen of the 21 skin cancer patients presented with palmoplantar hyperkeratosis as well. The mean latency period for carcinomas was estimated as 14 years.

In a mortality study from Lancashire, England, of a cohort of 478 patients who were given Fowler's solution, an excess of fatal and non-fatal skin cancers was compared to what would be expected based on age-, sex-, and calendar-year-specific rates for England and Wales (Cuzick *et al.*, 1982). Patients had been given Fowler's for periods ranging from 2 weeks to 12 years (average dose 250 mg arsenic per month). The mean total dose given was 1891 mg; the median dose was 448 mg. All but 13 patients had been given Fowler's solution as a treatment for skin complaints. The mean follow-up time was 20.3 years, and the mean age at treatment was 40 years. Forty-nine percent of the patients showed signs of arsenicism (hyperpigmentation, hyperkeratosis, or skin cancer). The median total dose causing the appearance of these signs was 672 mg; in people without such signs the median total dose was 448 mg. This difference was unlikely to be due to chance ($p < 0.001$).

Tay (1974) studied 74 patients with arsenic poisoning who had taken antiasthmatic herbal preparations containing arsenic trioxide (3 mg/day) and arsenic sulfide (10 mg/day). Hyperpigmentation and/or hyperkeratosis was reported in > 90 percent of the patients. Six patients (8 percent) were diagnosed with skin cancer. The medication used by the majority of patients (63 percent) was taken in pill form for the treatment of bronchial asthma. Each pill contained 22,000-ppm (22 mg/g) arsenic sulfide, and the recommended dosage was 10 pills per day. Sixty-five percent of the patients had hyperkeratosis of the palms and soles. Five patients who had shown signs of arsenicism for 5-20 years had malignant transformation of keratotic lesions. Four had developed Bowen's disease and multiple basal cell carcinomas on pre-existing keratoses, while one had developed a squamous cell carcinoma. Multiple basal cell carcinomas and Bowen's disease lesions developed in normal skin in these patients as well.

The most informative study of exposure to arsenic via contaminated drinking water was described in two papers by Tseng *et al.* (1968) and Tseng (1977), which involved a large population (> 100,000 persons) living on the southwest coast of Taiwan. Levels of arsenic in the artesian wells that provided drinking water for this population ranged from 0.001 to 1.82 mg/L, with average levels of around 0.4 to 0.6 mg/L. Analyses of a limited number of samples of water from these wells (Irgolic *et al.*, 1983, as cited by U.S. EPA, 1984) have shown arsenic to be present predominantly in the pentavalent inorganic form.

The inhabitants of this area began using these wells 45 years before the study was conducted. The investigators carried out a house-to-house medical survey of 40,421 exposed individuals, and demonstrated a dose-response relationship between prevalence of skin cancer and the arsenic content of drinking water supplies. A similar dose-response relationship was seen for duration of water intake. Overall prevalence rates for skin cancer, hyperkeratosis, and hyperpigmentation were 10.6, 71.0, and 183.5 per 1,000, respectively. The youngest patient with hyperpigmentation was 3 years old, the youngest with hyperkeratosis 4 years, and the youngest with skin cancer 24 years. Ninety-nine percent of those with skin cancer had multiple skin cancers. Of the total of 428 skin cancer cases found in Tseng's study, 238 skin cancers from 153 patients were examined histologically by Yeh *et al.* (1968). Of these, 58.4 percent were classified as intra-epidermal carcinomas (50.84 percent Bowen's disease and 7.56 percent Type B hyperkeratoses), 19.32 percent as squamous cell carcinomas, 15.12 percent as basal cell carcinomas, and 7.14 percent as mixed lesions. The combined forms were reported to consist of mixtures of superficial basal cell carcinomas, Bowen's lesions, and Jadassohn's epitheliomas.

A control population of 7,500 persons from nearby areas in Taiwan where arsenic levels in drinking water were very low (estimated by the author at < 0.001 mg/L to 0.017 mg/L but more likely < 0.04 mg/L, according to Greschonig and Irgolic, 1997) was also examined. This population was similar to the exposed population in age and sex distribution and with regard to occupation, diet and socioeconomic status. Most of the people involved in this study were engaged in farming, fishing or salt production, and their socioeconomic status was considered to be poor. Their diet was high in carbohydrates and low in animal protein and fat. No cases of hyperpigmentation, keratosis, or skin cancer were found in an examination of the 7,500 individuals who made up the control population.

Several U.S. studies described earlier (OEHHA, 1992a) which found no association between exposure to arsenic in drinking water supplies and occurrence of skin abnormalities are of interest in setting standards for arsenic contamination in U.S. water supplies. However, their findings cannot necessarily be considered to conflict with studies in other areas of the world, and particularly with the Taiwanese study used by the U.S. EPA for risk assessment purposes, as the levels of the arsenic in the drinking water of the exposed populations in each of the U.S. studies was considerably less than the levels reported by Tseng *et al* (1968, 1977) in Taiwan where there were a large number of people drinking water with over 0.6 mg/L of arsenic. Furthermore, the relatively small size of each population considered in the U.S. studies reviewed in the 1992 report means that these studies had insufficient statistical power to detect small increases in skin cancer rates which might have been present.

Karagas *et al.* (2001) collected data on 587 basal cell (BCC) and 284 squamous cell (SCC) skin cancer cases and 524 controls in a case-control study conducted in New Hampshire between 1993 and 1996. Arsenic exposure was estimated using neutron activation analysis of toenail clippings. Toenail arsenic concentrations ranged from 0.01 to 0.81 μg/g among control subjects, from 0.01 to 2.03 μg/g among BCC cases, and from 0.01 to 2.57 μg/g among SCC cases. Six exposure categories were analyzed and the odds ratios (OR) for SCC and BCC were close to unity for all but the highest category

(0.345-0.81 μg/g). Among individuals with toenail arsenic concentrations above the 97th percentile, the OR was 2.07 (95 percent C.I. 0.92-4.66) for SCC and 1.44 (95 percent C.I. 0.74-2.81) for BCC. While the risks of SCC and BCC do not appear significantly higher in most of the study subjects, the authors could not exclude the possibility of a dose-dependent increase in cancer at the highest exposure levels measured.

Yu *et al.* (2000) studied methylation status in 26 patients with arsenic induced skin lesions in southwestern Taiwan (2 BCC, 19 SCC, and 6 hyperkeratosis and/or hyperpigmentation). The study group was matched with age (within three years) and gender controls. The test and control populations had similar concentrations of arsenic in drinking water and excreted comparable urinary arsenic metabolite concentrations. There were significant differences in the percent of urinary inorganic arsenic, MMA, and DMA among the test cases compared with controls. Skin lesion cases had higher inorganic arsenic and MMA and lower DMA than controls. Their MMA/DMA ratio was also higher (0.24 ± 0.06 vs. 0.20 ± 0.04). Individuals with a higher percentage of MMA (>15.5 percent) had an OR for skin lesions of 5.5 (95 percent C.I. 1.22-24.81) compared with individuals with lower urinary MMA. These results suggest a role for methylation capacity in arsenic-induced skin disease.

Chen *et al*. (2003) conducted a case-control study of arsenic methylation and skin cancer risk in southwestern Taiwan. Seventy-six newly diagnosed skin cancer patients and 224 controls, all over 30 years old, were recruited for the study. The cancer cases were 29 percent Bowen's disease (BD), 33 percent basal cell carcinoma (BCC), and 47 percent squamous cell carcinoma (SCC). Primary and secondary methylation indexes (PMI and SMI) were defined as the ratios of urinary MMA^V/Asi and DMA^V/MMA^V, respectively. The cumulative arsenic exposure index (CAE) was defined as: CAE = Σ[(average As concentration of artesian well water in mg/L)i x (duration of of consuming artesian well water in years)i : unit of village]. Multiple logistic regression models were used to estimate the multivariate odds ratios (and 95 percent CIs) of skin cancer associated with arsenic methylation ability (PMI and SMI) and CAE. Skin cancer patients and controls were similar with regard to age, gender, smoking, and alcohol consumption. Given a low SMI (≤5), CAE > 15 (mg/L)yr was associated with an increased risk of skin cancer (OR, 7.48; 95 percent CI, 1.65-33.99) compared to a CAE ≤2 (mg/L)yr. Given the same level of PMI, SMI, and CAE, men had higher risk of skin cancer (OR, 4.04; 95 percent CI, 1.46-11.22) than women. Males in all strata of arsenic exposure and methylation ability had a higher risk of skin cancer than did women.

Internal Cancers

Results of the human investigations for arsenic ingestion with emphasis on internal cancers are described in the following section. Most studies fall into one of three categories according to the source of arsenic intake: drinking water, medicines, or wine substitutes. These sources are considered separately. Drinking water studies are further subdivided and presented by region of origin. The results of those epidemiological studies with quantitative exposure data are presented in Table 8 and in Figure 6.

Taiwan

The most extensive studies to date on the effects of ingested arsenic and 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. Consumption of artesian well water containing high levels of arsenic, however, has been linked to endemic rates of Blackfoot disease (BFD), a unique peripheral vascular disease caused by arsenic, which commonly ends with amputation of the affected distal parts of the extremities (Tseng, 1977). 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 Blackfoot disease endemic area (Tseng, 1977; Tseng *et al.*, 1968). Eventually, high rates of other internal cancers were found.

In a retrospective case-control study, Chen *et al.* (1986) examined potential risk factors related to significantly high mortality of cancers of the bladder, lung, and liver. In this study, the relationship between exposure to high arsenic artesian well water and malignant neoplasms of bladder, lung, and liver were examined. Cases were persons who died of bladder, lung or liver cancer, confirmed diagnostically either by biopsy or other tests. Controls were selected from the same geographical areas as the cases, frequency matched on age and sex. Adjustments for age, sex and other variables (smoking, tea drinking, vegetarianism, and frequency of consumption vegetables and of fermented beans) were performed by logistic regression analysis. The results indicated increasing trends in odds ratios (ORs) with increasing duration of intake of arsenic-containing artesian well water. The highest risks were seen for over 40 years exposure, with ORs of 4.10, 3.01, and 2.00 for bladder, lung, and liver cancer, respectively (see Table 8). Smoking, alcohol consumption, and other potential risk factors evaluated in the study were not found to be confounders in the arsenic-cancer associations.

Chen *et al.* (1988a) then examined the association between arsenic in artesian well water in relation to Blackfoot disease (BFD) and cancer, from a multiple risk factor perspective. The study area included the four townships in southwestern Taiwan where high rates of BFD had been described. Arsenic levels were reported to be high in water, soil and food, with estimates of arsenic ingestion by local residents of up to one mg per day. The study consisted of two parts. The first part compared all people living in the area with a diagnosis of BFD (N = 305) with healthy controls matched on sex, age and town of residence of the cases. The second part examined cancer and cardiovascular mortality in a cohort of people who had or developed BFD since 1968, totaling 789 patients and 7578 person-years of observation through 1984. Follow-up started in 1968, since this was the year death registration in Taiwan was computerized and completeness and quality of death certificate registration improved. Mortality of persons that died (N = 457) and were not lost to follow-up (N = 84) was compared to that of the general population of Taiwan using age and sex specific mortality rates from 1968 through 1983. Significantly high SMRs were found for cancers of the bladder (38.80), skin (28.46), lung (10.49) and liver (4.66). Elevated SMRs were found for the prostate (17.29) and kidney (19.53) but were based on only two and three deaths, respectively.

In a letter to *Lancet*, Chen *et al.* (1988b) briefly describe a steep dose response relationship between arsenic levels in artesian well water in 42 villages of the blackfoot disease endemic area of southwestern Taiwan and rates of bladder, lung, kidney and skin cancer, as well as prostate cancer for men. The study period (1973 through 1986) covered 899,811 person years of observation, and exposure was grouped in three categories based on arsenic levels from a survey of over 83,656 wells in all of Taiwan, covering 313 townships, conducted from 1962 to 1964. The exposure categories were <0.3 ppm, 0.3 to 0.59 ppm, and ≥0.6 ppm arsenic in drinking water. Mortality rates were age adjusted using the work population in 1976 as the standard. For all cancer sites in males the standardized mortality/100,000 individuals was: 128 (control); 154, <0.3 ppm; 258.9, 0.3 to 0.59 ppm; and 434.7, ≥0.6 ppm. For females the values were 85.5, 113.3, 182.6, and 369.4, respectively.

Age-adjusted mortality rates for various cancers were examined by Wu *et al.* (1989) for an area of southwestern Taiwan comprised of the 42 villages in six townships for which there were data on arsenic levels in well water. The arsenic content of the 155 wells sampled ranged from 10 to 1,750 µg/L (Natelson's method). The arsenic levels in well water were determined in 1964-1966 and the mortality and population data were for the period 1976-1986. The villages were divided according to their median arsenic levels in water into three exposure groups: <300, 300-590, and ≥600 µg/L. Death certificates were used to ascertain cause of death during the period 1973 through 1986. A significant dose-response relationship was found for cancers of the bladder, kidney, lung and skin for both men and women, and for prostate and liver for men. Although this was an ecological study, the findings are very strong and the dose-response relationships are very clear. In addition, because of the homogeneous nature of the villages studied, both in location and characteristics, all residents were thought to receive the same type of medical care, with corresponding similar reporting and accuracy of death certificates. Thus, diagnostic or reporting bias appeared unlikely.

Chen and Wang (1990) next used the ecological design to investigate cancer mortality rates in the arsenic-endemic areas of Taiwan compared to other areas of the country. This study is an extension of the Chen *et al.* (1988b) study described above. From 361 administrative areas in Taiwan, the 314 with arsenic water analyses were included in this study. The analyses were conducted by the Taiwan Provincial Institute of Environmental Sanitation from 1974 to 1976 using a standard mercuric bromide stain method. Among 83,656 wells tested, 18.7 percent had an arsenic concentration ≥50 µg/L and 2.7 percent had an arsenic concentration ≥350 µg/L. Urbanization and industrialization indices were included in the analysis to adjust for possible confounding effect of differing socioeconomic characteristics among the areas. Exposure measurements were derived from a national water survey of over 83,000 wells throughout Taiwan. Mortality data from 1972 to 1983 were used to evaluate 21 malignant neoplasms, using correlation analysis weighted by person-years at risk. Seven cancer sites were significantly associated with average arsenic levels in the water: bladder, lung, liver, kidney, skin and nasal cavity for both men and women, and prostate cancer for men. The results of multivariate analysis, presented in Table 7, indicate the increase in mortality per 100,000, which was predicted to occur for every 0.1 mg/L increase in arsenic concentration in water.

The main limitation of this study is its ecological design, in which groups are compared rather than individuals, with corresponding mean exposure levels obtained by averaging the arsenic measurements of the water samples from each geographical unit. Nevertheless, the results are consistent with those of other studies in the area (Chen *et al.*, 1985, 1986, 1988a,b; Wu *et al.*, 1989). The increase per unit arsenic measure (age-adjusted mortality/100,000 person-yr/0.1 ppm As) was highest for liver cancer among males, but not among females (6.8 versus 2.0), while it was similar for both sexes for the next two highest-rate cancers, bladder (3.9 for males, 4.2 for females), and lung (5.3 for both). It has been postulated that given the high rate of liver cancer in the area there may be some interaction between arsenic and the two established risk factors of aflatoxin exposure and hepatitis B carrier state.

Guo *et al.* (1997) used tumor registry data along with the same exposure data from the 1974 through 1976 nationwide water-quality survey used by Chen and Wang (1990). The authors used arsenic concentrations in drinking water from 243 townships with about 11.4 million residents. The annual incidence of bladder and kidney cancers for townships in 1980 through 1987 and subcategories of those cancer diagnoses were regressed against a model that included six variables for the proportions of wells in each of six categories of arsenic concentration in each township. Sex-specific models were adjusted for age and included an urbanization index and the annual number of cigarettes sold per capita. Regression models were weighted by the total population of each township. A total of 1,962 bladder, 726 kidney, 170 ureter, and 57 urethral cancers were included. Guo *et al.* (1997) found associations of high arsenic concentrations (more than 0.64 ppm) in both sexes with transitional-cell carcinomas of the bladder, kidney, and ureter and all urethral cancers combined, but they did not present relative risk estimates, so the results cannot be compared directly with other studies. Association of the township proportion of wells with arsenic at concentrations lower than 0.64 ppm was not significant, and some regression coefficients were negative. No association was found with cigarette sales, but a positive link was observed with urbanization. The overall crude annual bladder cancer incidence rate (2.15 per 100,000 population) reported by Guo *et al.* (1997) is far below that of comparable Asian populations, suggesting under-ascertainment of newly diagnosed bladder cancer in the voluntary national cancer registry. Cancer reporting is likely to be better in urbanized areas than in rural areas, such as the high arsenic regions of southwest and northwest Taiwan. Uncertainties in exposure estimates previously cited apply also to this study, and the type of ecologic analysis conducted is of questionable validity.

Chiou *et al.* (1995) investigated the relationship between internal cancers and arsenic in relation to Blackfoot disease. Patients (N = 263) and 2,293 healthy controls, all residents of the arsenic endemic area of southwestern Taiwan, were followed for seven years. After controlling for the effects of age, sex and smoking in the regression analysis, a dose-response relationship was observed between arsenic exposure from drinking well water and the incidence of bladder and lung cancer. Blackfoot disease patients were found to be at a significantly increased risk compared to controls after adjustment for cumulative arsenic exposure.

Chiou *et al.* (2001) studied the incidence of urinary tract cancers among 8,102 residents in an arseniasis-endemic area in northeastern Taiwan. Arsenic levels in the drinking water in this region ranged from less than 0.15 μg/L (undetectable) to 3590 μg/L. Exposure for each member of the cohort was assessed by measuring arsenic concentrations in the well associated with that particular household *at one point in time only*, although most households had used their current wells for at least ten years (Chen and Chiou, 2001). Each home was said to have its own well and that they had been in use for more than 50 years. The incidence of urinary tract cancers (kidney and bladder) were significantly increased in the cohort relative to the general population of Taiwan (SIR=2.05, 95 percent C.I. 1.22-3.24). The SIR for bladder cancer was 1.96 (95 percent C.I. 0.94-3.61) while the SIR for kidney cancer was 2.82 (95 percent C.I. 1.29-5.36). These results are based on nine subjects with bladder cancer, eight with kidney cancer and one with both. A significant dose response relationship was observed between urinary tract cancers, particularly transitional cell carcinoma (TCC) after adjusting for age, sex and smoking. The relative risks of developing TCC were 1.9, 8.2 and 15.3 for arsenic concentrations of 10.1 to 50.0 μg/L, 50.1 to 100 μg/L, and greater than 100 μg/L, respectively, although this analysis is based on very few cases. There were only one, two, and six diagnoses of TCC in the low, mid and high dose groups, respectively. As a consequence, the confidence intervals are huge and conclusions regarding a definitive dose-response relationship are difficult (Table 7). It should also be noted that while this study is valuable for causal inference, it is limited for dose-response assessment by the fact that exposure assessment was at one point in time only (Cantor, 2001).

Morales *et al.* (2000) evaluated the risk of cancers of the urinary bladder, liver, and lung associated with exposure to arsenic in drinking water, based on data from 42 villages in an arseniasis-endemic region of Taiwan. Excess lifetime risk estimates were made for several variations of the generalized linear model and for the multistage-Weibull model. The analysis was limited to persons ≥20 years of age and the entire Taiwanese population was used to calculate standardized mortality ratios (SMRs). Eight exposure intervals based on arsenic drinking water concentrations from 0-50 to 600+ μg/L were analyzed. The arsenic analyses for the study area were from 1964-1966 and the mortality data were collected for the 1973-1986 period. The mortality data for the study area were compared with those for all Taiwan for the same period. There appeared to be higher SMRs for bladder and lung at the higher exposure levels compared to the lower exposure range. There was no observed age dependency on the risk estimates and overall, females had higher SMRs than males. Liver cancer mortality was higher than expected but did not show a strong exposure-response relationship. Depending on the model and the referent population that was used, LED_{01} estimates ranged from 9 to 326 μg/L for bladder cancer, 6 to 63 μg/L for lung cancer, and 51 to 542 μg/L for liver cancer. For combined cancers, the LED_{01} estimates ranged from 2 to 148 μg/L. The LED_{01} is the lower bound (level unspecified) on the dose associated with a one percent response (ED_{01}).

Japan

A retrospective cohort study of a Japanese population, which between 1954 and 1959 used well water contaminated with arsenic from a dye factory, provided evidence of dose-response relationship with lung cancer and possibly other cancers (Tsuda *et al.*, 1995). Excess mortality was reported due to the following cancers among 113 persons exposed to arsenic above 1.0 mg/L (highest dose group): urinary tract (3 observed, 0.10 expected) SMR 31.18 (95 percent C.I. 8.62-91.75), lung (8 observed, 0.51 expected) SMR 15.69 (95 percent C.I. 7.38-31.02), liver (2 observed, 0.28 expected) SMR 7.17

(95 percent C.I. 1.28-26.05), and uterus (2 observed, 0.15 expected) SMR 13.47 (95 percent C.I. 2.37-48.63). The observed-to-expected ratios were near or below expectation among persons exposed to arsenic at less than 0.05 mg/L. Results in this low-exposure group were statistically unstable. Expected deaths numbered less than two for each cancer cause of death. Expected numbers of deaths were based on sex-, age-, and cause-specific mortality in Niigata Prefecture from 1960 to 1989.

South America

Bladder cancer mortality for the years 1986 through 1991 was investigated in the province of Córdoba, Argentina in an ecological study comparing counties previously having high, medium and low water levels of arsenic (Hopenhayn-Rich *et al.*, 1996c). The average water arsenic concentration for contaminated water sources in the two high-exposure counties was 178 μg/L (range 40 to 430 μg/L). The medium-exposure group comprised six counties with lower occurrence of arsenical skin disease and lower levels of arsenic water concentrations than the high-exposure group. Of 43 towns in the region with >120 μg As/L drinking water 22 were located in the six medium exposure counties vs. 15 in the two high exposure counties. Sixteen rural counties outside the "arsenical area" were classified as the low-exposure group although some elevated measures of arsenic had been reported in the area. Clear trends in bladder cancer mortality were shown with standardized mortality ratios (SMRs) of 2.14 for men (95 percent C.I. 1.78-2.53) and 1.82 for women (95 percent C.I. 1.19-2.64) in the two high exposure counties. The clear trends found in a population with a different ethnic composition and a high protein diet support the evidence from Taiwan that arsenic in drinking water is a cause of human bladder cancer. While it was made clear that exposure was not uniform within counties, it was noted that the findings were roughly consistent with risks predicted from the Taiwan studies. Increasing trends were also observed for kidney and lung cancer mortality as arsenic exposure increased, with the following SMRs for men and women respectively: kidney cancer, 0.87,1.33, 1.57 and 1.00, 1.36, 1.81; lung cancer, 0.92,1.54,1.77 and 1.24, 1.34, 2.16 (in all cases, $p<0.001$ in trend tests). There was a small positive trend for liver cancer but mortality was increased in all three exposure groups. To control for potential confounding by smoking, SMRs for chronic obstructive pulmonary disease (COPD) were derived. No differences were found for COPD between groups.

The categorization of exposure groups was based on clinical reports and surveys of arsenic in water, but unfortunately, the data were inadequate to estimate average arsenic concentrations in each exposure group. In addition, water arsenic measurements showed a wide range within the exposed groups. However, as indicated below, this was the first study outside of Taiwan conducted in a chronically arsenic-exposed population, showing similar dose-response relationships. For example, the study in Argentina showed positive trends for kidney and lung cancer SMRs with increasing arsenic exposure for males and females. The data in Taiwan showed positive regression coefficients (age –adjusted mortality/100,000 person-yr/0.1 ppm As) for a number of internal cancers, notably liver, lung, bladder, and kidney (Chen and Wang, 1990).

Smith *et al.* (1998) investigated cancer mortality in a population of around 400,000 people in a region of Northern Chile (Region II) exposed to high arsenic levels in drinking water in past years. Arsenic concentrations from 1950 to the present were obtained. Population-weighted average arsenic levels reached 570 μg/L from 1955 to 1969, and decreased to less than 100 μg/L by 1980. Cancer mortality for the years 1989 through 1993 in Region II was compared to the rest of Chile. The results indicated marked increases for bladder, kidney, lung, and skin cancer mortality in Region II, with corresponding SMRs of 6.0, 1.6, 3.8, and 7.7 for men, and 8.2, 2.7, 3.1, and 3.2 for women. All results were significant at the 95 percent confidence level, except liver cancer, with an SMR of 1.1 for both men and women. This study showed considerably elevated rates for the same cancers found to be consistently elevated in the Taiwanese studies except for liver cancer. Smoking survey data and mortality rates from COPD provided evidence that smoking did not contribute to the increased mortality from these cancers. These findings provide additional evidence that ingestion of inorganic arsenic in drinking water is indeed a cause of bladder and lung cancer. It was estimated that arsenic might account for seven percent of all deaths among those aged 30 and over. If so, the impact of arsenic on the population mortality in Region II of Chile is greater than any reported to date from environmental exposure to a carcinogen in a major population.

Table 8. Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

STUDY	XPOSURE INDEX	NUMBER EXPOSED	EFFECT MEASURE					
			Bladder		Lung		Liver	
Chen *et al*., 1986	**Years of water consumption**	**Controls**	**Cases**	**OR (adj.)**	**Cases**	**OR (adj.)**	**Cases**	**OR (adj.)**
E	0	136	17	1.00	20	1.00	20	1.00
	1-20	131	19	1.27	25	1.07	15	0.80
	21-40	50	10	1.63	9	1.46	9	1.14
	>40	51	23	4.10	22	3.01	21	2.00

STUDY	EXPOSURE INDEX	NUMBER EXPOSED		Age Standardized Mortality Rate (per 100,000)	As Level (µg/L)		
				Population	**<300**	**300-590**	**≥ 600**
Chen *et al*., 1988b	Village of residence, median arsenic levels of well water samples	899,811 person-yrs Observed 1973-1986					
			Male	3.1	15.7	37.8	89.1
			Female	1.4	16.7	35.1	91.5
			Kidney				
			Male	1.1	5.4	13.1	21.6
			Female	0.9	3.6	12.5	33.3
			Lung				
			Male	19.4	35.1	64.7	87.9
			Female	9.5	26.5	40.9	83.8
			Liver				
			Male	28.0	32.6	42.7	68.8
			Female	8.9	14.2	18.8	31.8
			Skin				
			Male	0.8	1.6	10.7	28.0
			Female	0.8	1.6	10.0	15.1
			Prostate				
			Male	1.5	0.5	5.8	8.4

Table 8 (Continued). Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

STUDY	XPOSURE INDEX	NUMBER EXPOSED	EFFECT MEASURE			
Wu *et al.*, 1989	Village of residence classified in three groups based on median arsenic levels in wells:	Person-yrs by exposure group & sex:		**Age Adjusted Mortality Ratio (per 100,000) As Level (µg/L)**		
E				**<300**	**300-590**	**≥ 600**
	1) <300 µg/L;	Group 1	Bladder			
	2) 300-590 µg/L;	Male: 248,728	*Male*	22.6	61.0	92.7
	3) ≥600 µg/L.	Female: 230,048	*Female*	25.6	57.0	111.3
		Group 2	*Male*	8.4	18.9	25.3
		Male: 138,562	*Female*	3.4	19.4	60.0
		Female: 127,502	Lung			
			Male	49.2	100.7	104.1
		Group 3	*Female*	36.7	60.8	122.2
		Male: 79,883	Liver			
		Female: 74,083	*Male*	47.8	67.6	86.7
		Kidney	*Female*	21.4	24.2	31.8
			Male	2.0	14.1	32.4
			Female	1.7	14.7	18.7

Chen and Wang, 1990	Average arsenic levels in water samples of all geographical units 73 percent had >5 percent of wells with >50 µg/L As; 14.7 percent had 5-14 µg/L; 11.5 percent had ≥15µg/L	340 geographical units with arsenic water measures. Analysis weighted by population in each group. Skin	**Coefficients from regression analysis (per 0.1 mg/L As increase)**		
			Cancer site	**Male**	**Female**
			Liver	6.8	2.0
			Bladder	3.9	4.2
			Lung	5.3	5.3
			Kidney	1.1	1.7
			Skin	0.9	1.0
			Nasal cavity	0.7	0.4

Table 8 (Continued). Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

STUDY	XPOSURE INDEX	NUMBER EXPOSED	EFFECT MEASURE				
Chen *et al.*, 1992 E	Village of residence classified in four groups, based on median arsenic levels of well water samples for each village	Person-years by sex and exposure group		**Deaths per 100,000 person-years of observation As Level (µg/L)**			
				<100	**100-290**	**300-590**	**≥600**
		Group 1: <100 µg/L	Bladder				
		Male 171,224	*Male*	11.1	9.1	27.4	46.0
		Female 157,775	*Female*	14.6	12.3	29.0	53.6
			Kidney				
		Group 2: 100-290 µg/L	*Male*	4.1	3.4	9.4	10.1
		Male 87,826	*Female*	1.3	6.2	12.5	19.9
		Female 81,032	Lung				
			Male	22.2	29.6	49.1	53.2
		Group 3: 300-590 µg/L	*Female*	19.6	27.1	35.3	56.6
		Male 138,562	Liver				
		Female 127,502	*Male*	22.1	29.6	33.2	43.1
			Female	10.1	14.8	16.5	9.9
		Group 4: ≥600 µg/L Male 69,561 Female 65,324					
Chiou *et al.*, 1995	Cumulative index for each subject: Σ (Ci × Di) Ci = median arsenic concentration of arsenic in wells of village Di = duration drinking water in that village	BFD patients, N=263 Healthy controls, N=2,256	**Cumulative Exposure (mg/L × yr)**	**Bladder Cancer**		**Lung Cancer**	
				‡	RR†	RR‡	RR†
			0	1.0	1.0	1.0	1.0
			0.1 – 19.9	2.1	1.6	3.1	2.7
			20+	5.1	3.6	4.7	4.0
			RR‡= relative risk after adjustment for age, sex, and smoking. RR RR†= relative risk after adjustments for age, sex, smoking, and BFD status (BFD patients had RR of 2.7 relative to non-BFD patients)				

Table 8 (Continued). Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

STUDY	XPOSURE INDEX	NUMBER EXPOSED	EFFECT MEASURE					
			Urinary organs			**Transitional cell carcinoma**		
			Cases	RR	95% CI	Cases	RR	95% CI
Chiou *et al.*, 2001	Arsenic concentration in well water (µg/L)	8,102 in the cohort 4,056 males 4,046 females						
E	<10.0		3	1.0		1	1.0	0.1-32.5
	10.1-50.1		3	1.5	0.3-8.0	1	1.9	0.7-99.1
	50.1-100.0		2	2.2	0.4-13.7	2	8.2	1.7-139.9
	>100.0		7	4.8	1.2-19.4	6	15.3	

STUDY	EXPOSURE INDEX	Observed/Expected Lung	Observed/Expected Urinary	Lung Cancer SMR	Lung Cancer 95% CI	Urinary Cancer SMR	Urinary Cancer 95% CI
Tsuda *et al.*, 1995	Arsenic concentration in well water (ppm)						
	<0.05-0.09	0/1.55	0/0.30	0.00	0-2.43	0.00	-
	0.05-0.09	1/0.43	0/0.08	2.33	0.12-13.39	0.00	-
	_1	8/0.51	3/0.10	15.69	7.38-31.02	30.0	-
		9/2.46	3/0.48	3.66	1.81-7.03	6.25	-

STUDY	EXPOSURE INDEX	Population in each exposure group:		SMRs	Bladder Male	Bladder Female	Kidney Male	Kidney Female	Lung Male	Lung Female
Hopenhayn-Rich *et al.*, 1996a and 1998 > TOTAL	Counties in study area grouped in three exposure levels: low, medium, high (high level mean = 178 µg As/L, no mean arsenic levels determined for medium and low groups)	Low	690,421	Low	0.80	1.22	0.87	1.00	0.92	1.24
		Medium	406,000	Medium	1.28	1.39	1.33	1.36	1.54	1.34
		High	273,014 Group	High	2.14	1.81	1.57	1.57	1.77	2.16
				Trend-test p-value	0.001	0.05	0.001	0.03	0.001	0.001

Table 8 (Continued). Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

STUDY	XPOSURE INDEX	NUMBER EXPOSED		EFFECT MEASURE	
Ferreccio *et al.*, 2000	Water As (µg/L) from 1958-1970			**Lung cancer OR adjusted for age and sex, full model**	
		Cases	Controls	*OR*	95 percent CI
E		11	92	1.0	
		3	62	0.3	0.1-1.2
		4	19	1.8	0.5-6.9
		22	51	4.1	1.8-9.6
	90-199	13	36	2.7	1.0-7.1
	200-399	23	44	4.7	2.0-11.0
	400-699	11	12	5.7	1.9-16.9
	700-999	64	103	7.1	3.4-14.8

STUDY	EXPOSURE INDEX	NUMBER EXPOSED		EFFECT MEASURE					
Bates *et al.*, 1995 30-59 60-89 0-10 10-29	Exposure Index 1 Cumulative dose (mg)	All Subjects		**All subjects**		**Never Smoked**		**Ever Smoked**	
		Cases	Controls	OR	95% CI	OR	95% CI	OR	95% CI
	<19	14	47	1.00		1.00		1.00	
	19 to <33	21	36	1.56	0.8-3.2	1.09	0.4-3.1	3.33	1.0-10.8
	33 to <53	17	39	0.95	0.4-2.0	0.68	0.2-2.3	1.93	0.6-6.2
	_53	19	38	1.14	0.7-2.9	0.53	0.1-1.9	3.32	1.1-10.3
	Exposure Index 2 mg/L x years	All Subjects		**All subjects**		**Never Smoked**		**Ever Smoked**	
		Cases	Controls	OR‡	95% CI	OR‡	95% CI	OR‡	95% CI
	<33	18	42	1.00		1.00		1.00	
	33 to <53	16	42	0.69	0.3-1.5	0.21	0.1-0.8	1.95	0.7-5.6
	53 to <74	16	40	.054	0.3-1.2	0.25	0.1-0.9	1.21	0.4-3.7
>	_74	21	36	1.00	0.5-2.1	0.91	0.3-3.2	1.41	0.5-4.3

‡OR Adjusted for sex, age, smoking (all subjects and smokers only), years of exposure to chlorinated surface water, history of bladder cancer infection, educational level, urbanization of the place of longest lifetime residence, and ever employed in high-risk occupation.

Table 8 (Continued). Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

Study	**xposure Index**	**Number Exposed**	**Effect Measure**					
Lewis *et al.*, 1999	Arsenic exposure index for each cohort member derived from years of residence and median As level in the given community.	4,058 members of cohort			**SMR by Exposure**			
			Cancers	**Low**	**Medium**	**High**	**Total**	**95% CI**
E		*Males* 2,092	Respiratory					
		Females 1,966	*Males*	0.32	0.96	0.44	0.57	0.38-0.82
			Females	0.44	0.66	0.22	0.44	0.16-0.95
	Low <1,000 ppb-years		Bladder/Other urinary					
	Medium 1,000-4,999 ppb-years		*Males*	0.36	-	0.95	0.42	0.08-1.22
	High 7,500 ppb-years		*Females*	1.18	-	1.10	0.81	0.10-2.93

Study	**Exposure Index**	**Shorter Latency**	**Longer Latency**	**Bladder Cancer**			
Kurttio *et al.*, 1999	Age, sex, smoking adjusted RR, 95% CI Finnish case-cohort study, As in well water.	Shorter Latency	Longer Latency	**Shorter Latency**[a]		**Longer Latency**[b]	
	As in water (μg/L)			**RR**	**CI**	**RR**	**CI**
		23	26	1		1	
	0.1-0.5	19	18	1.53	0.75-3.09	0.81	0.41-1.63
		19	17	2.44	1.11-5.37	1.51	0.67-3.38
	(log) continuous[c]		61	1.37	0.95-1.96	.96	0.59-1.55
	Dose of As (μg/day)						
		29	32	1		1	
	0.2-1.0 61	17	16	1.34	0.66-2.69	0.76	0.38-1.52
		15	13	1.84	0.84-4.03	1.07	0.48-2.38
<0.1	(log) continuous[c]		61	1.34	0.95-1.90	0.91	0.55-1.48
	Cumulative As dose (mg)						
>0.5	<0.5	16	27	1		1	
	0.5-2.0 61	20	21	1.61	0.74-3.54	0.81	0.39-1.69
		25	13	1.50	0.71-3.15	0.53	0.25-1.10
<0.2	(log) continuous[c]		61	0.92	0.57-1.47	0.78	0.51-1.20
>1.0							

[a] Exposure in the third to ninth calendar year prior to the cancer diagnosis
[b] Exposure in the tenth calendar year and earlier prior to the cancer diagnosis
[c] Result from the model using log-transformed exposure values

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>2.0

Table 8 (Continued). Summary of Epidemiological Studies of Arsenic Ingestion with Dose-Response Data for Internal Cancers

STUDY	XPOSURE INDEX	NUMBER EXPOSED		EFFECT MEASURE			
Kurttio *et al.*, 1999	Age, sex, smoking adjusted RR, 95% CI			**Kidney Cancer**			
E	Finnish case-cohort study, As in well water.	Shorter Latency	Longer Latency	**Shorter Latency [a]**		**Longer Latency [b]**	
	As in water (µg/L)			**RR**	**CI**	**RR**	**CI**
		23	25	1		1	
	0.1-0.5	12	9	0.78	0.37-1.66	0.33	0.14-0.77
		14	15	1.49	0.67-3.31	1.07	0.46-2.52
	(log) continuous [c]		49	1.16	0.80-1.69	0.72	0.38-1.36
<0.1							
	Daily dose of As (µg/day)						
>0.5	<0.2	26	27	1		1	
	0.2-1.0 49	13	11	1.08	0.52-2.25	0.55	0.25-1.21
		10	11	1.21	0.52-2.82	0.94	0.39-2.27
	(log) continuous [c]	49	49	1.10	0.77-1.58	.59	0.28-1.23
	Cumulative dose of As (mg)						
>1.0	<0.5	18	24	1		1	
	0.5-2.0	12	11	0.74	0.33-1.68	0.36	0.16-0.81
		19	11	0.80	0.42-1.86	0.47	0.21-1.04
	(log) continuous [c]	49	49	0.59	0.28-1.23	0.76	0.44-1.30
>2.0				[a] Exposure in the third to ninth calendar year prior to the cancer diagnosis [b] Exposure in the tenth calendar year and earlier prior to the cancer diagnosis [c] Result from the model using log-transformed exposure values			

Ferreccio *et al.* (2000) investigated the relationship between lung cancer and arsenic in drinking water in Northern Chile in a case-control study involving patients diagnosed between 1994 and 1996 and frequency-matched hospital controls. To avoid the problem of matching on exposure, eligible controls included all patients admitted to any public hospital in the whole study area. Each lung cancer case was matched to both a cancer and noncancer control. The study area included Regions I, II, and III, the names given to the three northernmost provinces. The population in Region II experienced high exposure to inorganic arsenic in past years from natural contamination of drinking water originating in the Andes mountains, while water sources in Regions I and III contained relatively little arsenic. The study identified 152 lung cancer cases and 419 controls. Participants were interviewed regarding drinking water sources, cigarette smoking, socioeconomic status, lifetime residential history, and occupation.

Since 1950, water companies have been required to carry out detailed chemical tests of the water including arsenic levels at least once a year. The investigators compiled data on arsenic concentrations from 1950 to 1994. Concentrations in earlier years were estimated based on measurements in the 1950s. Using lifetime residential histories, each participant was assigned the average water arsenic concentration for the county in which they resided for each year. Counties generally have just one important supply of water in this extremely dry desert part of Chile. Average arsenic water concentrations were calculated from 1930 to the present. In addition, the average arsenic water concentrations for the counties of residence were calculated for 1958 through 1970 when some of the highest exposures occurred. Lifetime (1930 to the present) average arsenic exposure and peak exposures (1958 to 1970) were examined as categorical variables. The lowest exposure categories were used as a reference to calculate ORs.

Logistic regression analysis revealed a clear trend in lung cancer odds ratios (95 percent C.I.s) with increasing concentration of arsenic in drinking water: 1, 0.3 (0.1-1.2), 1.8 (0.5-6.9), 4.1 (1.8-9.6), 2.7 (1.0-7.1), 4.7 (2.0-11.0), 5.7 (1.9-16.9), 7.1 (3.4-14.8) for arsenic concentrations ranging from less than 10 µg/L to 990 µg/L. Results of the analyses of various control groups showed very little difference in ORs across exposure categories. The investigators noted that relatively more controls were chosen from the highly exposed city of Antofagasta than from the lower exposure cities. The direction of this bias would result in underestimation of lung cancer risks for the highest exposures.

This was the first study to provide potentially useful dose-response data for arsenic ingestion and lung cancer. The only previous study with any dose-response information based on individual exposure data was the cohort study of Tsuda *et al.* (1995). However, the latter study only included three dose levels, and the number of cases was small, especially for the two lowest dose groups (no cases reported for <50 µg/L and one case for 50 to 990 µg/L). In addition, Ferreccio *et al.* (2000) is the only study of arsenic and cancer with essentially lifetime individual exposure assessment.

The study also provided evidence of synergy between ingested arsenic and cigarette smoking (Table 8). The lung cancer OR was 32.0 (7.2-198.0) for smokers exposed to more than 200 µg/L of arsenic in drinking water (lifetime average) compared to non-smokers exposed to less than 50 µg/L. The result for smokers is more than double the OR of 13.1, which would be expected from independent effects.

United States

Bates *et al.* (1995) linked 71 bladder cancer cases and 160 controls from the large National Bladder Cancer Study conducted in 1978 (the subsample of Utah residents), to arsenic levels in their water supplies. Overall, no increased risks were found with two cumulative arsenic intake indices used. However, among smokers only, positive trends in risk were observed for the exposure window of 30 to 39 years prior to diagnosis. Although exposures ranged from 0.5 to 160 μg/liter, most of them were very low (only 1.1 percent had levels greater than 50 μg/L), and the risk estimates obtained for smokers were much higher than those predicted from the studies in Taiwan. The authors concluded that bias or chance could account for the findings and that other confirmatory studies were needed.

An ecologic study of drinking water arsenic and mortality was investigated in a cohort of members of the Church of Jesus Christ of Latter-day Saints (LDS) in Millard County, Utah (Lewis *et al.*, 1999). The cohort was assembled from an earlier study (Southwick *et al.*, 1983) that consisted of 2,073 participants. Most of these individuals had at least 20 years of exposure history in their respective places of residence. The cohort was expanded to include all persons who lived for any length of time in the study area resulting in a total combined cohort of 4,058. More than 70 percent of the cohort had reached the age of 60 at the end of the follow-up period or by the time of their deaths. Approximately seven percent of the cohort was lost to follow-up. Arsenic concentrations in the drinking water supplies were based on measurements maintained by the state of Utah dating back to 1964. An arsenic exposure index was calculated from the number of years of residence and the median arsenic concentration of drinking water in a given community. The arsenic exposure index was categorized as low (<1,000 ppb-years), medium (1,000-4,999 ppb-years), and high (≥5,000 ppb-years). Data on confounding factors were not available; however, LDS members are prohibited from tobacco use and alcohol and caffeine consumption.

An apparent positive dose-response relationship for prostate cancer among males was found. SMRs for kidney cancer among males were increased in the medium and high exposures groups. Mortality from cancers of the gastrointestinal tract and respiratory system were lower for both male and females in the cohort versus the comparison population of Utah. It should be noted that the way in which cumulative dose has been estimated in this study makes it strongly correlated with age. As a result, the SMRs by low, medium, and high exposure are difficult to interpret with regards to dose response. Furthermore, the findings are consistent with lower smoking rates for the cohort compared to all of Utah. This is manifest in the SMRs for nonmalignant respiratory disease and the chronic bronchitis, emphysema, and asthma grouping. Because of the above, the study is not interpretable regarding lung cancer and arsenic. The same applies to bladder cancer where there is the additional problem of very small numbers. The median drinking water concentrations of arsenic in the study area ranged from 14 to 166 ppb.

Infante-Rivard *et al.* (2001) conducted a case control study to investigate the relationship between drinking water contaminants and childhood acute lymphoblastic leukemia (ALL). Cases (491) were matched on age, (within 24 months), sex, and region of residence at the calendar date of the case's diagnosis. There was one control per case.

Exposure was assessed using a municipality-exposure matrix for total and specific trihalomethanes, metals and nitrates based on information from parental interviews, historical data provided by the municipalities, and a tapwater survey carried out in 227 homes. Average level of exposure and cumulative exposure were used as exposure indices for pre and postnatal periods. An average arsenic level of five µg/liter was associated with an odds ratio (OR) of 0.94 (95 percent C.I. 0.49-1.81) for the prenatal period and 1.39 (95 percent C.I. 0.70-2.76) for the postnatal period. The number of cases and controls for the prenatal period was 18 and 19, respectively. Twenty cases and 14 controls were included in the postnatal period. When cumulative arsenic exposure was used, the odds ratios for the pre and postnatal periods were 0.70 (95 percent C.I. 0.39-1.25) and 1.14 (95 percent C.I. 0.59-2.21), respectively. Although the authors claim an association with arsenic in the abstract, the study is uninformative with regard to arsenic in view of the low odds ratio estimates and the wide confidence intervals.

Steinmaus *et al.* (2003) conducted a case-control study of bladder cancer and drinking water arsenic in the western U.S. The study area included six counties in western Nevada and Kings County, California. The cities of Hanford, CA, and Fallon, NV, which comprised 21 percent of the current population in the study area, have been the two largest populations in the U.S. exposed to drinking water As, at nearly 100 µg/L. A total of 265 bladder cancer cases were identified during the study period from 1994 to 2000. Of those who met the age and gender criteria for the study, 83 percent agreed to participate. Individual data on water sources, consumption patterns, and other factors were collected for 181 cases and 328 controls. Arsenic exposure was unknown for about 11 percent of the total person-yr that participants resided in the study area. Participants used water from 240 private wells within the study area and of these, records were available for 101 (42 percent), proxy measures were used for 64 (27 percent), and As concentrations were unknown in 75 (31 percent).

All the odds ratios were near 1.0 when exposure lags of five and 20 years were used. When exposures were lagged 40 years, odds ratios above 1.0 were seen for As intakes > 80 µg/d, however none of the 95 percent confidence intervals excluded the null value. For smokers with the highest 1-year exposures greater than 80 µg/d, an adjusted odds ratio of 3.67 (95 percent CI 1.43-9.42; linear trend, $P < 0.01$) was observed for exposures lagged 40 yr. The median intake in each of the three exposure categories was 0, 20, and 177 µg/d. These results provide some evidence that smokers who ingest arsenic at intake levels near 200 µg/d may be at increased risk of bladder cancer. The study also suggests a long latency period between arsenic exposure and cancer diagnosis.

Europe

In the report of Varsanyi *et al.* (1991), the distribution of arsenic concentrations in the drinking water of an area of Hungary is presented, together with the mortality rates of the population. The study region was that of Csongrad County in southern Hungary, including five towns and 54 villages with a total population of almost 500,000 people. Each town had its own water supply, mainly from ground water, and the arsenic content of 85 wells was measured during the study. Mortality rates were compared for two groups of villages, one with arsenic measures >50 µg/L and one with arsenic levels

<50 μg/L. Mortality data for 1987 was obtained from death certificates, and population figures from reports of the National Bureau of Statistics for 1987.

There were six towns included in each of the two comparison groups. In the exposed group, with a total population of 16,234, the arsenic concentrations ranged from 55 to 137 μg/L (average = 97 μg/L); in the unexposed group, totaling 15,832 people, levels ranged from 5 to 38 μg/L (average = 16 μg/L). There were no differences in mortality from neoplasms or diseases of the circulatory system between the two groups.

It is not clear how the towns included in the mortality rate comparisons were chosen, since the authors mention that almost 29,000 in the study area consumed drinking water above the permissible 50 μg/L arsenic content. However, for the analysis, a subgroup of six towns with around half the population was included. In addition, since comparison of mortality rates was only performed for large groups of diseases (all cancers, all cardiovascular), no inferences can be made with respect to individual target sites (e.g. bladder and lung cancer). Taken together, the SMR from all causes of deaths was reported to be somewhat higher in the exposed group, but did not reach statistical significance (SMRs not given).

In a case-cohort design, Kurttio *et al.* (1999) investigated the association of low arsenic exposure in Finnish well water and the risk of bladder and kidney cancers. Bladder and kidney cancer cases were identified during 1981 through 1995 within a registry-based cohort of all Finns who had lived at an address outside the municipal drinking water system during 1967 to 1980. The final study population consisted of 61 bladder cancer cases and 49 kidney cancer cases and what the investigators refer to as an age- and sex-balanced random sample of 275 subjects.

Estimates of arsenic exposure were determined in two periods. The first came from the second to ninth calendar years (shorter latency) and the second estimates from the tenth or earlier calendar years (longer latency) prior to the cancer diagnosis (or the respective year for referent persons). The daily dose of arsenic in drinking water was calculated from the arsenic concentration of well water and from the reported consumption of well water in the 1970s. The cumulative dose was defined as the integral of duration and intensity of exposure to arsenic in the well water. For the shorter latency period, cumulative dose was estimated from the beginning of the use of well water until two years before the cancer diagnosis while for the longer latency, the cumulative dose was calculated until 10 years before. The arsenic concentrations in the wells of the reference cohort ranged from less than 0.05 μg/L to 64 μg/L (median 0.14 μg/L).

The investigators stated that an increasing trend of arsenic in the drinking water and bladder cancer was observed with shorter latency but not with longer latency (Table 1). The age, sex and smoking adjusted risk ratios based on shorter latency increased from 1.53 (95 percent C.I. 0.75-3.09) in the 0.1 to 0.5 μg/L dose group to 2.44 (95 percent C.I. 1.11-53.7) in the greater than 0.5 μg/L dose group. The risk ratios for the longer latency period were 0.81 (95 percent C.I. 0.41-1.63) in the lower dose group and 1.51 (95 percent C.I. 0.67-3.38) in the higher dose group. Both data types appear to increase with increasing dose. Although based on small numbers, a synergistic effect was observed between bladder cancer and smoking. The risk ratios increased with increasing arsenic concentration (μg/L) from 1.10 (95 percent C.I. 0.19-6.24) to 10.3 (95 percent C.I.

1.16-92.5) among smokers. The risk ratios among nonsmokers were 0.95 (95 percent C.I. 0.25-3.64) for the lower dose group and 0.87 (95 percent C.I. 0.25-3.02) for the higher dose group. No evidence of an association between kidney cancer and arsenic in well water was observed.

Bangladesh

As noted above, the contamination of ground water with inorganic arsenic in Bangladesh has led to "the largest poisoning of a population in history" (Smith *et al*., 2000). In 1983 the first cases of arsenic-induced skin lesions were seen in patients from West Bengal, but in 1987 several were also seen from neighboring Bangladesh. The relatively recent occurrence of arsenic poisoning in the region was linked to the increasing usage of shallow tube-wells over the past 20 years. The health effects of chronic arsenic exposure develop slowly. The latency for arsenic-induced skin lesions is about 10 years (Mazumder *et al*., 1998), and small numbers of skin cancer cases have now started to appear. Due to the typical 20-year latency for arsenic-induced cancers, the future prevalence of skin and other more serious cancers is likely to be significant notwithstanding ongoing efforts to reduce exposure (Smith *et al*., 2000). Other non-cancer arsenic-induced adverse health effects such as hypertension and diabetes mellitus have also been observed recently in Bangladesh (Rahman and Axelson, 2001).

Arsenic in Medicines

Historically, several case reports have related lung cancer development with medicinal arsenic treatments or arsenic-related diseases (Heddle and Bryant, 1983; Robson, 1963; Goldman, 1973). In 1953, a series of twenty-seven cases with multiple skin cancers attributed to arsenic exposure was reported (Sommers and McManus, 1953). Of these cases, ten were diagnosed as also having internal cancers at various sites. The method for selecting the patients was not explained. Other case reports have observed an association of hepatic angiosarcoma in people who had previously been treated with Fowler's solution (potassium arsenite), a medicinal arsenic-containing tonic for skin complaints, psoriasis, malaria, anemia, epilepsy, and anxiety (Roat *et al*., 1982; Regelson *et al.,* 1968; Kasper *et al.,* 1984; Lander *et al.,* 1975; Kadas *et al.,* 1985). A review of 168 U.S. hepatic angiosarcoma deaths identified seven cases with a history of prolonged usage of Fowler's solution (Falk *et al*., 1981a,b).

Two Danish cohort studies (Andersen *et al*., 1973; Moller *et al*., 1975 from Bates) have also examined the association between internal cancers and arsenic exposure in patients with skin diseases that can be caused by inorganic arsenic. In a follow-up of 207 patients with Bowen's disease (Andersen *et al*., 1973), only one patient out of 33 with a history of arsenic medication was diagnosed with an internal tumor. Because no information on the expected number of cancers was presented, we were unable to calculate a relative risk estimate. The study of Moller *et al*., 1975 involved patients with multiple basal cell carcinomas. Of the 45 patients who had received treatment with arsenic-containing medications, four were diagnosed during the period of follow-up as having internal malignancies (SIR = 3.3, 95 percent C.I. 0.9-8.5).

Cuzick *et al.* (1982) examined a cohort of subjects in Britain that had taken Fowler's solution from 1945 through 1969. Slightly elevated numbers of respiratory cancer in conjunction with a small dose-response trend was found (SMRs 0.8, 1.1, 1.4, 1.8, $p=0.16$). Cumulative doses ranged from <500 mg to >2000 mg. A threefold increase in bladder cancer mortality (SMR 3.07; 95 percent C.I. 1.01-7.3) was reported after further follow-up of the cohort through 1990 (Cuzick *et al.,* 1992), strengthening the bladder cancer evidence previously reported. With one exception, the bladder cancer cases had received cumulative doses of less than 2,000 mg of arsenic. This is a relatively low cumulative dose, equivalent to drinking 2 liters/day of water with an arsenic concentration of 100 μg/L for 30 years. The most recent publication did not provide comparative respiratory cancer data. The most remarkable finding of this study is that all the cancer deaths (11 for all target sites, 7.1 expected) were among patients with arsenical skin disease (10 had keratoses), while none of those without signs died of cancer (although 6.3 were expected). This suggests that arsenical skin disorders may be markers of susceptibility to arsenic-related cancers. However, the absence of cases of cancer in those without skin lesions with 6.3 expected ($p = 0.004$) is peculiar and supports diagnostic bias as the more likely explanation.

Arsenic in Wine Substitutes

Reports of arsenic poisoning in German winegrowers date back to the 1930s. German investigators (Roth, 1957; Luchtrath, 1983) have stated that the cause of arsenic poisoning was the consumption of Haustrunk ("house-drink"), a wine substitute made from an aqueous infusion of already-pressed grape skins and containing 2 to 90 mg/L of arsenic trioxide. Insecticides containing arsenic trioxide were widely used in German vineyards until prohibited in 1942. Of twenty-seven Moselle vintners autopsied between 1950 and 1956, eleven had lung cancers and three had hepatic angiosarcomas (Roth, 1957). In two smaller autopsy series totaling twenty cases, another six cases of liver cancer were found, four hepatocellular carcinomas and two angiosarcomas (Falk *et al.*, 1981b). In a 1960 to 1977 series of 163 postmortem examinations of German winegrowers diagnosed based on cutaneous signs as having had chronic arsenic poisoning, Luchtrath (1983) found five cases with liver tumors, none of which were angiosarcomas. Since all the cases may have been heavy drinkers and forty-five had liver cirrhosis, the possibility that the hepatocarcinomas were alcohol-related cannot be discounted.

Overview of Cancer Epidemiology

Criteria for Causal Inference

Chance

Studies at high exposures have produced clearly increased risks of lung and bladder cancer such that chance is not an issue. For example, if we just consider the results from Taiwan and Chile, the tests of significance for lung and bladder cancer give p-values that are well below 0.001.

Bias

The most obvious potential confounding factor is cigarette smoking for cancers of the lung and bladder. There is no *a priori* reason to believe that smoking would have an association with arsenic ingestion. Confounding by smoking can be discounted as an explanation for the findings in the Taiwanese studies: the higher prevalence of smoking in the Blackfoot disease area (40 percent of males and females combined) was insufficiently different from that in Taiwan (32 percent) (Chen *et al.*, 1985). In the case-control study from the same area, the odds ratios were adjusted for smoking (Chen *et al.*, 1986).

If smoking were a major factor in increasing the risk of lung and bladder cancer, a concomitant increase in smoking-related diseases, such as chronic obstructive pulmonary disease (COPD) mortality, should be found. Smith *et al.* (1998) found that the mortality rates of COPD in Region II of Chile were similar to men in the rest of the country (SMR = 1.0, 95 percent CI 0.8-1.1), and were even lower for women (SMR = 0.6, 95 percent CI 0.4-0.7). In Argentina, COPD rates were under unity for men in all exposure groups and women in the medium and high exposure groups (Hopenhayn-Rich *et al.*, 1996c). Ferreccio *et al.* (2000) controlled for smoking in their lung cancer case-control study in Chile (Table 7). Odds ratios for lung cancer cases who had never smoked were 5.9 (95 percent C.I. 1.2-40.2) and 8.0 (1.7-52.3) for average cumulative exposure categories of 50 to 199 μg/L and greater than or equal to 200 μg/L inorganic arsenic, respectively (Table 9).

Based on the results of the aforementioned studies, confounding bias due to smoking does not explain the elevated risks of lung and bladder cancer in the studies of ingestion of inorganic arsenic in the drinking water.

Table 9. Interaction of Exposure to Arsenic in Drinking Water and Smoking on the Risk of Lung Cancer (Ferreccio *et al.*, 2000)

				Ever Smoked		
1930-1994 Average Cumulative As Exposure (μg/L)	Cases	Control	OR (95% CI)	Cases	Control	OR (95% CI)
≤49	2	63	1.0	20	103	6.1 (1.31-39.2)
50-199	11	59	5.9 (1.2-40.2)	39	66	18.6 (4.13-116.4)
≥200	17	67	8.0 (1.7-52.3)	62	61	32.0 (7.22-198.0)

Controversies regarding the Taiwanese studies

Despite the large magnitude of the associations, the presence of clear dose-response trends, and the consistency of the findings amongst several investigations, the data from Taiwan have been extensively questioned. For example, it has been suggested that arsenic may not be the sole cause of these cancers. Instead, it was postulated that certain fluorescent humic acid-like substances were responsible for at least some portion of these effects, since these substances were also found in high levels in the artesian wells, and they could cause a vascular disease similar to BFD (Carlson-Lynch *et al.*, 1994; Lu, 1990). Lu (1990) isolated three of these substances, which he initially described as ergot-like compounds, but later found to be chelates of humic acid and various metals. When one of these substances was injected into mice for 20 to 32 days, half of the animals developed ulceration, necrosis, and gangrene in the extremities. This was stated to resemble the pathogenesis of Blackfoot disease in the endemic region, not arsenic. However, Chen and Wang (1990) have pointed out that the pathology of Blackfoot disease appears to be different from that of the mouse lesions.

As is the case with several cancers that have occurred in the Blackfoot disease endemic region, the presence of Blackfoot disease itself has been shown to have a dose-related association with the presence of arsenic in water supplies (Wu *et al.*, 1989). Therefore, if the humic substances were the true cause of Blackfoot disease, their presence would also have to have been strongly correlated with arsenic levels in water supplies. If this were the case, the humic substances could be the putative confounding factor for cancers suggested above. The existence of the humic substances has not been shown to be confined mainly to the Blackfoot disease endemic areas, and no correlation of their concentration with arsenic levels with Blackfoot disease or any cancers has been shown. Indeed, humic compounds from the decay of plant matter are widespread contaminants of water supplies. In addition, peripheral vascular disease resembling Blackfoot disease has been reported in the Moselle vintners (Roth, 1957; Grobe, 1976) and in populations that used water supplies with high arsenic levels in Chile (Borgono and Greiber, 1971), Mexico (Cebrian, 1987), and Poland (Bencko, 1987). In short, there is little if any evidence to support the claim that humic substances are the cause of disease in the Blackfoot disease endemic area of Taiwan.

The Taiwanese findings have also been criticized for being primarily based on investigations using ecological study designs in which exposures are based on large group averages rather than on direct individual exposure data. In some or most cases, making a causal inference about individual phenomena based on observations of groups can result in a logical flaw known as the "ecological fallacy" (Morgenstern, 1982). However, ecological study data can become a message of significance not to be ignored, and the "ecological fallacy" is of much less concern when there is widespread exposure in a population with high risks (e.g., high arsenic water concentrations and high risks of specific cancers). In addition, other ecological studies in different regions of the world have provided substantial supportive evidence that ingested arsenic does indeed cause bladder cancer and lung cancer. Of these studies, the largest are two ecological mortality studies from South America. In the first one, mortality rates for lung and bladder cancer were found to be about twice the national average in two counties of Cordoba, Argentina, where a portion of the population was exposed to contaminated well water at average arsenic levels of 178 μg/L (Hopenhayn-Rich *et al.*, 1996c, 1998). In the second study, bladder and lung cancer mortality were 3 to 8 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 μg/L (Smith *et al.,* 1998).

In addition to these large ecological studies, two cohort investigations have identified associations between ingested arsenic and lung and bladder cancer, although the number of cases in both of these studies was small. In Namiki-cho, Nakajo-machi, Japan, an area contaminated by wastewater 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 (Tsuda *et al.*, 1995). Similarly, eight lung cancer cases were observed and 0.5 was expected. 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 (Cuzick *et al.,* 1992). A small dose response trend with arsenic ingestion and respiratory cancers was also found. In a case-control study in Chile with individual exposure data, lung cancer ORs ranged up to 8.9 for a 65-year concentration average exposure of 200 to 400 μg/L inorganic arsenic (Ferreccio *et al.*, 2000).

It has been hypothesized that the dose-response results from studies in Taiwan may not be directly applicable to other populations based on the supposedly poor nutritional status of the Taiwanese (Buchet and Lison, 2000; Carlson-Lynch *et al.*, 1994; Marcus and Rispin, 1988; Petito and Beck, 1990; U.S. EPA, 1988). Although a dietary assessment for Taiwan does not support the poor nutritional status hypothesis (Engel and Receveur, 1993), it has been argued that protein deficiencies may diminish the capacity of the Taiwanese to detoxify arsenic, thus making them more vulnerable to its toxic effects. This seems unlikely in view of recent findings on the carcinogenicity of DMA in experimental animals (Wei *et al.*, 2002). It has also been suggested that genetic differences may account for differing susceptibility to the carcinogenic effects of arsenic (Fowle, 1992; Smith *et al.*, 1992; U.S. EPA, 1988). Argentina, in contrast, has one of the world's highest rates of per capita beef consumption, 1.5 times that of the United States (General Agreement on Tariffs and Trade, 1994). In particular, the high-arsenic region of Cordoba is an important agricultural and beef-producing area, and animal protein is considered to be one of the basic foods of the population (Astolfi *et al.*, 1982; Beuschio *et al.*, 1980).

Concerning ethnic and possible genetic differences in Argentina, it should be noted that the Cordoba population increased sharply during the beginning of this century with a large influx of European immigrants. At that time, one-fifth of the total population consisted of persons of European descent (INDEC, 1993). In 1980, 49 percent of those age 65 years or more were foreign-born. The current inhabitants are to a great extent of Italian or Spanish origin. It is thus clear that the ethnicity and nutrition of the populations in studies from Argentina are different from those of the Taiwanese, and quite comparable with those of the United States and Europe. The results, however, are in general agreement with the findings in Taiwan. Therefore, susceptibility to arsenic specific to the Taiwanese, based on either ethnicity or nutrition seems unlikely. The potential for arsenic susceptibility will be further discussed later in this report.

The results of the aforementioned studies resolved several of the controversies surrounding the findings in Taiwan of a 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 the 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 (Chen *et al.*, 1986; Chiou *et al.*, 1995). Speculation that arsenic susceptibility resulting from ethnic or dietary differences is responsible for the increased cancer risks observed in Taiwan has not been substantiated. These studies have confirmed the associations between ingested arsenic and lung and bladder cancer that were identified by the earlier ecological 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. This issue is addressed below in the discussion of biological plausibility.

Consistency of the Results

Among the studies of people who have taken arsenical medications, the causal evidence they provide for anything other than skin cancer is weak. However, these studies had limited statistical power to detect cancer risks. The most informative investigation was the retrospective study of Cuzick *et al.* (1982), although the average follow-up of 20 years may have been inadequate. There was an elevated risk for bladder cancer, although this could have been due to chance. Consistency in the few studies of the Moselle vintners is difficult to judge, since relative risk estimates can be calculated for only one study (Luchtrath, 1983). However, there appears to be consistency in finding at least appreciable rates of lung cancer (Roth, 1957; Luchtrath, 1983). The drinking water studies from Taiwan, Japan, Argentina, and Chile are consistent in showing strong relations with arsenic exposure for mortality from a number of internal cancers, particularly cancer of the bladder and lung.

Strength of Association

Drinking water studies from Taiwan and Chile include large numbers of highly exposed subjects, and have shown very strong associations between ingestion of inorganic arsenic and the risk of cancers of the bladder and lung.

Evidence for Dose-Response Relationships

Studies from Taiwan, Japan, Argentina, and Chile have demonstrated strong dose-response relations with inorganic arsenic in the drinking water and cancers of the bladder and/or lung. Three studies with quantitative exposure data conducted in the United States and Finland did not demonstrate a positive dose-response trend. It should be noted, however, that these studies have limited power. In addition, the highest exposures to arsenic in these latter studies corresponded to the lowest and/or control dose groups from the former group of studies.

Temporality of Association

Known human carcinogens usually have a latent period from first exposure to cancer diagnosis of at least ten years before their effects become manifest. For many, the latency appears to be twenty years or more. This is the case in the studies of ingestion of inorganic arsenic and cancers of the bladder and lung, so the criterion of appropriate temporality of exposure and cancer outcomes is clearly met.

Biological Plausibility

Animal studies

Animal bioassays have so far not been conclusive concerning carcinogenic effects of inorganic arsenic. The epidemiological findings from Taiwan were initially criticized because they were not supported by toxicological 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 (IARC, 1987). More recently, arsenite and dimethylarsinic acid were shown to induce skin cancer in transgenic mice (Chen *et al.*, 2000). Chronic oral administration (p.o.) of a high dose of dimethyl arsenic acid induced urinary bladder cancers in male rats (Wei *et al.*, 1999, 2002). In addition, arsenite at 10 ppm in drinking water was recently shown to act as a cocarcinogen with UV radiation in mouse skin, which was postulated to occur through inhibition of DNA repair or enhancement of growth signaling (Rossman *et al.,* 2001). While it is true that the majority of animal testing has not shown arsenic to be a potent carcinogen, this does not mean that arsenic is not a human carcinogen. Since it is accepted that ingested arsenic can cause skin cancer and inhaled arsenic lung cancer, the lack of experimental corroboration of the epidemiologic data should not restrict causal inference for other cancer sites. Rather, failure to find positive results in most animal testing more likely reflects the differences in arsenic metabolism among various species. Moreover, arsenic is not the only chemical with limited carcinogenic potential in animal tests that is known to cause cancer in humans. Benzidine, for example, a well documented and highly potent human bladder carcinogen, does not induce bladder cancer in rats or mice (Wei *et al.*, 1999).

One experimental study by Shirachi *et al.* (1983) found an increase in kidney cancers when inorganic arsenic was administered to rats in conjunction with diethylnitrosamine (a known kidney carcinogen). A problem in the interpretation of this study is that these animals had appreciably less weight gain than the controls, possibly related to an arsenic-induced loss of appetite. This raised the possibility of an interaction between nutritional deficiency and the nitrosamine. For example, protein deficiency might have enhanced diethylnitrosamine carcinogenesis by a reduction in the level of detoxifying enzymes. However, nitrosamine metabolism appears to involve competing enzymatic pathways for activation and detoxification by denitrosation. A study in rats showed only 10 percent of an administered dose of dimethylnitrosamine to be denitrosated (Keefer *et al.*, 1987). Therefore, one might expect protein deficiency to inhibit carcinogenesis through reduced synthesis of P450. Since the converse happened in Shirachi *et al.* (1983), it provides limited evidence that arsenic may be a promoter, although the possibility of other effects of nutritional deficiency cannot be excluded.

Considering species differences in metabolism, rats (unlike humans) sequester arsenic in their erythrocytes, a process that may protect this species from arsenic induced cancers (Vahter, 1983). Mice have been shown to excrete arsenic more rapidly than humans do (Vahter, 1983). However, a more complete explanation of the species differences in carcinogenic response will require a better understanding of the mechanism(s) for the carcinogenic action of arsenic.

Arsenic has been shown to induce gene amplification in mouse cells in culture (Lee *et al.*, 1988). This evidence raised the possibility that arsenic may specifically amplify human oncogenes. If oncogene amplification were the major mechanism of arsenic's carcinogenic action in humans, then one would expect this to be a late stage effect. However, the latencies calculated for epidemiologic studies of arsenic carcinogenesis have ranged widely. For example, a study of smelter workers estimated a latency for respiratory cancers of about 10 years (Enterline and Marsh, 1982), whereas a comparable study estimated a mean period of 30.5 years (Wall, 1980). A mean latency of at least 23 years was estimated for a Japanese population exposed to arsenic in their water supply (Tsuda *et al.,* 1989), and two studies of people who had taken Fowler's solution estimated mean latencies of 14 and 18 years for skin cancer (Neubauer, 1947; Fiertz, 1965). There is evidence that inhaled arsenic may be retained in the lung for long periods after exposure ceases (Gerhardsson *et al.,* 1988) (see next section). Wright *et al*. (1990) observed that gene amplification was very rare in normal human cells such as mammary epithelial cells, keratinocytes, and diploid fibroblasts. No gene amplifications were seen in experiments involving more than 5×10^8 normal cells selected with three drugs known to reveal amplification in permanent cell lines. The authors concluded that the frequencies of gene amplifications may exceed 2×10^{-9} but were not detected due to lack of adequate selection conditions. Since gene amplification is common in tumors and cell lines but apparently rare in normal cells the steps in the origin of tumors or the immortalization of cell lines that lead to gene amplification are intriguing and may shed light on a mechanism of arsenic carcinogenicity. The rarity of gene amplification in normal cells may explain its possible role in the later stages of the carcinogenesis process when cells in tissues affected by arsenic may no longer be acting normally. Current research on the mechanistic evidence of arsenic carcinogenicity will be reviewed in a later portion of this document. Additional discussion of arsenic-induced cancer in animals can be found above (sections on Toxicology, Toxicological Effects in Animals, and Carcinogenicity).

Ingested inorganic arsenic lung tissue concentrations

Although most ingested arsenic is excreted in the urine, the biological plausibility that ingested inorganic arsenic might cause pulmonary disease is supported by limited evidence showing arsenic accumulation in the lungs (Brune *et al.*, 1980; Gerhardsson *et al.*, 1988). Several autopsy studies have linked exposure to inhaled arsenic in smelter workers with long-term persistence of arsenic in the lungs. In one study, exposed workers had arsenic concentrations in the lung six times higher than controls (47 μg/kg tissue versus 8 μg/kg). These increases were not seen consistently in the kidney or the liver, and the elevation in the lung did not decline significantly even as the time from retirement to death increased, suggesting a long biological half-life (Brune *et al.*, 1980).

Other human evidence indicates that ingested arsenic reaches the lungs. A fatal poisoning following arsenic ingestion by a three-year-old boy resulted in an arsenic concentration in the lungs of 7,550 µg/kg (Saady *et al.*, 1989). In another fatal case, the arsenic concentration in the lung was 2,750 µg/kg (Quatrehomme *et al.*, 1992). Mummified bodies preserved in Region II of Chile due to the very low humidity in the desert have been shown to retain arsenic in the lung tissue (Figueroa *et al.*, 1992). These mummies were from the same area where high concentrations of arsenic have been present in water for many years. Arsenic concentrations in six mummies averaged 5,400 µg/kg. From analysis of tissue remains, arsenic retention in these mummies was ranked by tissue type. Kidney, liver, nails, and lung tissue had the highest retention, above skin, intestines, hair, and muscles.

Animal studies have also assessed tissue concentrations of arsenic following various routes of exposure. One such study demonstrated that the lung tissue has a slower clearance of the arsenic after intravenous injection of As-DMA than the kidneys, blood, and liver (Vahter *et al.*, 1984).

It is also well known that inorganic arsenic binds to sulfhydryl groups and it has been suggested that arsenic concentrates in tissues with a high content of cysteine-containing proteins, including hair, nails, skin and the lungs (NRC, 1999). Considered overall, the data currently available from both animal and human studies indicate that ingestion of inorganic arsenic may result in increased lung tissue arsenic concentrations. This information increases biological plausibility for the findings concerning pulmonary effects of ingested arsenic.

In vitro *and* in vivo *studies of human cell response to inorganic arsenic*

Results of genotoxicity studies indicate arsenic does not cause point mutations, although it has been shown to induce chromosomal aberrations and sister chromatid exchanges when present during DNA replication (Basu *et al.*, 2001; Jacobson-Kram and Montalbano, 1985). The mechanism is not known, although it may involve interference with DNA repair enzymes through binding to their sulfhydryl groups. In this section, we focus on low exposure *in vitro* assays and studies involving human lung cells. To place concentrations in perspective, it might be noted that in human studies where urinary arsenic is measured, the concentrations of inorganic arsenic are on the order of 0.1 µM in those with low exposures, and on the order of 1 µM in the highly exposed. For example, in Chile, residents from a town with 600 µg/L of arsenic in their water had urinary levels of inorganic arsenic of 108 µg/L (about 1.5 µM), while residents from a town having 15 µg/L in their water had urinary levels of inorganic of 8.7 µg/L (a little over 0.1 µM) (Moore *et al.*, 1997b).

Table 10 presents results of the *in vitro* assays. Studies finding effects at surprisingly low concentrations include a study of human keratinocytes with increased proliferation at 0.001 µM (Germolec *et al.*, 1997), a study involving human lymphocytes reporting increased aneuploidy at this same concentration of 0.001 µM (Ramirez *et al.,* 1997), and another finding similar effects at 0.01 µM (Vega *et al.*, 1995). The first studies with lung cells reported effects at relatively high arsenic concentrations: increased heme oxygenase at 4 µM in human adenocarcinoma cell lines (Lee and Ho, 1994), and DNA single strand breaks and cross-link damage at 10 µM (Kato *et al.*, 1994). However, a recent study involving a human lung adenocarcinoma cell line reported hypermethylation in the p53 promoter at concentrations as low as 0.08 μM arsenite (Mass and Wang, 1997), and another study using normal human fibroblasts showed effects of arsenic on p53 and positive growth signaling (Vogt and Rossmann, 2001). These studies are important because they add biological plausibility to finding pulmonary effects in humans. They identify *in vitro* effects in various human cells including lung cells, and effects at doses commensurate with levels that may be present in human tissues at quite low levels of ingestion of inorganic arsenic.

Human Cancer Epidemiology Conclusions

Lung Cancer

Recent studies add to the evidence that ingestion of inorganic arsenic causes increased risks of lung cancer. Clear increased risks were found in ecological studies in both Argentina and in Chile. Confounding due to smoking could be excluded as the explanation in both populations. Increased lung cancer risks have been reported in a small study in Japan involving drinking water and a case-control study with individual exposure data from Chile. The biological plausibility that arsenic from ingestion might increase lung cancer risks is strengthened by the fact it is a confirmed lung carcinogen by inhalation. Taking this into account, there is now sufficient evidence to conclude that ingestion of inorganic arsenic is a cause of human lung cancer.

Bladder Cancer

There is sufficient evidence from several studies in several countries to conclude that ingestion of arsenic is a cause of human bladder cancer. Beyond the findings in Taiwan, the strongest additional evidence comes from large population studies in Chile and Argentina, each conducted with the a priori hypothesis that bladder cancer risks would be increased. Both studies found that the highest relative risks for internal cancer mortality associated with arsenic exposure were for bladder cancer. These ecological studies are supplemented by studies with individual data, in particular in Taiwan and in the Fowler's solution study in England. There is therefore ample evidence to conclude that inorganic arsenic ingestion is a cause of human bladder cancer.

Other Internal Cancers

While recent studies add to the existing evidence and make it probable that ingestion of arsenic can cause kidney cancer, the findings are not as strong as for bladder and lung cancer. The evidence concerning liver cancer has actually been weakened by recent studies, especially the lack of increased risks of liver cancer in Region II of Chile in the presence of dramatic increases in bladder and lung cancer mortality. It remains possible that arsenic increases the risk of primary liver cancer in the presence of a cofactor occurring in Taiwan. Aflatoxin and hepatitis are two possibilities. The possible misdiagnosis of secondary liver cancers as primary liver cancer on death certificates also warrants consideration as a possible explanation.

Table 10. Dose-Response Relationships of *In Vitro* As Exposure and Various Outcomes

Cell type/tissue	*Exposure Range*	*LOEL*	*Outcome*	*References*
In vitro				
1° Human keratinocytes	0.5-4 µM sodium arsenite	0.5 µM arsenite	Increase TNF Alpha, GM-CSF, TGF Alpha, mRNA transcripts, inc c-myc.	Germolec *et al.*, 1997
1° Human keratinocytes	0.001-0.004 µM sodium arsenite	0.001 µM arsenite	Suggestive increase in keratinocyte proliferation.	Germolec *et al.*, 1997
Cultured lymphocytes Bowen's Disease vs. controls	0, 0.5, 1.0, 2.0 µM sodium arsenite	0.5 µM arsenite	Increased SCEs (HFCs) and decreased RI for both cases and controls.	Hsu *et al.*, 1997
Human keratinocyte cell lines SCC-9, SIK, hEp	0.3, 1, 3, 10 µM sodium arsenate; 0.1, 0.3, 1, 3 µM sodium arsenite	EC_{50}s arsenite ≅ 1 µM, arsenate ≅ 2 µM	Suppression of differentiation markers involucrin, keratinocyte transglutaminase, filaggrin, and small proline-rich protein 1	Kachinskas *et al.*, 1997; Jessen *et al.*, 2001.
Human lung adenocarcinoma cell line A549	0.08-2 µM sodium arsenite; 30-300 µM sodium arsenate; 2-2000 µM DMA	0.08 µM arsenite/ 30 µM arsenate	Arsenite and arsenate but not DMA produced significant hypermethylation of a 341 bp fragment of the p53 promoter.	Mass and Wang, 1997
L5178Y/TK+/- lymphoma assay (heterozygote mouse lymphoma cell)	sodium arsenite 1-2 µg/ml; sodium arsenate 10-14 µg/ml; MMA 2,500-5,000 µg/ml; DMA almost 10,000 µg/mL		All four caused mutations.	Moore *et al.*, 1997b
Human lymphocytes	sodium arsenite 0.001-0.1 µM	0.001 µM	Increased aneuploidy, hyperploidy of chromosomes 1 & 7, inhibition of tubulin polymerization.	Ramirez *et al.*, 1997
Lymphocytes, lymphoblastoid cell line	0.1-10 µM sodium arsenite	0.5 µM	Increased incidence of SCEs	Rasmussen and Menzel, 1997

Table 10 (Continued). Dose-Response Relationships of *In Vitro* As Exposure and Various Outcomes

Cell type/tissue	***Exposure Range***	***LOEL***	***Outcome***	***References***
CHV79 WT/As/S27D-hamster hypersensitive/ human keratinocytes AG06, AG07, HeLa cells, meduloblastoma, and diploid fibroblasts HTB139	5 μM $NaAsO_2$ challenged (hamster), 0.5 μM HeLa cells, 0.05 μM AG06, 0.1 μM HTB139,		No inducible tolerance in human cells.	Rossman *et al.*, 1997
Human lung adenocarcinoma (CL3), (CL3R)	4 μM $NaAsO_2$ (300 ppb)		Expressed heme oxygenase	Lee and Ho, 1994
Human lymphocytes	1-50 μM sodium arsenite	1 μM	Induction of CAs, SCEs	Jha *et al.*, 1992
Human lymphocytes	10^{-10}-10^{-2} μM sodium arsenite	10^{-8} μM (0.01 μM)	Induction of CAs, Aneuploidy	Vega *et al.*, 1995
Human lymphocytes	0.5-2 μM sodium arsenite	1 μM, 2 μM respectively	SCE, CA respectively	Wiencke and Yager, 1992
Human lymphocytes	0, 3, 6, 9 μM sodium arsenite	3 μM	MN induction	Eastmond and Tucker, 1989
Mouse 3T6 cells	0.2-6.2 μM sodium arsenite	0.2 μM	Increase amplification in the dihydrofolate reductase gene.	Lee *et al.*, 1988
Cultured human alveolar cells (L-132)	5, 7.5, 10 mM DMA	10 μM DMA	DNA single strand breaks, DNA protein cross-link damage may be induced at AP sites (apurinic/apyrimidinic), damage thought to be caused by dimethylarsenic peroxyl radicals	Kato *et al.*, 1994
CHV79, G10, G12 cells	10 μM-10 mM DMAA and or 1-10 μM AsIII 6-24Hr	10 mM DMA	Slight mutagenesis in G10 & G12 cells at 10 mM DMA, higher if exposure was to both 10-100 μM DMA and 2.5-5.0 AsIII combined; 1-5 μM AsIII did not induce mutagenesis	Misawa and Horiike, 1996

Table 10 (Continued). Dose-Response Relationships of *In Vitro* As Exposure and Various Outcomes

Cell type/tissue	*Exposure Range*	*LOEL*	*Outcome*	*References*
In vivo				
Human urothelial cells	High exposure vs. low exposure; 670 vs. 15 µg/L in H_2O	50 µg/L total As in urine	Dose dependent increases in MN frequency from 50-700 µg/L urinary total As	Moore *et al.*, 1997a
Human urothelial, buccal cells, lymphocytes	High exposure vs. low exposure; 408.17 vs. 29.88 µg/L in H_2O	No breakdown of exposure groups given	About a four-fold increase in urothelial, buccal cell MN Increase in chromatid deletions, isochromatid deletions, percent cells with aberrations, CA/cell	Gonsebatt *et al.*, 1997
Transgenic mouse with v-Ha-Ras oncogene	Low dose TPA + 0.02% $NaAsO_2$ = 1.4 mM	Large dose	Increase number of skin papillomas, increased GM-CSF, TGF-alpha mRNA transcripts	Germolec *et al.*, 1997
MT- transgenic mice	N = 90 c57B1/6J mice and 140 female w/ metallothionein knock out (MT-)	500 µg As/L ad libitum for 26 mon, 2.0-2.5 µg As/day = 0.07-0.08 mg As/kg for 30 g mouse	Preliminary findings indicate incidence of tumors increased: GI: 14.4% vs. 12.9%; lung: 17.8-7.1%; liver: 7.8-5%; spleen 3.3-0.7%; bone: 2.2-0%; skin: 3.3-1.4%; reproductive system: 3.3-5%; eye: 1.1-0%. Indicates MT is not protective.	Ng *et al.*, 1998
Human lymphocytes	High exp. H_20 and urine: 0.13±0.09; 0.16±0.08 Low exp H_20 and urine: 0.02±0.02; 0.07±0.04	130 ppb	Significantly elevated SCEs in high exposure vs. low exposure.	Lerda, 1994

Table 10 (Continued). Dose-Response Relationships of *In Vitro* As Exposure and Various Outcomes

Cell type/tissue	*Exposure Range*	*LOEL*	*Outcome*	*References*
Male rats Male mice	Orally administered DMA; 1950 mg/kg and 1500 mg/kg respectively Morphologic changes examined in liver, spleen, kidney		DNA single strand breaks in lung after 12 hrs, no breaks in liver, kidney, and spleen. DNA damage caused by reaction between dimethylarsine and molecular oxygen (oxidative damage). No morphological changes within 24 d of DMA administration; marked increase of heterochromatin in endothelial cells of alveolar wall capillaries in mice 12-48 hrs after DMA administration, but not in the sinus endothelium of the liver. Lung-specific DNA protein cross-links	Okada and Yamanaka, 1994; Yamanaka *et al.*, 1989a,b
Pulmonary cells from *in vivo* experiments	Mn-SOD and GSH-Px elevated after administration of DMA		Suggests that DMA causes superoxide anion radical and hydrogen peroxide in lung, which might cause DNA strand breaks, and that superoxide anion radical is produced mainly in mitochondria-rich Clara and alveolar type-II cells. GSH markedly decreases in lungs after DMA, indicative of free-radical induced damage, GSH-R not activated. Also state that DMA has higher affinity for the nucleus than inorganic arsenic. Mechanism: dimethylarsine, which is metastable, gives electron to molecular oxygen to form $(CH3)_2As^*$ and O_2^- radicals rather slowly; $(CH3)_2As^*$ promptly reacts with molecular oxygen to produce dimethylperoxyl radical $[(CH3)_2AsOO\bullet]$ which is fairly stable, even in cells. This radical may attack DNA deoxyribose moieties to cause strand scissions in a manner similar to •OOH, since their properties seem analogous. The dimethylarsenic peroxyl radical rather than active oxygen species may play a major role in DNA strand breaks, presumably through formation of DNA adducts (Tezuka *et al.*, 1993).	Yamanaka *et al.*, 1990, 1991

Note: TNF = tumor necrosis factor; TGF = transforming growth factor; GM-CSF = granulocyte/macrophage colony stimulating factor; SCE = sister chromatid exchange;
CA = chromosome aberration; MN = mononuclei; AP = activating protein; SOD = super oxide dismutase.

Vulnerability of Infants and Children

There is some indication of differential toxic effects in children due to arsenic exposure in human studies on birth weight (Borzsonyi *et al*., 1992; Yang *et al*., 2003) birth weight and congenital malformations (Nordstrom *et al*., 1978, 1979a,b; Beckman and Nordstrom, 1982) and neurological development (IQ) (Siripitayakunkit *et al*., 1999; Calderon *et al*., 2001). Studies in Chile comparing communities exposed to high or low arsenic in their drinking water have indicated an association of arsenic exposure with elevated risks of fetal, neonatal, and postneonatal mortality (Hopenhayn-Rich *et al*., 2000). Ahmad *et al*. (2001) found higher risks of spontaneous abortion, stillbirth, or preterm birth in a community with high exposure to arsenic in drinking water versus a similar unexposed group in Bangladesh.

Arsenic is a known human carcinogen by inhalation and oral routes of exposure. The principal sites of cancer formation are skin, lung and urinary bladder. Lesser sites include liver and kidney (IARC, 1987; NRC, 1999). The data of Smith *et al*. (1998) indicate that childhood exposures to arsenic in drinking water may be associated with a significant increase in lung cancer in younger men aged 30-39 years. Recent work in mice (Waalkes *et al*., 2003) indicates a high vulnerability of the later stages of fetal development to the carcinogenicity of arsenic via maternal (transplacental) exposure to arsenic in drinking water.

Arsenic is teratogenic in mice, rats, hamsters, rabbits, and chicks. Arsenite (As^{III}) has been shown to cause reproductive and developmental effects at significantly lower doses than arsenate (As^{V}). The effects observed include increased fetal death, decreased fetal weight, and congenital anomalies. The anomalies most frequently reported include neural tube defects, eye defects, renal and gonadal agenesis, and skeletal malformations. Most studies have involved single high doses by gavage or injection. Maternal toxicity was often but not always observed in these studies (OEHHA, 1999a, 2000).

In addition to possible enhanced sensitivity to arsenic toxicity at multiple points in the developing child, infants and children would also experience higher exposure to environmental media containing arsenic, particularly drinking water. Consumption of water or food containing water (infant formula) is much higher on a body weight basis in infants and children than in adults (average total water consumption in infants less than one year of age is 163 mL/kg-d vs. 32.6 mL/kg-d for adults 20 –64 years; OEHHA, 2000, see also Table 20).

Concern for potential differential toxicity of arsenic compounds in children vs. adults is predicated on the carcinogenicity and developmental toxicity of arsenic compounds. The potential neurotoxicity of arsenic in children, possibly in combination with other environmental agents, is also a concern. Studies in mice (Meija *et al*., 1997) indicate combined effects of lead and arsenic on the central nervous system that were not observed with either metal alone.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Mode of Action

Barchowsky *et al.* (1996) investigated a possible mode of action in As-induced vascular disease, specifically the hypothesis that nonlethal levels of arsenic increase intracellular oxidant levels, promote nuclear translocation of *trans*-acting factors, and are mitogenic. Incubation of second passage vascular epithelial cells from porcine aorta with less than five µM arsenite for four hr increased the incorporation of [^{3}H]-thymidine into genomic DNA, while higher concentrations failed to stimulate or inhibit DNA synthesis. Within one hr exposure to five µM arsenite, oxidants accumulated ($P < 0.005$) and thiol status increased ($P < 0.001$). Concurrently there was increased nuclear retention of nuclear factor-κB (NF-κB) binding proteins and nuclear translocation of NF-κB also occurred in response to 100 µM H_2O_2. The antioxidants N-acetylcysteine and dimethylfumaric acid increased intracellular thiol status and prevented both oxidant formation and translocation of NF-κB binding proteins in response to arsenite. The results suggest that arsenite initiates vascular dysfunction by activating oxidant-sensitive endothelial cell signaling. Such dysfunction may induce an endothelial cell phenotype that is proinflammatory and retains monocytes in the vessel wall (Collins, 1993). The genes expressed by this phenotype, including those for adhesion molecules for proatherosclerotic monocytes, may contain requisite κB sites in their promoters (Collins *et al.*, 1995; Mackman, 1995).

Parrish *et al.* (1999) studied the effects of low As^V or As^{III} concentrations (0.01-10 µM) on rabbit renal slices. The precision-cut slices were exposed for up to eight hr. Cytotoxicity was assessed by intracellular K^+ levels. Neither arsenical induced overt toxicity. No alterations in expression of the heat shock proteins Hsp 60, 70, or 90 were seen. However, increased heme oxygenase-1 (Hsp 32) was seen with a four hr treatment of As^{III} but not As^V (≥ 0.1 µM, $P < 0.05$). Both As^{III} and As^V induced DNA binding of activator protein (AP-1) at 2-4 hr exposure. Neither arsenical altered the DNA binding of ATF2, but both forms enhanced the DNA binding of Elk-1. The enhanced DNA binding activity of AP-1 and Elk-1 was correlated with increased gene expression of *c-fos* at two hr, *c-myc* at six hr, but not *c-jun*. The results indicate that short-term arsenic exposure causes alterations in signaling pathways and gene expression in the rabbit kidney. The authors suggest that AP-1 may play a role in the regulation of several genes implicated in renal fibrosis and note that arsenic has been reported to initiate renal tubulointerstitial fibrosis (Prasad and Rossi, 1995). Alternatively, longer-term exposures of human keratinocytes to arsenic appear to inactivate at least certain AP-1 dependent gene expressions (Jessen *et al.*, 2001).

Menzel *et al.* (1999) isolated at least four arsenic-binding proteins induced by treatment of human lymphoblastoid cells with 10 µM arsenite. Two of the proteins were tentatively identified as tubulin and actin; the identities of the remaining proteins are unknown. The authors speculate that activation of As^{III} receptor protein could influence gene expression directly or indirectly via nuclear DNA binding proteins such as activator protein-1 (AP-1), NF-κB, or another family of nuclear transcription regulators.

Lynn *et al.* (2000) studied arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. Human aorta cells (VSMCs) were treated four hr at one to10 μM arsenite and apparent DNA strand breaks detected by single-cell alkaline electrophoresis. DNA strand breaks were increased by formamidopyrimidine-DNA glycosylase (Fpg) and decreased by diphenylene iodinium, superoxide dismutase, catalase, pyruvate, DMSO, or D-mannitol. Extracts from arsenite-treated cells exhibited an increased capacity for producing superoxide in the presence of NADH. Conversely, addition of arsenite to untreated cell extracts did not increase superoxide production. The authors concluded that arsenite activates NADH oxidase producing superoxide and oxidative DNA damage in vascular smooth muscle cells. Such DNA-damaged cells may initiate an atherosclerotic plaque that may be considered a benign smooth muscle cell tumor.

Bau *et al.* (2002) observed a marked increase in the number of DNA strand breaks (DSB) in arsenite treated human cells using Fpg and proteinase K (PK) digestion. Arsenite concentrations were low, 0.25 μM-4 hr, and did not affect cell viability. A 0.25 μM-72 hr treatment did not affect cell survival, whereas 2 μM-72 hr did. The cell types tested arsenite μM concentration and fold DSB increase were: umbilical vein endothelial cells (2, 3.7), vascular smooth muscle cells (1, 3.2), leukemia HL60 cells (0.25, 3.1), leukemia NB4 cells (0.25, 2.5), fibroblasts (2, 1.9). Oxidized guanine products were found in all As^{III}-treated human cells examined. DNA-protein cross-links were also seen in arsenite treated NB4 and HL60 cells. In umbilical vein endothelial cells the induction of oxidized products was sensitive to inhibitors of nitric oxide (NO) synthase but not to the oxidant modulators, catalase or diethyldithiocarbamate. Vascular smooth muscle cells showed the opposite effect, whereas oxidized products and DNA-protein cross-links in NB4 and HL60 cells were sensitive to calcium, NO synthase, oxidant, and meyloperoxidase. The authors note that in addition to oxidative DNA damage, NO, peroxynitrite, and reactive oxygen species also attack other molecules such as lipids and proteins and these altered molecules may interfere with signal transduction and transcription factors. While such reactions may influence the carcinogenic process, they may also play a role in chronic non-cancer effects such as vascular toxicity.

Animal Studies

Experimental animals appear to be less sensitive to the toxic effects of arsenic than humans, and care must be exercised in extrapolating to safe human exposures from animal data. The studies discussed above that may be suitable for human risk assessment are the developmental toxicity study in hamsters by Hanlon and Ferm (1986) which gave a PBPK adjusted LOAEL of 2.8 mg As/kg-d; the chronic (2 yr) dog study of Byron *et al.* (1967) with a NOAEL of 3.1 mg/kg-d; and the chronic (1 yr) monkey study of Heywood and Sortwell (1979) with a NOAEL of 2.8 mg-kg-d.

Human Studies

Of the studies reviewed above bearing on the noncarcinogenic effects of arsenic in humans, those of Chiou *et al.* (1997b) on cerebrovascular effects and of Chen *et al.* (1996a) on ischemic heart disease seem most suitable for quantitative assessment. Both studies involve relatively large populations of humans exposed to arsenic via drinking water. The study results are summarized in Tables 11 and 12, respectively.

Table 11. Cerebrovascular Disease and Ingested Arsenic (Chiou *et al.*, 1997b)

					Percent CI (95% CI)
Unadjusted					
< 0.1	1004	9	0.9	4	0.4
0.1-50	3436	65	1.9	41	1.2
50.1-299.9	1808	38	2.1	29	1.6
≥ 300 µg/L	698	19	2.7	17	2.4
Adjusted for age and sex					
<0.1			0.9		0.4
0.1-50			2.2 (1.3-3.7)**		1.3 (0.6-2.8)**
50.1-299.9			2.4 (1.4-4.3)***		1.7 (0.8-3.8)***
≥ 300 µg/L			3.1 (1.6-6.1)***		2.6 (1.1-6.1)***
Adjusted for age, sex, smoking, alcohol					
<0.1			0.9		0.4
0.1-50			2.3 (1.3-3.9)***		1.3 (0.6-2.9)***
50.1-299.9			2.5 (1.4-4.5)***		1.8 (0.8-3.9)***
≥ 300 µg/L			3.2 (1.6-6.4)***		2.8 (1.2-6.6)***
Cumulative Dose Unadjusted					
< 0.1	1378	12	0.9	7	0.5
0.1-4.9	5498	100	1.8	68	1.2
≥ 5.0 (mg/L)yr	1208	27	2.2	20	1.6

Table 11 (Continued). Cerebrovascular Disease and Ingested Arsenic (Chiou *et al.*, 1997b)

					Percent CI (95% CI)
Adjusted for age and sex					
< 0.1			0.9		0.5
0.1-4.9			2.2 (1.1-3.6)*		1.3 (0.6-2.8)*
≥ 5.0 (mg/L)yr			2.5 (1.2-4.4)*		1.6 (0.6-3.6)*
Adjusted for age, sex, smoking, alcohol					
< 0.1			0.9		0.5
0.1-4.9			2.0 (1.1-3.7)**		1.4 (0.6-2.9)*
≥ 5.0 (mg/L)yr			2.3 (1.2-4.8)**		1.7 (0.7-4.1)**

[a] 95 percent confidence intervals (CI) are based on the values given by Chiou *et al*. 1997b in Tables 3 and 4. (*) indicates P < 0.05; (**) , P < 0.01; and (***), P < 0.001.

Table 12. Ischemic Heart Disease Mortality and Ingested Arsenic (Chen *et al.*, 1996a)

					Adjusted Percent ISHD
< 0.1	467	3	0.64	1.00	0.64
0.1-9.9	313	1	0.32	2.5 (0.5-11.4)	1.60
10.0-19.9	434	3	0.69	4.0 (1.0-15.6)*	2.56
≥ 20.0	386	9	2.33	6.5 (1.9-22.2)**	4.16

[a]Model 3 includes adjustment for age, sex, cigarette smoking, body mass index, serum cholesterol and triglycerides, and hypertension and diabetes disease status. (*) indicates P < 0.05; (**), P < 0.01.

The data in Table 11 were analyzed using the U.S. EPA benchmark dose software (bmds, beta version 1.1b). The doses employed were the midpoints of the dose ranges given and points 50 percent below or above the lower and upper inequalities, respectively. The data were best fit using the quantal linear regression (QLR) dose-response equation. Since the responses were of the order of 0.1 to 2 percent, the values calculated were for the 1 percent response, ED_{01} and LED_{01}, rather than the usual 5 or 10 percent response values. The LED_{01} is the 95 percent lower bound on the ED_{01}. The analysis of the CVD and CI data is presented in Table 13. The values for cerebral infarction were marginally better fit by the dose-response equation than those for CVD. Due to the severity of these and other endpoints analyzed below, the uncertainty in the dose assignments, and the fact that the chosen points of departure or LED's were generally two-fold or more above concurrent control levels, the LED is considered equivalent to a LOAEL for the purposes of this risk assessment.

Similarly, the analysis of the ISHD data from Table 12 is presented in Table 14. In this case, the data are also adequately fit by the QLR dose-response equation, and the LED_{01}, based on ISHD mortality, should also be considered a LOAEL for this endpoint. In both these analyses the cumulative arsenic dose metric of (mg/L)yr and resultant benchmark doses would need to be divided by 70 yr to yield lifetime drinking water concentrations of arsenic.

The Chen *et al.* (1995) data on the association of hypertension (HT) and cumulative arsenic intake via drinking water are analyzed below in Table 15. As above the QLR dose-response equation fit the unadjusted data well but was somewhat less than adequate for the adjusted prevalence values. The acceptable criterion for the X^2 goodness of fit test for the benchmark dose is $P > 0.05$. In the case of arsenic induced hypertension, the 10 percent effect level was chosen due to the higher background and greater dose response range. For HT the LED_{10} is considered an appropriate LOAEL for risk assessment. In the case of the adjusted data set removal of the highest cumulative dose allows an acceptable fit of the QLR equation with an LED_{10} of 7.4 (mg/L)yr. The data of Rahman *et al*. (1999) are also analyzed in Table 15. Both crude and adjusted data sets were well fit by the QLR with P values much greater than 0.1. The best fit LED_{10} value of 5.8 (mg/L)yr from Bangladesh is quite similar to the best fit value of 7.2 (mg/L)yr from the Taiwan study.

Table 13. Benchmark Dose Analysis of Cerebrovascular Disease and Arsenic Ingestion (Chiou *et al*., 1997b)

						LED$_{01}$
0.05, 25, 175, 450 μg/L	CVD, unadjusted	9/1004, 65/3436, 38/1828, 19/698	4.24	0.12	359	189
	CVD age, sex adj.		6.32	0.04	293	164
	CVD age, sex, smoking, alcohol		6.98	0.03	274	156
As above	CI unadjusted	4/1004, 41/3436, 29/1828, 17/698	4.15	0.12	268	166
	CI age, sex adjusted		4.88	0.09	245	155
	CI age, sex, smoking, alcohol adjusted		5.32	0.07	233	149
0.05, 2.5, 7.2 (mg/L)yr	CVD unadjusted	12/1378, 100/5498, 27/1208	2.73	0.099	5.1	3.0
	CVD age, sex adjusted		5.78	0.02	4.2	2.5
	CVD age, sex, smoking, alcohol adjusted		3.93	0.05	4.8	2.8
As above	CI unadjusted	7/1378, 68/5498, 20/1208	2.03	0.15	5.9	3.5
	CI age, sex adjusted		2.99	0.08	6.3	3.6
	CI age, sex, smoking, alcohol adjusted		3.10	0.08	5.4	3.2

Table 14. Benchmark Dose Analysis of Ischemic Heart Disease and Arsenic Ingestion (Chen *et al.*, 1996a)

						LED_{01} (mg/L)yr
0.05, 4.9, 15.0, 30.0	467, 313, 434, 386	0.64, 1.6, 2.56, 4.16	0.26	0.88	8.27	5.53

Table 15. Benchmark Dose Analysis of Hypertension and Arsenic Ingestion (Chen *et al.*[a], 1995; Rahman *et al.*[b], 1999)

						LED_{10}
0, 3.1, 8.6, 12.8, 16.6, 27.8a	119, 82, 94, 104, 98, 236	5.0, 4.9, 12.8, 22.1, 26.5, 29.2	4.3	0.37	8.8	7.2
As above	As above	Adjusted* 5.0, 3.9, 11.5, 17.0, 19.0, 14.5	10.4	0.03	21.1	14.4
0, 0.5, 3.0, 7.5, 15.0b	114, 238, 693, 279, 271	7.9, 5.5, 12.0, 14.3, 22.9	3.5	0.32	8.4	6.3
As above	As above	Adjusted** 7.9, 6.3, 11.8, 17.4, 23.7	1.6	0.66	7.6	5.8

*Multivariate-adjusted odds ratios including the risk factors age, sex, disease status of diabetes and proteinuria, body mass index (BMI), and fasting serum triglyceride levels were used to adjust the percent prevalence values for HT. ** Adjusted for age, sex, BMI.

Similarly, the data of Lai *et al.* (1994) and Rahman *et al*. (1998) are analyzed in Table 16. In this case, both unadjusted and multivariate-adjusted prevalences were adequately fit by the QLR dose-response model. EDs and LEDs were determined for the 1 and 5 percent response levels. The LED_{05} for the adjusted values appears the best choice for the chronic LOAEL for arsenic induced diabetes mellitus. In addition to the values noted above, an estimated LOAEL of 20 (mg/L)yr for peripheral vascular disease from Tseng *et al.* (1996) is also included in this analysis.

Table 16. Benchmark Dose Analysis of Arsenic Ingestion and Diabetes Mellitus (Lai *et al*., 1994[a]; Rahman *et al*., 1998[b])

						LED_{05}
0, 7.45, 22.6 (mg/L)yr[a]	108, 284, 326	Unadjusted 0.9, 7.0, 14.4	0.7	0.4	7.7	6.0
As above	As above	Adjusted* 0.9, 5.9, 9.4	1.8	0.2	12.8	8.8
0, 0.25, 0.75, 1.5 mg/L[b]	854, 78, 68, 14	Unadjusted 2.9, 8.9, 11.8, 21.4	0.90	0.64	0.34	0.21
As above	As above	Adjusted** 2.9, 7.2, 34.2, 61.3	3.34	0.19	0.11	0.08
As above	512, 33, 43, 14 males only	Unadjusted 2.7, 12.0, 18.6, 21.4	1.30	0.52	0.23	0.14

*Multivariate-adjusted odds ratios including risk factors of age, sex, body mass index, and physical activity level were used to adjust percent prevalence values for diabetes mellitus.
**Adjusted for age and sex only.

The arsenic-induced skin keratosis and hyperpigmentation data of Mazumder *et al.* (1998) were analyzed as above; the results are given in Table 17. For both male and female skin keratosis data sets, adequate fits were obtained by the quantal-linear relation with lower bound values (LED_{01}) of 49.6 µg/L for males and 124 µg/L for females. Adequate fits could not be obtained for both hyperpigmentation data sets with the models available in the benchmark dose program; however, the dose-response graphs appeared to be linear in the lower exposure groups with respective LED_{01}s of 18.9 and 34.7 µg/L. It appears that a single dose level (125 µg/L) was largely responsible for the failure of the statistical test. Since the dose response appeared to be adequate visually above and below this point the LED_{01} values for hyperpigmentation were retained in Table 17 for comparison. Mazumder also included an assessment of skin keratosis and hyperpigmentation prevalence by dose per body weight and an analysis of these data is also given in Table 17. Using the dose metric of µg/kg-d the skin hyperpigmentation data were still unable to be fit by the BMDS models. Therefore only the skin keratosis endpoint will be used in the subsequent development of a health protective value for arsenic-induced noncancer effects.

Table 17. Benchmark Dose Analysis of Arsenic in Drinking Water and Skin Lesions (Mazumder *et al*., 1998)

						LED_{01} (LED_{05})
25, 75, 125, 175, 275, 425, 650 ppb [a]	Male, 1559, 385, 274, 235, 442, 246, 320	Keratosis, 0.2, 1.5, 1.6, 4.7, 4.9, 9.0, 8.9	7.9	0.16	58.5	49.6
1.6, 9.0, 44.4 µg/kg-d	Male, 520, 520, 520	Keratosis, 0.8, 4.2, 11.0	1.5	0.23[c]	(13.1)	(9.4)
25, 75, 125, 175, 275, 425, 650, 1200 ppb	Female, 1908, 386, 313, 259, 505, 269, 335, 118	Keratosis, 0, 0.4, 1.2, 2.3, 2.0, 2.7, 3.1, 8.3	8.7	0.27	156	124
1.6, 9.0, 44.4 µg/kg-d	Female, 636, 636, 636	Keratosis, 0.8, 2.2, 3.5	2.3	0.13	16.4	10.0
25, 75, 125, 175, 275, 425 ppb	Male, 1559, 385, 274, 235, 442, 246	Hyperpigmentation, 0.4, 3.2, 11.0, 7.8, 13.1, 15.7	23.8	0.0001[d]	21.5	18.9
1.6, 9.0, 44.4 µg/kg-d	Male, 520, 520, 520	Hyperpigmentation, 0.4, 6.9, 15.2	7.5	0.006	1.17	0.55
25, 75, 125, 175, 275, 425 ppb	Female, 1908, 386, 313, 259, 505, 269	Hyperpigmentation, 0.3, 0.8, 5.7, 5.1, 6.5, 9.5	13.5	0.0092[d]	40.8	34.7
1.6, 9.0, 44.4 µg/kg-d	Female, 636, 636, 636	Hyperpigmentation, 0, 2.9, 5.9	6.5	0.011	4.2	2.0

[a] Values are the midpoints of exposure level ranges from Mazumder *et al.* (1998).

[b] Goodness of fit statistic, criterion = 0.05.

[c] Log-logistic regression was used on this data set; all others were fit using quantal-linear regression (QLR).

[d] Goodness of fit statistic inadequate even with top two exposure levels removed, criterion = 0.05

Despite limitations of the data of Siripitayakunkit *et al.* (1999) on neurodevelopmental toxicity in children exposed to arsenic in drinking water, the data set was subjected to quantitative analysis. The data given in Table 6 on hair arsenic versus IQ were adjusted to quantal format with midpoints assigned for analysis as shown in Table 18.

Table 18. IQ versus Hair Arsenic for Selected Children Aged Six to Nine in Thailand: Data Adjusted to Quantal Format (Siripitayakunkit *et al.*, 1999)

				7.5
65	0	3	7	6
75	5	18	39	11
85	10	40	81	36
100	25	74	105	38
115	4	10	12	4
125	0	1	0	0
N	44	146	244	95
Mean IQ ± SD	**95.11 ± 11.0**	**92.67 ± 13.66**	**90.76 ± 11.66**	**88.89 ± 14.44**

The data were subjected to a continuous BMR analysis using polynomial (CPR) and power (CP) dose-response models of EPA's Benchmark Dose Software (BMDS). Both models gave acceptable fits to the data ($P > 0.05$) although the CPR model gave a better visual fit in the lower exposure range. The results of the latter analysis are summarized in Table 19. Using the LED value, a slope or potency can be derived as follows: - 0.025/0.75 = - 0.033/µg As/g hair, or a decrease of 3.3 IQ points per µg/g increase in hair arsenic. Although there are problems relating hair arsenic to arsenic intake via environmental media (Hindmarsh, 2002), Kurttio *et al*. (1998) have estimated that an increase of 10 µg As/L in drinking water corresponds to a 0.1 µg/g increase in hair arsenic. Thus these two relations can be combined as follows:

$$-3.3\ (\text{IQ}/\mu\text{g/g hair}) \times 0.01\ (\mu\text{g/g hair}/\mu\text{g/L water}) = -\ 0.033\ \text{IQ}/\mu\text{g/L}$$

Alternatively this can be expressed as minus one IQ point per 30 µg/L increase in drinking water arsenic. The neurodevelopmental risk estimate developed above for arsenic appears to be less than that of lead. From the OEHHA Lead PHG document (OEHHA, 1997), the daily lead intake that corresponds to a blood lead level of concern of 10 µg/dL is 28.6 µg/d or 29 µg/L for a 1 L/d tap water intake for a 1-2 year old infant. This value was used as a "NOAEL" in the calculation of the lead PHG with an overall margin of safety of 15. Possibly a more relevant comparison may come from the assessment of lead as a toxic air contaminant (OEHHA, 1996). In this assessment the results of prospective cohort studies indicated a potential mean decrease of 1.39 IQ points per µg Pb/m^3 of air. Assuming 50 percent absorption of inhaled lead and a 10 m^3/d inhalation rate, the corresponding water concentration would be 2.5 to 5 µg/L at 1 L/d intake. Thus as little as 2 µg Pb/L might be associated with a loss of one IQ point. By this comparison, lead would be 8 to 15-fold more developmentally neurotoxic to children than arsenic.

Table 19. Continuous BMR Analysis of IQ vs. Hair Arsenic in Thai Children Aged Six to Nine (Based on Data of Siripitayakunkit *et al*., 1999)

					X^2 Residual
	0.5	95.1	11.0	94.5 ± 12.7	1.98
	1.5	92.7	13.7	93.0 ± 12.7	-3.46
	3.5	90.8	11.7	90.6 ± 12.7	1.73
	7.5	88.	14.4	89.0 ± 12.7	-0.25
ED_{05}	3.45 µg/g				
LED_{05}	1.65 µg/g				
P	>0.05				
$ED_{02.5}$	1.45 µg/g				
$LED_{02.5}$	0.75 µg/g				
P	> 0.05				

*Note: Continuous dose response model: $Y= a + bX + cX^2$, Y = IQ, X = hair arsenic; X^2 residual = observed – expected values/standard deviation

As noted above, the studies of Siripitayakunkit *et al.* (1999) and Kurttio *et al.* (1998) were used to derive a relation between arsenic concentration in drinking water and IQ loss in children of – 0.033 IQ/µg As/L. The Kurttio *et al*. study had an estimated water consumption rate of 1.3 L/d (weighted average of exposed subjects aged 2 to 83). Children may have considerably higher rates of water consumption and a more health protective calculation would take this into account. In Table 20, projected total water consumption values for infants and children up to eight years of age are presented. These values are based largely on OEHHA (2000). The 95th percentile of water consumption at five to eight years of age ranges up to 3.5 L/d, a value 2.7-fold higher than estimated in Kurttio *et al*. (1998). The projected loss in IQ is 0.9 units at 10 µg As/L assuming this higher water consumption rate. The effective LOAEL for a one point IQ loss would be 11 µg As/L using this child-based calculation, versus 30 µg/L for adults.

Table 20. Adjustment of Developmental Neurotoxicity Health Protective Level for Infant and Child Water Intake

								8 yr
Body weight, kg	4.45	7.03	9.25	12.82	15.55	17.75	19.72	26.19
L/d, average	0.73	1.15	0.70	0.97	1.17	1.34	1.48	1.97
L/d 75%	0.85	1.34	0.85	1.18	1.43	1.63	1.81	2.41
L/d 90%	1.06	1.68	1.09	1.51	1.83	2.09	2.32	3.08
L/d 95%	1.22	1.92	1.25	1.73	2.10	2.41	2.67	3.54
L/d 99%*	0.64	1.01	1.32					
IQ change at 10 μg As/L								
L/d, average	-0.18	-0.29	-0.17	-0.24	-0.30	-0.34	-0.38	-0.50
L/d 75%	-0.22	-0.34	-0.22	-0.30	-0.36	-0.41	-0.46	-0.61
L/d 90%	-0.27	-0.42	-0.28	-0.38	-0.46	-0.53	-0.59	-0.78
L/d 95%	-0.31	-0.49	-0.32	-0.44	-0.54	-0.61	-0.68	-0.90
L/d 99%*	-0.16	-0.26	-0.34					

Total water intakes for average to 95th percentiles based on distributions of L/kg-d in OEHHA 1999, infant 99 percentile estimates based on breast milk intake corrected for water content (Lucas *et al*. 1987); adjustments in IQ based on 1.3 L/d estimated water intake from Kurttio *et al*. (1998) and the resultant slope of – 0.033 IQ/μg As/L and the relation 0.01 μg As/g hair/μg As/L water. Body weights vs. age from regressions in Price *et al*. (2003).

Carcinogenic Effects

Mode of Action

The mechanisms for arsenic carcinogenicity are unknown. Because arsenic does not cause point mutations in experimental systems, some investigators have postulated that these results are consistent with theories of sub-linearity for arsenic dose-response relationships. However, inference of sub-linearity from simple toxicological considerations is at best speculative without support from empirical data from human studies. Since there may be several mechanisms involved, multiple interactions with other factors both extrinsic and intrinsic, and variations in genetic susceptibility, inference from *in vitro* experiments and mechanistic theories cannot predict the shape of dose-response relationships for incidence rates of long latency diseases with complex multistage and multifactorial etiologies such as cancer. In addition, no information has been produced to identify the range of arsenic exposures in which meaningful sub-linearity might occur for any postulated theoretical mechanisms.

As with other major causes of human cancer, it is not likely that mechanisms allowing for valid predictions of dose-response relationships for low levels of arsenic will be identified in the near future. Indeed, mechanistic theories to date do not even predict why such high rates of bladder and lung cancer would occur in humans exposed to arsenic at levels not much higher than the current drinking water standards. Until they do, it is futile to begin to use such theories to postulate what might be happening below the as-yet detectable effect levels in humans. This is not to say that mechanistic research is not important. However, this research involves a long-term investment that may take decades and as such will not provide the methods for determining permissible exposure limits for arsenic in drinking water in the near future. It is also noteworthy that for many established causes of human cancer, the dose-response relationships found in epidemiological studies are linear, whether or not the particular agents involved cause point mutations (e.g., asbestos, chromium VI, beryllium, and nickel subsulfide; OEHHA, 1999).

There is quite extensive human evidence concerning dose-response relationships for arsenic methylation. As discussed previously, there is substantial evidence that inorganic arsenic was present in urine in approximately similar proportions to methylated forms at all levels of exposure from very low to very high (Hopenhayn-Rich *et al.*, 1993). Subsequent studies have confirmed these findings (Hopenhayn-Rich *et al.*, 1996b,c; Vahter *et al.*, 1995a). Considering all the evidence, it can be concluded that some sub-linearity in cancer dose-response relationships could be supported by the human methylation data if inorganic arsenic is the main carcinogenic agent. However, the sub-linearity would be very slight, and there is no evidence from methylation patterns that would support a threshold below which there would be no cancer risks.

In 1997 the U.S. EPA convened an expert panel to evaluate a number of issues surrounding arsenic carcinogenicity (U.S. EPA, 1997a). The panel considered the following potential modes of action (MOAs):

- <u>Chromosomal Abnormalities</u>. Although arsenic at low concentrations does not induce mutations at single gene loci, arsenic or its metabolites are genotoxic in that they can cause changes in chromosome structure, chromosome number, and sister chromatid exchanges (SCEs). Chromosome aberrations including micronuclei induction have been observed both *in vitro* and *in vivo* in rodents and in humans (Jha *et al.*, 1992; Dulout *et al.*, 1996; Warner *et al.*, 1994). In using information on chromosomal abnormalities to define a MOA for arsenic carcinogenicity the panel thought that such aberrations could be produced either by errors in DNA repair or errors in DNA replication. SCEs are produced by errors in DNA replication although SCEs have not been reported in studies of humans exposed to As. Aneuploidy can be caused by a number of processes and how arsenicals induce aneuploidy is unknown. Arsenite causes cell transformation in rodent and human cells *in vitro*. In SV40-transformed human keratinocytes, arsenite induces gene amplification at the dihydrofolate reductase (dhfr) locus, but does not cause amplification of SV40 sequences. This suggests that As may affect checkpoint pathways such as p53 rather than directly damaging DNA. The panel concluded that many of the genotoxic effects of As are consistent with the type of genomic instability resulting from interference with p53 or other pathways involving DNA repair or cell cycle control. For example, Chang *et al.* (1998) investigated the expression of bcl-2, p53, and Ki-67 arsenic-induced skin cancers. Bcl-2 is a proto-oncogene encoding an inner mitochondrial membrane protein that preserves cells from death by apoptosis. P53 gene mutations are the most common genetic abnormalities in human cancers, including non-melanoma skin cancers. This gene encodes a nuclear phosphoprotein involved in the inhibition of cell proliferation. The Ki-67 antigen is a reliable marker of tumor growth. The authors examined bcl-2, p53, and Ki-67 expression in human subjects with Bowen's disease (BD, N=30), basal cell carcinoma (BCC, N=12), and squamous cell carcinoma (SCC, N=8) using immunohistochemistry to assess the control of cell proliferation and cell death (apoptosis). Basal cell carcinoma expressed bcl-2 strongly and homogeneously, but none of the SCC expressed bcl-2 and only 40 percent of the BD expressed bcl-2 homogeneously or focally. P53 and Ki-67 were expressed in all of the arsenical skin cancers and in perilesional normal skin. Bcl-2 expression in arsenic induced skin cancer is related to the phenotype of cell origins, positive in tumors from germinative basal cells such as BCC and negative in keratinocyte-derived SCC. BD is composed of two cell types of variable ratio.

- DNA Repair. Arsenite may act as a co-mutagen and/or inhibitor of DNA repair. It has been found to enhance mutagenesis by ultraviolet irradiation (UV) in *E. coli* and of methyl methanesulfonate (MMS) and methyl nitrosourea (MNU) in Chinese hamster cells. As compounds inhibit the repair of DNA damage induced by x-rays and UV, the post-replication repair of UV-induced damage, and the completion of repair of MNU-induced damage, potentiate X-ray and UV-induced chromosomal damage in peripheral human lymphocytes and fibroblasts (Jha *et al.*, 1992, Vogt and Rossman, 2001). As also acts synergistically with diepoxybutane, a DNA cross-linking agent, to cause chromosomal aberrations (Weincke and Yager, 1992). The inhibition by arsenite of the completion of DNA excision repair may result from effects on DNA ligation (Li and Rossman, 1989b; Lee-Chen *et al.*, 1994), but, neither DNA ligases nor DNA polymerases alpha or beta are inhibited at As concentrations which inhibit DNA repair in cells. Hu *et al.* (1998) measured the arsenic inhibition of several purified human DNA repair enzymes, including DNA polymerase β, DNA ligase I and DNA ligase III and found them to be mostly insensitive to arsenic, with IC_{50} values ranging from 6-30 mM with arsenite and 30-175 mM with arsenate. Some enzymes were activated by lower concentrations of As, e.g., DNA ligase III (4.4-fold by 5 mM As III and 9.5-fold by 20 mM AsV). The effects of As on DNA repair do not appear to be mediated by As induced enzyme inhibition. Arsenic may affect cellular redox levels, accessory proteins, or cellular control of DNA repair processes via interference with p53 expression.

- DNA Methylation. Alterations in cytosine DNA methylation are common to a number of human tumors (Counts and Goodman, 1995; Jones, 1996; Issa *et al.*, 1997). Zhao *et al.* (1997) have proposed that the early events in As-induced carcinogenesis result from aberrant gene expression subsequent to DNA hypomethylation, caused by continuous methyl depletion. They induced transformation in rat liver epithelial-cell line (TRL 1215) by chronic exposures to low levels of sodium arsenite (125, 250, or 500 nM). Transformation was dependent on As dose and duration of exposure. Global DNA hypomethylation occurred concurrently with transformation and with significantly reduced intracellular SAM concentrations. Hypomethylation was also dependent on As dose level and exposure duration. Aberrant gene activation of the c-*myc* oncogene was also detected. Activation of c-*myc* can induce transformation without mutation via overexpression (Leder *et al.*, 1986). Arsenic-induced DNA hypermethylation has also been proposed as a mode of carcinogenic action. When it affects the promoter region of expressed genes, hypermethylation can stop transcription of the gene (Meehan *et al.*, 1992; Eden and Cedar, 1994). Several tumor suppressor genes have been transcriptionally inactivated by promoter methylation. Thus, DNA hypermethylation may induce carcinogenesis by inactivating tumor suppressor genes or by inactivating genes involved in DNA repair. In a study of lung cancer cells exposed to increasing levels of As, Mass and Wang (1997) found increased levels of overall DNA methylation and increased methylation of the p53 tumor suppressor gene promoter. As has also been observed to increase cytosine-DNA methyltransferase activity. The mechanism of As-induced hypermethylation is unknown. One hypothesis is that arsenic acts as a differentially specific inhibitor of the many SAM-dependent methyltransferases. In the presence of the inhibition of As-sensitive MTases, transient increases in unutilized SAM may drive functioning As-resistant MTases to overmethylate their substrates, resulting in DNA hypermethylation. Preliminary studies indicate that cytosine methyltransferase activity can be maintained at arsenite concentrations of 10 mM, and hence may represent an As-resistant MTase. These findings tend to support a role for DNA methylation abnormalities as a mode of action of arsenic carcinogenesis (Goering *et al.*, 1999; Zhong and Mass, 2001).

- Oxidative Stress. Active oxygen species can cause tumor promotion and free radical generating substances can promote tumors via their ability to induce cell proliferation. Free radicals can also directly damage DNA. Free radicals are also implicated in arsenic-induced cancers. Dimethyl arsine (DMAH) induced lung-specific DNA strand breaks in mice via the peroxy radical and other oxygen species produced during DMAH metabolism (Yamanaka and Okada, 1994). A number of other findings point to an oxidative stress MOA: addition of superoxide dismutase a can block arsenite-induced genotoxicity in human lymphocytes (Nordenson and Beckman, 1991); α-tocopherol (Vitamin E) protects human fibroblasts from arsenite toxicity (Lee and Ho, 1994); an X-ray sensitive, catalase-deficient CHO cell variant is hypersensitive to killing and micronucleus induction by arsenite, and micronucleus induction can be blocked by catalase (Wang and Huang, 1994). Arsenic induces a number of proteins that are induced by and protect against oxidative stress including metallothionein (Albores *et al.*, 1992). Oxidative stress can also be induced by arsenic through glutathione (GSH) depletion. Arsenite reacts with GSH, and GSH is required for As metabolism via chemical reduction and enzymatic reductive methylation. Trivalent inorganic As and organic arsenicals can also inhibit GSH reductase (Styblo *et al.*, 1997).

- Cell Proliferation. As noted above, As does not interact directly with DNA. There is evidence from both *in vitro* and *in vivo* studies that As can increase cell proliferation in pluripotential cells of one or more target tissues. Exposure of humans to high levels of As produces skin keratoses, which may evolve to invasive squamous carcinomas. As-induced cell proliferation has not been studied in regard to internal As-induced cancers (lung, bladder, liver, and kidney). In rodents, exposure to dimethylarsinic acid (DMA) after pretreatment with five carcinogens yielded increased incidences of lung, bladder, liver and thyroid tumors (Yamanaka *et al.*, 1996; Yamamoto *et al.*, 1995; Wanibuchi *et al.*, 1996). Administration of DMA without prior carcinogens pretreatment resulted in increased cell proliferation in the urinary bladder (Wanibuchi *et al.*, 1996), liver (Yamamoto *et al.*, 1995), and kidney (Murai *et al.*, 1993). Proliferation rates in lung and thyroid have not been studied. Despite limited data, the panel considered increased cell proliferation to be a likely mode of As-induced cancers.

- <u>Co-Carcinogenicity</u>. DMA has been reported to induce bladder, liver, or lung tumors in three in animals when administered with other carcinogens. Alternatively, there are no convincing animal data showing that any form of arsenic is carcinogenic when administered alone. Some human studies indicate a higher incidence of lung cancer in arsenic-exposed smokers than in similarly exposed nonsmokers (Hertz-Picciotto *et al.*, 1992; Chiou *et al.*, 1995) and in As-exposed miners also exposed to radon gas (Xuan *et al.*, 1993).

The panel agreed "arsenic and its metabolites do not appear to directly interact with DNA. Had there been evidence for such a mode of action, it would likely have led to the conclusion that tumor induction was linear with dose over the dose range from the lowest point of observation for tumors. The conclusion that there does not appear to be any direct interaction of arsenic with DNA does not rule out a linear dose-response relationship at lower doses. However, all identified modes of action would lead to nonlinear responses for cancer."

Recently Kitchin (2001) reviewed recent advances in the MOAs above; in addition, evidence supporting those based on altered growth factors, p53 gene suppression, and gene amplification. He concluded that three MOAs have a degree of supporting evidence in both human and animal experimental systems and in human tissues that warrant serious consideration: chromosomal abnormalities; oxidative stress, and a continuum of altered growth factors, cell proliferation, and promotion.

Hei *et al.* (1998) evaluated the mutagenicity of sodium arsenite in an AL cell assay. The AL hamster-human hybrid cells contain a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11. Chromosome 11 encodes cellular surface markers that render AL cells sensitive to killing by specific monoclonal antibodies. Arsenite concentrations between 0.5 and 2.0 μg/mL yielded linear dose responses for $HPRT^-$ and $S1^-$ mutants with one and five day treatments. The authors observed suppression of $S1^-$ mutants by 0.1 percent DMSO, a free radical scavenger. Arsenite alone gave an induced $S1^-$ mutant yield of 195.5×10^{-5} versus 35.5×10^{-5} with 0.1 percent DMSO ($P < 0.01$). Thus, the mutagenic activity of arsenite in this assay depends at least in part on reactive oxygen species for its activity.

In a subsequent study (Liu *et al.*, 2001) using the same AL cells and the fluorescent probe 5', 6'-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate it was observed that arsenite induced up to a three-fold increase in intracellular oxyradical production within five minutes of treatment. Concurrent exposure of cells to arsenite and the radical scavenger DMSO reduced fluorescent intensity to control levels. The study provides additional evidence that reactive oxygen species, particularly hydroxyl radicals, play a causal role in As genotoxicity in mammalian cells.

Several arsenic species (As^{III}, As^{V}, MMA^{V}, DMA^{V}, $MAs^{III}O$, and $DMAs^{III}I$) were evaluated for their ability to mobilize iron from horse spleen ferritin *in vitro* (Ahmad *et al.*, 2000). Dimethylarsinic acid (DMA V) and iododimethylarsinous acid ($DMAs^{III}I$), at 10 mM each, significantly released iron from ferritin with or without ascorbic acid (10 mM). The release induced by $DMAs^{III}I$ without ascorbate was 29.8 nM Fe^{+2}/min vs. 0.00 and with ascorbate was 282.0 nM/min vs. 30.5 nM/min for ascorbate alone ($P < 0.001$). A significant release was also seen with DMA V and ascorbate, 58.1 vs. 30.5 for ascorbate alone ($p < 0.001$). The As-induced release of iron from ferritin in tissues *in vivo* could increase oxidative stress since free iron catalyzes oxidative reactions, which damage DNA, lipid, and protein via reactive oxygen species.

Arsenite was observed to increase the mRNA transcripts and secretion of transforming growth factor-α (TGF-α), granulocyte macrophage-colony stimulating factor (GM-CSF), and tumor necrosis factor-α (TNF-α) in primary human keratinocytes *in vitro* (Germolec *et al.* 1997). *In vivo* studies in Tg.AC transgenic mice treated with 0.02 percent sodium arsenite in their drinking water for 10 weeks showed increases in GM-CSF and TGF-α mRNA transcripts in the epidermis at clinically normal sites. Immunohistochemical staining localized TGF-α overexpression to the hair follicles. Injection of neutralizing antibodies to GM-CSF after tetradecanoyl phorbol acetate (TPA) application reduced the number of papillomas in arsenic-treated Tg.AC mice. Samples of skin lesions obtained from human subjects exposed to arsenic in their drinking water also showed similar alterations in growth factor expression. The evidence suggests that arsenic acts via simulation of keratinocyte-derived growth factors and as a co-promoter (Germolec *et al.*, 1998).

Vega *et al.* (2001) compared the effects of several arsenic species (As^{III}, As^{V}, $MAs^{III}O$, MMA^{V}, $DMAs^{III}GS$, DMA^{V}) in human keratinocyte cultures. The relative toxicities were: $As^{III} > MAs^{III}O > DMAs^{III}GS, > DMA^{V} > MMA^{V} > As^{V}$. The trivalent arsenicals increased cell proliferation in the 0.001 to 0.01 μM range with inhibition at concentrations > 0.5 μM. The pentavalent arsenicals did not stimulate cell proliferation. Exposure to low doses of trivalent arsenicals stimulated secretion of the growth-promoting cytokines, GM-CSF and TNF-α. DMA^{V} was observed to reduce cytokine secretion.

The arsenic-mediated stimulation of growth factors in other target tissues is less well documented than for skin. Simeonova and Luster (2000) reported that a human urinary bladder epithelial cell line also responds to arsenic by moderately enhanced growth. Histological examination and immunostaining have shown that hyperplasia occurred in urinary bladder epithelial cells following *in vivo* exposure to arsenite. Gene expression induced by arsenite was evaluated in UROsta cells, a human uroepithelial cell line, using cDNA microarrays. At 50 μM arsenite 16 genes were activated, seven of which were also activated at 10 μM. The activated genes included AP-1, c-myc, EGR-1 (early growth response), NGF (nerve growth factor), GADD153, GADD45 (growth arrest and DNA damage), BCL-2 (binding protein), BAG-1 (repair associated protein, cytoskeleton). The authors conclude that arsenic may initiate cell-signaling pathways leading to transcription factors and the induction of a series of genes involved in the regulation of cell metabolism and mitosis.

Chen *et al.* (2001) applied cDNA microarray technology to an evaluation of the genetic events of As-induced malignant transformation in a normal rat liver TRL1215 cell line. The cells were continuously cultured in medium containing 0, 125, 250, and 500 nM sodium arsenite for at least 18 weeks. Oncogenes and tumor-suppressor genes showing significant increases over control cells were *c-myc*, *s-myc*, *c-H-ras*, *c-met*, *erbB2*, *erbB3*, *TGF-β3*, *Rb*, and *WT1* ($P < 0.05$). *N-myc* showed a significant decrease while *c-jun* was increased ($P > 0.05$) and *p53* was unchanged. Arsenic–induced overexpression of c-myc, c-jun, and Rb, all of which are important in cell-cycle regulation, indicates this may be a key target of arsenic action. The cell-cycle-related genes cyclin C, cyclin D1, cyclin D2, and *PCNA* were also overexpressed in arsenite-transformed cells ($P < 0.05$). Cyclin D3 was significantly decreased and cyclin E, p21-waf1, and p27-Kip1 were unchanged. In addition, other cell cycle genes were also upregulated (wee1 tyrosine kinase, prothymosin-α, and *O*-6-methylguanine-DNA methyltransferase). Among 588 genes examined, approximately 80 (~13 percent) were aberrantly expressed. In addition to the tumor suppressor, onco- and cell-cycle genes noted above, signal transduction, stress response, apoptosis, cytokine production and growth factor and hormone-receptor production genes were also affected.

In a similar study in human fibroblast cells (HFW) exposed to 5 μM arsenite for up to 24 hr, 568 genes were used to examine mRNA profile changes (Yih *et al*., 2002). Of 133 target genes selected for further analysis, 94 were induced by arsenite, while 39 were repressed. The genes were involved in signal transduction, transcriptional regulation, cell cycle control, stress responses, and proteolytic enzymes.

Rea *et al*. (2003) studied the alteration of gene expression by inorganic arsenic in cultured human keratinocytes from normal epidermis, a premalignant lesion, and a malignant tumor. The malignant SCC9 cell line was treated with 2 μM arsenite or 6 μM arsenate. These As concentrations had no effect on cell growth or total protein and produced nearly equivalent suppressive effects on differentiation under the test conditions. Expression analysis showed that about 30 percent of the genes (3576/ ~12,000) were expressed with no treatment. Of those present, seven percent (254 transcripts) were either induced (87) or suppressed (167) at least two-fold by arsenite treatment. Similar results were seen with arsenate, five percent induced or suppressed. A number of induced genes reflected an adaptive response to reactive oxygen including heme oxygenase 1 (HO1, 11-32-fold), NAD(P)H quinone oxidoreductase (2.3-2.6-fold), and thioredoxin reductase (3-fold). The effects noted with arsenite and arsenate were approximately the same except in a few cases. The SCC9 genes with altered transcription, particularly suppression, included kinases, phosphatases, transcription factors, and other factors of signaling pathways. In general, considerably more genes were affected in the normal (hEp) cells than in premalignant (SCC12F2) or malignant (SCC9) cells. This may indicate a decreased regulatory flexibility in the latter cells from oncogene activation and loss of cell-cycle checkpoints and tumor supressors. Genes indicative of reactive oxygen generation were detected at the earliest time period, indicating that these may drive subsequent cellular responses. Unlike some agents that produced transient HO1 induction, arsenicals produced sustained induction. Overall, the results of this study appear to support a role for oxidative stress as a possible mode of arsenic carcinogenic action.

These studies reveal a remarkable complexity of As-induced alterations in gene expression, possibly reflecting the broad range of toxic effects seen at higher organizational levels.

Hamadeh *et al.* (1999) exposed human keratinocytes (HaCaT) to 1-1,000 nM arsenite for 14 days. Cell viability was not affected. Arsenic exposure caused a dose- and time-dependent decline in p53 protein levels at concentrations above 10 nM. Concomitant increases were seen in mdm2 levels, suggesting possible disruption of a p53-mdm2 loop regulating cell cycle arrest. Arsenite, arsenate, and phenylarsine oxide were all active in this respect, whereas MMA^V and DMA^V were inactive. In a subsequent study Hamadeh *et al.* (2002) observed that exposure of normal human keratinocytes to 0.005-5 μM AsIII for 24-48 hr simultaneously modulated DNA repair, cell proliferation and redox-related gene expression.

Vogt and Rossman (2001) evaluated the effects of arsenite on cell signaling in cultured W138 normal human fibroblasts. Cells treated long-term for 14 days at 0.1 μM arsenite exhibited a modest (3-fold) increase in p53 expression, while only a toxic concentration (50 μM arsenite) increased p53 after short-term exposure (18 hr). When cells were exposed to ionizing radiation (6 Gy), p53 and p21 protein concentrations were increased 12 and nine-fold, respectively, after four hours. Both long-term and short-term arsenite exposures suppressed radiation-induced increase in p21 abundance. Long-term arsenite exposure of irradiated cells caused a six-fold increase in p53 whereas short-term exposure resulted in only a slight increase over radiation alone. In addition, long-term low dose arsenite exposure resulted in increased expression of cyclin D1. Conversely, short-term high dose arsenite caused a decrease in cyclin D1 abundance. The authors note that in cells treated with arsenite, p53-dependent increase in p21 expression, which would function to cell cycle progression after DNA damage, is deficient. Concurrent low-dose exposure to arsenite would enhance positive growth signaling via cyclin D1. Thus arsenite might play a comutagenic and cocarcinogenic role via disruption of the anti-growth circuit promoting the replication of a DNA-damaged template.

Okoji *et al*. (2002) studied the effect of sodium arsenite in drinking water on DNA hypomethylation in methyl-deficient C57BL/6J mice. Ninety male mice (15/dose group) were administered sodium arsenite in drinking water at 0 (methyl sufficient diet), 0, 2.6, 4.3, 9.5, or 14.6 mg sodium arsenite /kg-d (methyl deficient diet). Dosing was *ad libitum* for 130 days. Dose-related effects on the liver included steatosis and microgranulomas. Sodium arsenite increased genomic hypomethylation at several cytosine sites within the promoter region of Ha-ras. Eleven methylation sensitive enzymes were used to examine the region 474/976. Arsenite treatment resulted in reductions of five of the 11 restriction sites examined when compared to methyl-deficient animals not treated with arsenite (EcoRII, StuI, AluI, AluII, XhoI). The study indicates that arsenite exposure induces Ha-ras hypomethylation. Such altered methylation in the regulatory region of the gene may contribute to arsenic-induced cancer via increased expression and cell cycle dysregulation.

Yamanaka *et al*. (2001) have observed that nine hr after oral administration of DMA to mice (N = 3-5) at 50 and 100 mg/kg significant concentrations of 8-oxo-2'-deoxyguanosine (8-oxodG) were found in the urine ($P < 0.05$ and <0.001, respectively vs. control). Mice administered 400 ppm DMA in drinking water ad libitum for four weeks showed increased levels of 8-oxodG in arsenic target tissues of lung, liver, spleen, kidney, urinary bladder, and skin, but only lung and liver increases were statistically significant ($P < 0.05$). Similar treatment with arsenite did not give significant increases of 8-oxodG in urine. The authors postulate that DMA itself does not induce DNA damage but rather its metabolites, possibly the dimethylarsenic peroxy radical formed by reaction of the dimethylarsine metabolite with oxygen. Dimethylarsine has been observed as an exhaled product of mice exposed to DMA (Yamanaka *et al*., 1989).

Hong *et al*. (2001) observed that arsenic trioxide is a potent inhibitor of the SMRT corepressor. Many nuclear receptors are bipolar in action being able to either repress or activate the expression of target genes. Repression is accomplished through the recruitment of a complex of auxiliary proteins or corepressors that mediate the repression process. The corepressor protein SMRT and its paralog, N-CoR, play a key role in the process by serving as the principal point of contact of the corepressor complex within the nuclear receptors. The ability to recruit SMRT may play a key role in leukemogenesis by the PML-retinoic acid receptor α (RARα) oncoprotein, an aberrant nuclear hormone receptor implicated in human acute promyelocytic leukemia (APL). Using a mammalian two-hybrid assay with CV-1 cells exposed to 20μM arsenite, the authors observed an inhibited interaction between SMRT and T3R (thyroid hormone receptor). The effects of arsenite were mediated, in part through activation of an MAP kinase cascade and resulted in phosphorylation of the corepressor. In addition, dissociation of SMRT from its nuclear receptor partners, and relocation of SMRT out of the nucleus into the cytoplasm were observed. The authors suggest that this previously unrecognized effect of arsenic, the ability to inhibit corepressor function, be added to the potential mechanisms by which arsenic induces toxic, oncogenic, and antineoplastic effects.

Dong (2002) observed arsenite-induced transformation of JB6 Cl 41 cells when exposed to 0.5-25 μM arsenite, whereas no transformation was seen at 50-100 μM arsenite. At a higher exposure concentration of 200 μM arsenite or arsenate apoptosis resulted (44.5 and 61.5 percent, respectively). Arsenite induced phosphorylation of extracellular signal-regulated protein kinases (Erks) and c-Jun NH_2-terminal kinases (JNKs). The author found that inhibition of Erks activation with dominant-negative Erk2-K52R stable transfectants blocked arsenite-induced cell transformation. Alternatively a dominant-negative mutant JNK1 blocked the induction of apoptosis by arsenite (four percent) or arsenate (seven percent) compared to vector-transfected control cells (31.5 and 40.5 percent, respectively). Arsenic also induced AP-1 and nuclear factor kappa B (NF-κB) in different cell culture models. Expression of a dominant-negative inhibitory kappa Bα blocked arsenic-induced activation of NF-κB and apoptosis. The results suggest separate dose-dependent modes of action for the oncogenic and anti-neoplastic effects of arsenic.

Tran *et al.* (2002) studied the effect of sodium arsenite on DNA damage induced by benzo[a]pyrene (BaP) in vivo. Three groups of 20 SPF female Sprague-Dawley rats, about 250 g each, were injected by the intra-mammilary route with: group one, BaP 100 μg/gland x 8 glands; group two, arsenite 2.5 μmol/gland x 8; group three, BaP + arsenite x 8, 800 μg/400 μL DMSO + 20 arsenite/400 μL water, total dose. The animals were sacrificed on days 1, 3, 5, 10, and 27 and the mammary tissues collected for DNA adduct measurement. The DNA adducts in group one (BaP) reached a maximum level by day 5 and fell to 13 percent of this level by day 27. Adduct levels in group three (BaP-As^{III}) also reached a maximum of 80 percent that of group one on day five but 84 percent of this amount still remained at day 27. The authors conclude that arsenite inhibits the repair of BaP-induced DNA adducts.

In view of the broad range of potential MOAs for arsenic-induced carcinogenicity, it would appear prudent, in a public health sense, to assume a linear dose response. Since arsenic has a number of target sites and may very likely act via more than one mechanism simultaneously in different sites, it would seem overly optimistic to assume any particular form of nonlinear dose response for arsenic induced cancer in human populations exposed to low levels.

Biomarkers

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. Biological markers of effect of toxic human exposures have the potential to allow exploration of dose-response relationships at levels of exposure lower than those which can be assessed by traditional epidemiological studies involving the ultimate disease end-point. 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.

Bladder Cell Micronucleus

The frequency of micronucleated cells in exfoliated bladder and buccal cells was examined in a case-control study in Nevada (Warner *et al.*, 1994). This study involved 18 subjects whose well water contained on average 1312 μg/L of arsenic, and 18 age- and sex- matched controls whose well water averaged 16 μg/L. Exposed subjects had a 1.8-fold increase in bladder cell micronuclei, but the differences were largely confined to males.

Moore *et al.* (1997a) conducted a cross-sectional study confined to male participants in view of the extensive exfoliation of squamous cells as well as transitional bladder cells that occurs in females. There were 70 high-exposure participants (average urinary arsenic 616 μg/L) and 55 low-exposure participants (average urinary arsenic 66 μg/L). The prevalence of micronuclei increased three-fold (95 percent CI 1.9-4.6) from the lowest exposure quintile (less than 53.8 μg/L arsenic in urine) to those in the second highest exposure quintile (414-729 μg/L urinary arsenic). Surprisingly, those in the highest exposure quintile (more than 729 μg/L urinary arsenic) did not have any increase in micronucleus prevalence. This finding is not fully explained, but could be due to cytostasis or cytotoxicity at these high exposure levels. The centromeric probe classification of micronuclei suggested that chromosome breakage increased 7.5-fold in the third exposure quintile (137-414 μg/L urinary arsenic). It is noteworthy that the prevalence of micronuclei in bladder cells was elevated even in the second to lowest quintile of exposure (urinary arsenic levels between 53.9 and 137.3 μg/L, prevalence ratio 2.1, 95 percent CI 1.4-3.4), which raises the possibility that arsenic has genotoxic effects on bladder cells at relatively low levels of exposure, similar to consumption of drinking water containing 50 μg/L of arsenic, the current drinking water standard.

Water low in arsenic (45 μg/L) was provided to 34 highly exposed participants in a cross-sectional study in Chile (Hopenhayn-Rich *et al.*, 1996c). Mean urinary arsenic levels in this sub-group decreased from 742 to 225 μg/L during the intervention. Bladder cell micronucleus (MNC) prevalence decreased from 2.63/1000 to 1.79/1000 cells post-prevalence. This finding is not fully explained, but could be due to cytostasis or cytotoxicity at these high exposure levels. The centromeric probe classification of micronuclei suggested that chromosome breakage was the major cause of micronucleus formation. Micronuclei formed by breakage increased 7.5-fold in the third intervention ($p<0.05$) (Moore *et al.*, 1997b). When the analysis was limited to individuals previously having subcytotoxic urinary arsenic levels (<700 μg/L), the change between pre- and post-intervention MNC was more pronounced: from 3.54 to 1.47/100 cells, respectively ($p=0.002$). The changes primarily occurred among smokers, suggesting that smoker's bladder cells could be more susceptible to genotoxic damage caused by arsenic. The reduction in bladder cell MNC prevalence with reduction in inorganic arsenic intake provides further evidence that arsenic is genotoxic to bladder cells.

Although these results are based on a small number of cases and represent preliminary findings, the higher prevalence of genetic changes and mutations in the high-exposure group compared to the low-exposure group (that could not be accounted for by differences in the stage and grade of the tumor groups) is consistent with the hypothesis that highly exposed tumors may behave more aggressively. It is possible that the tumors in highly exposed individuals could be more fatal, explaining the high bladder cancer mortality found in arsenic-exposed regions of the world. This is consistent with preliminary evidence from both Argentina and Chile where arsenic in drinking water has a stronger relationship to bladder cancer mortality than bladder cancer incidence. Although confirmatory studies are needed, the results also suggest that ingested inorganic arsenic might have genotoxic effects in bladder cells at low exposure levels.

Susceptibility to Arsenic Health Effects

Variability in human response to arsenic is an important topic, which has not been widely studied. We consider here phenotypic markers of arsenic susceptibility related to metabolism, genotypic markers, and potential variation in susceptibility related to nutritional facts.

Phenotypic markers of arsenic susceptibility

In general, little is known about factors influencing arsenic toxicity and metabolism in humans. It is known that after ingestion, inorganic arsenic in pentavalent form is reduced to As+3 and subsequently methylated in the liver and elsewhere to the less toxic and more readily excreted metabolites, methylarsonic acid (MMA) and dimethylarsenic acid (DMA). In epidemiologic studies, these metabolites are analytically measured in urine to assess individual exposure and their ability to methylate and detoxify the arsenic they ingest. It has been hypothesized that at low doses, most of the inorganic arsenic ingested is readily methylated and excreted, but at high doses, the capacity to methylate may be overwhelmed and a smaller portion of arsenic detoxified. As discussed previously, several investigations have provided substantial evidence that a threshold for arsenic methylation does not exist. However, more inter-population variability has been noted in the second step of methylation including intriguing differences in the proportion of arsenic excreted as MMA. The apparent variability within populations in arsenic methylation warrants further study since it may relate to cancer risks at the level of the individual.

Genotypic markers of arsenic susceptibility

There is evidence that genetic susceptibility risk factors combined with environmental exposure can determine a significant proportion of cancer development (Bell *et al.*, 1993). For this reason, susceptibility gene status, in both cases and controls, has been focused upon as an effect modifier of disease development in relation to exposure. In general, cancer susceptibility genes are those that encode for proteins involved in the metabolic pathway from exposure to disease. They are part of the protective mechanism against cancer development caused by environmental carcinogens (Zhong *et al.*, 1993). Members of the glutathione S-transferase family (GST) are important candidates for involvement in cancer susceptibility because they may regulate an individual's ability to metabolize environmental carcinogens (Seidegard *et al.*, 1988). Two genes encode the cytosolic enzymes GST-u (GSTM1; chromosome 1p13.3) and GSTTø (GSTT1; chromosome 22q11.2) that regulate the conjugation of many different carcinogenic agents to glutathione. Carriers of homozygous deletions in the GSTM1 and GSTT1 genes ("null" genotypes) have an absence of GSTu and GSTø activity due to transcription of a truncated protein (Seidegard and Pero, 1988). These deletion variants have been useful for molecular epidemiology studies of cancer because they divide study subjects into two well-defined susceptibility classes: those who are and those who are not able to detoxify potential carcinogens by GSTM1 and GSTT1 regulated pathways.

Although the mechanisms involved in arsenic metabolism are not well understood, it has been shown that glutathione (GSH) plays an important role in both the reduction and methylation of arsenate (Thompson, 1993). Experimental studies have shown that GSH elevation is a natural reaction to arsenic insult, presumably as a protective mechanism (Li and Rossman, 1991) and that GSH depletion prior to treatment with arsenic leads to interference with arsenic metabolism, including inhibition of methylation in the liver and decrease in the elimination rate of arsenic metabolites (Hirata *et al.*, 1990). Since glutathione is involved in arsenic metabolism, it is possible that the inter-individual variation in methylation capacity relates to GSTM1 and GSTT1 genotypes. Reduced levels of GSTs in those with the null genotypes may increase susceptibility by decreasing methylation efficiency, leading to exposure of tissues to the more toxic forms of arsenic. Other possible mechanisms can be proposed for interactions of the GSTM1 and GSTT1 polymorphism in lung cancer causation by arsenic. Arsenic has been shown to produce toxic effects common to oxidative stress (e.g. induction of heme oxygenase) (Keyse *et al.*, 1990; Lee and Ho, 1994; Taketani *et al.*, 1991). These effects are thought to be mediated by the binding of arsenic metabolites (e.g., As +3) to critical sulfhydryl groups within proteins (Abernathy and Ohanian, 1992). GSTT1 deficiency could therefore directly exacerbate the oxidant effects of arsenic exposure. Alternatively, since arsenic may inhibit the repair of DNA damage induced by lung carcinogens (Li and Rossman, 1989b; Rossman, 1981), GSTT1 deficiency may act synergistically with arsenic by allowing greater levels of lung carcinogens from cigarette smoking to bind to DNA. Cigarette smoke contains known substrates for GSTT1 (e.g. ethylene oxide) and promotes peroxidation of lipids that may also be detoxified through GSTT1 (Ketterer *et al.*, 1989). The apparently synergistic effect of arsenic and smoking could be due in part to such effects.

A common mutation, C to T at codon 667, in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, has been shown to reduce the activity and thermolability of the reductase enzyme. MTHFR catalyzes the reduction of 5,10- methylene THF to 5-methyl THF, which is the point of entry of folate coenzymes in the remethylation of homocysteine to methionine. This reaction increases the availability of the major methyl donor, *S*-adenosylmethionine. If mutations are present in this gene, the availability of methyl groups may be diminished affecting the rate at which arsenic can be methylated and metabolized. Since folic acid is a cofactor for the reaction, the mutation also increases dependence on folic acid for adequate remethylation because of the reduced enzyme activity. Those affected may have to increase their folate intake concentrations for normal levels of remethylation, especially at times of rapid growth. Alternatively, individuals that are wild type for the gene may be at risk for having an excess of methyl groups available for DNA methylation, causing increased gene expression. Recently a second mutation in the gene, C to A at codon 1298, has been identified which in combination with the 667 C to T mutation accounts for an even greater decrease in methyl donor availability (Van der Put *et al.*, 1998; Weisberg *et al.*, 1998). Since alteration of DNA methylation by arsenic has been proposed as one of its possible modes of carcinogenic action, underlying mutations in methyl metabolism may predispose affected individuals to the adverse effects of arsenic exposures.

Arsenic Effects and Nutritional Susceptibility

Nutritional factors have long been postulated to relate to arsenic toxicity. In rabbits fed low amounts of methionine, choline, and protein, a marked decrease in the urinary excretion of DMA and an increased arsenic retention was noted (Vahter and Marafante, 1987). Biswas *et al.* (1999) reported the reduction of arsenic induced cytotoxicity after short-term dietary administration of selenium in mice. Similarly, Poddar *et al.* (2000) found that ferrous sulfate administration with or before exposure to sodium arsenite resulted in a reduction of arsenic induced clastogenic effects *in vivo* in mice.

Relative malnutrition in Taiwanese populations exposed to arsenic, including the possibility that low intake of protein and methionine might reduce arsenic methylation, has been discussed (Engel, 1993; Yang and Blackwell, 1961). Low intake of certain micronutrients including zinc (Engel, 1993) and selenium (Levander, 1997) might increase arsenic caused disease risks. Studies in Taiwan found increased skin cancer risks associated with a staple diet of dried sweet potatoes (Hsueh *et al.*, 1995) and also with low levels of serum B-carotene (Hsueh *et al.*, 1997). These and other nutritional factors warrant investigation as possible susceptibility factors or risk modifiers. However, it is noted that increased cancer risks, including lung cancer risks, have been found in populations in different countries having different diets. Also, preliminary results from Smith *et al.* (submitted) have recently found a high prevalence of skin lesions due to arsenic in drinking water in a small study of 11 families in Chile, who have high fruit, vegetable and meat intake. Moreover, in West Bengal, preliminary results from blood assays of patients with skin lesions show no association with selenium, methionine, nor with retinol (vitamin A) (Mazumder *et al.*, submitted).

Arsenic Essentiality

The potential cancer risk due to inorganic arsenic in drinking water needs to be considered with regard to the possibility that arsenic is a beneficial micronutrient. Signs of arsenic deprivation, including depressed growth and abnormal reproductive function have been suggested for the rat, goat, pig, and chicken (U.S. EPA, 1988). These data provided the first indication of the possibility that arsenic, at least in inorganic form, is an essential nutrient. It should be noted that arsenic has neither been tested for essentiality in humans nor has it been found to be required for any essential biochemical processes (NRC, 1999). However, as pointed out below, these studies cannot be used to support the essentiality of arsenic.

Low arsenic intake (0.03 ppm of diet) was associated with elevated perinatal mortality and depressed the growth of rat pups compared to a high dose group with arsenic at 4.5 ppm of diet (Nielsen, 1975). Uthus *et al.* (1983) also noted growth depression in a three-generation study in rats with males more susceptible to the effects than females. Reduced fertility and litter size were also observed among animals fed a diet low in arsenic. However, these results were not consistently seen among animals fed a low arsenic diet that was casein-based rather than corn-based (Uthus *et al.*, 1983).

Depressed growth and elevated perinatal mortality rates have been demonstrated among low arsenic intake goats and mini pigs (Anke *et al.*, 1976; Anke, 1986, 1991). Only 58 percent of low arsenic intake goats and 62 percent of low arsenic intake mini pigs produced offspring, compared with 92 percent and 100 percent of the controls. The majority of the breeder goats fed the low-arsenic diets were said to have died suddenly between the 17th and 35th day of their second lactation. Addition of two µg of arsenic to the diet of chicks fed a low-arsenic diet stimulated growth in these animals as compared to controls (Nielsen, 1980). The average body weight was 894 g with arsenic supplementation and 747 g in arsenic-deprived chicks at four weeks of age.

Based on the aforementioned experimental data, various estimates of human nutritional requirements have been made ranging from 12 to 25 µg/day (Uthus, 1993). However, the relevance of the experimental animal data to humans is unclear, and OEHHA does not consider the evidence adequate to presume that there is a human dietary requirement for arsenic. No human arsenic deficiency syndrome has yet been reported, even through many water supplies contain less than 2.5 μg/L.

Quantitation of Cancer Risks

Skin cancer was the first cancer to be linked to arsenic ingestion (Tseng *et al.*, 1968). However, since arsenic causes squamous and basal cell skin cancers, not malignant melanoma, skin cancer is not an important cause of death from arsenic ingestion. Furthermore, to ascertain incidence is very difficult since tumor registries do not usually include skin cancer. In any case, tumor registries do not exist in most arsenic exposed regions of the world. So far, skin cancers due to arsenic have not been reported in the absence of nonmalignant skin lesions. Bladder cancer is the most sensitive endpoint for chronic consumption of arsenic induced mortality via the drinking water as far as relative risk is concerned. Lung cancer has been strongly linked to arsenic ingestion in studies in Taiwan, Chile, and Argentina. While the relative risk is lower than that for bladder cancer, the number of lung cancer cases that occur in arsenic exposed populations is larger than for bladder cancer and the absolute risks of lung cancer are therefore greater. The present risk assessment will therefore focus primarily on lung cancer, but will also calculate cancer potency estimates for both lung and bladder cancer combined.

Contribution of Lung Cancer to Overall Arsenic Cancer Mortality Risks

Table 21 presents data from population studies in Taiwan (Chen *et al.*, 1988b), and studies in Argentina (Hopenhayn-Rich *et al.*, 1996c, 1998) and Chile (Smith *et al.*, 1998) comparing the excess deaths (observed minus expected) from cancers related to arsenic in drinking water. In each country, excess lung cancer deaths were the major contributor to excess risk, ranging from 41 percent for women in Taiwan to 79 percent for men in Chile. These data suggest that, of the variety of different cancer effects caused by ingesting inorganic arsenic, lung cancer plays the most important role in mortality and should be the primary outcome of interest for risk assessment and standard setting for arsenic in drinking water. In fact, pooling data from the three countries suggests that arsenic is responsible for more lung cancer deaths than all other arsenic-caused cancers combined. This evidence indicates that the risk of lung cancer will be the largest component in the derivation of cancer potency estimates for ingested inorganic arsenic.

Calculation of Carcinogenic Potency

The relative risk model is utilized in the dose-response assessment (Wright *et al.*, 1997). The lifetime excess lung and bladder cancer risks for a population exposed to arsenic through the drinking water can be determined from the following equation $R_x = R_o(xB)$ where R_x represents the predicted risk to persons with exposure level x, R_o presents the background lifetime risk of dying from lung or bladder cancer without arsenic exposure, and B represents the slope from the linear relative risk model. To determine the slope B, the relative risk estimates from each selected study will be plotted against the corresponding exposure categories of arsenic in the drinking water in units of µg per liter. The y-intercept will be forced through a relative risk of one. Issues concerning the use of the relative risk model with linear extrapolation will be presented in the section on uncertainties.

Table 21. Excess Deaths (Observed Minus Expected) for Lung, Bladder, Kidney, and Skin Cancer Deaths in Three Populations Exposed to Arsenic in Drinking Water

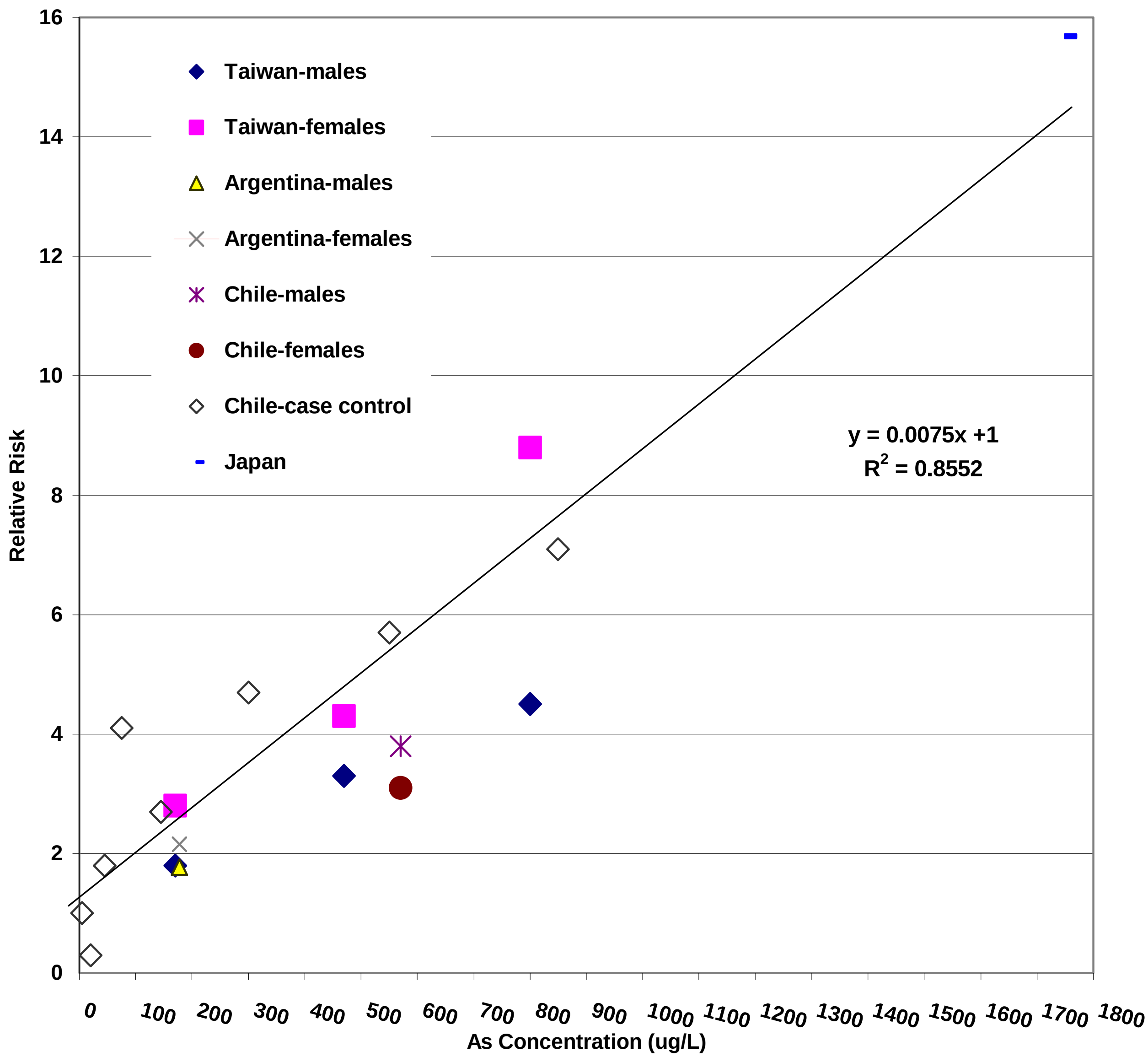
			Women	
				Percent of total excess
Argentina* (high exposure)				
Lung cancer	307	77	84	71
Bladder cancer	70	7	12	10
Kidney cancer	19	5	12	10
Skin cancer	3	<1	11	9
Total	399		119	
Chile† (Region II)				
Lung cancer	401	79	105	56
Bladder cancer	78	15	56	30
Kidney cancer	14	3	22	12
Skin cancer	17	3	5	3
Total	510		188	
Taiwan‡				
Lung cancer	228	50	177	41
Bladder cancer	152	34	157	36
Kidney cancer	37	8	60	14
Skin cancer	37	8	42	10
Total	454		436	

*Hopenhayn-Rich *et al.*, 1996, 1998 (high exposure); †Smith *et al.*, 1998; ‡ Chen *et al.*, 1985.

Selection of Studies for Lung Cancer Potency Estimates

The results of all studies that provided quantitative exposure data including high concentrations of arsenic in the drinking water and death from lung cancer are depicted in Figure 1. A linear regression analysis forced through a y-intercept equal to one demonstrated that the combined results are highly correlated (R^2=0.86) and consistent with a linear dose-response relationship. Included in this graph are lung cancer relative risk estimates from Chen *et al.* (1988b) in Taiwan, Hopenhayn-Rich *et al.* (1998) in Argentina, Ferreccio *et al.* (2000) and Smith *et al.* (1998) in Chile, and Tsuda *et al.* (1995) in Japan. When available, the relative risk estimates for males and females have been incorporated separately. Although Tsuda *et al.* (1995) calculated relative risk estimates for both males and females, we combined results due to the small numbers of lung cancer deaths involved. Only seven lung cancer deaths were observed among males and one lung cancer death among females for the entire cohort. All deaths were from the highest arsenic exposed group. Hopenhayn-Rich *et al.* (1998) calculated lung cancer mortality for three dose groups; low, medium and high. However, a crude estimate of arsenic in the drinking water was available only for the highest dose group. Consequently, these latter results were the only data included from the study. The data utilized from Ferreccio *et al.* (2000) involve the average water arsenic concentrations during the peak years of exposure from 1958 to 1970, and were the logistic regression findings adjusted for age, sex, smoking, work in a copper smelter, and socioeconomic status.

Figure 1: Lung Cancer Relative Risk Estimates by Arsenic Concentration - All studies combined (Chen et al. 1988b; Hopenhayn-Rich et al., 1988; Ferreccio et al., 2000; Smith et al.,1998; and Tsuda et al.,1995)



Figures 2 and 3 present linear regressions (y-intercept = 1) of lung cancer relative risk by arsenic drinking water concentration for males and females separately. Data from Chen *et al.*, 1988; Hopenhayn-Rich *et al.* (1998) and Smith *et al.* (1998) were available for inclusion in the analysis. Results were highly correlated between lung cancer and arsenic exposure, although more so for males (R^2=0.99) than females (R^2 = 0.71). Both graphs demonstrate increasing linear dose-response trends, however, the slope of the regression analysis for females (0.0076) is greater than that calculated for males (0.0046). This difference between males and females appears to be driven by results from Taiwan. Lung cancer relative risk estimates were similar for males and females in the populations studied by Hopenhayn-Rich *et al.* (1998) and Smith *et al.* (1998). Likewise, the case-control study of Ferreccio *et al.* (2000) found that the lung cancer OR for males as compared to females was 1.1 (95 percent CI 0.6-4.8). Results from this latter study are plotted in Figure 4. Note that the linear regression analysis gives a slope (0.0082) similar to that calculated for the combined study results for females (0.0076).

We have no explanation for the fact that the lung cancer relative risks differ between men and women in Taiwan as compared to South America. Rather than ignore this difference, we will base our lung cancer risk estimation on potency estimates derived for each sex separately. Each of the studies selected for the risk assessment (Figures 2 and 3) have been discussed in the Hazard Identification portion of this document and their results have been presented in detail in Table 8. The reasons for their inclusion are that they provide quantitative exposure data for the relationship between lung cancer and arsenic concentrations in drinking water including persons with high levels of exposure, were of adequate latency and power to detect an effect, and were not subject to confounding bias. No other studies meet these selection criteria.

Dose-Response Calculations for Lung Cancer

As presented in the previous section, the relative risk estimates for lung cancer from Chen *et al.*, 1988b, Hopenhayn-Rich *et al.,* 1998, Smith *et al.,* 1998 were plotted against the corresponding exposure categories of arsenic in the drinking water in units of µg per liter for each sex separately. The y-intercepts were forced through a RR of 1 for 0 µg/L of arsenic in the drinking water. Figures 2 and 3 show the best fitting lines of all results combined for males and females, respectively. The slopes of these lines are equal to 0.0046 $(\mu g/L)^{-1}$ for males and 0.0076 for females $(\mu g/L)^{-1}$. These results mean that for the populations under study, the excess relative risks are 0.0046 and 0.0076 for every µg/L of arsenic in the drinking water for males and females, respectively.

Conversion of the risk estimates to a lifetime excess cancer risk requires having an estimate of background lifetime risk from lung cancer. Before presenting the calculations involved, the issue of incorporation of background rates of cancer into a risk assessment and the choice of which background rates are most appropriate in the present risk assessment will be addressed.

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Figure 2: Lung Cancer Relative Risk by Arsenic Drinking Water Concentration-Results for Males Combined (Chen et al., 1988b; Hopenhayn-Rich et al., 1998; and Smith et al., 1998)

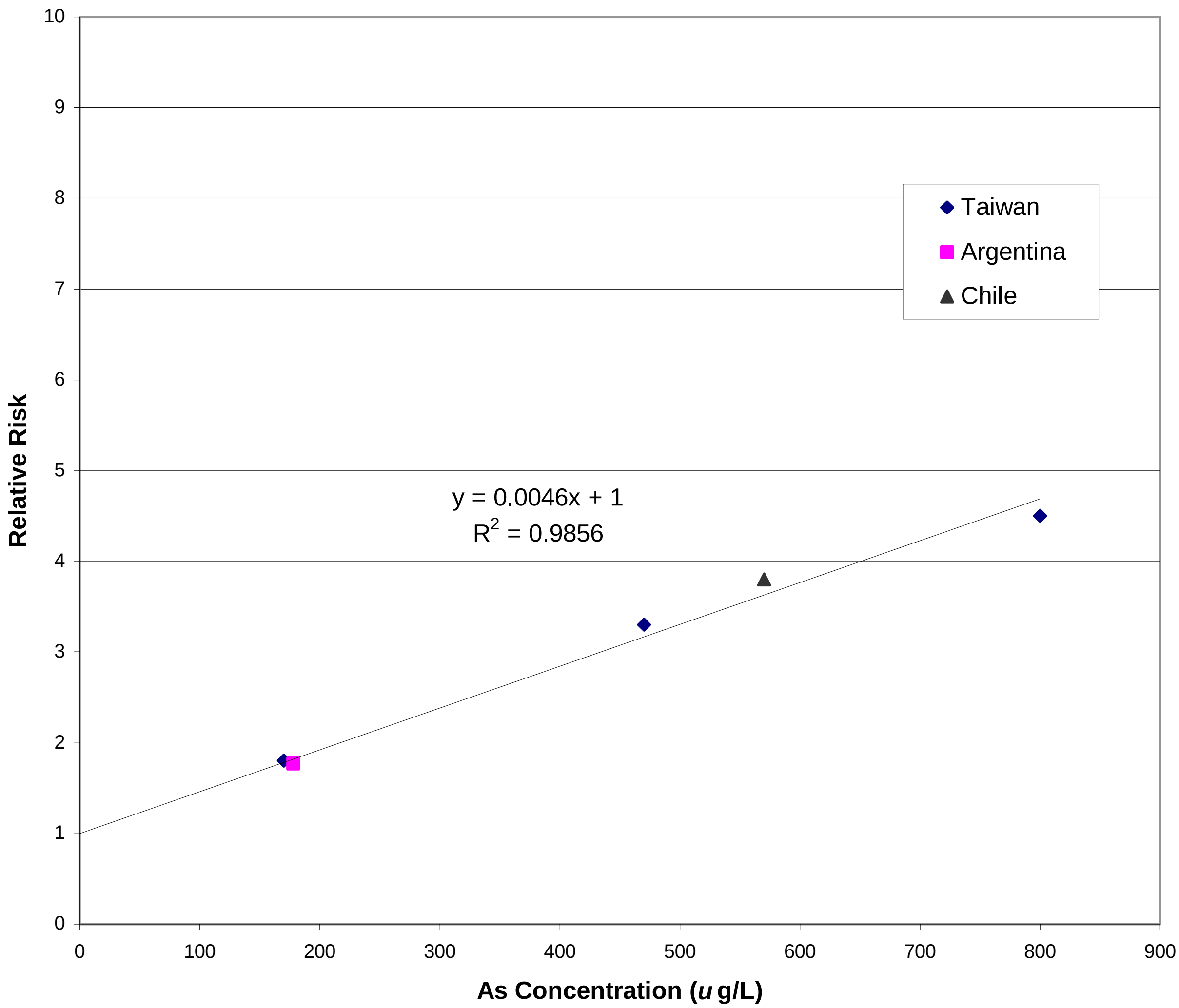
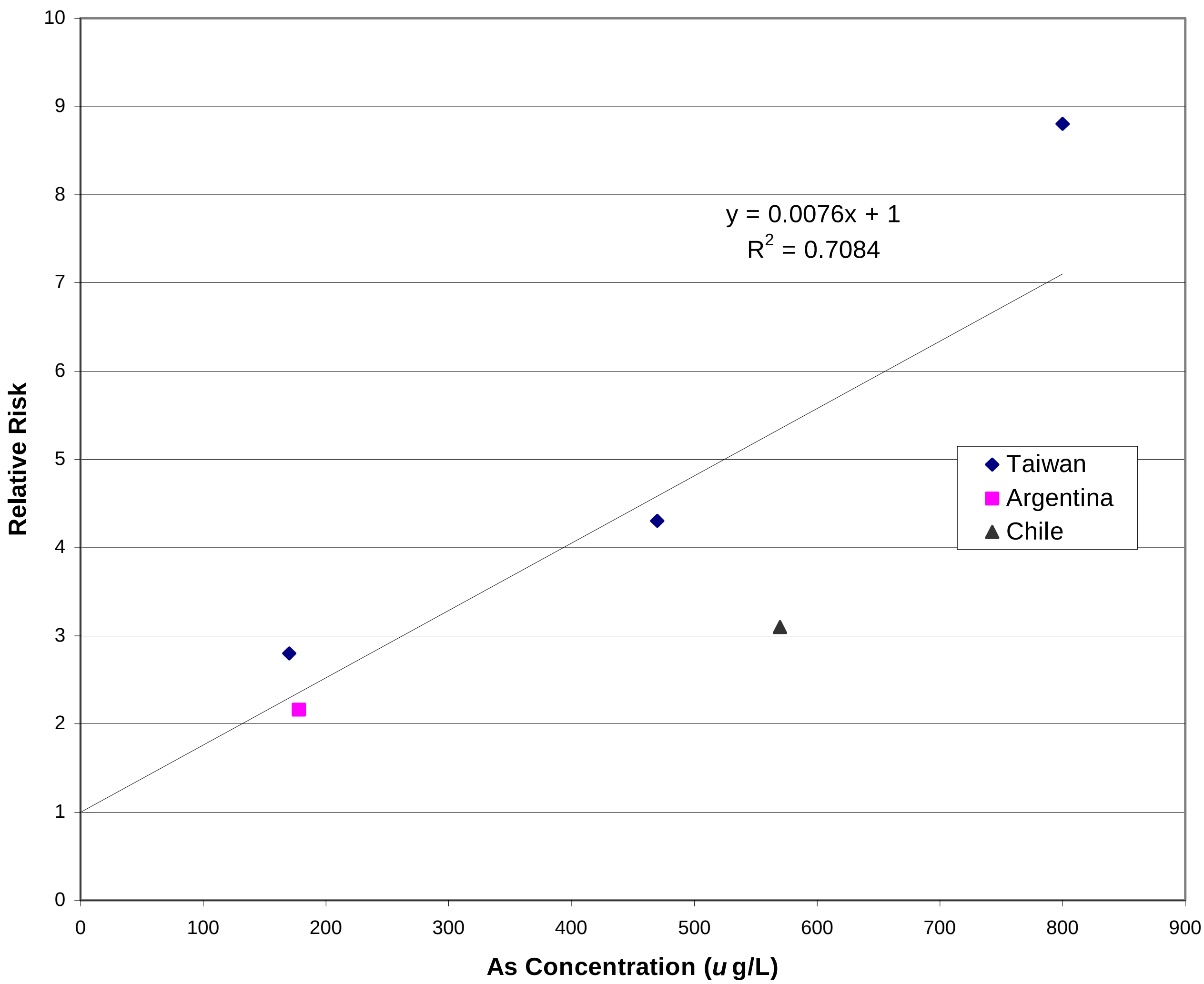


Figure 3: Lung Cancer Relative Risk Estimates by Arsenic Drinking Water Concentration-Results for Females Combined (Chen et al., 1988b; Hopenhayn-Rich et al., 1998; Smith et al., 1998)



Incorporation of Background Rates

It is necessary to have an estimate of background lifetime risks from lung cancer when applying cancer relative risks generated from one study to another population. One question is whether to take background lung cancer risks in the past, currently, or in the future. Current death rates occur in different birth cohorts which may not have experienced the same age-specific mortality rates in the past, and who may not in the future. There is therefore no simple answer to what the "background rates" of cancer are. Life-table analysis has to make specific assumptions about future mortality rates, which may not be justified. A much simpler approach is recommended, and has been used in this risk assessment. An estimate of background risks of lung cancer mortality may be obtained by dividing the number of lung cancer deaths during a certain period in time by the number of deaths of all causes during the same period. The implicit assumption is that current mortality rates in each birth cohort represent lifetime mortality rates. While life-table analysis may appear to a more sophisticated approach, the assumptions involved means there is no gain in validity, but there is clearly a loss in transparency compared to the straightforward approach we use here. The estimates of background rates we present can easily be checked by going to Vital Statistics publications and dividing the number of lung cancer deaths in a given year by the total number of deaths in the same year.

A question arises when one considers what to do in assessing current cancer risks in the U.S., since lung cancer mortality rates among U.S. citizens may have increased due to changes in smoking habits. In addition, a question arises as to how to estimate the risks from ingestion of inorganic arsenic for some other population such as Californians. The relevant biological question involves interaction. If the ingestion of inorganic arsenic acts to increase lung cancer risk in an additive manner that is independent of the local population's background lung cancer risks, then the background rates of these cancers in the population under consideration would not enter the calculations. Rather, the background rates of lung cancer in the populations in the arsenic studies would be used to estimate additive risks in those populations. The additive approach identifies risks from inorganic arsenic, which would not change over time nor from place to place with background incidence of cancer.

On the other hand, if inorganic arsenic acts in a multiplicative manner with the background causes of lung cancer in a population, then the background rates of lung cancer will have a direct bearing on the risks one would estimate for inorganic arsenic in drinking water for that population. For example, if the background rates were twice that of the population in which the study occurred then the risks estimated for inorganic arsenic would be doubled.

The following should be noted with regard to the multiplicative approach. Estimated risks from inorganic arsenic would vary over time and from place to place with variation in background rates of lung cancer. Since smoking is the main determinant of lung cancer incidence in the general population, and since smoking rates are now falling over time (www.dhs.ca.gov/tobacco/documents/adult smoking, youth smoking etc.), this means that calculated risks for exposure to inorganic arsenic would also fall without any change in inorganic arsenic levels. The implication is that estimated risks for inorganic arsenic, using the most recent lung cancer rates, would need to be adjusted periodically with decreasing background rates for lung cancer. Acceptable levels for inorganic arsenic exposure would increase over time if the same lifetime cancer risk yardstick were maintained.

There are obvious policy disadvantages to this approach. However, the critical question is biological validity since, if the real risk from exposure to inorganic arsenic were falling over time due to the reduction in background lung cancer rates, this is relevant to risk management decisions.

As indicated above, the question is one of interaction. In particular, since smoking accounts for 80 to 90 percent of the incidence of lung cancer in most countries, the question concerns the interaction between ingested inorganic arsenic and cigarette smoking in causing lung cancer.

Consideration of synergy between arsenic and cigarette smoking

Hertz-Picciotto and Smith (1993) have published evidence suggesting a synergistic effect between inhaled arsenic and cigarette smoking in causing lung cancer. More recently, the case-control study of Ferreccio *et al.* (2000) provides evidence that ingested inorganic arsenic acts synergistically with inhaled cigarette smoke in causing lung cancer. Although limited somewhat by non-ideal control group selection, this study utilized individual exposure data and demonstrated additional evidence that lung cancer in Northern Chile is strongly related to arsenic concentrations in drinking water. The relative risk estimate (in this case odds ratios adjusted for age, sex, smoking and SES) was 7.1 (95 percent C.I. 3.4-14.8) for those with the highest average water concentrations (Table 8; Figure 4). Marked increases in lung cancer risks (up to an odds ratio of 8.0, 95 percent C.I. 1.7-52.3) were evident among non-smokers, but the combination of having smoked cigarettes and high average arsenic water concentrations resulted in a relative risk estimate of 32 (95 percent C.I. 7.2-198.0). These data were presented earlier in Table 9 and are depicted graphically in Figure 5.

Although based on small numbers, other epidemiological evidence of synergy between ingestion of inorganic arsenic and smoking comes from the cohort of Tsuda *et al.* (1995). The non-smokers exposed to ≥ 50 ppb arsenic had a mortality rate of 0.31/1000 person-yrs. The mortality for smokers not exposed to ≥ 50 ppb was 0/1000 person-yrs. However, for the smokers exposed to ≥ 50 ppb arsenic the lung cancer mortality was 3.7/1000 person-years. The excess fraction of cases attributable to both smoking and arsenic exposure was 0.92. Dividing the cohort into low, medium, and high exposed groups, the smokers had SMRs of 0, 3.72, and 18.73, respectively, whereas the non-smokers had SMRs of 0, 0, and 10.14, respectively.

Figure 4: Lung Cancer Odds Ratios by Arsenic Drinking Water Concentration, 1958-1970 (Ferreccio et al., 2000)

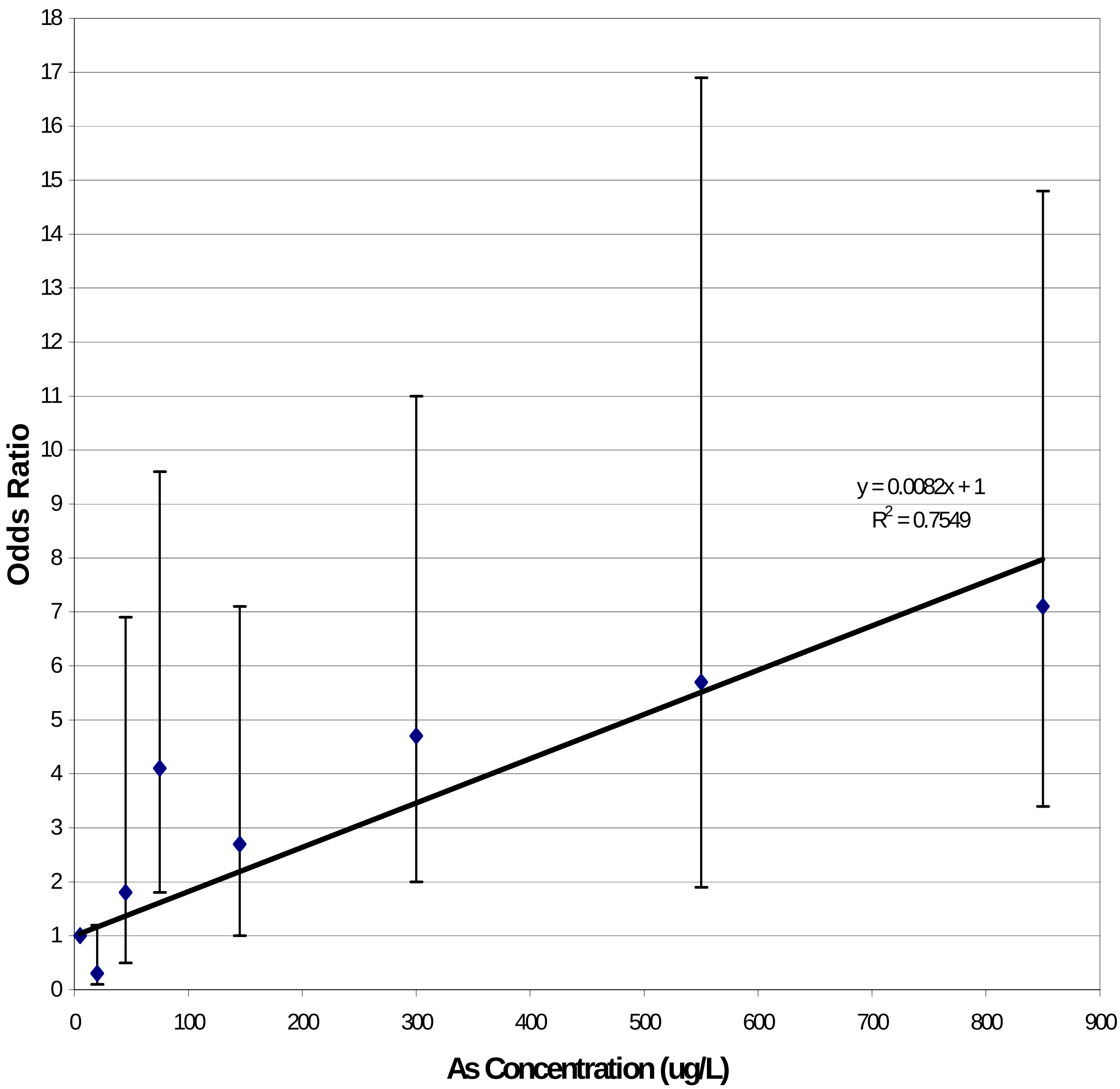
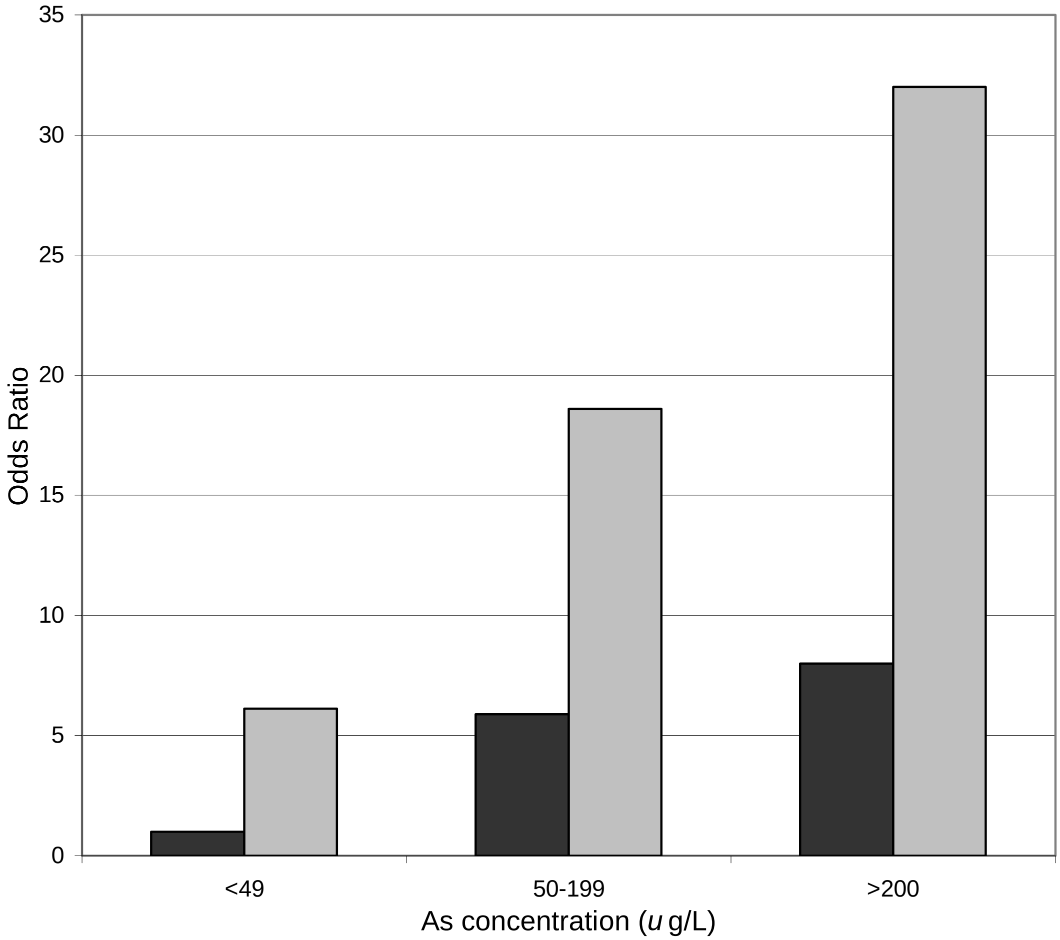


Figure 5: Lung Cancer Risk (OR) Associated with As Concentration in Drinking Water and Smoking (Ferreccio et al., 2000)



Conclusion Regarding Background Rates

Considering the evidence that the effects of arsenic and smoking are synergistic, this suggests that the lung cancer risk estimates from inorganic arsenic exposure depend on the background rates of lung cancer. It is therefore appropriate to incorporate the current lung cancer mortality rates for the U.S. and California in the risk assessment.

The numbers of all cause, lung cancer deaths in the U.S. and California for both sexes based on data from 1996 are presented in Table 24 (NCHS, 1998). The background U.S. lifetime risk of death from lung cancer for both sexes combined was estimated at 66 per 1000, obtained by dividing the number of deaths from lung cancer in 1996 by the total number of all cause deaths in 1996. The result was multiplied by 1000 to give a background rate of lung deaths per 1000 deaths in the U.S. population. The background lung cancer mortality rates are higher for males than for females (79 versus 52 per 1,000). This higher lung cancer mortality is largely the result of a greater percentage of male than female smokers. The background lifetime risk of death from lung cancer in California for both sexes combined is estimated at 61 per 1,000. The mortality rate for males is again higher than for females (66 versus 55 per 1,000). It should be noted that cancer rates are not in steady state and each birth cohort will experience different risks. However, the above methods are useful since they can be derived in a very straightforward manner. Any full life table analysis would have to make assumptions about which birth cohort is being described and about future lung cancer rates. As a result, it would not be superior to the simple method used here, and would suffer the disadvantage that it would be complicated for a reader to validate the life-table analyses.

Adjustment for Differences in Drinking Water Consumption

Data on daily drinking water consumption are applied to the calculations of the lifetime added lung cancer risk to reflect the risk from drinking one liter of water per day. Table 21 presents findings for drinking water consumption from various arsenic studies. The populations from Chile had an average daily drinking water consumption rate for both sexes combined of 2.5 L/day. Males and females were found to drink 2.6 and 2.2 L/day, respectively. In Argentina, the average daily water consumption was 1.9 L/day. Males drank an average of 2.0 L/day and females drank 1.7 L/day. The U.S. EPA has previously assumed a daily drinking water intake of 3.5 L/day for males and 2 L/day for females in Taiwan (U.S. EPA, 1988). However, we know of no field studies on which these drinking water consumption rates are based. In the absence of such data, we used averages of the findings from South America by sex to approximate the drinking water consumption rates in the populations included in the risk assessment. The average daily intake is estimated at 2.3 and 2.0 liters for males and females, respectively, based on the case-control studies in Argentina and Chile (Table18). The adjustment factors per L/day are therefore, 0.43 for males (1 liter/2.3 liters) and 0.50 for females (1 liter/2.0 liters).

Table 22. Drinking Water Consumption from Epidemiological Investigations of Arsenic Outside the U.S.

	Drinking water consumption (L/day)		
	Males	Females	Total
Chile – case control[a]	2.6	2.2	2.4
Argentina – case control[b]	2.0	1.7	1.9
India – case control[c]	2.6	2.1	2.4
Chile - Biggs *et al.*, 1997	San Pedro	Toconao	
	2.5	2.3	
Chile - Moore *et al.*, 1997a	-	-	2.6

[a]findings from participants in the lung cancer case-control study of Ferreccio *et al.* (2000)
[b]preliminary findings from on-going bladder case-control study; 28 females and 149 males
[c]preliminary findings from on-going skin cancer case-control study; 73 females and 143 males

Estimate of Lifetime Added Lung Cancer Risk

Table 23 gives the estimates of lifetime added lung cancer risk based on current U.S. and California background lung cancer mortality rates and lifetime consumption of arsenic in the drinking water for males and females. Risks were calculated for exposure to the current U.S. standard of 50 µg/L arsenic in drinking water and 10 µg/L (permissible exposure level proposed by WHO, 1981). Based on the equation $R_x = R_o(xB)$, the lifetime added risk of lung cancer for males resulting from exposure to 50 µg/L inorganic arsenic per day is estimated at 7.8 per 1,000 when national background mortality rates are utilized. R_o equals the background lifetime lung cancer mortality risk per 1,000 in the U.S. of 79, x equals a daily consumption over a lifetime of 50 µg/L of arsenic in the drinking water, *B* equals the slope of 0.0046 $(\mu g/L)^{-1}$, and 0.43 is the adjustment factor from 2.3 liters to one liter of drinking water (79 x 50 x 0.0046 x 0.43). Using the same formula, the estimate of lifetime added lung cancer risk for females drinking one liter of water per day containing 50 µg/L inorganic arsenic is 9.9 per 1,000. The estimates of lifetime added lung cancer risk for males and females ingesting 10 µg/L inorganic arsenic per day are 1.6 and 2.0 per 1,000, respectively. Despite the higher background lung cancer mortality rate among men compared to women in the U.S. population, the higher relative risk estimates for lung cancer and the lower drinking water consumption rates for women result in a slightly higher lifetime added lung cancer risk estimate for females than males.

When California background lung cancer mortality rates are used to calculate the potency estimates, the lifetime added risk of lung cancer for males resulting from exposure to 50 µg/L inorganic arsenic per day is estimated at 6.5 per 1,000. The estimate of lifetime added lung cancer risk for females in California exposed to 50 µg/L inorganic arsenic per day is 10.5 per 1,000. The estimates of lifetime added lung cancer risk for males and females ingesting 10 µg/L inorganic arsenic per day are 1.3 and 2.1 per 1,000, respectively. The potency estimates based on California rates are slightly higher for females compared to the results calculated using U.S. background lung cancer mortality, while those for males are slightly lower.

Table 23. Estimates of Excess Lung and Bladder Cancer Risk Due to Arsenic in Drinking Water*

			California	
				Females
				0.0076
				55
				0.50
				10.5
				2.1
				1.6
				16.8
				3.4

* Based on data from Chen *et al*. 1985, 1988b; Hopenhayn-Rich *et al.*, 1996, 1998; Smith *et al,* 1998; and Ferreccio *et al*, 2000.

Figure 6: Bladder Cancer Relative Risk Estimates by Arsenic Drinking Water Concentration - All Studies Combined (Chen et al., 1988b; Hopenhayn-Rich et al., 1996c, 1998; Smith et al., 1998)

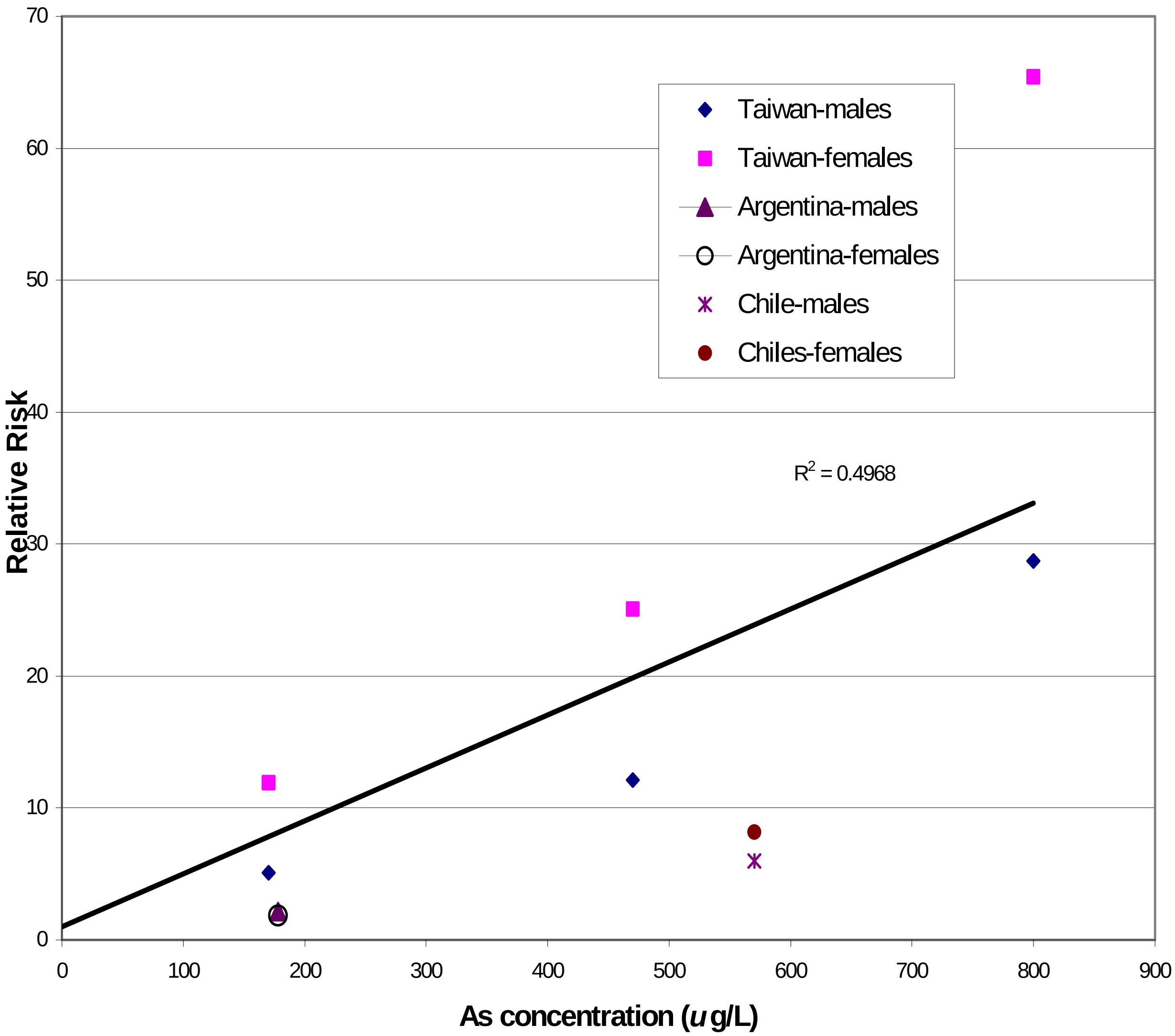


Table 24. Numbers of All Causes and Lung Cancer Deaths in California and the U.S. in 1996 (NCHS, 1998) with Estimated Death Rates per 1,000 Persons

	Number of Deaths		Death Rate per 1,000[a]
California			
All	223,447	13,601	61
Males	114,552	7,597	66
Females	108,895	6,004	55
United States			
All	2,314,690	152,015	66
Males	1,163,569	91,620	79
Females	1,151,121	60,395	52

[a]Number of deaths/all cause deaths x 1,000)

Selection of Studies for Bladder Cancer Potency Estimate

The relative risks for bladder cancer mortality from each study with data for high-levels of arsenic in the drinking water are depicted in Figure 6 (Chen *et al.*, 1988b; Hopenhayn-Rich *et al.*, 1996c, 1998; Smith *et al.*, 1998). Note that these same populations were considered in the lung cancer risk assessment. Results from Japan (Tsuda *et al.*, 1995) were excluded since results for kidney and bladder cancer mortality were combined. Although the bladder cancer relative risks from each individual study plotted in Figure 6 increase with increasing arsenic concentration in the drinking water, the results taken as a whole vary ($R^2 = 0.50$ when the y-intercept is forced through 1.0). Bladder cancer mortality was much higher in the Taiwanese studies compared to the results from South America. In addition, the bladder cancer relative risks for Taiwanese women were approximately twice that observed in Taiwanese men. No significant differences in bladder cancer relative risk estimates were found between men and women in the studies from Argentina and Chile. There is no explanation currently available for the difference in results from studies in Taiwan compared to South America, nor for the difference observed between the sexes.

Dose-Response Calculations for Lung and Bladder Cancer

Because of the degree of variation between the relative risks for bladder cancer and arsenic in the drinking water as shown in Figure 6, it is not possible to calculate a slope and apply the relative risk model to estimate the lifetime added bladder cancer risk with as much precision as for lung cancer. We demonstrated in Table 21 that the excess lung cancer deaths from the population studies in Taiwan, Argentina, and Chile were the major contributor to the excess cancer risk, ranging from 41 percent for women in Taiwan to 77 percent for men in Argentina. The percentage of total excess deaths resulting from bladder cancer in these same studies ranged from 10 percent for females in Argentina to 36 percent for females in Taiwan. Table 25 also shows the excess deaths from lung and bladder cancer from each of these cohorts by sex. In order to incorporate the added risk of dying from bladder cancer in the overall estimate of lifetime added cancer risk, we have calculated the ratio of the total excess lung and bladder cancer deaths relative to the excess lung cancer deaths from all studies combined (excess lung cancer deaths plus excess bladder cancer deaths divided by the excess lung cancer deaths). These ratios were 1.3 for men and 1.6 for females.

Table 25. Excess Deaths (Observed Minus Expected) from Lung Cancer (LC) and Bladder Cancer (BC) Related to Arsenic in Drinking Water

				Women		
						(Excess LC deaths + BC deaths)/Excess LC deaths
Argentina*						
Lung cancer	307	4.4	1.2	84	7	1.1
Bladder cancer	70			12		
Chile (Region II)[+]						
Lung cancer	401	5.1	1.2	105	1.9	1.5
Bladder cancer	78			56		
Taiwan[#]						
Lung cancer	228	1.5	1.7	177	1.1	1.9
Bladder cancer	152			157		
TOTALS						
Lung cancer	936	3.1	1.3	366	1.6	1.6
Bladder cancer	300			225		

[*]Hopenhayn-Rich *et al.*, 1996, 1998 (high exposure group); [+]Smith *et al.*, 1998; [#]Chen *et al.*, 1985

The estimates of lifetime added lung and bladder cancer risk for males and females exposed to arsenic in the drinking water are shown in Table 23. These results were obtained by multiplying the lifetime added lung cancer risks by the ratios reflecting the additional risk of death from bladder cancer relative to the excess lung cancer deaths in the study populations. The estimates of lifetime added lung and bladder cancer risk per 1000 persons exposed to 50 µg/L of arsenic in the drinking water were 10.1 for males (1.3 x 7.8) and 15.8 (1.6 x 9.9) for females when U.S. background lung cancer mortality data are used. Exposure to a daily intake of 10 µg/L reduces these risk estimates to 2.1 per 1,000 in males and 3.2 per 1,000 in females. The average estimate of lifetime added lung and bladder cancer risk for both sexes combined is 13.0 in 1,000 when the arsenic exposure is 50 µg/L per day and 2.7 per 1,000 at 10 µg/L per day.

The estimates of lifetime added lung and bladder cancer risk for males and females based on background lung cancer mortality for California are also presented in Table 23. The lifetime added lung and bladder cancer risk per 1,000 persons exposed to 50 µg/L of arsenic in the drinking water were 8.5 for males (1.3 x 6.5) and 16.8 (1.6 x 10.5) for females. Exposure to a daily arsenic intake of 10 µg/L reduces these risk estimates to 1.7 per 1000 in males and 3.4 per 1,000 in females. The average estimates of lifetime added lung and bladder cancer risk for both sexes combined are essentially the same as the estimates based on U.S. rates, i.e., 12.7 in 1,000 when the arsenic exposure is 50 µg/L per day and 2.6 per 1000 at 10 µg/L per day.

Overall Cancer Risks

The estimate of lifetime added lung and bladder cancer risk resulting from exposure to 50μg/L of arsenic in the drinking water over a lifetime is approximately 13 per 1,000 persons or approximately one in 100 (with about 55 percent of the risk attributable to lung cancer). This result is strikingly similar to the estimate made in our earlier risk assessment (Smith *et al.*, 1992) that was based solely on data from Taiwan (Chen *et al.*, 1988b; Wu *et al.*, 1989). The lifetime risk of dying from cancer of the liver, lung, kidney or bladder from drinking one L/day of water at 50 µg/L of arsenic was found to be as high as 13 per 1,000 (with about 25 percent of the risk attributable to liver and kidney cancers). Likewise, the National Research Council has recently concluded that ingestion of inorganic arsenic causes both bladder cancer and lung cancer, and that the combined cancer mortality risks for all cancer sites associated with drinking 50 µg/L of arsenic could be on the order of one in 100 exposed persons (NRC, 1999) or even more (NRC, 2001).

The lifetime added cancer risk estimates are based on a fixed consumption of one liter of water per day. Daily water intakes will vary by individual. To estimate the lifetime added lung and bladder cancer risk resulting from consumption of greater than or less than one L/day, one needs to multiply the daily intake by the fixed estimate. For example, the U.S. EPA (1988) once assumed that the daily drinking water consumption in the U.S. was two L/day. Therefore, the lifetime added lung and bladder cancer risk from exposure to arsenic at 50 µg/L would be approximately 26 per 1,000 rather than 13 per 1,000.

It should be noted that the present analysis does not reflect the total impact of ingested arsenic from water. Arsenic also causes nonfatal bladder and skin cancers that would further contribute to the cancer risk, and is also a cause of kidney cancer (see Risk Characterization section below).

Potency estimates from animal studies

This is an exercise to compare the carcinogenic activity of arsenic in animals to the unit risk estimated from epidemiology studies, 2.7×10^{-4} per µg/L (see page 157). Here, to make this comparison, the transplacental carcinogenicity assay in mice of Waalkes *et al.* (2003) is used to predict water concentrations associated with de minimis cancer risk. In making these calculations, it is recognized that the identification of rodent models for arsenic carcinogenicity has been difficult, and it is unclear the degree to which the findings in this study are representative of humans. Also, because gestation in rodents may be a period of high sensitivity to arsenic carcinogenesis it is difficult to extrapolate the findings from exposure in the transplacental period to, say, adult exposure. Four different assumptions regarding age susceptibility to cancer are applied below to estimate four different unit risk values from the Waalkes *et al*. study.

In the Waalkes *et al.* study, pregnant female C3H mice, 10 per group, were given sodium arsenite at concentrations 0, 42.5 or 85 ppm ad libitum from day eight to 18 of gestation. Offspring were weaned at four weeks and then randomly placed in separate groups of males and females according to maternal exposure level. Males were observed for the next 74 weeks, females for the next 90 weeks. The authors calculated that dosage levels of 42.5 and 85 ppm corresponded to 9.55 and 19.13 mg arsenic/kg/day for the exposure period, or a total dose of 95.6 and 191.3 mg/kg. The authors note a biological half-life of inorganic arsenic of four days and that some translactational exposure may have occurred.

Treatment-related proliferative lesions were observed in male liver, adrenals, and lung and in female ovary, oviduct, lung, and uterus. Typically, tumors but not hyperplastic lesions provide the basis for cancer potency calculation. Table 26 gives tumor incidences that were notably increased. To predict water concentrations associated with de minimis cancer risk, the sites with the strongest responses are emphasized - male liver and female ovary. Although the incidence of adrenal tumors in males is high, malignancies were not observed. The dose response analysis of ovarian tumors is restricted to treatment-related tumors arising from the same cell type, in this case adenoma and carcinomas.

Table 26. Tumor Incidences from the Transplacental Mouse Bioassay of Waalkes et al. (2003)

			High Dose
Male			
Liver carcinoma	3/24 (p=0.0006)	8/21 (p=0.049)	14/23 (p=0.0007)
Liver adenoma and/or carcinoma	10/24 (p=0.0016)	11/21 --	20/23 (p=0.0014)
Adrenal cortical adenoma	9/24 (p=0.001)	14/21 (p=0.049)	21/23 (p=0.0001)
Female			
Total ovarian tumor	2/25 (p=0.015)	6/23 (p=0.098)	9/24 (p=0.015)
Ovarian adenoma or adenocarcinoma	1/25 (p=)	4/23 (p=0.15)	7/24 (p=0.021)
Lung adenoma or carcinoma	2/25 (p=0.099)	3/23 --	6/24 (p=0.11)
Lung carcinoma	0/25 (p=0.0086)	1/23 --	5/24 (p=0.023)

[1]Beneath the control group incidence is the trend test p-value reported by study authors. Beneath the arsenic-treated groups are Fisher Exact test p-values for pairwise comparison incidences in control and treated animals.
[2]Ovarian tumors: Control – one adenoma and benign granuloma cell tumor; low dose – three adenomas, one adenocarcinoma, one benign and one malignant granuloma cell tumor; high dose – seven adenomas, one luteoma, one hemangiosarcoma. No one animal had multiple types of ovarian tumor.

Unit risk is the slope at low doses of the curve relating water concentration to cancer risk. Here this is calculated from cancer potency estimated by fitting the multistage model (see below) to the animal cancer bioassay data.

Typically in PHG calculations, benchmark dose calculations are used to estimate the drinking water concentration associated with de minimis risk. However, in this exercise the comparison is being made between unit risk estimated from animal studies and the unit risk estimated earlier from human data. Thus in the interest of parsimony the cancer potency, or "cancer slope factor," estimated by fitting the multistage model to the bioassay data will be converted to the human unit risk. Cancer potency in units $(mg/kg\text{-}day)^{-1}$ is converted to unit risk, in units µg/L, by taking into account water consumption (WC, L/day) and human body weight (70 kg), as follows:

$$\text{Unit risk} = \text{cancer potency} \times (\text{WC} \div 70\ \text{kg}) \times (1\ \text{mg} / 1{,}000\ \mu\text{g})$$

In these calculations, mean water consumption (2.076 L/d) is assumed.

The multistage model gives the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) as follows (CDHS, 1985; U.S. EPA, 1987; Anderson and U.S. EPA, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_j d^j)]$$

with constraints

$$q_i > 0 \text{ for all } i.$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter q_0 represents the background lifetime incidence of the tumor, and q_1, or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1^*, the upper 95 percent confidence bound on q_1 (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units mg/kg-d, the parameters q_1 and q_1^* are given in units $(mg/kg\text{-}d)^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977).

Because the Waalkes *et al.* study did not last for the lifetime of the animals, a correction for the shortened experiment is made. To estimate potency in animals (q_{animal}) from experiments of duration T_e, rather than the natural lifespan of the animals (T), it is assumed that lifetime incidence of cancer increases with the third power of age:

$$q_{animal} = q_1^* \times (T/Te)^3, \text{ for } Te < T$$

Following Gold and Zeiger (1997) and U.S. EPA (Anderson and U.S. EPA, 1983), the natural lifespan of mice and rats is assumed to be two years. So, for experiments lasting T_e weeks in these rodents, with $T_e < 104$ weeks,

$$q_{animal} = q_1^* \times (104/Te)^3.$$

Study length is calculated here to be the 20-day gestation, plus the four-week preweaning, plus the subsequent observation period. Thus, for males, the study length is 80.86 weeks (=1/7 x (20 + (4+74) x 7)), or 566 days. The study length for females is similarly calculated to be 96.86 weeks, or 678 days. The lifetime of animals to be used in the dose corrections for shortened experiments will include the gestation period. The standard lifetime used in risk potency estimation is 104 weeks for mice. Adding on the 20-day gestation period gives 106.86 weeks, or 748 days.

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. In the PHG program, dose in units of milligram per unit bodyweight to the ¾ power is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-fourth power when animal potency is expressed in units (mg/kg-day)$^{-1}$:

$$q_{human} = q_{animal} \times (bw_h / bw_a)^{1/4}.$$

Human body weight (bw_h) is assumed to be 70 kg here.

In calculating dose the transplacental design of the Waalkes *et al*. experiment had to be taken into account. Four methods of dose averaging were employed to obtain doses in animal studies to apply in cancer potency estimation, and to estimate effective human dose.

Method 1: Average daily dose during lifetime of animal.

This assumes that there is no inherent age sensitivity to the carcinogenic stimulus and that the time of exposure during the study does not influence the outcome. To calculate average dose, the total dose applied is divided by the number of days the animals lived.

Low dose males: 95.6 mg/kg ÷ 566 days = 0.1689 mg/kg-day

High dose males: 191.3 mg/kg ÷ 566 days = 0.3380 mg/kg-day

Low dose females: 95.6 mg/kg ÷ 678 days = 0.1410 mg/kg-day

High dose females: 191.3 mg/kg ÷ 678 days = 0.2822 mg/kg-day

Method 2: Cancer susceptibility differs by age.

In recognition of possible increased susceptibility during gestation, it is assumed in this approach that susceptibility differs by age at exposure. For this example, it is assumed that the animals are a factor of 10 more sensitive than adults during gestation. To calculate "effective" dose received during the study, the average daily dose is multiplied by 10, since the animals were only exposed during gestation. Thus, effective doses are 1.689 and 3.38 mg/kg-day for high and low dose males, and 1.410 and 2.822 mg/kg-day for high and low dose females, respectively.

For humans, following draft U.S. EPA (2002) Supplemental Guidance, it is assumed during the period of prenatal and two years postnatal exposure that the child has ten times the sensitivity of the adult; from age three to 15, the child has three times the susceptibility of an adult; and at age 16 the child and adult have the same sensitivity.

Assuming humans are exposed to the same ppb concentration throughout life, the following factor is applied to the human potency estimated from mice by this method:

$$\{(10 \times ([9 \text{ mo.}/12 \text{ mo.}]) + 2) + (3 \times [15 - 2]) + (70 - 15)\}/70 = 1.736$$

Method 3: Doll-Armitage adjustment. 3rd power of age.

By this method the inherent differences in tissue susceptibility with age are not addressed, but time from dose to observation is. The Armitage and Doll (1954) mathematical description of carcinogenesis as expressed by Crouch (1983) and Crump and Howe (1984) allows for the analysis of data sets with variable dosing over time. The model assumes that cancer derives from a single cell after it has undergone a series of transformations. The model has been used to describe cancer dose response data in animal bioassays as well as in the general population.

Assumptions are required for the application of the Doll-Armitage model regarding: 1) the mathematical relationship between applied dose and the probability that a "stage transition" has occurred, 2) the stage affected by the carcinogen and 3) the number of "stages". Here, a linear relationship is assumed between dose and cell transformation, and arsenic is assumed to affect an early stage of the cancer process.

As discussed by Crouch (1983), if the probability per unit time of the stage transformation depends linearly on dose rate (*d(t)*), and the carcinogen only affects a single "stage," the probability of tumor by time T_e under Armitage and Doll (1954) becomes

$$P(Te) = 1\text{-}\exp[-(A + BD)]$$

with

$$D = \frac{1}{T^m \cdot \beta(m - j + 1, j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt$$

where T_e is the time to observation, and β is Euler's beta function. Following Anderson *et al.* (1983), the natural lifetime of the test animal, T, is assumed to be two years for rats and mice. The integer *m* (the number of "stages") specifies the rate of increase in incidence with time and *j* is the "stage" affected by the carcinogen. For this example arsenic is assumed to act early in the process only (*j* = 1). For *j* = 1, the solution to the equation describing the equivalent constant dose correction factor becomes

$$\frac{(T_e - a)^m - (T_e - b)^m}{T^m}$$

for a given time interval from *a* to *b*.

The value of *m* is often assumed to be 3.0 in analysis of animal data and characterization of cancer potency; this assumption was made for the purposes of this report since no contrary information was available.

Thus according to this approach the weighting factors applied to average dose for male and female mice are as follows:

Male: $\{[(566 - 8)^3 - [(566 - 18)^3]/ 566^3 \} \div \{10 \times 1/566\} = 0.0506/0.01767 = 2.8640$

Female: $\{[(678 - 8)^3 - [(678 - 18)^3]/ 678^3 \} \div \{10 \times 1/678\} = 0.0426/0.01767 = 2.8861$

Method 4: Pregnancy specific potency.

The final method only addresses susceptibility to arsenic during gestation. For the mouse gestation period of 20 days, doses received by animals during pregnancy are:

Low dose: 95.6 mg/kg ÷ 20 days = 4.78 mg/kg-day

High dose: 191.3 mg/kg ÷ 20 days = 9.565 mg/kg-day

A variant on the Methods 2 and 4 would be to assume that the most sensitive period is during organogenesis. In this case human exposures from day eight to the end of the first trimester and mouse exposures from days 5 or 6 to, say, day 15 would be weighted the most.

Table 27. Unit Risk ([μg/L]$^{-1}$) Predicted from Different Dose Averaging Methods Applied to the Waalkes *et al.* Transplacental Bioassay*

				Method 4
Ovarian adenoma and adenocarcinoma	6.4×10^{-4}	1.1×10^{-4}	2.2×10^{-4}	2.0×10^{-5}
Male liver adenoma or carcinoma	4.4×10^{-3}	7.7×10^{-4}	1.6×10^{-3}	1.6×10^{-4}
Male adrenal tumors	7.4×10^{-3}	1.3×10^{-3}	2.6×10^{-3}	2.7×10^{-4}

As may be seen in Table 27, method 2, which accounts for the greater sensitivity early in life and also the potential carcinogenicity in adulthood, gives estimates within an order of magnitude of that obtained from the analysis of human data – 2.7×10^{-4} (μg/L)$^{-1}$. In view of the uncertainties involved in both approaches, the results appear to be consistent with most of the unit risk estimates in the 1×10^{-4} to 1×10^{-3} (μg/L)$^{-1}$ range.

CALCULATION OF PROPOSED PHG

Non-Cancer Effects

The calculations of a public health protective concentration (C) of arsenic in water based on the non-cancer toxicity endpoints discussed above are summarized in Table 28. Three example calculations are given below each based on different dosimetry. Based on the Hanlon and Ferm (1986) study with a PBPK-adjusted oral minimally effective dose estimate of 2.8 mg/kg-d for fetal malformation, the value C is calculated as follows:

$$C = \frac{2.8 \text{ mg/kg-d} \times 60 \text{ kg} \times 0.2}{1000 \text{ UF} \times 2 \text{ L/d}} = 0.0168 \text{ mg/L} = 17 \text{ µg/L}$$

In this case 60 kg is the adult female body weight, 0.2 (20 percent) is the default relative source contribution for arsenic from drinking water; and the uncertainty factors are 10 for LOAEL to NOAEL, 10 for interspecies variation, and 10 for severity of effect. (A 1,000-fold UF is frequently used in extrapolating developmental and reproductive toxicity from animals to humans.) The daily total water intake is assumed to be 2 L/day.

Similarly, the LED_{01} values from the human studies of vascular toxicity and arsenic concentration in drinking water are used as follows:

$$C = \frac{166 \text{ µg/L} \times 0.2}{30 \text{ UF}} = 1.1 \text{ µg/L}$$

In this case the LED_{01} for cerebral infarct (Chiou *et al.*, 1997) is multiplied by a relative source contribution of 0.2 (20 percent) and divided by the UF product of 10 for point of departure (POD) to NOAEL and3 for interindividual differences. The use of 10 for the POD is due to the severity of effect, in this case stroke. No corrections are needed for body weight and water consumption because the exposure was to an adult human via the water route.

The LED_{01} for ischemic heart disease mortality (Chen *et al.*, 1996) and estimates of cumulative arsenic intake via drinking water were used in the following calculation:

$$C = \frac{5.5 \text{ (mg/L)yr} \times 0.2}{70 \text{ yr} \times 30 \text{ UF}} = 0.00052 \text{ mg/L} = 0.52 \text{ µg/L}$$

In this case the cumulative dose metric of (mg/L)yr is multiplied by the same relative source contribution of 0.2, then divided by the default 70 yr lifetime and the 30 UF product (10 for POD to NOAEL, 3 for interindividual differences to obtain the health protective concentration of arsenic in drinking water (0.5 µg/L rounded). Like cerebral infarct, ischemic heart disease mortality uses a UF of 10 for the POD to NOAEL adjustment, instead of three, to account for the severity of effect.

In Table 28 a number of relevant non-cancer endpoint calculations are summarized for comparison. The UF/Risk column lists the uncertainty factor applied to the dose response criterion, which along with the relative source contribution is used in the calculation of the health protective value as noted in the examples above. Also provided in the last two columns is a risk-based calculation, which uses the reciprocal of the LED as a slope in a linear extrapolation similar to a cancer potency calculation. These calculations are provided for comparison. Thus for the CVD endpoint the calculated health protective value of 0.0038 mg/L or 3.8 ppb is twice the $1x10^{-4}$ risk-based value of 1.9 ppb.

Table 28. Calculation of Health Protective Drinking Water Arsenic Concentrations Based on Non-Cancer Toxicity

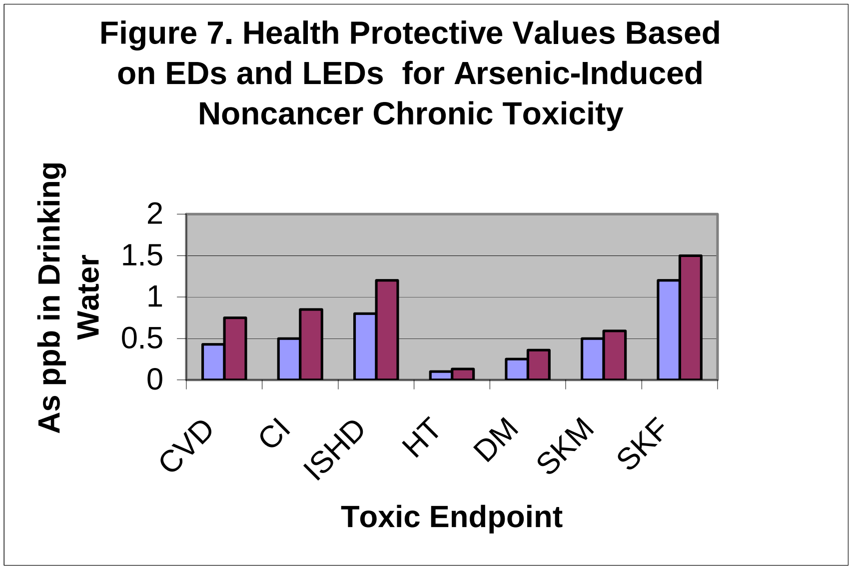
					Health Protective Concentration, (mg/L)
Hamster Hanlon & Ferm, 1986a	Fetal malformation	LOAEL, PBPK adjusted	2.8 mg/kg-d	1000	0.017
Dog Byron *et al.*, 1967	Death, anorexia, listlessness, weight loss, slight to moderate anemia	NOAEL	1.25 mg/kg-d	100	0.088
Rat Byron *et al.*, 1967	Decreased survival, weight loss, bile duct enlargement	NOAEL	3.12 mg/kg-d	100	0.22
Rhesus monkey Heywood & Sortwell, 1979	Sudden death without other clear clinical signs, possible CNS effects	LOAEL	2.8 mg/kg-g	1000	0.02
Human Tseng, 1977, Tseng *et al.*, 1968	Skin hyperpigmentation and keratoses	RfD US EPA	0.3 µg/kg-d	3	0.0021
Human Mazumder *et al.*, 1998	Skin keratosis	LED01	50 µg/L	10 1E-4*	0.0010 0.0005
Human Mazumder *et al.*, 1998	Skin keratosis	LED05	9.4 µg/kg-d	10 1E-4*	0.0066 0.0007
Human Chiou *et al.*, 1997	Cerebral infarct	LED01 LED01	166 µg/L 3.5 (mg/L)yr	30 1E-4* 30 1E-4*	0.0011 0.0016 0.000330 0.0024

					Health Protective Concentration, (mg/L)
	Cerebrovascular disease	LED01 LED01	189 μg/L 3.0 (mg/L)yr	10 1E-4* 10 1E-4*	0.0038 0.0019 0.00086 0.0021
Human Chen *et al.*, 1996	Ischemic heart disease mortality	LED01	5.5 (mg/L)yr	30 1E-4*	0.00052 0.00078
Human Chen *et al.*, 1995	Hypertension	LED10	7.2 (mg/L)yr	10 1E-4*	0.0020 0.00010
Human Rahman *et al.*, 1999	Hypertension	LED10	5.8 (mg/L)yr	10 1E-4*	0.0016 0.00008
Human Lai *et al.* 1994	Diabetes mellitus	LED05	8.8 (mg/L)yr	10 1E-4*	0.0025 0.00025
Human Rahman *et al.*, 1998	Diabetes mellitus	LED05	0.21 mg/L	10 1E-4*	0.0042 0.00042
Human Wang *et al.*, 2002	Carotid atherosclerosis (subclinical)	LOAEL estimated	20 (mg/L)yr	10	0.006
Human Tseng *et al.* 1996	Peripheral vascular disease	LOAEL estimated	20 (mg/L)yr	10	0.006
Human Siripitayakunkit *et al.* 1999	Developmental neurotoxicity, IQ deficit in children	LOAEL estimated & LED025	30 μg/L	30 1E-2*	0.0002 0.0003

*Note: Risk calculations assume low dose linearity, where x is the point of departure fraction, e.g., 0.01 for 1 percent, and risk is the criterion, e.g., 1E-4: risk*70yr/[(x/LEDx)*1000] for the cumulative dose metric and risk/x/LEDx for the water concentration metric.

The human studies analyzed by benchmark response (BMR) methodology appear to give the best basis for determining a health protective concentration for arsenic in drinking water. This is because they involve a dose response methodology that utilizes more of the available data and the health effects analyzed are of public health concern. In Figure 7 the LED and ED values for the various noncancer endpoints analyzed are compared. While the studies vary in quality from a statistical viewpoint, the ED/LED appear to be quite consistent across the wide range of endpoints, populations, studies, and methods employed.

Legend for Figure 7. Benchmark Dose Analysis of Noncancer Toxicity and Arsenic Exposure from Drinking Water: Cerebrovascular Disease (CVD), Cerebral Infarct (CI), Ischemic Heart Disease Mortality (ISHD), Hypertension (HT), Diabetes Mellitus (DM), Skin Keratosis in Males (SKM), Skin Keratosis in Females (SKF). In all cases the higher bar represents the ED value and the lower bar the LED value from the analysis of the respective endpoint.



The study by Chiou *et al*. (1997) appears to be the most robust of the studies evaluated. If the four values from this study (CVD and CI using the arsenic concentration, and cumulative arsenic exposure metrics) are averaged, a geometric mean (Gmean) of 1.0 µg/L is obtained. The single most representative value from the study is for CVD by the cumulative exposure metric, 0.86 µg/L. If the values for CI (0.33 ppb), ISHD (0.52 ppb), HT (2.0 ppb) and CVD (0.86 ppb) are averaged, a Gmean of 0.74 µg/L is obtained. A slightly higher value can be derived by including the data of Lai *et al.* (1994) and Rahman *et al*. (1998) on diabetes mellitus (1.21 ppb, µg/L). Calculated values for peripheral vascular disease and skin effects are somewhat higher at about 6 ppb. Thus, a level of 0.00086 or 0.0009 mg/L (rounded) (0.9 ppb) based on cerebrovascular disease (Chiou *et al*., 1997) would represent a suitable health protective value for non-cancer adverse health effects due to chronic intake of arsenic in drinking water.

$$C = \frac{3.0\ (\text{mg/L})\text{yr} \times 0.2}{70\ \text{yr} \times 10\ \text{UF}} = 0.00086\ \text{mg/L} \cong 0.9\ \text{ppb}$$

Cancer Effects

Lung and Bladder Cancer

Based on the unit risk for lung cancer derived from Figure 1, the health protective concentration (C) associated with the negligible cancer risk level (R) of $1x10^{-6}$ for consumption of inorganic arsenic in drinking water can be calculated as follows:

$$C = \frac{R}{CSF \times BR \times WCA} = \mu g/L$$

where,

R = *de minimis* cancer risk level (10^{-6});
CSF = cancer slope factor;
BR = background cancer rate;
WCA = water consumption adjustment between the two populations.

Therefore,

$$C = \frac{1x10^{-6}}{0.0075\ (\mu g/L)^{-1} \times 0.066 \times 0.5} = 4.0x10^{-3}\ \mu g/L\ (ppb)$$

Alternatively, the unit risk for lung and bladder cancer for combined sexes from Table 19 (U.S. estimate, bottom of table) of $2.7x10^{-4}$ $(\mu g/L)^{-1}$ can be used directly (i.e., 2.7/1000/10 µg/L), to calculate the health protective concentration:

$$C = \frac{1x10^{-6}}{2.7x10^{-4}\ (\mu g/L)^{-1}} = 3.7x10^{-3}\ \mu g/L = 4x10^{-3}\ \mu g/L\ (rounded)$$

If the California lung cancer background rate of 0.061 were used instead of the national rate, the value of C would be 10 percent higher, or $4.4x10^{-3}$ ppb. Also, if the Chilean populations exposed to arsenic had similar water consumption rates (L/kg-d), then removal of the water adjustment factor would lead to even lower values of C. These values are based on the data of Chen *et al.*, 1985, 1988b; Hopenhayn-Rich *et al.*, 1996, 1998; Smith *et al*., 1998; and Ferreccio *et al.*, 2000.

The above calculations assume low dose linearity of response. The concentrations associated with theoretical lifetime added lung cancer risks of $1x10^{-5}$ and $1x10^{-4}$ would be 0.04 and 0.4 µg/L (ppb), respectively. The actual risks at low concentration are not likely to exceed these estimates and may actually be lower or zero. The recent study of bladder cancer in the western U.S. by Steinmaus *et al*. (2003) would indicate a lower potency for this cancer but also an increased risk for smokers as seen with lung cancer. The data overall are insufficient to determine the shape of the dose-response relation below the region of direct observation, although theoretical arguments have been advanced to support low dose sublinearity (see discussion in next section). Based on these calculations a public health goal (PHG) of 4×10^{-6} mg/L, or 0.004 ppb (4 parts per trillion) is developed for arsenic in drinking water.

It should be noted that this level, associated with a *de minimis* lifetime cancer risk and based on data from Taiwan, Chile, and Argentina, is only two-fold higher than the value proposed by OEHHA in 1992 (OEHHA, 1992a), which was based on data from Taiwan, skin cancer risk, and semi-quantitative risk estimates for internal cancers. Although the shape of the cancer dose response relation at arsenic concentrations below the current MCL of 50 µg/L has been a contentious issue for many years, it seems likely that this relation is linear. Since there are non-water exposures to arsenic, primarily from food, it is likely that the low-level dose response for waterborne arsenic is incremental and linear for lung and bladder cancers (Crawford and Wilson, 1996; also see discussion below).

RISK CHARACTERIZATION

Non-Cancer Effects

There are a number of uncertainties associated with the non-cancer health protective water concentration of arsenic derived above. The use of human data, while more relevant, presents the usual problems of uncertain dosimetry. The studies of Chiou *et al.* (1997) and Chen *et al.* (1995, 1996) on vascular effects indicate a clear dose response for presumably causal effects. In general the application of the benchmark dose method is superior to that based on identifying a single dose NOAEL/LOAEL in that it uses more of the available data. The application of benchmark dose methodology to these and other data sets may be subject to uncertainty since exposures in these studies were often not assessed on an individual basis. Also there are methodological uncertainties. The current analysis uses an extra risk approach assuming that the background rates of disease endpoints in the exposed populations are the same as in the unexposed or control populations. The extra risk formulation is $[P(d) - P(0)]/[1- P(0)]$ where $P(d)$ is the proportion of subjects exposed to dose (d) that have an adverse response, and $P(0)$ is the background or control. This is a public-health-conservative approach that gives higher risk estimates for more common endpoints with higher background levels (U.S. EPA, 1996, 2000b). An alternate approach uses an additional risk formulation $[P(d) - P(0)]$ and dose specific background adjustments. The values obtained by this approach are generally within 50 percent of the values obtained by the current extra risk analysis. Of the nine data sets analyzed by the additional risk-dose-specific background method five gave higher (less health protective) values, three gave lower values, and one gave an identical value. The alternative values in the order presented in Table 23 are: skin keratosis 68 µg/L; CI, 2.0; CVD, 3.1; ISHD, 11.3; HT 10.6, 7.8; DM 6.5 (mg/L)yr, 209 µg/L.

The calculation of health protective values generally employed the default relative source contribution of 20 percent (0.2). Arsenic intake from the diet is likely the major source with estimates for North American diets ranging from 8 to 14 µg/d for inorganic arsenic (Yost *et al.*, 1998). The median drinking water concentration of arsenic from the ACWA (1995) study of small water systems was 2 µg/L. At 2 L/d water consumption this would be equivalent to an intake of 4 μg/d and a calculated RSC range of 22 to 33 percent. Higher levels of arsenic would increase the RSC and higher dietary contributions would decrease it. Also the diet may contain organic arsenic species which present uncertain risks. Since these estimates seemed close to the default, we decided to use the default to simplify the assessment.

The health protective values calculated in this assessment were not very different than those based on earlier evaluations and similar endpoints, e.g., the value based on U.S. EPA's oral RfD for arsenic of 0.0003 mg/kg-d (U.S. EPA, 1998). In general, the uncertainty factor used in the benchmark dose based assessments was 10, which was comprised of three (or half a log unit) for extrapolation from the chosen point of departure (POD) to an assumed no-effect level and three for inter-individual variation. In cases where the endpoint involved potentially incapacitating morbidity or mortality, i.e., stroke and heart attack, an additional UF of three was included for POD to NOAEL to account for severity of effect (total UF = 30). Lower UFs would give a higher health protective value. However, OEHHA is not convinced that lower values are justified at present. The effect level chosen for the different endpoints was based on the degree of response and was generally lower for the more serious effects, e.g., one percent each for cerebral infarct, ischemic heart disease mortality, and cerebrovascular disease.

Cancer Effects

Sources of Uncertainty in the Quantitative Risk Estimates

Quantitative cancer risk estimation for low-level environmental exposure to carcinogens involves many uncertainties. Nevertheless, systematic, logical and informed approaches to decision making about carcinogens in the environment call for quantitative assessments, because the absence of clearly definable thresholds does not permit identification of "safe" levels of exposure. Unfortunately, due to the frequent lack of sufficient data, assumptions have to be made in order to complete quantitative assessments of cancer risk. While these assumptions are based on scientific judgment, they may result in a higher degree of implied certainty in the overall assessment than is warranted. In order to capture this uncertainty in quantitative assessment the final results are often limited to a single significant figure. The estimate of cancer potency for environmental exposure levels may include zero since it is possible that a threshold or low dose sublinearity are present. Thus the estimate that lifetime exposure to 10 μg/L of inorganic arsenic in drinking water might increase cancer risks by as much as 2.5 per 1,000 should be qualified by stating that the increased lung cancer risks could fall below 2.5 per 1,000, but are unlikely to be higher.

Assumption that the Exposure-Response is Linear at Low Levels of Exposure.

The major single source of uncertainty in quantitative cancer risk estimation is the shape of the dose-response curve. It has been argued that the dose-response relationship between ingested arsenic and cancer may not be linear, rather a threshold or sublinear response may exist (Carlson-Lynch *et al.*, 1994; IARC, 1987; Lu, 1990). If this were the case, the assumption of linearity would overestimate risks. Currently, evidence is limited that a threshold or significant sublinear dose-response mechanism exists for ingested arsenic and cancer.

Dose-Response Relationships: Linear or Sublinear?

Figure 1 provides information showing that existing data do not support postulating departures from linearity. In fact, the study with the best exposure data, that of Ferreccio *et al*. (2000) suggests supralinearity at low dose, if anything. Nevertheless, the confidence intervals are broad (Figure 4), and therefore we would not use this study to propose supralinearity as the shape of the dose-response relation. We merely make this observation to support the point that the major question about linearity or non-linearity is in the direction of possible under-estimation of risks at low doses, which would pertain if the dose-response relationship were indeed supralinear.

Most other studies have employed ecological groupings rather than individual exposure data. However, quite extensive dose-response data are available for inhalation of inorganic arsenic and lung cancer risks.

It is reasonable to propose that the shape of the dose-response curve for lung cancer caused by arsenic inhalation would be similar to that for lung cancer and other cancers caused by ingestion of inorganic arsenic. Interestingly, as with water arsenic, the main question is whether or not dose-response relationships might be supralinear or linear (Hertz-Picciotto and Smith, 1993). There is no evidence to suggest sublinearity in the dose-response relationship. The findings using air measurements of arsenic inhalation were consistent with supralinearity in six studies conducted in three countries. One possible explanation is consistent overestimation of exposure at high air concentrations due to work practices to avoid exposure. This explanation is supported by one study that found supra-linearity using air measurements for exposure, but linearity when urine measurements of arsenic were used (Enterline *et al.*, 1987). Urine arsenic concentrations reflecting absorbed dose may give a better estimate of inhaled dose than measurements of air concentrations using fixed samplers. This would occur if workers tended to avoid the dustiest environments as much as possible during their workday. We believe this is the most likely explanation, and that the true dose-response relationship between inhaled arsenic dose and lung cancer risks is linear in the observable range, rather than supralinear. In any case, although the smelter studies involve a different pathway of exposure, they provide some evidence against expecting sublinearity in dose response for arsenic ingestion and lung cancer.

Examination of Epidemiological Evidence for a Threshold or Sublinearity Concerning Arsenic in Drinking Water

Two ecological analyses have suggested that the relationship between arsenic water concentrations and cancer occurrence in Taiwan is sublinear or has a threshold. Brown and Chen (1995) reanalyzed the Taiwanese data and concluded that there could be a threshold or sublinearity in the arsenic and cancer dose-response relationships. However, the reanalysis appears to have involved reclassifying village exposure and deleting villages according to post hoc criteria. A further ecological analysis has been presented for bladder cancer incidence data in Taiwan (Guo *et al.*, 1997). The investigators used a novel method for ecological data analysis. Superficial examination of the results suggests a threshold for arsenic water levels and bladder cancer. However, the unusual methods used were not accompanied by any results allowing the comparison of findings with other studies in Taiwan. Indeed, they would appear to be in conflict with them. In addition, it is not possible to derive relative risk estimates from this study. Furthermore, crudely derived estimates suggest that the model fitting was not appropriate. For these reasons, this study provides little, if any, evidence for nonlinearity in dose-response relationships for arsenic induced bladder cancer, let alone evidence of a threshold.

In contrast to these unusual ecological reanalyses of data from Taiwan, results of other epidemiological studies, including further studies in Taiwan, demonstrate it is unlikely that there is marked sublinearity and provide no evidence for a threshold. Skin cancer prevalence in Taiwan increased according to duration of residence in the area, duration of consumption of high-arsenic artesian well water, average arsenic water levels, and cumulative dose (Hsueh *et al.*, 1995) and possible skin cancer effects have been observed at low doses in the U.S. (Karagas *et al.*, 2001). Similar findings have been reported for lung and bladder cancer (Chiou *et al.,* 1995, 2001). Although variables were for the most part categorized into three levels, the findings generally demonstrated a monotonic dose-response relationship for both cancers by duration of exposure, average arsenic concentration in drinking water, and cumulative exposure. The results of the Chilean lung cancer case-control study with individual exposure data (Ferreccio *et al.*, 2000) are suggestive of supralinearity at low doses (< 200 µg As/L) with both cumulative and peak exposure and lung cancer risk but suffer from broad confidence intervals (Figure 4).

Although some disagree (e.g., Gebel, 2000), apart from two unusual ecological analyses of Taiwanese data, there are no data supporting either sublinearity or a threshold. This does imply that the results of these analyses should be excluded. However, in the absence of data supporting them, it is important to note that findings in various ecological studies, and limited findings with some individual data studies, support a monotonic dose-response relationship in the ranges of exposure considered thus far. In particular, the findings of the Chilean lung cancer case-control study (Ferreccio *et al.*, 2000) with individual exposure data are supportive of linearity over the entire dose range (Figure 4).

Arsenic Methylation Studies

The idea that a threshold exists for the carcinogenic effects of arsenic is most commonly based on the methylation process and the earlier belief in the relatively lower toxicities of MMA and DMA compared to 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 supposedly 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 (Hopenhayn-Rich *et al.*, 1993). On average, the results indicated that regardless of the internal or absorbed dose, the average proportions of inorganic arsenic, MMA and DMA (around 20 percent, 15 percent and 65 percent, 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 unexposed populations in Argentina, Finland, Nevada, and Taiwan have confirmed these findings and have provided substantial evidence that a threshold for arsenic methylation does not exist (Hopenhayn-Rich *et al.*, 1993; Hsueh *et al.*, 1998; Kurttio *et al.,* 1998; Warner *et al.,* 1994).

Arsenic methylation patterns were investigated in this cross-sectional study of two towns in Chile (Hopenhayn-Rich *et al.*, 1996a). The study included 122 people from a town exposed to high levels of arsenic and 98 people in a neighboring town with low levels of arsenic. Arsenic levels in drinking water were 600 µg/L and 15 µg/L, respectively. The corresponding mean urinary arsenic levels were 580 µg/L and 60 µg/L, of which 18.4 percent and 14.9 percent were inorganic arsenic, respectively. The main differences were found in the monomethylarsonate (MMA) to dimethylarsinate (DMA) ratio. High exposure, smoking, and being male were associated with higher MMA/DMA, while longer residence in the exposed town (Atacameño), ethnicity, and being female were associated with lower MMA/DMA. Overall, there was no evidence of a threshold for methylation capacity, even at very high exposures. This study, which is the largest study conducted involving metabolites of arsenic to date, confirmed conclusions made in our earlier publications that the methylation threshold hypothesis was not supported.

Hopenhayn-Rich *et al.* (1996b) conducted an intervention study of 73 participants (from the above cross-sectional study in Chile), who were provided with water of lower arsenic content (45 µg/L) for two months. Total urinary arsenic levels fell from an average of 636 µg/L to 166 µg/L. There was a small decrease from 17.8 percent to 14.6 percent in the percent of urinary arsenic in inorganic form consistent with what might be predicted from the cross-sectional study. Other factors such as smoking, gender, age, years of residence, and ethnicity were associated mainly with changes in the MMA/DMA ratio. The main difference was found for smokers, where practically all of the smokers showed a decrease in the MMA/DMA ratio. Much more variability was seen in the non-smokers. Overall, the changes in the observed percent inorganic arsenic and in the MMA/DMA ratio did not support an exposure-based threshold for arsenic methylation in humans. In addition, both this study and the cross-sectional study indicate that most of the inter-individual variability in the distribution of urinary metabolites remains unexplained.

Although the original methylation threshold hypothesis has been refuted, several other issues have been raised which 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^{III}) may be considerably more toxic than DMA (Thompson, 1993). If this is true, highly exposed populations such as those involved in the Taiwanese studies would not be able to "detoxify" arsenic as well 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 (Del Razo *et al.*, 1997; Hopenhayn-Rich *et al.*, 1996a,b), the association between elevated proportions of MMA and cancer has not been firmly established. The recent observation by Zakharyan *et al*. (2001) that human monomethylarsonic acid (MMA^{V}) reductase has a Km in the high mM range suggests that little MMA^{III} is produced relative to As^{III} and that MMA^{III} may not play a greater role in arsenic toxicity and carcinogenicity than As^{III}. Thus, based on our current knowledge, a sublinear dose-response relationship that would significantly affect the risk estimates based on linearity cannot be substantiated.

In addition to criticisms aimed at the linear dose-response model, questions have been raised about the comparability of the Taiwanese study population to citizens of the U.S. 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 (Carlson-Lynch *et al.*, 1994). 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 with sufficient protein. 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 (Vahter and Marafante, 1987). To what degree this effect occurs in humans is unknown, however. 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 (Hopenhayn-Rich *et al.*, 1996c). In addition, Mushak and Crocetti (1995) have estimated that less than one percent 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 (Yang and Blackwell, 1961) 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 (Engel and Receveur, 1993). Thus, it is unlikely that dietary deficiencies 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.

Finally in this section, uncertainties in dose-response modeling are discussed. This risk assessment is based on a simple linear dose-response assessment using relative risks and arsenic water concentrations. This model has been widely used in epidemiology for many years. Linearity is often seen in the observable range of exposures, as is the case here with lung cancer and arsenic in drinking water. However, a variety of mathematical models can be used. Morales *et al.* (2000) used 13 different mathematical models on the ecological data from Taiwan to provide a wide range of risk estimates for arsenic in drinking water, some higher than provided here and some lower. However, there is neither biological nor empirical basis for many of the models they used, so judgments based on the range of possible values have limited use.

Morales *et al.* (2000) also focused on Taiwan findings, leaving out unexposed comparison populations. This was due to a fear of confounding in comparing rural with general populations that include many urban dwellers. However, there is no basis for this concern. If anything, one would expect lung cancer (and bladder cancer) rates to be higher in urban dwellers than among rural dwellers since the smoking epidemic started and expanded most rapidly in urban dwellers. Furthermore, exposure to industrial carcinogens is more likely in urban than rural populations. By contrast, there must be a major concern about comparisons within the region containing the exposed villages. The reason for this is that many of those classified as having low exposures, based on measurements from wells used at one point in time only, could well have been exposed to arsenic in wells they drank from at earlier points in time, or when visiting other locations. Hence, the first three models used by Morales *et al.* (2000) based on inappropriate dose-response estimation without an unexposed comparison population do not warrant further consideration. Yet we reiterate that their findings include risk estimates both higher and lower than presented here. In the case of lung cancer, the water concentrations they linked to a one in 100 risk estimate ranged from eight µg/L to 396 µg/L. Considering men and women separately, of their 26 estimates of water concentrations producing a one in 100 risk of lung cancer, eight were below 50 µg/L and 11 were above 100 µg/L. We have estimated here a lung cancer risk of about 0.8 per 100 for men and one in 100 for women at 50 µg/L, which is near the middle of their range of estimates.

It should also be noted that there are serious disadvantages to the non-transparency of mathematical models that might be applied to epidemiological data. The linear dose-response model with relative risks can be used without accessing the original data, and in a manner that is easily reproducible by the reader of a risk assessment. Most epidemiological studies present data using this model, and the study publications themselves provide all the data required. Obtaining the original data, then using what on the surface may appear to be more sophisticated mathematical models, does not necessarily increase the accuracy of the risk assessment results. In fact, the converse can be the case; clearly mathematical modeling makes a risk assessment non-transparent since the reader is not able to check the findings presented.

Assumption that the Exposure Estimates are Representative of Actual Exposures.

There are some uncertainties regarding the actual exposures to arsenic in the drinking water. For example, the concentrations of arsenic in studies from Taiwan were based on measurements made at one point in time. In addition, the major ecological studies from Taiwan used in this risk assessment did not have individual data concerning which well(s) a person may have drank from either at present or in the past. However, repeated measures of arsenic in the drinking water have been made over the past 50 years in all major water sources in Region II of Chile. In the study of Ferreccio *et al.* (2000), lifetime residential histories were obtained from each participant. These could be linked to the water sources that were used in their county of residence. In this extremely dry desert region, most counties only had one major source of water. Thus, the quality of exposure data varies from study population to study population, yet the dose-response relationships for lung cancer, the major contribution to risk, are remarkably consistent. In any case, while it is possible that the exposures to inorganic arsenic in the drinking water studies were higher or lower, it is unlikely that actual exposures would be more than five times higher or lower than estimated. Consequently, the degree of uncertainty in the estimates of exposure is relatively low.

Uncertainties regarding the impact of the background rates of lung cancer

As discussed earlier, there are two main alternative approaches to human health risk assessment concerning background rates of cancer in the population for which estimates are derived. One is to ignore them. This approach basically assumes that the exposure of interest has an independent impact. In other words, the risks due to an exposure are based on that exposure alone and do not interact in either a synergistic or an antagonistic way with the background causes of the disease in the population. This is the most common approach to risk assessment, although it is often used without awareness of the assumption involved, that the exposure of interest acts independently of other causes of the disease in the population.

The alternative is based on noting that if a given exposure is synergistic with the background causes of a disease, then the impact of a given exposure will be increased in populations with high background exposures to the other agent, and therefore high background rates of the disease. If relative risk is used in a risk assessment, then it is apparent that the parameter itself is influenced by the background rate of disease. The relative risk estimates the rate of a disease in a population exposed at a given level divided by the background rate in those unexposed. However, what is often not appreciated is that if you derive relative risk estimates from one population, they are not transportable to another with a different background rate of disease unless the exposure of interest in synergistic with background causes to the extent the rate of disease is multiplicative with background rates. For example, if at a given level of exposure the relative risk is 1.5, then the impact on risk estimates for a population with twice the background rate of disease as another would be twice as great.

In this risk assessment we have noted evidence that arsenic is synergistic with smoking, the main background cause of lung cancer worldwide. Since the U.S. has a high background rate of lung cancer, this results in higher risk estimates for arsenic than if an independent risk model were used. Furthermore, in this model, risk among non-smokers would be less than among smokers. Since smoking is decreasing in the California population (www.dhs.ca.gov/tobacco/documents), population risks due to arsenic should reduce over time. The major uncertainty in this is that it is possible that risks are overestimated if it turns out that arsenic has an independent (nonsynergistic) effect in causing lung cancer. Furthermore, the risk will be overestimated if whatever synergy may exist with smoking is less than multiplicative with the background causes of disease.

Finally in this section, there are uncertainties related to the simple method of estimation of background rates of mortality and incidence used in this risk assessment. While estimates could be refined with lifetable analyses, these would not necessarily make the risk assessment more accurate. At the same time, they would certainly make the risk assessment more complicated to check. As with modeling dose-response, the goal of transparency of methods used in risk estimation greatly outweighs the questionable increase in validity by using models that are more complex. Any risk assessment requires projecting into the future. We do not know what the background rates will be in the future. At any one point in time, they are a complex combination of age effects, birth cohort effects, and changes in exposure to risk factors. It seems better to take a cross-sectional snapshot with simple current data, than to pretend that life-table analysis, which must itself use current or past age-specific rates to project into the future, would increase accuracy.

Assumption that lung and bladder cancer mortality are the greatest contributors to the cancer potency estimates

We have assumed that the risks of lung and bladder cancer mortality will be the largest contributing factors in the derivation of the cancer potency estimates and that the exclusion of skin and kidney cancer deaths will not greatly affect the results. Table 21 shows the excess deaths for lung, bladder, kidney, and skin cancer due to arsenic in the drinking water from the three populations included in the risk assessment. Note that the percentages of lung and bladder cancer deaths combined range from 84 percent to 96 percent for men and 77 percent to 86 percent for women. The percentages of kidney and skin cancer deaths combined range from less than 6 percent (Argentina) to 16 percent (Taiwan) for men and 10 percent to 14 percent for women. Deaths from kidney cancer vary from approximately two to three times higher among women as compared to men in the three regions studied. No explanation is currently available for this difference.

To illustrate the impact on the cancer risk estimates, we incorporated the added risk of dying from kidney and skin cancer in the same manner as that applied for bladder cancer. The ratio of the total excess cancer deaths relative to excess lung cancer deaths from all studies combined (excess lung, bladder, kidney, and skin cancer deaths divided by excess lung cancer deaths) is 1.5 (1,363/936) for males and 2.0 (743/366) for females. The estimates of lifetime added lung, bladder, kidney, and skin cancer risk are shown in Table 29. The potency estimates obtained based on lung and bladder cancer deaths are presented for comparison. The calculations were made using California background lung cancer mortality rates. When all cancer sites are included, the estimate of lifetime added cancer risk per 1,000 persons exposed to 50 μg/L arsenic in the drinking water is 9.8 for men (6.5 x 1.5) and 21 for women (10.5 x 2). For a lifetime exposure to 10 μg/L, the risks are 2.0 per 1,000 for men and 4.2 per 1,000 for women. These estimates are 25 percent higher than the potency calculations based on lung and bladder cancer mortality for women and 18 percent higher for males. The larger estimate for women relative to men is the result of the greater number of excess kidney cancer deaths in each of the populations included in the risk assessment. The estimate of lifetime added cancer risk per 1,000 persons exposed at 50 μg/L for both sexes combined is 15.4 when all cancer sites are included versus 12.7 when the potency estimate is based solely on lung and bladder cancer mortality (approximately 20 percent higher).

Table 29. Estimates of Excess Lung and Bladder Cancer Risk Due to Arsenic in the Drinking Water Compared to All Internal Cancers Combined Using California Background Lung Cancer Mortality Rates*

			Lung, Bladder, Kidney, Skin Cancer	
				Females
				21.0
				4.2
			15.4	
			3.1	

*Hopenhayn-Rich *et al*., 1996, 1998; Smith *et al*., 1998; Chen *et al*., 1985.

Uncertainties regarding the use of background mortality rates rather than background incidence rates in the potency calculations

Because lung cancer is highly fatal, we have assumed that mortality gives a reasonable estimate of incidence. It should also be noted that the relative risks from each of the studies included in the risk assessment are based upon cancer mortality not cancer incidence. There is evidence that arsenic may have a greater impact (in terms of relative risk) on bladder cancer mortality than it does on bladder cancer incidence. Preliminary results from Argentina and Chile suggest that arsenic may cause bladder tumors that are more aggressive, and if so, more fatal than non-arsenic related bladder cancers. If this is the case, incorporating background bladder cancer incidence in the calculation of cancer potency in place of background bladder cancer mortality will overestimate the true risks.

Table 30 shows lung and bladder cancer cases and deaths for men and women in the state of California in 1996. The ratios of lung cancer cases to lung cancer deaths are 1.2 for men and 1.3 for women, while the ratios of bladder cancer cases to bladder cancer deaths are 5.6 for men and 4.0 for women. To examine how the potency estimates might change when incidence rates are used, we incorporated background lung and bladder cancer incidence data for California into the risk assessment and compared these results to the added lifetime risk based on lung and bladder cancer mortality. As stated previously, the studies included in the risk assessment examined cancer mortality rather than incidence. We approximated what the incidence of bladder and lung cancer might be in these populations by multiplying the excess deaths (Table 21) by the ratios of lung and bladder cancer incidence to lung and bladder cancer deaths for California, respectively (Table 29). These results were used to calculate the ratios of excess lung and bladder cancer cases divided by the excess lung cancer cases in order to incorporate the added risk of developing bladder cancer in the overall estimate of lifetime added cancer risk.

Table 30. Comparisons of Background California Cases and Deaths of Lung and Bladder Cancer in 1996 with Approximate Total Excess Cancer Cases from Arsenic Studies in this Risk Assessment*

Lung Cancer									
Number of Cases in California 1996		Number of Deaths in California 1996		Ratio of California Cases to Deaths		Total Excess Deaths from All Arsenic Studies		Approx. Number of Total Cases for All Arsenic Studies	
Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
9,384	7,787	7,597	6004	1.2	1.3	936	366	1,123	476
Bladder Cancer									
Number of Cases in California 1996		**Number of Deaths in California 1996**		**Ratio of California Cases to Deaths**		**Total Excess Deaths from All Arsenic Studies**		**Approx. Number of Total Cases for All Arsenic Studies**	
Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
4,024	1,314	720	326	5.6	4.0	300	225	1680	900

*Hopenhayn-Rich *et al*., 1996, 1998; Smith *et al*., 1998; and Chen *et al*., 1985.

When background lung cancer incidence is incorporated into the calculations, the estimate of lifetime added lung cancer risk per 1,000 persons exposed to 50 µg/L arsenic in the drinking water is 7.8 for men (6.5 x 1.2) and 13.6 for women (10.5 x 1.3) (Table 31). For a lifetime exposure to 10 µg/L, the risks are 1.6 per 1,000 for men and 2.7 per 1,000 for women. These results are 20 percent higher (i.e., 1.2-fold) than the results based on background lung cancer mortality for men and 30 percent higher (i.e., 1.3-fold) than the potency estimates for women. The estimates of lifetime added lung and bladder cancer risk for males and females based on background lung and bladder cancer incidence for California were also derived from the values in Table 30 and are presented in Table 31. The lifetime added lung and bladder cancer risks per 1,000 persons exposed to 50 µg/L of arsenic in the drinking water were 19.5 for males (7.8 x 2.5) and 39.4 (13.6 x 2.9) for females. Exposure to a daily intake of 10 µg/L reduces these risk estimates to 4.0 per 1,000 in males and 7.8 per 1,000 in females. These estimates are about two and a half times higher than the potency calculations based on lung and bladder cancer mortality. The average estimates of lifetime added lung and bladder cancer risk for both sexes combined are 29.4 in 1,000 when the arsenic exposure is 50 µg/L per day and 5.9 per 1,000 at 10 µg/L per day. These estimates are about two times higher. However, as noted above, relative risk estimates from studies of bladder cancer mortality may lead to overestimation of risks incorporating bladder cancer incidence.

Table 31. Estimate of Excess Lung and Bladder Cancer Risk due to Arsenic in the Drinking Water Using Background Cancer Incidence*

		Females
		10.5
		2.1
		1.3
		13.6
		2.7
		2.9
		39.4
		7.8
	29.4	
	5.9	

*Hopenhayn-Rich *et al*., 1996, 1998; Smith *et al*., 1998; and Chen *et al*., 1985.

Assumption that daily water consumption rates in the study populations were approximately two liters per day

Estimates on average daily drinking water consumption from the study populations included in the risk assessment were not obtainable. Instead, we used data on daily drinking water intake from various arsenic studies in similar regions in South America. No data were available from Taiwan. Previous risk assessments of arsenic have assumed that Taiwanese males drink on average 3.5 liters of water per day throughout their lives (U.S. EPA, 1998). This estimate by U.S. EPA appears to be based on the assumption that men in this population performed heavy outdoor work in a very hot climate. It was not derived from actual field studies. Taiwanese females were said to consume two liters of water per day. Note that we used this same assumption of drinking water intake for females in the present risk assessment. Even if the amount of water consumed per day were greater in the populations on which the risk assessment is based relative to the assumption of 2.3 liters/day for males and 2.0 liters/day for females, the actual amounts consumed are not likely to be more than two or three times greater. As a result, the uncertainty in the drinking water consumption rates and its subsequent impact on the cancer risk assessment is quite low.

It is interesting to note that the participants from a current study in India (West Bengal) where the climate is more similar to that of Taiwan compared to Argentina and Chile had drinking water consumption rates similar to the results from South America (Table 22). The water intake for men was 2.6 liters/day and 2.1 liters/day for females. The evidence suggests that uncertainties about the volume of water consumption in the study populations are not very important.

Assumption that the observed lung and bladder cancer risks in the study populations are not greatly influenced by inorganic arsenic in food

Little information is available concerning arsenic concentrations in food sources in the study populations. All evidence to date suggests that the overriding exposure to inorganic arsenic was from the drinking water. However, local contamination of food sources could lead to a small overestimate of the cancer risks if the concentration in food is correlated with that in local water sources. This would mean that the exposures to inorganic arsenic would be underestimated, and hence the risks overestimated. However, if food is widely distributed so that its arsenic concentration is not correlated with that in local water sources, then all doses would be underestimated, but the slope of the relative risk dose-response relationship would not be affected.

Note that the food intake of inorganic arsenic in the U.S. does not impact the cancer potency estimates derived in this risk assessment because we have calculated the *incremental* lifetime lung and bladder cancer risks resulting from the ingestion of inorganic arsenic in the drinking water. Considered overall, there is no basis for attributing much uncertainty in arsenic risk estimates for drinking water to arsenic contamination of food.

SUMMARY AND COMPARISON OF RECENT RISK ASSESSMENTS

This risk assessment has derived a PHG of 4 ppt based on a unit risk of 2.7×10^{-4} $(\mu g/L)^{-1}$ and a negligible theoretical lifetime cancer risk level of 1×10^{-6}. The unit risk was based on linear regression analysis of lung and urinary bladder cancer mortality data in epidemiological studies in Taiwan, Chile, and Argentina and background mortality rates for these cancers in the United States. Other estimates of unit risks include: 2.6×10^{-4} $(\mu g/L)^{-1}$ based on California mortality rates; 3.1×10^{-4} $(\mu g/L)^{-1}$ based on the sum of lung, bladder, skin, and kidney cancer mortality; and 5.9×10^{-4} $(\mu g/L)^{-1}$ based on lung and bladder cancer incidences rather than mortality. Unit risk estimates based on a transplacental carcinogenicity assay in mice were generally in the 1×10^{-4} to 1×10^{-3}

$(\mu g/L)^{-1}$ range for various tumors and dose averaging methods. Thus the range of plausible PHGs based on these unit risks is 1.7 to 3.8 ppt. The latter figure rounded to one significant figure is considered the most robust estimate in this assessment (see discussion of sources of uncertainty above).

The National Research Council in their "Arsenic in Drinking Water: 2001 Update" (NRC, 2001) concluded that fitting the additive Poisson model with a linear term for dose to the lung and urinary bladder cancer incidence data from southwestern Taiwan to estimate ED_{01} values at specific As levels of interest (i.e., 3, 5, 10, and 20 μg/L) was the preferred analytical approach to assessing human cancer risk. They estimated excess lifetime risks/10,000 people exposed to 10 μg As/L of 14 to 18 for lung cancer and 23 to 12 for bladder cancer in males and females, respectively. Assuming linear low dose extrapolation, these values would correspond to unit risks of 1.2×10^{-4} to 2.3×10^{-4} $(\mu g/L)^{-1}$ for lung cancer and 1.4×10^{-4} to 1.8×10^{-4} $(\mu g/L)^{-1}$ for bladder cancer. These values are quite close to our estimate of a combined incidence-based unit risk (i.e., the sum of the NRC figures = 6.7×10^{-4} vs. 5.9×10^{-4} $(\mu g/L)^{-1}$). The NRC, while acknowledging that the analysis of the Chilean data (Ferreccio *et al*. 2000) "is the only study available for risk assessment that has individual estimates of exposure on all subjects for more than 40 years," faulted the study for "methods used for control selection." NRC concluded that the Ferreccio *et al.* (2000) study "can be used in a quantitative assessment of risk of arsenic in drinking water, along with data from other selected studies." In effect, that is what the present assessment has done.

The U.S. EPA (2001) in their Final Rule on arsenic in drinking water assumes an average water consumption of 1.0 and 1.2 L/d for tap and total water and 90th percentile values of 2.1 and 2.3 L/d, respectively. For cancer risks the Agency has essentially used risk estimates for lung and bladder from Morales *et al*. (2000). That assessment is similar in approach to the NRC assessment described above and is based entirely on data from Taiwan. At 10 μg As/L, U.S. EPA estimates the mean population cancer risk as 2.41×10^{-4} to 2.99×10^{-4} and the 90th percentile upper bounds as 5.23×10^{-4} to 6.09×10^{-4}. Assuming low dose linearity of dose response these values would correspond to unit risks of 2.41×10^{-5} to 6.09×10^{-5} $(\mu g/L)^{-1}$, equivalent to negligible risk drinking water concentrations of 16 to 40 ppt. Thus the U.S. EPA risk estimates are about four to ten times less than those of OEHHA in the present document. As noted elsewhere in this document, Morales *et al*. (2000) estimated a broad range of risks depending on the mathematical model that was fit to the data sets and what comparison population was used. For lung cancer without a comparison population, Morales *et al.* (2000) estimated LED_{01}s (lower bounds on the ED_{01}s) of 213 to 396 μg/L. With a Taiwanese comparison population, these values were 6 to 196 μg/L and with a southwestern Taiwanese comparison population 8 to 181 μg/L. The combined tumor LED_{01} estimates with a Taiwanese comparison population ranged from 2 to 106 μg/L, values which if extrapolated to negligible risk levels would bracket the estimates in the present assessment (i.e., 0.2 to 11 ppt).

OTHER REGULATORY STANDARDS

ATSDR has derived a chronic oral MRL of 0.0003 mg As/kg-d for inorganic arsenic. This MRL is based on a NOAEL of 0.0008 mg As/kg-d observed in a large Taiwanese population exposed to arsenic via drinking water (Tseng, 1977; Tseng *et al.*, 1968; ATSDR, 1997).

U.S. EPA has derived a chronic and oral reference dose (RfD) of 3×10^{-4} mg/kg-d based on the NOAEL of 0.0008 mg/kg-d and a LOAEL of 0.014 mg/kg-d (Tseng, 1977; Tseng *et al.*, 1968). The adverse effects noted in the study were skin keratoses and hyperpigmentation, and possible vascular effects (U.S. EPA, 1998).

U.S. EPA has established a primary national drinking water regulation (NPDWR or MCL) of 0.05 mg/L. An oral slope factor of 1.5 $(mg/kg\text{-}d)^{-1}$ and a drinking water unit risk of 5×10^{-5} $(\mu g/L)^{-1}$ have also been determined, giving 10^{-4} to 10^{-6} risk levels of 2 to 0.02 ppb (U.S. EPA, 1998). The Maximum Contaminant Level Goal (MCLG) for arsenic in drinking water was set at zero. U.S. EPA more recently proposed a national primary drinking water regulation (MCL) of 0.01 mg/L (10 ppb) and a health-based non-enforceable MCLG of zero for arsenic in drinking water (U.S. EPA, 2001). This rule was subsequently withdrawn for additional evaluation, then reinstated.

U.S. EPA has also established ambient water quality criteria for arsenic of 0.0022 ppb for ingestion of water and aquatic organisms, and 0.0175 ppb for ingestion of aquatic organisms only.

The World Health Organization has adopted a guideline for drinking water quality of 0.01 mg/L (10 ppb) based on a lifetime skin cancer risk of 6×10^{-4} (WHO, 1993).

The State of California currently has an MCL of 50 ppb for arsenic in drinking water. In 1992 OEHHA proposed a recommended public health level (RPHL) of 0.002 ppb for arsenic in drinking water based on a negligible risk estimate for lifetime extra risk of skin and internal cancers (OEHHA, 1992).

The oral cancer potencies of 2.7×10^{-4} $(\mu g/L)^{-1}$ and 9.5 $(mg/kg\text{-}d)^{-1}$ derived in this document are comparable to the inhalation potencies of 3.3×10^{-3} $(\mu g/m^3)^{-1}$ and 12.0 $(mg/kg\text{-}d)^{-1}$ derived earlier by this office (OEHHA, 1999).

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