EPA

TOXICOLOGICAL REVIEW

OF

INGESTED INORGANIC ARSENIC

(CAS No. 7440-38-2)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

July 2005

NOTICE

This document is an **IRIS review draft for the Science Advisory Board (SAB)**. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to inorganic arsenic. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of inorganic arsenic.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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Reviewers

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

List of Abbreviations

8-OHdG	8-hydroxydeoxyguanosine
AIC	Akaike Information Criterion
ATSDR	Agency for Toxic Substances and Disease Registry
As^{III}	arsenite
As^{V}	arsenate
BFD	blackfoot disease
BMD	benchmark dose
BMD_{10}	benchmark dose at 10% effect
BMDL	lower 95% confidence limit on the benchmark dose
BMDS	benchmark dose software
BMI	body mass index
BrdU	bromodeoxyuridine
CA	chromosome aberrations
CASRN	CAS registry number
CCR	chromate copper arsenate
CHO	Chinese hamster ovary
CI	confidence interval
COPD	chronic obstructive pulmonary disease
DAAC	diseases of the arteries, arterioles and capillaries
DHLP	dihydrolipoic acid
DMA^{III}	dimethylarsenous acid
DMA^{V}	dimethyl arsinic acid
DMA	dimethyl arsenic-used when the oxidative state is unknown or not specified
DMPS	2,3-dimercaptopropane-1-sulfonic acid
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
ED	effective dose
EKG	electrocardiogram
eNOS	endothelial nitric oxide synthase
EPA	Environmental Protection Agency
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GI	gastrointestinal
GM-CSF	granulocyte macrophage-colony stimulating factor
GSH	glutathione
GSSG	oxidized glutathione
HSDB	Hazardous Substance Data Base
IGF-1	insulin-like growth factor 1
IGFBP-1	IGF-1 binding protein
IRIS	Integrated Risk Information System
ISHD	ischemic heart disease

JNCV	Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure
LD_{50}	lethal dose 50%
LOAEL	lowest-observed-adverse-effect-level
MH-PR	Mantel Haenzel-weighted prevalence ratios
MMA^{III}	monomethylarsonous acid
$MMA^{III}O$	methylarsine oxide
MMA^{V}	monomethyl arsonic acid
MMA	monomethyl arsenic-used when oxidative state is unknown or not specified
MN	micronuclei
MNU	*N*-methyl-*N*-nitrosourea
NCI	National Cancer Institute
NO	nitric oxide
NOAEL	no-observed-adverse-effect-level
NRC	National Research Council
ODC	ornithine decarboxylase
PBPK model	physiologically based pharmacokinetic model
PKG	phosphoglycerate kinase
PNP	purine nucleoside phosphorylase
RBCs	red blood cells
RfD	oral reference dose
RfC	inhalation reference concentration
ROS	reactive oxygen species
RR	relative risk
SAM	S-adenosylmethionine
SCE	sister chromatid exchange
SMR	standard mortality ratio
SOD	superoxide radical dismutase
TGF-α	transforming growth factor-alpha
TMAO	trimethylarsine oxide
TNF-α	tumor necrosis factor-alpha
TPA	12-*O*-tetradecanoyl phorbol-13-acetate
USGS	U.S. Geological Survey
UVR	ultraviolet radiation

1. INTRODUCTION

This assessment is based on the EPA-sponsored reviews "Arsenic in Drinking Water" and "Arsenic in Drinking Water, 2001 Update" published by the National Research Council in 1999 and 2001, respectively. The NRC arsenic committee took into consideration presentations at the committee's public meetings, submitted public comments, and the comments made by technical experts on the draft NRC arsenic reports. The conclusions, recommendations and final content of the NRC (1999, 2001) reports rest entirely with the committee and the National Research Council. This IRIS document has undergone review by EPA health scientists from several program offices and regional offices.

The goal of this IRIS assessment is to provide a summary, in the format needed for the IRIS system, of the key science analysis and recommendations for risk assessment methodology developed by the NRC. This assessment also incorporates EPA's review of literature on arsenic's health effects published after the publication of the NRC 2001 update. This new information is incorporated into the assessment text as appropriate, but did not indicate a need for any fundamental changes vis-a-vis the recommendations developed by the NRC. The quantitative cancer risk assessment in this assessment tracks NRC recommendations on cancer dose response modeling and risk assessment. This assessment does include additional sensitivity analysis on effects of modeling assumptions on estimated cancer risk. The NRC text recommends such sensitivity analysis. The recommended cancer unit risk estimate in this IRIS assessment is based on the NRC's preferred approach (as presented in the Summary of the 2001 Update). The NRC did not develop quantitative risk assessments for noncancer health effects of arsenic. By comparison this IRIS assessment does develop a reference dose (RfD) to address noncancer health effects of arsenic.

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment. The majority of arsenic exposures discussed in this Toxicology Review are from oral waterborne arsenic exposure. Although inhalation exposure is already in IRIS, it will not be addressed or revised in this report.

The RfD provides quantitative information for noncancer dose-response assessments. It is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime.

The carcinogenicity assessment in this report provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk

from oral exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The unit risk is the quantitative estimate in terms of either risk per μg/L drinking water or risk per μg/m3 air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for inorganic arsenic has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). EPA guidelines that were used in the development of this assessment may include the following: Guidelines for the Health Risk Assessment of Chemical Mixtures (U.S. EPA, 1986a), Guidelines for Mutagenicity Risk Assessment (U.S. EPA, 1986b), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996), Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998a), Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), (proposed) Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995), Science Policy Council Handbook: Peer Review (U.S. EPA, 1998b, 2000a), Science Policy Council Handbook: Risk Characterization (U.S. EPA, 2000b), Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c) and Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (U.S. EPA 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk also was considered in the development of this document. The relevant literature was reviewed through December, 2004.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Properties

Arsenic is a metalloid that can exist in the -3, 0, +3, and +5 oxidation states. The arsenite (+3) and arsenate (+5) forms are the primary forms found in drinking water. The chemical and physical properties of arsenic are listed in Table 2-1 below.

Table 2-1. Chemical and Physical Properties of Arsenic and Selected Inorganic Arsenic Compounds (ATSDR, 2000; Merck Index, 1989)

	As	As_2O_3	As_2O_5	$NaAsO_2$	Na_2HAsO_4
CAS No.	7440-38-2	1327-53-3	1303-28-2	7784-46-5	7778-43-0
Valance	0	+3	+5	+3	+5
Molecular Weight	74.9	197.8	229.8	129.9	185.9
Synonyms	Metallic arsenic, gray arsenic	Arsenic trioxide, arsenolite, white arsenic (+3)	Arsenic pentoxide, arsenic acid anhydride (+5)	Sodium arsenite (+3)	Disodium arsenate (+5)
Physical State (25°C)	Solid	Solid	Solid	Solid	Solid
Boiling Point (°C)	613 (sublimes)	465	---	---	---
Melting Point (°C)	817 @ 28 atm	312	315 (decompose)	---	86.3
Density	5.727	3.738	4.32	1.87	1.87
Vapor Pressure (20°C)	---	---	---	---	---
Water Solubility (g/100 mL)	Insoluble	3.7 @ 20 °C; 11.5 @ 100 °C	150 @ 16 °C; 76.7 @ 100 °C	Very soluble	Very soluble
Log Octanol/Water Partition – Coefficient (log K_{ow})	---	---	---	---	---
Taste Threshold	---	---	---	---	---
Odor Threshold	---	---	---	---	---
Conversion Factor	---	---	---	---	---

--- No data available.

Uses

The metalloid, arsenic, is used as an alloying constituent in metallurgy for hardening copper and lead alloys (HSDB, 2005). It also is used in glass manufacturing (decolorizing and refining agent), as component of electrical devices, in the semiconductor industry, and as a catalyst in the production of ethylene oxide. Other arsenic compounds are used in preserving hides, as a mordant in the textile industry, medicinals, pesticides, pigments, and wood preservatives. Approximately 90% of the domestic consumption of arsenic is in the production of wood preservatives (e.g., chromate copper arsenate [CCR]; ATSDR, 2000), which is currently being phased out.

Occurrence

Because arsenic naturally comprises 0.0005 % (2 μg/g) of the earth's crust, where it is the twentieth most abundant element, background levels of arsenic occur in all environmental media (ATSDR, 2000; Merck Index, 1989). Therefore, low concentrations of arsenic are a natural component of water, food, soil, and air. However, industrial activities such as refining and smelting operations have increased the concentration of arsenic in the environment, often resulting in toxic concentrations of arsenic in soil, air, and water (Adams et al., 1994). In addition, certain geographic areas have high levels of arsenic in their underground rock formation, which can be leached and cause high arsenic concentrations in drinking water (ATSDR, 2000).

The highest background arsenic levels found in the environment are in soils, at a mean concentration of approximately 5,000 parts of arsenic per billion parts of soil (ppb) (ATSDR, 2000). Food and drinking water also typically contain arsenic concentrations of 20 to 140 ppb (highest in shellfish and other marine foods) and <50 ppb, respectively (ATSDR, 2000). The majority of surface and ground waters contain less than 10 μg arsenic/L (although levels of 1,000-3,400 μg/L have been reported, especially in areas of the western U.S.), and several studies suggest that most (>90%) ground and surface drinking water systems contain less than 5 μg/L of arsenic (ATSDR, 2000). Mean arsenic concentrations in ambient air have generally been found to range from <0.001 to 0.003 micrograms per cubic meter (μg/m^3) in remote areas and from 0.02 to 0.03 μg/m^3 in urban areas, but can range much higher, e.g., up to 2.5 μg/m^3 near nonferrous metal smelters (ATSDR, 2000).

Environmental Fate

Arsenic as a free element (0-oxidation state) is rarely encountered in the environment (HSDB, 2005). Under normal conditions, arsenic is present as soluble inorganic arsenate (+5-oxidation state) because it is more thermodynamically stable in water than arsenite (+3-oxidation state).

Arsenic is largely immobile in agricultural soils, and tends to remain and concentrate on the soils surface indefinitely (ATSDR, 2000). However, the downward migration of arsenic is much greater in sandy soil compared to clay loam (ATSDR, 2000). The most influential parameter affecting arsenic mobility is the iron content of the soil. Leaching of arsenic from polluted soil is generally low (ATSDR, 2000).

3. TOXICOKINETICS

3.1. ABSORPTION

The majority of arsenic exposures discussed in this Toxicology Review are from oral waterborne arsenic exposure. Although inhalation exposure also is common, it will not be addressed specifically in this report. Dermal exposure and exposure from food, however, need to be addressed as they are possible confounding variables in epidemiology studies. There is a lack of information on the bioavailability of inorganic arsenic in various types of food (NRC, 1999; 2001). Although there have been no studies performed on the rate of inorganic arsenic absorption through intact human skin, systemic toxicity in people dermally exposed to inorganic arsenic solutions indicates the skin as an exposure route (Hostynek et al., 1993). The systemic absorption via the skin appears to be low (NRC, 1999) as is demonstrated by an *in vitro* study by Wester et al. (1993) where 2-6% of radiolabeled arsenate (as a water solution) was absorbed by human and rhesus monkey skin over a 24-hour period. Mouse dorsal skin was demonstrated to absorb 30% (Rahman et al., 1994) through similar *in vitro* testing. This study also demonstrated that 60-90% of the absorbed arsenic was retained in the skin. NRC (1999) suggests this indicates that inorganic arsenic binds externally to skin and hair.

Water soluble forms of inorganic arsenic (both trivalent and pentavalent) are readily absorbed (about 80-90%) in experimental animal models as well as humans (Pomroy et al., 1980; Freeman et al., 1995). Monomethyl arsonic acid (MMA^V) and dimethyl arsinic acid (DMA^V) also appear to be well absorbed in humans and experimental animals (75-85%; Stevens et al., 1977; Buchet et al., 1981; Yamauchi and Yamamura, 1984; Marafante et al., 1987; Yamauchi et al., 1988). Gastrointestinal absorption of low-solubility arsenic compounds such as arsenic trisulfide, lead arsenate, arsenic selenide gallium arsenide (Mappes, 1977; Marafante et al., 1987; Webb et al., 1984; Yamauchi et al., 1986), and of arsenic-contaminated soil (Freeman et al., 1995), is much lower than soluble inorganic arsenic. However, the degree of absorption for arsenic-contaminated soil is dependent on the form of arsenic in the soil and on the type of soil.

Harrington et al. (1978) compared a group of people in Fairbanks, Alaska who had arsenic contaminated water (345 µg/L) in their home, but drank only bottled water, to a group of people who had less than 50 µg arsenic/L in their home water. The results demonstrated that the group with high arsenic in their water had the same average concentration of total arsenic metabolites in their urine (i.e., 43 µg/L) as the group with less than 50 µg/L in their

home water (i.e., 38 μg/L in urine), indicating external absorption via bathing or other exposure sources. In addition, the group with high arsenic in their water had elevated hair arsenic concentrations associated with bathing, suggesting that arsenic was bound externally to hair and probably also to skin during washing with arsenic-rich water.

3.2. DISTRIBUTION

The retention and distribution of arsenic are determined mainly by its chemical properties. While both arsenite (As^{III}) and arsenate (As^{V}) bind to sulfhydryl groups, As^{III} has approximately a 10-fold greater affinity for sulfhydryl groups than As^{V} (Jacobson-Kram and Montalbano,1985), which has properties similar to those of phosphate. Cellular uptake rates and resulting tissue concentrations are substantially lower for the pentavalent than for the trivalent forms of arsenic, and in terms of cellular efflux of methylated species, DMA appears more readily excreted than MMA (NRC, 2001). Liu et al. (2002) found arsenite to be transported into cells by aquaglycoporins 7 and 9, which have been associated with the transport of glycerol. Arsenate, however, has been suggested to be transported by the phosphate transporter (Huang and Lee, 1996). Retention of arsenic can vary not only by its form, but also by tissue (Thomas et al., 2001). Other factors that affect the retention and distribution of arsenic include: the species, dose level, methylation capacity, valence form, and route of administration.

Transportation in Blood

Once arsenic is absorbed it is transported in the blood to organs throughout the body. It is generally bound to sulfhydryl groups of proteins and low-molecular-weight compounds such as glutathione (GSH) and cysteine (NRC, 1999). Binding of As^{III} to GSH has been demonstrated by several investigators (Anundi et al., 1982; Scott et al., 1993; Delnomdedieu et al., 1994a; Delnomdedieu et al., 1994b). Because of species differences in binding characteristics of arsenic, the retention in the blood varies among species. Arsenic elimination in humans is triphasic with half-times of 1 hour, 30 hours, and 200 hours (Mealey et al., 1959; Pomroy et al., 1980). Rats retain arsenic in the blood considerably longer than other species because DMA^{III} accumulates in red blood cells, apparently bound to hemoglobin (Odanaka et al., 1980; Lerman and Clarkson, 1983; Vahter, 1983; Vahter, 1984).

Shiobara et al. (2001) demonstrated that the uptake of DMA in the blood was dependent both on the chemical forms and animal species. DMA^{III} and DMA^{V} were incubated with rat, hamster, mouse, and human red blood cells (RBCs). DMA^{V} was only minimally taken up or the uptake was very slow in all animal species tested. DMA^{III}, on the other hand, was efficiently taken up by the RBCs in the following order: rats > hamsters > humans. Mice RBCs were less efficient at the uptake of DMA^{III} and also followed a different pattern from the other species. Rat RBCs retained the DMA^{III}, but hamsters effluxed DMA^{III} in the form of DMA^{V}. Humans also effluxed DMA^{III} as DMA^{V}, but the rate of uptake of DMA^{III} and efflux of DMA^{V} was much slower than in hamster RBCs.

In CHO cells, the rate of uptake was $DMA^{III} > MMA^{III} > As^{III}$ (Dopp et al., 2004); the pentavalent forms are taken up at a much lower rate than the trivalent forms. Stevens et al. (1977) calculated a half-time of 90 days in rat RBCs after a single oral dose of 200 mg/kg. Lanz et al. (1950) also reported a high retention of arsenic in the blood of cats, although less than in the rat; however, they did not determine if DMA was the cause. A single iv dose of 5.8 μg As/kg (in the form of $^{73}As^{V}$) body weight administered to two male chimpanzees had a half-life plasma elimination rate of 1 hour and a half-life elimination rate from red blood cells of about 5 hours (Vahter et al., 1995b).

The concentration of arsenic in plasma and red blood cells will vary depending on heath status and exposure level. Heydorn (1970) reported that heathy, presumably unexposed people, had similar arsenic concentrations in their plasma and whole blood (2.5 μg/L). This indicates that there was very little accumulation in the red blood cells. However, Taiwanese people exposed to arsenic-rich water had plasma levels of 15 μg/L and whole blood levels of 22 μg/L. Blackfoot disease patients and their families had levels of 30 μg/L and 60 μg/L in the plasma and red blood cells, respectively, indicating an increasing accumulation of arsenic in the red blood cells. De Kimpe et al. (1993) showed that chronic hemodialysis patients had greater serum (12 μg/L) and erythrocyte (9.5 μg/L) arsenic levels than that of controls (0.38 μg/L and 3.2 μg/L, respectively). Similarly, Zhang et al. (1996) reported that mean total arsenic levels were greater in hemodialysis patients (6.5 μg/L), compared to other types of patients (5.1 μg/L), and controls (0.96 μg/L). The ratio between plasma and red blood cell arsenic concentrations also may depend on the exposure form of arsenic (NRC, 1999). Delnomdedieu et al. (1995) demonstrated that As^{III} is taken up more readily by red blood cells in rabbit than As^{V}, MMA, or DMA.

Tissue Distribution

The distribution of inorganic arsenic (mainly As^{III}) in the skin, hair, oral mucosa, and esophagus is most likely due to the binding of the inorganic form of arsenic with sulfhydryl groups of keratin in these organs. In studies using rats and mice where the transfer of methyl groups from S-adenosylmethionine (the proposed mechanism of arsenic metabolism; see Metabolism section below) was chemically inhibited, the concentration of arsenic in most tissues (especially the skin) increased (Marafante and Vahter, 1984). This also is evident in the tissue distribution in the marmoset monkey, which does not methylate inorganic arsenic (Vahter et al., 1982). In contrast to rodent models, however, marmoset monkeys also accumulate arsenic in their testes, mainly in the spermatogenetic epithelium, and liver (Vahter et al., 1982).

The longest retention of inorganic arsenic in mammalian tissues during experimental studies has been observed in the skin (half-time more than a month; Marafante and Vahter, 1984), hair, squamous epithelium of the upper gastrointestinal tract (oral cavity, tongue, esophagus, and stomach wall), epididymis, thyroid, skeleton, and the lens of the eye (Lindgren et al., 1982, 1984; Vahter et al., 1982). All the aforementioned tissues, with the exception of the skeleton, contained higher concentrations of As^{III} than As^{V} within a short

period after the individual addition of either. The calcified areas of the skeleton (in mice) had an immediate accumulation and long-term retention of As^V, most likely a reflection of the similarities between As^V and phosphate causing a substitution of phosphate by As^V in the apatite crystals in bone.

Hughes et al. (2003) estimated that a steady-state of whole-body arsenic was established after nine repeated daily doses of 0.5 mg As/kg as radioactive arsenate in adult female B6C3F1 mice. Twenty-four hours dosing, the whole-body burden of arsenic following 9 days of exposure was about twice that observed after a single dose; however the rate of elimination was slower after the repeated dose. Accumulation of radioactivity was highest in the bladder, kidney, and skin. The loss of radioactivity was greatest in the lungs, but was slowest in the skin. Atomic absorption spectrometry revealed an organ-specific distribution of arsenicals. MMA was detected in all tissues except the bladder. DMA was found in the highest percentage in the bladder and lung after a single per os exposure, with increases after repeated exposure. Inorganic arsenic was predominant in the kidney. After a single per os exposure of arsenate (0.5 mg As/kg), DMA was the predominant form of arsenic in the liver, but after nine repeat exposures the percentage of DMA decreased while the percentage of inorganic arsenic increased. A trimethylated form of arsenic also was detected in the liver.

Kenyon et al. (2005) examined the time course for tissue distribution of different arsenic species after a single oral dose of 0, 10, or 100 micromole As/kg as sodium arsenate to adult female B6C3F1 mice. The concentration of all forms of arsenic were lower in the blood compared to the other organs across all doses and time points. The concentration of inorganic arsenic in the liver and kidney were similar at both dose levels with peak concentrations observed 1 hour after dosing. For the first 1 to 2 hours, inorganic arsenic was the predominant form in the liver and kidney for both dose groups; however, at the later times, DMA was the predominant form. Kidney measurements 1 hour after dosing demonstrated that MMA was 3-4 times higher compared to the other tissues. DMA concentrations in the kidney reached their peak 2 hours after dosing. DMA was the predominant form measured in the lungs at all time points following exposure to 10 micromole As/kg as arsenate. DMA concentration in the lung were greater than or equal to those of the other tissues beginning at 4 hours. The study could not distinguish the different valence states of the MMA or DMA compounds.

Human subjects also have demonstrated a high concentration of arsenic in tissues with a high content of cysteine-containing proteins, including the hair, nails, skin, and lungs. Levels in these tissues of human subjects exposed to background levels of arsenic ranged from 0.01 to 1.0 mg/kg of dry weight (Liebscher and Smith, 1968; Cross et al., 1979; Das et al., 1995). Benign and malignant skin lesions from 14 patients with a minimum of 4 years exposure to inorganic arsenical medication had a greater arsenic level (0.8 to 8.9 ppm) than normal skin or malignant skin lesions from 6 subjects with no history of arsenic intake (0.4 to 1.0 ppm; Scott, 1958). In West Bengal, India, where the district average of arsenic in the drinking water is 200-700 μg/L, arsenic concentrations in the skin scale, hair, and nails were

1.9-5.5, 3.6-9.6, and 6.1-23 mg/kg dry weight, respectively (Das et al., 1996). However, the amount of arsenic in the skin, hair, and nails resulting from an external exposure cannot be determined.

Marmoset monkeys do not accumulate arsenic in the ocular lens or the thyroid (Vahter et al., 1982). However, administration of ^{14}C labeled DMA to mice demonstrated accumulations in these tissues. In fact, the tissues with the longest retention of DMA were the lens of the eyes, thyroid, lungs, and intestinal mucosa (Vahter et al., 1984). Methylated arsenic (both MMA and DMA), however, has a shorter tissue retention than inorganic arsenic with ^{14}C labeled-DMA concentration decreasing rapidly in most tissues in mice (Vahter et al., 1984).

Trivalent and pentavalent inorganic arsenic, as well as methylated metabolites have been demonstrated to cross the placenta at all stages of the gestational period (Hanlon and Ferm, 1977; Lindgren et al., 1984; Hood et al., 1987) with tissue distribution of arsenic similar between the mother and the fetus. The marmoset monkey (known not to methylate arsenic) had somewhat less placental transfer after administration of arsenite than mice (Lindgren et al., 1984). The arsenic concentrations in the cord blood was similar to that observed in maternal blood (an average of 9 μg/L) in pregnant women living in a village in northwestern Argentina, where the arsenic in the drinking water is approximately 200 μg/L (Concha et al., 1998b). This also was noted in pregnant women with no known arsenic exposure where an average of 2-3 μg/L was measured in the cord blood and maternal blood (Kagey et al., 1977). Women living near smelters also have been determined to have an increased concentration of arsenic (Tabacova et al., 1994). Although the fetus is exposed to arsenic, it may be more in the form of DMA (at least in late gestation), as 90% or more of the arsenic in the urine and plasma of newborns and mothers (at time of delivery) was in the form of DMA. In any case, the fetal toxicity of arsenic has yet to be clarified.

Intracellular Distribution

Rabbits and mice exposed to radiolabeled arsenic had the majority of the arsenic in the nuclear and soluble fractions when measured in the liver, kidneys, and lungs (Marafante et al., 1981; Marafante and Vahter, 1984). The marmoset monkey had a different intracellular distribution with approximately 50% of the arsenic in the microsomal fraction in the liver (Vahter et al., 1982; Vahter and Marafante, 1985). However, chemical inhibition of arsenic methylation in rabbits did not alter the intracellular binding of arsenic (Marafante and Vahter, 1984; Marafante et al., 1985). An increase in tissue arsenic concentration (especially in the liver) was associated with an increase in the arsenic concentration in the microsomal fraction in the liver in rabbits fed diets with low concentrations of methionine, choline, or proteins, which leads to a decrease in arsenic methylation (Vahter and Marafante, 1987). The levels of arsenic in the microsomal fraction of the liver in these rabbits was similar to those observed in the marmoset monkey (Vahter et al., 1982), therefore, indicating nutritional factors may play a role in the subcellular distribution of arsenic.

3.3. METABOLISM

Upon entering the system arsenate can be reduced to arsenite. A substantial fraction of absorbed As^V is rapidly reduced to As^{III}, probably mainly in the blood, in mice, rabbits, and marmoset monkeys (Vahter and Envall, 1983; Vahter and Marafante, 1985; Marafante et al., 1985). This reduction also might occur in the stomach or intestines, but quantitative experimental data are not available. GSH may play a role in the reduction of As^V (NRC, 1999). Cysteine and dithiothreitol (DTT) have been shown to reduce pentavalent arsenic in purified rabbit liver enzyme preparations (Zakharyan et al., 1995) and rat liver cytosols (Buchet and Lauwerys, 1985; Styblo et al., 1996).

Although pentavalent arsenic can be directly reduced, arsenate reductase enzymes have been detected in the human liver (Radabaugh and Aposhian, 2000), which has been subsequently characterized as a purine nucleoside phosphorylase (PNP) (Gregus and Németi, 2002; Radabaugh et al., 2002). This enzyme requires a thiol and a heat-stable cofactor for activation. According to Radabaugh et al. (2002), dihydrolipoic acid (DHLP) is most active naturally occurring thiol in mammalian systems required for the enzymatic reduction of arsenate to arsenite.

Németi et al. (2003), however, observed the reduction of arsenate to arsenite by PNP *in vitro* only. PNP did not appear to a major player in the reduction of arsenate to arsenite in either human erythrocytes or in rats *in vivo*. Németi and Gregus (2004; 2005) further demonstrated that human erythrocytes contain a PNP-independent As^V-reducing mechanism that requires a supply of GSH, NAD, and a substrate to either one or both of the following enzymes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or phosphoglycerate kinase (PGK). This also was demonstrated to be the mechanism of reduction in rat liver cytosol (Németi and Gregus, 2005), however, another unidentified enzyme in the liver cytosol also had the capacity to reduce As^V. A further study (Gregus and Németi, 2005) demonstrated that GAPDH was capable of As^V reductase activity, but that PGK served as an auxiliary enzyme when 3-phosphoglycerate is the glycolic substrate.

The oxidative methylation of trivalent arsenicals, as well as, the reduction of pentavalent arsenicals is catalyzed by an arsenic (+3 oxidation state) methyltransferase, which is encoded by the AS3MT gene (Waters et al., 2004a). In chimpanzees, which do not methylate arsenic, Li et al. (2005) found a frameshift mutation in the gene code that caused a deletion in the gene for arsenic (+3 oxidation state) methyltransferase leading to an inactive truncated protein. The addition of GSH increased the yield on mono- and di-methylated arsenicals, but suppressed the production of TMAO (Waters et al., 2004a). Thomas et al. (2004) discovered a similar arsenic methyltransferase in the rat liver, which they designated cyt19 because an orthologous *cyt19* gene encodes an arsenic methyltransferase in the mouse and human genome. Glutathione alone does not support recombinant rat *cyt19* catalytic function, but when added to the reaction mixture containing other reductants, the rate of arsenic methylation increases (Waters et al., 2004b). According to Waters et al. (2004b), *cyt19* may posses both As^{III} methyltransferase and As^V reductase activities. In the presence of

exogenous or physiological reductant, *cyt19* was found to catalyze the entire sequence to convert arsenite to its methylated metabolites. Drobna et al. (2004) linked the genetic polymorphism of *cyt19*, with other cellular factors and to the interindividual variability in the capacity of primary human hepatocytes to retain and metabolize As^{III}.

Dithiols (e.g., reduced lipoic acid) have been indicated in recent years to be more active than GSH for providing a reducing environment for methylation by MMA^{III} methyltransferase (Zakharyan et al., 1999). An MMA^{V} reductase also has been detected in rabbit liver (Zakharyan and Aposhian, 1999), hamster tissues (Sampayo-Reyes et al., 2000), and human liver (Zakharyan et al., 2001). In any case, arsenate is rapidly reduced to arsenite; therefore, chronic exposure to arsenite and arsenate should result in fairly similar metabolite distribution in the body unless the reducing capacity is exceeded by acute high-dose exposures (Vahter, 1981; Lindgren et al., 1982).

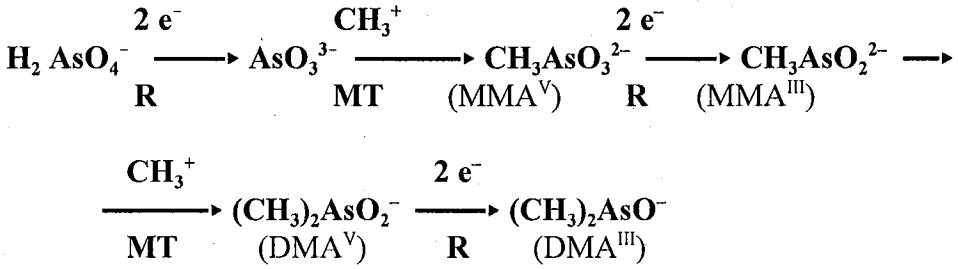
Humans and most experimental animal models methylate inorganic arsenic to MMA and DMA, with the amounts differing by species as determined by urinary metabolites. The methylated metabolites historically have been considered less acutely toxic, less reactive with tissue constituents, less cytotoxic, and more readily excreted in the urine than inorganic arsenic (Buchet et al., 1981; Vahter and Marafante, 1983; Vahter et al., 1984; Yamauchi and Yamamura, 1984; Marafante et al., 1987; Moore et al., 1997a; Rasmussen and Menzel, 1997; Concha et al., 1998a; Hughes and Kenyon, 1998; Sakurai et al., 1998). Monomethylarsonous acid (MMA^{III}) and dimethylarsenous acid (DMA^{III}), however, have recently been demonstrated to be more cytotoxic in Chang cells (a human liver cell line; Petrick et al., 2000, 2001), CHO (Dopp et al., 2004), and cultured primary rat hepatocytes (Styblo et al., 1999b, 2000) than As^{III}, As^{V}, MMA^{V}, or DMA^{V}.

Methylation is important in the distribution in tissues and in the excretion of arsenic. It has been demonstrated that inhibition of arsenic methylation results in increased tissue concentrations of arsenic (Marafante and Vahter, 1984; Marafante et al., 1985).

Arsenic Methylation

In vitro studies using rat liver preparations indicate that the methylating activity is localized in the cytosol with S-adenosylmethionine (SAM) as the main methyl donor for As^{III} (Marafante and Vahter, 1984; Buchet and Lauwerys, 1985; Marafante et al., 1985; Styblo et al., 1995, 1996; Zakharyan et al., 1995). The proposed metabolic pathway for arsenic is demonstrated in Figure 3.1.

Figure 3-1. Proposed metabolic pathway for the methylation of inorganic arsenic in humans. R = reductase; MT = methyltransferase; e^- = electron



The main products of methylation are MMA^V and DMA^V, which are readily excreted in the urine (Marcus and Rispin, 1988). MMA^{III} and DMA^{III} have recently been detected in human urine (NRC, 2001). However, most publications do not differentiate the valence state of MMA or DMA. Le et al. (2000a,b) as well as Del Razo et al. (2001) noted that the concentration of trivalent metabolites in the urine may be underestimated because they are easily oxidized after collection. However, Le et al. (2000a,b) discovered 32-127 µg/L of MMA^{III} and 21-38 µg/L of DMA^{III} in the urine of a population from Inner Mongolia, China, who were exposed to 510-660 µg/L (0.46 µM) of arsenic via the drinking water. MMA^{III} and DMA^{III} have recently been demonstrated to be more cytotoxic in Chang cells (a human liver cell line; Petrick et al., 2000) or cultured primary rat hepatocytes (Styblo et al., 1999b, 2000) than As^{III}, As^V, MMA^V, or DMA^V. Petrick et al. (2001) estimated LD_{50}s of 112 µmol/kg and 29.3 µmol/kg for As^{III} and MMA^{III}, respectively, therefore, indicating the toxicity of intermediates in the metabolism of arsenic.

A small percent of DMA^{III} may further be methylated to trimethylarsine oxide (TMAO) in mice, hamsters, and humans (for review, see Kenyon and Hughes, 2001). TMAO is detected in the urine following DMA exposure, but has not been detected in the blood or tissues of mice exposed intravenously to DMA (Hughes et al., 2000) or in the urine of mammals exposed to inorganic arsenic. This may be due to rapid clearance of DMA and MMA from cells (Styblo et al., 1999a; Lin et al., 2001); however, most analytical methods are not optimized for the detection of TMAO and thus it could be present and not detected. Styblo et al. (1999b) reported that DMA was the only metabolite detected in rat or human hepatocytes incubated with DMA or a glutathione complex of DMA.

Although the kinetics of arsenic methylation *in vivo* are not fully understood, it is believed that the liver may be the primary source of arsenic methylation. Marafante et al. (1985) discovered that DMA appeared in the liver prior to any other tissue in rabbits exposed to inorganic arsenic. It also has been demonstrated that oral administration of inorganic arsenic favors methylation more than either subcutaneous or intravenous administration (Charbonneau et al., 1979; Vahter, 1981; Buchet et al., 1984) presumably because the arsenic will pass through the liver first after oral administration. However, liver disease (i.e., alcoholic, postnecrotic, or biliary cirrhosis, chronic hepatitis, hemochromatosis, and steatosis)

increased the ratio of DMA to MMA in the urine following a single injection of sodium arsenite (Buchet et al., 1984; Geubel et al., 1988), indicating a more efficient methylation of arsenic. In addition, the site of methylation may depend on the rate of reduction of As^{V} to As^{III}. Isolated rat hepatocytes readily absorbed and methylated As^{III}, but not As^{V} (Lerman et al., 1983). Kidney slices, on the other hand, produced five times more DMA produced from As^{V} than As^{III} (Lerman and Clarkson, 1983). Therefore, it is likely that any arsenate not initially reduced can be methylated in the kidney for urinary excretion.

There have been unequivocal results with *in vitro* studies on the methylation capacity of different tissues. Buchet and Lauwerys (1985) reported the liver in rats as the main organ for methylation with the methylating capacities in the red blood cells, brain, lung, intestine, and kidneys as insignificant in comparison. Arsenite methyltransferases from mouse tissues demonstrated that the testes had the highest amount of methylating activity, followed by the kidney, liver, and lung (Healy et al., 1998). Aposhian (1997) determined that the amount of methyltransferases vary in different tissues and animal species, methylating capacities *in vitro*, however, do not necessarily reflect *in vivo* methylation (NRC, 1999). Arsenite bound to tissue component can be methylated and released. This may explain the initial rapid phase (immediate methylation and excretion) followed by a slow elimination phase (continuous release of bound arsenite through methylation) (Marafante et al., 1981; Vahter and Marafante, 1983) as described below under **Elimination**.

Species Differences in the Methylation of Arsenic

There is considerable variation in inorganic arsenic methylation among mammalian species (NRC, 1999). Humans, rats, mice, dogs, rabbits, and hamsters have been shown to have efficient methylation of arsenic to MMA and/or DMA. Rats and hamsters also appear to methylate administered DMA to TMAO more efficiently than other species (Kitchin et al., 1999; NRC, 1999; Yamauchi and Yamamura, 1984) with about 10% urinary arsenic present as TMAO after exposure to DMA in the drinking water (100 mg/L) of male rats (Yoshida et al., 1998). Humans (mainly exposed to background levels or exposed at work) have been estimated through a number of studies to excrete 10-30% of the arsenic in its inorganic form, 10-20% as MMA, and 55-75% as DMA (see Hopenhayn-Rich et al., 1993 for review). A study of urinary arsenic in a population in northern Argentina exposed to arsenic via drinking water demonstrated an average of only 2% MMA in the urine (Concha et al., 1998a; Vahter et al., 1995a). This may indicate variations in methylation depending on route of exposure, level of exposure, and possible nutritional or genetic factors.

The rabbit (Marafante et al., 1981; Vahter and Marafante, 1983; Maiorino and Aposhian, 1985) and hamster (Charbonneau et al., 1980; Yamauchi and Yamamura, 1984, 1985; Marafante and Vahter, 1987) are more comparable to human with respect to arsenic methylation than other experimental animals. However, in general rabbits and hamsters excrete more DMA and less MMA than humans. However, Flemish giant rabbit (De Kimpe et al., 1996) and New Zealand rabbits (Bogdan et al., 1994) excrete MMA in amounts similar to humans. Mice and dogs, efficient methylators of arsenic, excrete more than 80% of the

administered dose as DMA within a few days (Charbonneau et al., 1979; Vahter, 1981). Guinea pigs (Healy et al., 1997), marmoset monkeys (Vahter et al., 1982; Vahter and Marafante, 1985), and chimpanzees (Vahter et al., 1995b) do not appear to methylate inorganic arsenic. In addition, no methyltransferase activity was detected in these species (Zakharyan et al., 1995, 1996; Healy et al., 1997; Vahter, 1999b). Li et al. (2005) found a frameshift mutation in the gene code of chimpanzees that caused a deletion in the gene for arsenic (+3 oxidation state) methyltransferase leading to an inactive truncated protein. Healy et al. (1999) determined marked variations in the activity of methyltransferases and Vahter (1999a) determined a difference in methylation efficiency between different species. The variations in methyltransferase activity and methylation efficiency are probably the underlying reason for the cross-species variability in methylation ability as all the species had ample arsenate reductase activity (Vahter, 1999b; NRC, 2001). However, methyltransferases have only recently been detected in human hepatocytes (Zakharyan et al., 1999; Styblo et al., 1999a).

3.4. ELIMINATION

The major route of excretion for most arsenic compounds by humans is via the urine with a biological half-time of about 4 days, which is slightly shorter following exposure to As^{V} than to As^{III} (Yamauchi and Yamamura 1979; Tam et al. 1979; Pomroy et al. 1980; Buchet et al. 1981). Six human subjects, who ingested radiolabeled ^{74}As-arsenate, excreted 38% of the dose in the urine within 48 hr and 58% within 5 days (Tam et al., 1979). The data indicate a three compartment exponential function, with 66% excreted with a half-time of 2.1 days, 30% with a half-time of 9.5 days, and 3.7% with a half-time of 38 days (Pomroy et al., 1980). Buchet et al. (1981) supported this information by exposing three subjects orally to 500 µg of arsenic in the form of arsenite in water. Buchet's results indicate about 33% of the dose was excreted in the urine within 48 hr, and 45% within 4 days. The methylated metabolites, MMA and DMA, are excreted in the urine faster than the inorganic arsenic. In humans, about 78% of MMA and 75% of DMA were excreted in the urine within 4 days of ingestion (Buchet et al., 1981). In mice, the half-time of MMA and DMA was found to be about 1 hr following iv administration (Hughes and Kenyon, 1998).

Rats have a slow whole body clearance of DMA (Vahter et al., 1984), even though they are efficient at methylating inorganic arsenic to DMA, because a significant portion of the DMA produced is retained in the erythrocytes (Odanaka et al., 1980; Lerman and Clarkson, 1983; Vahter, 1983; Vahter et al., 1984). The rat also has extensive biliary excretion of inorganic arsenic which is about 800 times greater than observed in the dog and 37 times that of the rabbit.

Although absorbed arsenic is removed from the body mainly via the urine, small amounts of arsenic are excreted via other routes (e.g., skin, sweat, hair, and breast milk). Although arsenic has been detected to a low degree in the breast milk of women in northwestern Argentina (i.e., 2 µg/kg), breast-feeding caused a decrease in the

concentration of arsenic in the urine of the newborn child (Concha et al., 1998c). Women in the Philippines were determined to have about 19 μg/kg arsenic in their breast milk. The average concentration of arsenic in sweat induced in a hot and humid environment was 1.5 μg/L, and the hourly loss was 2 μg (Vellar, 1969). With an average arsenic concentration in the skin of 0.18 mg/kg, Molin and Wester (1976) estimated that the daily loss of arsenic through desquamation was 0.1-0.2 μg in males with no known exposure to arsenic.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A physiologically based pharmacokinetic (PBPK) model for exposure to inorganic arsenic (orally, intravenously, and intratracheally) in hamsters and rabbits has been developed by Mann et al. (1996a). It contains tissue compartments for lung (naso-pharynx, tracheo-bronchial, pulmonary), plasma, RBCs, liver, gastrointestinal (GI) tract, skin, lungs, kidney, keratin, and combined other tissues. Oral absorption of As^{III}, As^{V}, and DMA (pooled III and V oxidation states) is modeled as a first-order transport directly from the GI contents into the liver. Distribution to tissues is diffusion-limited, based upon literature values for capillary thickness and pore sizes for each tissue. Reductive metabolism of As^{V} to As^{III} is modeled as a first-order process in the plasma. Oxidative metabolism of As^{III} to As^{V} is modeled as first-order processes in the plasma and kidneys. Methylation of inorganic As species to MMA (pooled III and V oxidation states) and then to DMA is modeled as saturable Michaelis-Menten processes in the liver. Urinary, biliary, and fecal excretion of As^{III}, As^{V}, MMA, and DMA are modeled as first-order processes. Parameters for absorption, tissue partition, metabolism, and biliary excretion were estimated by fitting the model to literature data on the urinary and fecal excretion of total arsenic from rabbits and hamsters administered various arsenic compounds by iv, oral gavage, or intratracheal instillation (Marafante et al, 1985; Marafante et al., 1987; Charbonneau et al., 1980; Yamauchi and Yamamura, 1984). The model was found to simulate accurately the excretion of arsenic metabolites in the urine of rabbits and hamsters, and was additionally validated with reasonable fits to liver, kidney, and skin concentrations in rabbits and hamsters (Marafante et al, 1985; Marafante and Vahter, 1987; Yamauchi and Yamamura, 1984).

Mann et al. (1996b) extended the PBPK model for humans by adjusting physiological parameters (organ weights, blood flows) accordingly and re-estimating absorption and metabolic rate constants by fitting the model to literature data on the urinary excretion of total arsenic following a single oral dose of As^{III} or As^{V} in human volunteers (Buchet et al., 1981; Tam et al., 1979). The extended model was validated against empirical data on the urinary excretion of the different metabolites of inorganic arsenic following repeated oral intake of arsenite, intake of inorganic arsenic via drinking water, and occupational exposure to arsenic trioxide (Buchet et al., 1981; Vahter et al., 1986; Harrington et al., 1978; Valentine et al., 1979). The model predicted a slight decrease (i.e., about 10% with an increase in dose of about 1,000 μg) in the percentage of DMA in urine with increasing single-dose exposure (highest dose of arsenic at 15 μg/kg of body weight),

especially following exposure to As^{III}, and an almost corresponding increase in the percentage of MMA. The model demonstrated that adults drinking water containing 50 μg/L had a higher urinary excretion of arsenic than an occupational exposure of 10 $\mu g/m^3$ (Mann et al., 1996b).

Yu (1999a,b) also developed a PBPK model for arsenic in humans. It contains tissue compartments for lung, skin, fat, muscle, combined kidney and richly perfused tissues, liver, intestine, GI contents, stomach contents, and bile. Oral absorption of As^{III}, As^{V}, and DMA (pooled III and V oxidation states) is modeled as a first-order transport from the GI contents into the intestinal tissue. Distribution to tissues is flow-limited. Reductive metabolism of As^{V} to As^{III} is modeled as a first-order, glutathione-dependent process in the intestinal tissue, skin, liver and kidney/rich tissues. Oxidative metabolism of As^{III} to As^{V} is not modeled. Methylation of inorganic As species to MMA (pooled III and V oxidation states) and then to DMA is modeled as saturable Michaelis-Menten processes in the liver and kidney/rich tissues. Urinary, biliary, and fecal excretion of As^{III}, As^{V}, MMA, and DMA are modeled as first-order processes. Parameters for absorption, tissue partition, metabolism, and biliary excretion were estimated by fitting the model to literature data on tissue concentrations of total arsenic from a fatal human poisoning (Saady et al., 1989) and blood, urine, and fecal elimination of total arsenic following oral administration (Odanaka et al., 1980; Pomroy et al., 1980). The model was not validated against external data and fits to the data sets used for parameter estimation were not provided.

Gentry et al. (2004) adapted the model proposed by Mann et al. (1996a) to different mouse strains by adjusting physiological parameters (organ weights, blood flows) accordingly and re-estimating absorption, partition, and metabolic rate constants by fitting the model to literature data on urinary excretion of various arsenic species following iv administration of MMA to B6C3F1 mice (Hughes and Kenyon, 1998) or single oral administration of As^{III} or As^{V} to B6C3F1 mice (Kenyon et al., 1997; Hughes et al., 1999). Additionally, the description of methylation in the model was refined to address the uncompetitive inhibition of the conversion of MMA to DMA by As^{III}. The PBPK model was then validated using data from a single oral administration of As^{V} (Hughes et al., 1999) and 26-week drinking water exposure of As^{III} to C57Black mice (Moser et al., 2000) . This data was adequately fit by the model without further parameter adjustment. Ng et al. (1999) had found arsenic-induced tumors in C57Cl/6J mice, while numerous other mouse strains (Swiss CR:NIH[S], C57Bl/6p53[+/-], and C57Bl/6p53[+/+], and Swiss CD-1) have not resulted in a significant increase in arsenic-induced tumors. The Gentry et al. model was unable to explain the different outcomes in the mouse bioassay.

The Mann et al. (1996a,b) and Gentry et al. (2004) models are well-documented, validated against external data, and appear to capture the salient features of arsenic toxicokinetics in rodents and humans. The information provided by these different models may help understand the mode(s) of action involved in carcinogenesis along with possible reasons that humans are apparently more susceptible to the carcinogenic effects of arsenic.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

The toxicity of arsenic is dependent on its valence state (i.e., trivalent or pentavalent) with trivalent arsenic appearing to be 4 times more acutely toxic than pentavalent arsenic. However, symptoms are not dependent on valence state. There have been a number of epidemiology studies conducted on the health effects of arsenic. Some of the limitations of the epidemiology studies include not specifying the form of arsenic, difficulties with the exposure assessment, and/or not examining possible confounding variables. However, the pentavalent form of inorganic arsenic predominates in water and soil (Buchet and Lison, 2000). Although many of the epidemiology studies attempt to examine major confounders such as age or smoking status, few if any address nutritional effects such as selenium and/or zinc deficiencies, which may enhance arsenic toxicity (Gebel, 2000). This section will focus mainly on the epidemiology studies conducted on the effects of chronic ingestion of arsenic in drinking water. The studies address the concentration of arsenic in the well water, but few if any address exposure through bathing or possible exposure through food consumption.

Cancer Effects

There are many epidemiology reports that examined the association between arsenic and cancers. Each study is conducted in a different manner (i.e., prevalence studies, cross-sectional studies, case-controls, cohort, and ecological studies) with its own limitations, but the majority found an association between arsenic and cancer (type varied by study). Table 4-1 summarizes the epidemiology studies giving their type and cancer results. Arsenic has been associated with cancer as far back as 1887 when Hutchinson reported an unusual number of skin tumors in patients treated with arsenicals. The association between skin cancer and arsenic has been reported in a number of studies since then (Neubauer, 1947; Sommers and McManus, 1953; Roth, 1957; Robson and Jelliffe, 1963; Fiertz, 1965; Tseng et al., 1968; Tseng, 1977; Kjeldsberg and Ward, 1972; Jackson and Grainge, 1975; Popper et al., 1978; Prystowsky et al., 1978; Reymann et al., 1978; Nagy et al., 1980; Falk et al., 1981; Roat et al., 1982; Robertson and Low-Beer, 1983; Yu, 1984; Yu et al., 1984; Chen et al., 1985; Wu et al., 1989; Chen et al., 1988; Cuzick et al., 1992; Hopemhayn-Rich et al., 1996a and 1998; Smith et al., 1998; Ahmad et al., 1999; Hinwood et al., 1999; Ma et al., 1999; Tsai et al., 1999; Karagas et al., 2001; Kuorkawa et al., 2001; and Tucker et al., 2001).

Several studies have demonstrated an association between arsenic and cancer and/or disease in populations in Taiwan. This population has been extensively studied due to the switch from surface water wells to artesian (ground water) wells for drinking water in the 1920s to improve the microbiology and reduce salinity of the water. The artesian wells have since been discovered to be contaminated with naturally occurring arsenic resulting in widespread exposure.

Tseng et al. (1968) and Tseng (1977) demonstrated an increase in the occurrence of skin lesions with high level (≥0.60 ppm) of exposure to arsenic from artesian wells in villages of southwestern Taiwan. The increases were seen across both sexes and different age groups. This was an ecological study examining the prevalence of skin cancer, keratosis, and hyperpigmentation in 37 villages in Taiwan containing a total of 40,421 inhabitants who consumed drinking water from artesian wells, which unknowingly was contaminated with high concentrations of naturally occurring arsenic. Three exposure groups were investigated: 0.0-0.29, 0.30-0.59, and ≥0.60 ppm. Hyperpigmentation (183.5/1000) had the highest prevalence, followed by keratosis (71.0/1000), and skin cancer (10.6/1000). There was a clear-cut increase in the prevalence of skin cancer from low to high exposure groups in both sexes of three age groups. In 20-39 year old the prevalence were 1.3, 2.2, and 11.5 from the low- to high-dose group. For the same dose groups the prevalence was 4.9, 32.6, and 72.0 in the 40-59 year age group and 27.1, 106.2, and 192.0 in the 60 years and over age group.

Chen et al. (1985) studied 84 villages in Southwestern Taiwan known to be endemic for Blackfoot-disease and other types of skin lesions. Mortality rates of the 84 villages from 1968-1986 were compared to national and sex-specific rates. Of these villages, 31 used only artesian wells (100 to 200 m deep), 27 used both artesian and shallow wells, 24 used only shallow wells, and 2 used surface water. Although no exposure measurements were taken, the authors cited an earlier study (Chen et al. 1962) that reported the arsenic content of artesian well water measured in BFD-endemic areas in 1959 to 1962 to range from 0.35 ppm to 1.14 ppm with a median of 0.78 ppm and the shallow well water to have arsenic content between 0.00 and 0.30 ppm, with a median of 0.04 ppm (Chen et al. (1962) actually reported the arsenic content of BFD-endemic shallow wells to be between 0.00 and 0.25 ppm, with a median of 0.03ppm).

Standardized Mortality Ratios (SMRs) of the 84 villages for the period 1968-1982 were computed for various cancers by gender. National Taiwan rates were used to derive the standard. The SMRs of the villages, regardless of exposure, for various cancers separated by sex are listed in Table 4-1. Cancers of the small intestine, esophagus, nasopharynx, rectum, stomach, and thyroid were not statistically significantly associated with arsenic exposure.

Table 4-1. SMRs in Taiwan by Sex. From Chen et al. (1985).
(95% confidence intervals in parentheses.)

	Males	Females
Bladder cancer	11.0 (9.3-12.7)	20.1 (17.0-23.2)
Kidney cancer	7.7 (5.4-10.1)	11.2 (8.4-14)
Skin cancer	5.3 (3.8-6.9)	6.5 (4.7-8.4)
Lung cancer	3.2 (2.9-3.5)	4.1 (3.6-4.7)
Liver cancer	1.7 (1.5-1.9)	2.3 (1.9-2.7)
Colon cancer	1.6 (1.2-2.0)	1.7 (1.3-2.1)
Leukemia	1.4 (1.0-1.8)	0.9 (0.5-1.3)

Chen et al. (1985) also found that the age-specific cumulative mortality ratios for cancers of the bladder, kidney, skin, lung, liver, and colon in the BFD-endemic area were higher than in Taiwan for all age groups and that the SMRs in the BFD-endemic areas generally increased with increasing BFD relevance rates. Finally, the authors observed a dose-response association between BFD endemicity (defined as follows: hyperendemic when BFD rate was greater than 5.0/1000; endemic when BFD prevalence rate was between 0.1 and 5.0/1000; and nonendemic where no BFD cases were reported) and the SMRs for cancer of the bladder, kidney, skin, lung and liver, but not colon cancer. Although cigarette smoking has been related to cancers of the kidney and bladder, the high SMRs observed in the BFD-endemic regions are not readily explained by the relatively higher smoking rate in the BFD-endemic area than in Taiwan (40% versus 32%).

Chen et al. (1988) analyzed the mortality of residents in the BFD-endemic area based on 899,811 person-years observed from 1973 to 1986. Age-adjusted mortality rates (per 100,000) for various cancers were calculated, with results displayed in Table 4-2.

Table 4-2. Age-Adjusted Mortality Rates (per 100,000) in Taiwan
from Chen et al. (1988)

Cancer site	Sex	BFD-endemic area			General population in Taiwan
		>0.6*	0.30-0.59*	<0.30*	
All Sites	M	434.7	258.9	154.0	128.1
	F	369.4	182.6	113.3	85.5
Liver	M	68.8	42.7	32.6	28.0
	F	31.8	18.8	14.2	8.9
Lung	M	87.9	64.7	35.1	19.4
	F	83.8	40.9	26.5	9.5
Skin	M	28.0	10.7	1.6	0.8
	F	15.1	10.0	1.6	0.8
Prostate	M	8.4	5.8	0.5	1.5
Bladder	M	89.1	37.8	15.7	3.1
	F	91.5	35.1	16.7	1.4
Kidney	M	21.6	13.1	5.4	1.1
	F	33.3	12.5	3.6	0.9

*Well-water arsenic concentrations (ppm).

Significantly higher age-adjusted mortality from the various cancers occurred among residents in the BFD-endemic region than in the general Taiwanese population. In addition, according to Chen et al. (1988), there was a significant dose-response relation between the arsenic level in drinking water and age-adjusted mortality for cancers of the bladder, kidney, skin, prostrate, lung, and liver.

Wu et al. (1989) investigated 42 villages in southwestern Taiwan with contaminated drinking water sources. These included 27 of the villages in the four townships investigated by Chen et al. (1985) plus an additional 15 villages in two other townships. Water samples were collected from 155 wells of the 42 villages where shallow and/or deep well water was being used in 1964-1966. The arsenic content of well water samples ranged from 0.010 ppm to 1.752 ppm with two clusters at levels of 0.05 ppm-0.25 ppm and 0.45-0.65 ppm.

Death certificates of people who died from cancer during 1973-1986 were coded for underlying causes of death, and age-adjusted mortality rates were computed. The resulting mortality rates were categorized into three groups according to their median arsenic levels of well water (<0.30 ppm (20 villages); 0.30-0.59 ppm (15 villages); and

≥0.60 ppm (7 villages). Mortality from a number of different types of cancers was found to increase with increasing arsenic concentrations for both sexes among residents aged 20 years or more. The authors performed a Mantel-Haenszel χ^2 test to test for significance of the trends. Table 4-3 shows the age-adjusted mortality rates (per 100,000) from various cancers and all sites combined that were significant. No significant trends were found for mortality from leukemia, and cancers of the nasopharynx, esophagus, stomach, colon and uterine cervix with increasing arsenic levels.

Table 4-3. Model Parameters and Potency Index (Wu et al. 1989)

Cause of death	Median arsenic levels in well water (ppm) and significance levels							
	Males (467,173 person-years)				Females (431,633 person-years)			
	<0.30	0.30-0.59	≥ 0.60	p	<0.30	0.30-0.59	≥ 0.60	p
All sites	224.56	405.12	534.61	†	162.22	277.20	487.20	†
Bladder	22.64	61.02	92.71	†	25.60	57.02	111.30	†
Kidney	8.42	18.90	25.26	†	3.42	19.42	57.98	†
Skin	2.03	14.01	32.41	†	1.73	14.75	18.66	†
Lung	49.16	100.67	104.08	†	36.71	60.82	122.16	†
Liver	47.78	67.62	86.73	‡	21.40	24.18	31.75	NS
Prostate	0.95	9.00	9.18	‡	-	-	-	-

‡ < 0.05; † < 0.001, based on the trend test of the extension of the Mantel-Haenszel χ^2 test.

NS: not significant

Chen et al. (1992) used the Armitage-Doll model to develop cancer potency indices for residents in an endemic area of chronic arsenism. The study area and population were the same as in Wu et al. (1989). The age-specific mortality rate for a disease was calculated as:

$I(t,d) = B(t) + H(t,d)$, where $I(t,d)$ was the age and cause-specific mortality rate, $B(t)$ the background mortality rate, where the reference was the general Taiwan population, and $H(t,d)$ the mortality rate due to the arsenic exposure at age t and dose rate d, which was assumed to have the form $H(t,d) = a \times d \times t^k$.

The authors estimated the parameters a and k using maximum likelihood methods. Table 4-4 shows the results. The potency index represents the excess lifetime risk from an

intake of 10 μg/kg/day[111] of arsenic. The authors point out that the risk for males and females was within a factor of two indicating no gender difference and also that the potency of the four internal cancers were comparable, differing only by a factor of four.

Table 4-4. Estimated values for Chen et al. (1992)

Disease	Sex	a	k	Potency index
Liver cancer	Male Female	3.3 E-8 1.0 E-8	2.6 2.8	0.0043 0.0036
Lung cancer	Male Female	2.2E-10 2.2E-9	4.0 3.4	0.012 0.013
Bladder cancer	Male Female	6.3E-11 1.4E-11	4.5 4.9	0.012 0.017
Kidney cancer	Male Female	1.1E-12 2.4E-9	5.2 3.3	0.0042 0.0048

Guo et al. (1997) used tumor registry data and information provided by the nationwide water-quality survey conducted during 1974-1976. Data on arsenic concentrations were gathered from 243 township containing 11.4 million residents. Regression models were created to compare the incidence of bladder, kidney, and subcategories of those cancers diagnoses and six variables for the proportions of wells in each of six categories of arsenic concentration (<0.05 ppm; 0.05-0.08 ppm; 0.09-0.16 ppm; 0.17-0.32 ppm; 0.33-0.64 ppm; and >0.64 ppm) in each township. Adjustments for age, urbanization index, and annual number of cigarettes sold per capita were included in the sex-specific models. For high arsenic concentration levels (>0.64 ppm), there was a strong association with transitional-cell carcinomas of the bladder, kidney, ureter, and all urethral cancers combined for both sexes. A significant association was not found for arsenic concentrations below 0.64 ppm. Guo et al. (1997) did not present relative risk estimates so the results cannot be directly compared with other studies. Additionally, no association was found with cigarette sales. Yet, there was a positive association found with urbanization. A crude incidence rate of 2.15 per 100,000 was determined for bladder cancer, which is far below that of the comparable Asian population, suggesting under-ascertainment of newly diagnosed bladder cancer in the voluntary national cancer registry.

In another study by Guo (2004), arsenic exposure was associated with an increase in mortality from lung cancer. Arsenic was measured in 138 villages from a census survey conducted by the government. Certificates of death that occurred in the village between

[1] Chen et al. (1992) give the units as μg kg day; it is assumed that what is meant is μg/kg/day, although μg/day is an alternate possibility.

January 1, 1971 and December 31, 1990 were reviewed, and 673 male and 405 female mortality cases of lung cancer were identified. Multi-variate regression models were applied to assess association between arsenic levels in drinking water and mortality of lung cancers using village as a unit. After adjusting for age, arsenic levels above 0.64 mg/L were associated with a significant increase in the mortality of lung cancer in both genders, but no significant effect was observed at lower levels. Post-hoc analyses confirmed such a dose-response relationship.

Tsai et al. (1999) used SMRs for deaths occurring from 1971 to 1994 in the blackfoot disease endemic area of southwest Taiwan. The median arsenic concentration in the artesian well water was stated to be 0.78 ppm (range 0.25 to 1.14 ppm). Soil in this area also contained high arsenic levels (median ~7.2 mg/kg, range 5.3 to 11.2 mg/kg). Comparison between both local (similar habits) and national (more stable age and sex rates) reference groups demonstrated an association between arsenic and liver, respiratory, bone, skin, bladder, and kidney cancer, as well as lymphoma in both males and females (see Table 4-5 below). In addition, colon, leukemia and prostate cancers were increased in males.

Table 4-5. Statistically Significant ($p<0.05$) SMRs for Cancers in a Blackfoot Disease Endemic Area of Taiwan Compared to Local and National Controls (Tsai et al., 1999)

Cause	Local Reference SMR*		National Reference SMR*	
	Males	Females	Males	Females
Liver	1.83 (1.69-1.98)	1.88 (1.64-2.14)	1.83 (1.69-1.98)	1.87 (1.64-2.14)
Respiratory				
Nasal	3.00 (2.14-4.09)	4.98 (3.33-7.15)	3.66 (2.61-4.98)	5.10 (3.41-7.32)
Laryngeal	1.78 (1.20-2.55)	4.76 (2.53-8.15)	1.76 (1.19-2.52)	3.76 (2.00-6.43)
Lung	3.10 (2.88-3.34)	4.13 (3.77-4.52)	2.64 (2.45-2.84)	3.50 (3.19-3.84)
Bone	2.46 (1.77-3.34)	2.25 (1.56-3.14)	2.33 (1.67-3.16)	2.18 (1.51-3.05)
Skin	4.83 (3.74-6.15)	5.68 (4.41-7.21)	5.97 (4.62-7.60)	6.81 (5.29-8.63)
Bladder	8.92 (7.96-9.96)	14.07 (12.51-15.78)	10.50 9.37-11.73)	17.65 (5.70-19.79)
Kidney	6.76 (5.46-8.27)	8.89 (7.42-10.57)	6.80 (5.49-8.32)	10.49 (8.75-12.47)
Lymphoma	1.63 (1.23-2.11)	1.70 (1.18-2.37)	1.42 (1.07-1.84)	1.43 (1.00-1.99)
Colon	1.49 (1.20-1.83)	1.42 (1.13-1.76)	1.35 (1.09-1.66)	Not significant
Leukemia	1.34 (1.04-1.70)	Not significant	1.34 (1.04-1.70)	Not significant
Prostate	2.52 (1.86-3.34)	-	1.96 (1.44-2.59)	-

* 95% confidence interval

Morales et al. (2000) calculated excess lifetime risk estimates for the arsenic-exposed populations in southwestern Taiwan using the same data set used by Wu et al. (1989) and Chen et al. (1992) for residents from 42 villages. However, Morales et al. limited their investigation to the study of bladder, liver, and lung cancer. It was assumed that the number of deaths due to cancer follow a Poisson distribution. The models they considered for the hazard function were of the form: $H(x,t) = h_o(t) \times g(x)$, where $h_o(t)$ represents the instantaneous hazard of dying of cancer at time t for the unexposed population and g(x) is a modifying function to account for an exposure x. The authors developed nine different generalized linear models (GLMs) by considering different forms

for the age effect $h_o(t)$ (linear, regression, and spline), the dose effect g(x) (linear, quadratic, exponential linear, and exponential quadratic), and dose transformation (linear, logarithmic, and square root); a tenth model considered was the multistage-Weibull (MSW). Each of the ten models was fit to the data to determine the constants implicit to the form used for the age and dose effect functions; this process was completed three times, once without consideration of a comparison population, once with considering all of Taiwan as the comparison population, and once considering southwestern Taiwan as the comparison population. The resultant models were compared and ordered using the Akaike information criterion (AIC).

The authors completed their analysis by displaying, for each of the three cancer types, the concentrations of arsenic that would result in a number of different risk measures, including a 1% excess risk (ED_{01}), a 5% excess risk (ED_{05}), and a margin of exposure $MOE_{01}(50)$ which represents the ratio of a point of departure, here taken to be ED_{01}, to an environmental exposure of interest, here taken to be 50 μg/L. The authors state that "the concentrations are reported in U.S. equivalent concentrations of arsenic in drinking water, based on conversions that account for the average weight and average water intake for a male living in the United States compared to a male living in Taiwan." No further details are provided on how this conversion was carried out.

The risk statistics of the best-fitting models vary from model to model, as well as according to whether a comparison population was used. Table 4-6 illustrates the range of estimates for each of the three possible comparison populations.

Table 4-6. Risk Statistics of Best Fitting Models from Morales et al. 2000

Concentrations (μg/L) without Comparison Population								
	Bladder		Lung		Liver		Combined	
	M	F	M	F	M	F	M	F
ED_{01}	351-395	244-365	227-364	256-396	573-864	657-824	163-169	120-267
MOE_{01} (50)	7.0-12.7	4.9-7.3	4.5-7.3	5.1-7.9	11.5-17.3	13.1-16.5	3.3-3.4	2.4-5.3
ED_{05}	1181-1439	796-828	1171-1345	879-898	-	-	703-720	492-605
Concentrations (μg/L) with Taiwanese Comparison Population								
	Bladder		Lung		Liver		Combined	
	M	F	M	F	M	F	M	F
ED_{01}	22-164	17-88	11-196	8-116	239-895	331-551	3-106	2-53
MOE_{01} (50)	0.4-3.3	0.3-1.8	0.2-3.9	0.2-2.3	4.8-17.9	6.6-11.0	0.1-2.1	0-1.1
ED_{05}	504-852	293-455	925-1276	448-579	1089	-	111-544	54-273
Concentrations (μg/L) with Southwestern Taiwanese Comparison Population								
	Bladder		Lung		Liver		Combined	
	M	F	M	F	M	F	M	F
ED_{01}	21-185	19-136	10-181	10-113	119-779	455-597	3-98	2-55
MOE_{01} (50)	0.4-3.7	0.4-2.7	0.2-3.6	0.2-2.3	2.4-15.6	0.2-2.3	0.1-2.0	0-1.1
ED_{05}	649-959	452-624	768-936	520-608	1608	-	93-506	63-284

Data from Tables 8, 9, and 10 of Morales et al. (2000); includes best-fitting of the GLM models plus the MSW model.

Kayajanian (2003) performed a re-analysis of the Taiwan data presented in NRC (1999) (data stated to be from pgs 308-309, Table A10-1). In this analysis, villages were grouped by fives: the lowest five, the next lowest five, etc. For each exposure group the total number of lung, liver, and bladder cancers were put in the numerator, while the denominator contained the totaled man-years associated with these cancers. The results were expressed as fraction as cancers/1000 man-years. The lowest-dose group was found to have a 3 times higher ratio than the next lowest group (1.65 compared to 0.53). The difference was statistically significant with a $p<0.001$. The number of cancers/1000 woman-years was 3 times higher in the lowest dose group compared to the second and third lowest exposure groups (1.62; 0.51, and 0.52, respectively; $p<0.001$). The arsenic concentration in the lowest dose group was 10 to 32 μg/L, 42 to 60 μg/L in the second lowest dose group, and 65 to 110 μg/L in the third lowest dose group. In Morales et al. (2000), the lowest dose group was considered 0 to 100 μg/L, which would not allow for the same results to be noted.

Kayajanian (2003) further tested the observation using mortality from cancer (lung, liver, bladder, kidney, colon, and melanoma) in Utah with four exposure groups (0 to <25; 25 to <75; 75 to <150; and 150to175 μg/L. Although there were no significant differences in the males, the second and third lowest doses were lower than the lowest dose group (2.68, 1.99, and 1.83 cancers per 100 people, respectively). In females, the 25 to <75 μg/L group had results significantly lower than the 0 to <25 μg/L group (2.63 compared to 0.642 cancers per 100 people, respectively). Data by Cuzick et al. (1982) was also re-evaluated and demonstrated that regardless of time from exposure (subjects were intentionally dosed for medicinal purposes), the <500 mg dose group had fewer cancer mortalities than expected. As the doses increased, however, the observed outweighed the expected.

Chiou et al. (2001) conducted a cohort study to evaluate the association between transitional cell carcinoma (TCC) of the bladder and arsenic exposure from drinking water. The study population consisted of 8,102 residents (4,056 men and 4,046 women) in northeastern Taiwan, where each subject's individual arsenic exposure was estimated based on the arsenic concentration in his or her own well water. A total of 3,901 households provided well water samples (85.1%). Arsenic concentrations ranged from undetectable (<0.15 μg/L) to 3.59 mg/L. However, 1136 subjects in 685 households no longer had wells and provided no data on arsenic exposure. Cancer occurrence was ascertained from annual personal interviews, and through hospital records, national death certification, and cancer registries. The study was initiated in October 1991 and follow-up concluded on December 31, 1996. During the follow-up period, there were 18 new cases of urinary tract cancers, including 11 TCCs. There were 15 cases with urinary cancer and 10 cases with TCC that had data on arsenic exposure. Standardized incidence ratios (SIRs) were estimated from the general population of Taiwan. The exposure data were stratified into 3 duration groups (<20.0, 20.1-29.9, and ≥40.0 years) and 4 arsenic levels (≤10.0, 10.1-50.0, 50.1-100.0, and >100.0 μg/L). Cox's proportional hazards regression analysis was conducted to estimate multivariate-adjusted relative risks and 95% confidence intervals. Overall, there was a significantly increased incidence of urinary cancers of the study cohort compared to the general population (SIR=2.05, 95% CI 1.22-3.24). A significant dose-response relationship was also observed after adjustment for age, sex, and cigarette smoking. The multivariate-adjusted relative risks for TCC were 1.9 (95% CI 0.1-32.5), 8.2 (95% CI 0.7-99.1), and 15.3 (95% CI 1.7-139.9), for arsenic concentrations of 10.1-50.0, 50.1-100, and >100 μg/L, respectively, compared to the reference level of ≤10.0 μg/L. The multivariate-adjusted relative risks for total urinary cancers were 1.5 (95% CI 0.3-8.0), 2.2 (95% CI 0.4-13.7), and 4.8 (95% CI 1.2-19.4), for arsenic concentrations of 10.1-50.0, 50.1-100, and >100 μg/L, respectively, compared to the reference level of ≤10.0 μg/L. The duration of exposure was not associated with urinary tract cancers. Since each household in this study had its own drinking water well, individual exposure estimates were much more precise than previous studies in other endemic areas of Taiwan.

Although there were a number of studies performed on populations in Taiwan, there also were a number of studies that examined other geographic regions and found associations between arsenic and cancer. There was a significant ($p<0.001$), dose-related

increase in mortality for both males and females in Córdoba, Argentina for low-, medium-, and high-exposure groups (Hopenhayn-Rich et al., 1996a). The exposure levels for the groups were not specified; however, the average level for the high exposure was 178 μg/L. Water usage data also was not available. In males, respective cancer mortalities (and 95% CI) were: kidney, 0.87 (0.66-1.10), 1.33 (1.02-1.68), and 1.57 (1.17-2.05); lung, 0.92 (0.85-0.98), 1.54 (1.44-1.64), and 1.77 (1.63-1.77); and bladder, 0.80 (0.66-0.96), 1.42 (1.14-1.74), and 2.14 (1.78-2.53). In females the respective mortalities (and 95% CI) were: kidney, 1.00 (0.71-1.37), 1.36 (0.94-1.89), and 1.81 (1.19-2.64); lung, 1.24 (1.06-1.42), 1.34 (1.12-1.58), and 2.16 (1.83-2.52); and bladder, 1.21 (0.85-1.64), 1.58 (1.01-2.35), and 1.82 (1.19-2.64). SMRs were established for deaths occurring between 1986 and 1991 in regions that were determined to have high, medium, or low arsenic in the drinking water up through the 1970s (in the 1970s aqueducts from rivers low in arsenic were built for water consumption) compared to all of Argentina.

The SMRs for liver cancer in all three exposure levels in males and females were significantly elevated, but the dose-response trend was less apparent (males, 1.54, 1.80, and 1.84 for low, medium and high exposure, respectively, $p=0.06$; females, 1.69, 1.87, and 1.92, respectively, $p=0.14$). The SMR for skin cancer was elevated in high exposure females (i.e., 2.78, 95% CI 1.61-4.44) and all male exposure groups (i.e., 2.04, 95% CI 1.38-2.89; 1.49, 95% CI 0.83-2.45; and 1.49, 95% CI 0.71-2.73 for low, medium, and high exposures, respectively), but did not follow a dose-related trend. Since there was only a small number of deaths from skin cancer and most skin cancer is nonfatal, this may underestimate the relationship between arsenic and skin cancer in this study. There was no relationship observed between arsenic exposure and stomach tumors. Although the study could not account for any possible confounding factors due to lack of data on individuals, the study attempted to account for smoking by examining chronic obstructive pulmonary diseases (COPD) since 80% of COPD mortality is associated with smoking. Since there was no association between COPD and arsenic, it was concluded that smoking was not a significant confounder.

Cuzick et al. (1992) studied the excessive mortality of bladder cancer in 478 patients medically treated for skin complaints, malaria, anemia, epilepsy or anxiety with potassium arsenite for various lengths of time (2 weeks to 12 years) during 1945-1969 in England and Wales. There was an excessive occurrence of bladder cancer deaths than expected ($p < 0.05$; 5 observed/1.6 expected) adjusting for age-, sex-, and calendar-year. No bladder cancer deaths were found in the first 5 years. The SMR for patients receiving doses greater than 500 mg of arsenic was calculated to be 5.00 (95% CI 2.0-15). There were no excessive lung cancer deaths observed. Smoking was ruled out as a possible factor in the excess of bladder-cancer deaths as the SMR for bladder cancer in relation to smoking was 0.91 (95% CI 0.74-1.1) using circulatory disease as indicators.

A cohort of 113 people exposed to arsenic concentrations greater than 1.0 mg/L by industrially contaminated drinking water for 5 years in villages of Niigata Prefecture, Japan was used by Tsuda et al. (1995) to determine the SMRs in Table 4-7. The expected

number of deaths were based on sex-, age-, and cause-specific mortality from 1960 to 1989.

Table 4-7. SMRs in Japanese Cohort (Tsuda et al. 1995)

Cancer	Observed deaths/Expected deaths	SMR
Urinary-tract	3/0.10	31.18 (95% CI = 8.62-91.75)
Lung	8/0.51	15.69 (95% CI = 7.38-31.02)
Liver	2/0.28	7.17 (95% CI = 1.28-26.05)
Uterine	2/0.15	13.47 (95% CI = 2.37-48.63)

Bladder cancer risks were determined from 117 bladder cancer cases and 266 population-based controls in Utah after relatively low-level exposure to arsenic in drinking water (Bates et al., 1995). Study participants were surveyed in 1978 for the National Bladder Cancer Study sponsored by the National Cancer Institute. Based on residential-history information and water-sampling information from public water sources, individual exposures to arsenic in drinking water were determined, and it was reported that 92% (81 towns) of the towns in Utah had arsenic concentrations of less than 10 μg/L; only one town had concentrations greater than 50 μg/L. There were no associations between bladder cancer and arsenic exposure levels. Odds ratios were calculated using cumulative dose (defined as the product of micrograms per liter and years of exposure) and were 1.00; 1.56, 95% CI 0.8-3.2; 0.95, 95% CI 0.4-2.0; and 1.41, 95% CI 0.7-2.9 for a relative lifetime exposure of less than 19 mg, 19 to 33 mg, 33 to 53 mg, and greater than 53 mg, respectively. The study suggests that smokers had increased risk in time-window analyses of exposures for years 20-29 (16 cases in the highest exposure group) and 30-39 years (9 cases in the highest exposure group) prior to the surveys.

Arsenic exposure was related to an increased risk of mortality in both males and females 30 years of age or older from Northern Chile when compared to the National mortality data (Smith et al., 1998). The SMRs (and 95% CI) for cancer mortalities in males and females were: bladder, 6.0 (4.8-7.4) and 8.2 (6.3-10.5), respectively ($p<0.001$); lung, 3.8 (3.5-4.1) and 3.1 (2.7-3.7), respectively ($p<0.001$), kidney, 1.6 (1.1-2.1) and 2.7 (1.9-3.8), respectively ($p=0.012$ and <0.001, respectively); and skin 7.7 (4.7-11.9) and 3.2 (1.3-6.6), respectively ($p<0.001$ and $=0.016$, respectively). There was no increased risk of mortality from liver cancer in either males or females (SMR = 1.1 with a 95% CI of 0.8-1.5 for both; $p>0.3$) associated with arsenic exposure. Concentrations of arsenic in drinking water were well-documented and had been high in all major population centers of Region II, especially before 1975. The population-weighted average in the years 1950-1974 was 420 μg/L with a maximum of 870 μg/L measured in Antofagasta (the largest city) between 1955 and 1969. The arsenic exposure in all cities or towns (8 examined) exceeded the standard of 50 μg/L from 1950 until 1990. The mortality rate was obtained during the period of 1989 and 1993. The reported number of excessive lung cancer deaths among men (400.8) was about 5 times that for bladder cancer (77.5). Among women, the number of excessive lung cancer deaths (105) was about twice that for bladder cancer (56.2). The study examined the SMRs by age and demonstrated that the greatest excessive risk for lung

cancer and COPD occurred in the 30-39 age group for both males and females (observed/expected = 5.0 and 60.0, respectively), which would most likely be associated with exposure during childhood. In addition, females had the greatest excessive risk of death from liver (observed/expected = 2.5) and kidney (observed/expected = 30) cancers in this age group. The study had limited information on smoking and could not separate the results by age or sex, but did not demonstrate any regional differences in smoking habits that may explain the increased cancer risk. The study estimates that 9.3% of all male and 4.9% of all female deaths from bladder, kidney, lung, and skin cancer may be attributable to arsenic exposure. Although this study has the limitations of an ecological study design, several strengths were identified. There were well-defined population exposures, compared with several other exposed populations, and mortality to COPD was low, indicating low smoking rates in this population.

Kurttio et al. (1999) established an association between arsenic and bladder cancer by evaluating the relative risks (RR) in 61 bladder cancer cases diagnosed between 1981 and 1995 in Finland who drank water from drilled wells between 1967 and 1980 compared to an age and sex balanced reference cohort of 275 subjects. The dose-dependent relationship between arsenic and bladder cancer was only noted when exposure was calculated as beginning of the use of the well until 2 years before diagnosis (short latency; RR ratios of 1, 1.53 (95% CI 0.75-3.09), and 2.44 (95% CI 1.11-5.37) for concentrations in the water of <0.1, 0.1-0.5, and ≥0.5 μg/L, respectively; RR ratios of 1, 1.34 (95% CI 0.66-2.69), and 1.84 (95% CI 0.84-4.03) for daily doses of arsenic of <0.2, 0.2-1.0 and ≥1.0 μg/day). When exposure was considered from the beginning of the use of the well until 10 years before diagnosis (long latency), there was no association with an increase in risk for bladder cancer. Smoking also was associated with bladder cancer with a possible synergistic effect observed between arsenic and smoking on the risk of developing bladder cancer. There was no relationship between kidney cancer and arsenic exposure. Misclassification of exposure was possible during the study due to recall bias since the study was interested in water consumption from the 1970s. In addition, the study did not attempt to measure arsenic intake from dietary sources or nutritional factors which may effect the metabolism and carcinogenicity of arsenic.

Chiou et al. (2001) performed a cohort study to examine the relative risk of transitional cell carcinomas in relation to ingested arsenic of 8,102 residents in northeastern Taiwan. Estimations of individual exposure was based on the arsenic concentration in their well water and questionnaire information on duration of consumption. The occurrence of urinary tract cancers were ascertained by follow-up interviews, community hospital records, national death certification profile, and cancer registry profile. Multivariate-adjusted relative risk and 95% confidence intervals (CI) were estimated using Cox proportional hazards regression analysis. There was a dose-dependent trend for both transitional cell carcinomas ($p<0.05$; 1, 1.9, 95% CI 0.1-32.2; 8.1, 95% CI 0.7-98.2; and 15.1, 95% CI 1.7-138.5 for arsenic concentrations in the well water of 0-10.0, 10.1-50.0, 50.1-100.0, and >100.0, respectively) and all urinary cancers ($p<0.01$; 1, 1.6, 95% CI 0.3-8.4; 2.3, 95% CI 0.4-14.1; and 4.9, 95% CI 1.2-20.0, respectively).

A cohort of residents from Millard County, Utah was assembled using historical documents of the Church of Jesus Christ of Latter-Day Saints for establishing an association between arsenic in the drinking water and mortality outcome (Lewis et al., 1999). SMRs were calculated using residence history and median drinking water arsenic concentrations. Lewis et al. (1999) evaluated the SMR by low (<1000 ppb-year), medium (1000-4999 ppb-year), and high (≥5000 ppb-year) exposures per sex, as well as all exposures combined by sex. Arsenic exposure took into consideration the number of years living in a certain area and the concentration of arsenic for that specific area to obtain a cumulative estimate of exposure. The level of arsenic in the drinking water for the different towns ranged from a mean of 18.1 to 190.7 ppb (μg/L). The low dose was selected based on 20 years of exposure for most cancers to manifest with exposure to 50 ppb for a cumulative arsenic exposure of 1000 ppb-years. Although all endpoints were examined, only cancer endpoints are referred to at this point.

Lewis et al. (1999) conducted a retrospective cohort mortality study of 4,058 residents of Millard County, Utah. Drinking water in this region is derived from wells where arsenic concentrations range from undetectable up to a few hundred micrograms per liter. Lewis et al. (1999) calculated SMRs using residence history and median drinking water arsenic concentrations (i.e., low, <1000 ppb-year; medium, 1000-4999 ppb-year; and high, ≥5000 ppb-year), exposures by sex as well as all exposures combined by sex. Arsenic exposure took into consideration the number of years living in a certain area and the concentration of arsenic for that specific area to yield a cumulative estimate of exposure. For several causes of death, statistically significant increases in SMRs occurred in a generally uneven pattern based on exposure level. Such as the significant ($p \leq 0.05$) associations between arsenic and mortality overall in males from prostate cancer (i.e., SMR=1.07, 1.70, and 1.65 for low, medium, and high exposure). Females had a significant association between melanomas and arsenic exposure. The SMR for all females was 1.82 (95% CI, 0.50-4.66), and was 5.30 (no CI provided) in the low exposure group. Results were not provided for the medium and high exposure groups. While there were no other significant associations between specific cancers and arsenic exposure in females, there were increased SMR values for biliary passage and liver cancer (i.e., SMR=1.42 all females; 95% CI 0.57-2.93), kidney cancer (i.e., SMR=1.60 all females; 95% CI 0.44-4.11), and all other malignant neoplasms (i.e., SMR=1.34; 95% CI 0.84-2.03). This study did not look at any other possible confounding factors, such as smoking, on the mortality. However, smoking was rare in this population due to the churches prohibition of the use of tobacco, alcohol, or caffeine. NRC (2001) details several limitations to this study mainly on the exposure estimates. In addition, comparison rates used in the analysis were for the state of Utah with the study cohort composed of Mormons who have strict religious prohibitions. Although smoking rates in the state of Utah are low (12-13%), this rate is expected to increase the rates of several cancers (e.g., lung). That fact and the rural setting of the cohort were possible contributors to the deficits observed in SMRs for urinary and pulmonary cancers.

A case-control study using patients diagnosed with lung cancer between 1994 and 1996 in northern Chile and frequency-matched hospital controls provides evidence that consumption of inorganic arsenic from drinking water is associated with human lung cancer (Ferreccio et al., 2000). Control selection was considered a major weakness by the study authors and included two types of control. First, patients were selected who had been diagnosed with cancers that are generally not suspected to be related to arsenic and second patients were selected with diagnosis other than cancer. This limitation of the controls likely biased the estimates towards lower odds ratios. There was a clear dose-related increase in the odds ratio when adjustments were just made for age and sex (1; 1.5, 95% CI 0.4-4.6; 4.0, 95% CI 1.4-12.1; 4.6, 95% CI 2.2-10.0; and 8.0, 95% CI 3.8-17.0 for arsenic water concentrations of 0-10, 10-29, 30-49, 50-199, and 200-400, respectively) or when adjustments also included smoking, socioeconomic status, and working in copper smelting (1; 1.6, 95% CI 0.5-5.3; 3.9, 95% CI 1.2-12.3; 5.2, 95% CI 2.3-11.7; and 8.9, 95% CI 4.0-19.6, respectively). The study also demonstrated a greater than additive effect between arsenic exposure and smoking (odds ratios in never smoked for arsenic concentrations ≤49 [considered the referent category], 50-199, and ≥200 μg/L were 1; 5.9, 95% CI 1.2-40.2; and 8.0, 95% CI 1.7-52.3, respectively; odds ratios in ever smoked group were 6.1, 95% CI 1.31-39.2; 18.6, 95% CI 4.13-116.4; and 32.0, 95% CI 7.22-198.0, respectively). In addition, peak exposure in the cases mainly occurred 20-40 years prior to diagnosis. This study has individual estimates of exposure on all subjects for more than 40 years. Because of the time to onset of lung cancer, this is a study strength for causal association. Other strengths include an acceptable response rate, unbiased ascertainment of exposure, information of individual data on potential confounding factors for lung cancer, appropriate analysis of study data, and adequate study size.

A number of studies on the association between arsenic exposure and cancer have appeared in the peer-reviewed literature after the NRC (2001) report was published. These are discussed below.

Tucker et al. (2001) analyzed data describing prevalent skin cancer from a cross-sectional study in a region of Inner Mongolia with increased concentrations of arsenic in drinking water. The 1992 study examined a total of 3,179 persons in three villages and the well-water-use histories for these individuals. Water samples were collected from 184 of the 187 local wells and analyzed for arsenic content. The median age of the participants was 29 years with an average well-use history of 25 years. Arsenic in drinking water ranged from below detection (10 μg/L) to 2,000 μg/L. Skin cancer was observed in eight subjects. Several statistical models (frequency weighted, simple linear regression, hockey stick, and maximum likely estimate) were used to analyze data. Two measures of exposure were used with each model. A dose-response relationship was found for skin cancer.

Karagas et al. (2001) measured arsenic levels in toenail clippings from subjects with basal cell carcinoma (587), squamous cell carcinoma (284), or controls (524). The study demonstrated that there was only an increase in squamous cell carcinomas (odds ratio= 2.07 with a 95% CI of 0.92 to 4.66) and basal cell carcinomas (odds ratio=1.44 with

a 95% CI of 0.74 to 2.81) in the subjects with the highest (i.e., 0.345-0.81 μg/g) levels of toenail arsenic. Toenail arsenic levels from 0.009-0.344 μg/g did not have elevated odds ratios. However, arsenic measured in toenail clippings may result from external exposure as well as internal exposure and typically relates exposure from a two week period occurring approximately a year prior to sampling. Therefore, the exposure measured in the toenail clippings did not occur during the critical period for development of skin cancer (NRC, 2001).

A re-analysis of the Wu et al. (1989) and Chen et al. (1992) data sets by Lamm et al. (2003) used exposure to arsenic as a continuous variable, while simultaneously stratifying on the type of well used as a drinking water source (i.e., artesian, shallow, or mixed). Artesian wells (14 villages) all had medians > 325 μg/L, while shallow wells (19 villages) had medians < 325 μg/L and mixed wells (9 villages) had medians both greater than and less than 325 μg/L. This approach determined that there was no increase in bladder cancer mortality rates below 400 μg/L in any of the well types, and that a significant dose-response relationship between arsenic exposure and bladder cancer rates was present in only the artesian wells (median levels ranging from 350-934 μg/L). The median arsenic levels for the shallow and mixed wells ranged from 10-717 μg/L. The authors suggest two possible reasons for their findings: 1) arsenic is acting as a high-dose carcinogen, or 2) a co-carcinogenic factor is present only in artesian wells and not the shallow/mixed wells. However, this study has several limitations of its own. First the classification into village well type was based on median arsenic concentrations such that well type and arsenic concentration are not independent variables. In addition, the study did not consider age or smoking status as factors since the data were not available. These limitations were suggested by the U.S. EPA (2004a). In addition, the U.S. EPA (2004a) suggests that the use of linear regression may not be appropriate because bladder cancer rates are not normally distributed, and that a Poisson regression would have been more appropriate.

Because Morales et al.'s (2000) analysis of the Taiwan data suggest that excessive cancer mortality may occur in many populations where the drinking water standard for arsenic is set at 50 μg/L (the current drinking water standard for arsenic in the U.S.) and Lamm et al. (2003) suggest that this was not the case, Lamm et al. (2004) performed an ecological study examining the relationship between bladder cancer mortality in white males from 133 counties in the United States and arsenic exposure through drinking water. The study was designed to be analogous to the Wu et al. (1989) Southwest Taiwan Study. Arsenic exposure from groundwater arsenic data collected by the U.S. Geological Survey (USGS) for 133 US counties. These counties exclusively used groundwater as a source of drinking water, had arsenic concentrations ≥3 μg/L, and reported at least one white male bladder cancer death. White male bladder cancer mortality data from 1950-1979 were extracted from National Cancer Institute (NCI) and EPA (NCI/EPA 1983). The study included over 4500 US white male bladder cancer deaths, and the data suggest that there was no increase in bladder cancer mortality in the 3-60 μg/L range of arsenic exposure. Standardized mortality ratios (SMR) that were stratified by median arsenic groundwater

concentrations, ranged from 0.95 (95% CI, 0.89-1.01) in the lowest exposure group (3.0-3.9 μg/L) to 0.73 (95% CI, 0.41-1.27) in the highest exposure group (50.0-59.9 μg/L). The composite totals found an SMR of 0.94 with 95% CI of 0.90-0.98.

A major limitation in the Lamm et al. (2004) study was the lack of information about the counties or specifics indicating that the counties were similar in nature. If the counties with higher concentration of arsenic in their groundwater had younger males or better medical facilities, results would tend to show lower mortality in these areas. Other potential confounding factors that were not addressed include: diet, use of alternative water sources, variation of intake, age, duration of exposure, occupation, income, smoking status, and migration. In addition, exclusion of counties without bladder cancer mortality may also bias the results. U.S. EPA (2004a) discusses these limitations and also states that the authors overanalyzed the data and that the method of calculating the expected number of cases was unconventional.

Steinmaus et al. (2003) conducted a case-control study of bladder cancer in relation to arsenic in the drinking water in six counties of western Nevada and Kings County in California, selected because the counties contained the largest population historically exposed to approximately 100 μg/L of arsenic in their drinking water. The 181 cases presented in the article were 20-85 years old with primary bladder cancer diagnosed between 1994 and 2000. Controls (328 identified, 248 of which were interviewed) were matched to cases by 5-year age groups and gender and were selected by random digit dialing (for controls under 65) or using the Health Care Financing Administration (for controls over 65 years old). Arsenic exposure was assessed for each subject by linking the residence within the study area to a water arsenic measurement for that residence. Fluid intake (L/day), estimated by interviewing cases and controls or their relatives, was multiplied to the arsenic concentration (μg/L) to determine average daily arsenic intake (μg/day).

Overall, Steinmaus et al. (2003) did not find an association between bladder cancer risk and arsenic exposure, with an adjusted odds ratio of 0.94 (95% CI, 0.56-1.57; $p=0.48$) for intakes greater than 80 μg/day. The adjusted odds ratios were not significant when the data were stratified by exposures using the highest 1-year, 5-year, 20-year averages or cumulative exposures, or by the length of exposures (5 years, 20 years or 40 years). However, for smokers with arsenic exposures of 40 or more years to > 80 μg/day (median intake 177 μg/day), the odds ratio was 3.67 (95% CI, 1.43-9.42; $p<0.01$). These results support the hypothesis that arsenic and cigarette smoke act synergistically in causing bladder cancer, and suggest that the latency of arsenic-caused cancer may be greater than 40 years. This study had several limitations, particularly in non-matching characteristics of the cases and controls involving income, education, and smoking. Other limitations involved possible exposure misclassification (because more next-of-kin of cases were interviewed than controls) and lack of exposure data for 31% of the private wells (accounting for 11% of the person-years). Because of the long latency period for bladder cancer and the small number of cases, it is unlikely that a statistically significant risk for

bladder cancer would be detected in the low levels of arsenic exposure among never smokers. U.S. EPA (2004) reported that for a moderate (2.0-3.0 relative risk) association, a sample size of 178,000 to 535,000 would be needed. In addition, cases that chose not to participate in the study were more likely to live in areas of known arsenic contamination than nonparticipating controls. The authors did not have arsenic levels for 31% of the private wells, which accounted for 11% of the person-years. The authors gave this group a value of zero for the wells. Zero exposure also was applied to residents living outside the study area and those using bottled water or filters to remove arsenic. In doing so they bias the results towards the null effect. Because of the long latency period for bladder cancer and the small number of cases, it is unlikely that a statistically significant risk for bladder cancer would be detected in the low levels of arsenic exposure among never smokers. The sample size needed to detect a very weak (1.15 relative risk) association would be 17,045,000. U.S. EPA (2004) reported that for a moderate (2.0-3.0 relative risk) association, a sample size of 178,000 to 535,000 would be needed.

Tollestrup et al. (2003) conducted a retrospective cohort study to determine whether childhood exposure to ambient arsenic was associated with increased mortality rates. The cohort was comprised of children who had lived within 2 miles of a copper smelter and arsenic refinery (American Smelting and Refining Company) in Ruston, Washington, for at least 2 years from 1907-1932. The subjects were identified from school census records, and included 1,827 boys and 1,305 girls with an age limit of 14 years. Exposure intensity was calculated as the total number of days spent at a residence within 1 mile of the smelter stack, and grouped by the number of years spent at the residence: 0 ≤ 1.0 year, 1.0-3.9 years, 4.0-4.9 years, and ≥ 10.0 years. A total of 3,336 potential subjects were identified, and 196 were excluded because they had worked at the smelter. Crude mortality rates were based on person-years of follow-up, and calculated for 10 general causes of death. The highest crude mortality rate for boys was for ischemic heart disease in all exposure intensity groups, but no evidence of a dose-response relationship was found. The 2nd highest mortality rate for boys was for malignant neoplasms, with a range of 12.5/10,000 person-years to 21.9/10,000 person-years. A dose-response was observed only for the mortality rate for "external causes," such as motor vehicle accidents. Cox proportional hazard ratios adjusted for year of birth found only one exposure group (≥ 10.0 years) for which the mortality rations were significantly higher than 1.00. These included all causes of death (1.52, 95% CI 1.23-1.86), ischemic heart disease (1.77, 95% CI 1.21-2.58), and external causes (1.93, 95% CI 1.03-3.62). Although girls also had the highest crude mortality rates for malignant neoplasms and ischemic heart disease, no dose-response relationships were observed. This study did not find consistent patterns of adverse health effects from childhood exposure to ambient arsenic at levels much lower than occupational settings.

A deficiency of the Tollestrup et al. (2003) study was the truncation of the study period to 1932 which could result in exposure misclassification. Other limitations include ambiguous exposure data (exposures to arsenic were not chronic and were unknown since air and soil levels were not quantified), poor follow-up (34.7% of boys and 46.5% of girls

were not found after their last date of exposure), the use of crude mortality rates, and lack of information on smoking within the cohort and on family members who worked at the smelter and could have brought arsenic into the household.

Chen et al. (2004) investigated the relationship between ingested arsenic and lung cancer and the effect of smoking on the relationship. A total of 2,503 residents in southwestern and 8,088 residents in northeastern Taiwan were followed for an average period of 8 years. These were areas where residents had been drinking well water contaminated with high concentrations of arsenic until the establishment of public water systems. Questionnaires were administered to all participants in the study eliciting information on residential and occupational history, history of drinking well water, cigarette smoking and alcohol consumption. Water measurements taken in the 1960s of shared artesian wells in the southwestern area were used in conjunction with information derived from the questionnaire to derive an average arsenic concentration for each participant, which was used as an exposure metric in subsequent analysis. Average arsenic concentrations for participants in the northeastern region, who derived their drinking water from shallow wells, were determined by direct measurement of individual wells. The incidence of lung cancer was ascertained from national registry data for the period the period January 1985-December 2000. During the follow up period of 83,783 person-years, 139 lung cancer cases were diagnosed.

After adjusting for cigarette smoking and other risk factors such as age, alcohol consumption, and years of schooling, a significant ($p < 0.001$) increasing trend in lung cancer was shown to result from increasing average levels of arsenic in well water. With levels <10 μg/L as the referent, relative risks (with 95% confidence intervals) for those consuming drinking water with arsenic concentrations of 10-99, 100-299, 300-699, and ≥700 μg/L, were respectively, 1.09 (0.63-1.91), 2.28 (1.22-4.27), 3.03 (1.62-5.69), 3.29 (1.60-6.78). It was further shown that 32% to 55% of lung cancer cases were attributable to both arsenic exposure and cigarette smoking. The synergism was shown to be additive; multiplicative interaction was not statistically demonstrated.

NRC (2001) made a series of recommendations for conducting the dose-response assessment:

- Since the formula used by Morales et al. (2000) to compute ED_{01} did not easily accommodate the incorporation of the baseline cancer risk based on the U. S. population, the ED calculation should be done according to a formula presented in Appendix II of the BEIR IV analysis of lung cancer associated with radon exposure (NRC 1988).
- Morales et al. (2000) estimated risk using no comparison population, the entire Taiwanese population as a comparison population, and the southwestern Taiwanese population as a comparison population. The NRC recommended using a comparison population in risk estimation.

- Because of the biological plausibility and consistency with other approaches to quantitative risk assessment, the additive Poisson model with a linear dose should be used in the risk assessment.
- Since the dietary intake of arsenic in the Taiwanese population is higher than in the United States population, a constant concentration of arsenic should be added to the exposure rates for all individuals in the study villages. The Committee recommended use of a background rate in food of 30 μg/day, assuming a 50 kg weight for a Taiwanese person.

With these assumptions, the Subcommittee used the southwestern Taiwanese data set (Chen et al., 1985, 1982; Wu et al., 1989) to perform maximum likelihood estimates of the excess lifetime risk (incidence per 10,000 people) of lung and bladder cancer for populations exposed to various concentrations of arsenic in drinking water. National Taiwan data was used as background incidence. The results are shown in Table 4-8, where it was further assumed that the typical U.S. resident weighs 70 kg, compared with 50 kg for the typical Taiwanese, and that the typical Taiwanese drinks just over 2 L of water per day, compared with 1 L per day in the United States.

Table 4-8. NRC 2001 Maximum Likelihood Estimates of Excess Lifetime Risk (per 10,000 people)

Arsenic Concentration (μg/L)	Bladder Cancer		Lung Cancer	
	Females	Males	Females	Males
3	2.3	2.0	1.8	1.7
5	3.8	3.2	3.0	3.0
10	7.5	6.8	6.2	6.1
20	15	13	12	12

Confidence Intervals not provided.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS - ORAL AND INHALATION

4.2.1. Prechronic and Chronic Studies

Wei et al. (1999 and 2002) demonstrated that 10-week-old male F344/DuCrj rats (36/group) administered 12.5, 50, or 200 ppm dimethylarsenic acid (DMA; a major metabolite of inorganic arsenic) in their drinking water for 104 weeks had no affect on the morbidity, mortality, body weights, hematology, or serum biochemistry. Reductions in electrolyte concentrations in the urine were related to an increase in urinary volume resulting from an increased water consumption in the 50- and 200-ppm groups. There was no difference in the urinary pH between control and treated rats.

4.2.2. Cancer Bioassays

Cancer bioassays with inorganic arsenic have obtained negative results with mice, rats, hamsters, rabbits, beagles, and cynomologus monkeys (for review see Kitchin, 2001; NRC, 1999). However, the following studies have observed increases in tumors in animals exposed to arsenic.

Transplacental-Mice

Timed pregnant female C3H/HeNCr (C3H) mice (10/group) were administered 0 (control), 42.5, or 85 ppm arsenite in their drinking water *ad libitum* from Day 8 to Day 18 of gestation (Waalkes et al, 2003). Strain and doses used in the experiment were determined through preliminary short-term testing that determined C3H mice to be the most sensitive to arsenic toxicity of the three strains tested (i.e., C3H, C57BL/6NCr, and B6C3F1/NCr), and the preliminary test indicated that a dose of 100 ppm was unpalatable and resulted in approximately 10% reduced growth in the offspring. The doses used in this study did not affect maternal water consumption or body weight in the dams. It was estimated that the pregnant females consumed 9.55 to 19.13 mg arsenic/kg/day for a total dose of 95.6 to 191.3 mg arsenic/kg.

Offspring were weaned at 4 weeks and received no additional exposure to arsenic. Male and female offspring (25/sex/group) were observed for the next 74 or 90 weeks, respectively. Males were sacrificed at 74 weeks due to high mortality in the high-dose group beginning at 52 weeks. Both the 42.5- and 85-ppm males had a significant increase in the incidence of hepatocellular carcinomas (12.5% in controls vs. 38.1% in 42.5-ppm and 60.9% in 85-ppm groups) and adrenal cortical tumors (37.5% in controls vs. 66.6% in 42.5-ppm and 91.3% in 85-ppm groups), which followed a significant ($p \leq 0.001$), dose-related trend. In addition, the 85-ppm group had a significant increase in the multiplicity (tumor/mouse) for both hepatocellular carcinomas (0.13, 0.42, and 1.30, respectively) and adrenal tumors (0.71, 1.10, and 1.57, respectively) which also had a significant ($p \leq 0.02$), dose-related trend. Although there was no differences in the incidence of hepatocellular adenomas in males, the multiplicity of hepatocellular adenomas (0.71, 1.43, and 3.61, respectively) followed a significant ($p<0.0001$), dose-related trend.

Males and females had an increase in lung tumors (8.0%, 13.0%, and 25.0%, respectively, in females, 0%, 0%, and 13.0%, respectively, in males), which followed a significant ($p \leq 0.03$), dose-response trend. In addition, females had increases in the incidence of benign ovarian tumors, which reached statistical significance in the 85-ppm group. Although a significant increase was not observed in malignant ovarian tumors, the total incidence (benign plus malignant) of ovarian tumors was significant in the 85-ppm group and followed a significant ($p=0.015$), dose-related trend (8% in control vs. 26% in 42.5-ppm and 37.5% in 85-ppm groups). There was an increase in uterine tumors that was not significant and did not follow a dose-response, but was accompanied by a significant ($p=0.0019$), dose-related increase in hyperplasia with a significant increase occurring at

both doses. Females also had a dose-related increase in hyperplasia of the oviduct. The number of tumor-bearing males and the number of males bearing malignant tumors was significantly increased in both dose groups and followed a significant (p=0.0006 and 0.0001, respectively), dose-related trend. Females had a slight increase in tumor-bearing animals, which did not reach statistical significance and did not appear to be dose-related. The number of females bearing malignant tumors was significantly increased for both dose groups, but not in a dose-dependent manner.

Waalkes et al. (2004a) followed this same procedure (except offspring were observed for 104 weeks), but 25 male and 25 female offspring from each exposure group (0, 42.5, or 85 ppm in the drinking water from gestational days 8 to 18 with no additional exposure after birth) were exposed to acetone or 12-*O*-tetradecanoyl phorbol-13-acetate (TPA; 2 μg/0.1 ml in acetone) twice a week to a shaved area of dorsal skin for 21 weeks after weaning in an attempt to promote skin tumors. However, very few skin lesions occurred and were not associated with arsenic exposure either in the absence or presence of TPA. As was noted in Waalkes et al. (2003), there was a dose-dependent increase in the incidence and multiplicity of hepatocellular adenomas and carcinomas in treated males, both in the absence (adenomas: 41.7, 52.2, and 90.5% for the 0-, 42.5-, and 85-ppm exposure groups, respectively; carcinomas: 12.5, 34.8, and 47.6%, respectively; total incidence:50, 60.9, and 90.5%, respectively; multiplicity: 0.75, 1.87, and 2.14 respectively) and presence (adenomas: 34.8, 52.2, and 76.2%, respectively; carcinomas: 8.7, 26.0, and 33.3%, respectively; total incidence:39.1, 65.2, and 85.7%, respectively; multiplicity: 0.61, 1.44, and 2.14 respectively) of TPA with a statistically significant increase noted with 85-ppm arsenic. Arsenic only caused a dose-dependent increase in hepatocellular adenomas and carcinomas in the presence of TPA in females (adenomas: 8.3, 18.2, and 28.6% for the 0, 42.5, and 85 ppm exposure groups with TPA exposure, respectively; carcinomas: 4.2, 9.1, and 19.0%, respectively; total incidence:12.5, 27.3, and 38.1%, respectively; multiplicity: 0.13, 0.32, and 0.71 respectively) with a statistically significant increase in total incidence and multiplicity for the 85-ppm group.

There also was an increase in ovarian adenomas in treated female offspring regardless of whether they were treated with TPA (0, 22.7, 19.0%, respectively) or acetone (0, 17.4, and 19.0%, respectively). There was no effect on the incidence of ovarian carcinomas. This was accompanied by increases in the incidence of uterine epithelial hyperplasia (cystic) and total uterine proliferative lesions, which increased in severity with dose. There also was a dose-dependent increase in oviduct hyperplasia.

Male offspring exposed to arsenic had an increase in the incidence and multiplicity of cortical adenomas of the adrenal glands. The increases were statistically significant for both arsenic exposure groups, but was only related to dose in the absence of TPA (p=0.020). Incidences were as follows: 37.5, 65.2, and 71.4% for the 0-, 42.5-, and 85-ppm dose groups, respectively, in the absence of TPA and 30.4, 65.2, and 57.1%, respectively, with TPA treatment. Multiplicities also were statistically significantly increased in arsenic exposed male offspring with a significant dose-dependent trend both in

the absence (0.58, 2.13, and 2.19, respectively; p=0.0014) or presence (0.54, 1.65, and 1.62, respectively; p=0.016) of TPA.

Lung adenomas were increased in a dose-dependent manner in females exposed to TPA (4.2, 9.1, and 28.6%, respectively; p=0.018), but not in the absence of TPA (4.2, 8.7, and 9.5%, respectively; not significant). Males only had a statistically significant increase (5-fold increase) in lung adenomas in the 42.5-ppm group exposed to TPA.

Arsenic caused a statistically significant increase in the tumor multiplicity of all tumors in males (with or without TPA), which was not dependent on dose. Although females also had an increase in the tumor multiplicity of all tumors, the only statistically significant increase occurred in the 85-ppm group exposed to TPA. The increase in females exposed to TPA also appeared to be dose-dependent The statistically significant increase observed in the multiplicity of malignant tumors in males was greater in the absence of TPA, but was dose-dependent in the presence of TPA. In females, there was also an increase in the multiplicity of malignant tumors in arsenic treated mice (regardless of TPA exposure), but the results did not reach statistical significance nor were they dose-dependent.

Rat-Oral

Wei et al. (1999 and 2002) demonstrated that 10-week-old male F344/DuCrj rats (36/group) administered 50, or 200 ppm dimethylarsenic acid (DMA; a major metabolite of inorganic arsenic) in their drinking water for 104 weeks developed bladder tumors (mainly carcinomas) and papillary or nodular hyperplasia in a dose-dependent manner. Controls and rats administered 12.5 ppm did not develop any bladder tumors or hyperplasia. There was a significant ($p<0.05$) increase in 5-bromo-2'-deoxyuridine (BrdU) labeling of morphologically normal epithelium of the bladder in the 50- ($p<0.05$) and 200-ppm ($p<0.01$) groups (Wei et al., 2002). There was no significant increase in any other tumor type related to DMA treatment. There appeared to be a dose-related increase in subcutis fibromas (i.e., 4% in controls, 12% in 12.5-ppm group, and 16% in both the 50- and 200-ppm groups). Data indicate that multiple genes are involved in the stages of DMA-induced urinary bladder tumors. Wei et al. (2002) further indicate that reactive oxygen species (ROS) may play an important role during the early stages of DMA carcinogenesis.

Shen et al. (2003) administered TMAO, an organic metabolite of inorganic arsenic, to male F344 rats for 2 years via their drinking water at concentrations of 0, 50, or 200 ppm. Daily intakes were estimated to be 0, 638, and 2475 mg/kg, respectively. From 87 weeks of treatment on, there was an increase in the incidence and multiplicity of hepatocellular adenomas in rats sacrificed or dead. Incidences of 14.3, 23.8, and 35.6%, respectively, were reported. The respective multiplicities were 0.21, 0.33, and 0.53. The results were statistically significant in the 200-ppm dose group.

Other

Transgenic models also have been developed to examine arsenic carcinogenesis. Arsenic exposure (200 ppm sodium arsenite in drinking water for 4 weeks) in transgenic (Tg.AC) mice containing activated H-ras did not induce skin tumors alone, but in the group also administered subsequent skin painting with TPA an increase in the number of papillomas was noted in arsenite treated mice compared to TPA alone. Thus, it was suggested that arsenite may be a "co-promoter" in skin carcinogenesis (Germolec et al., 1997; Luster et al., 1995).

Ten ppm of either sodium arsenite or DMA^V (cacodylic acid) administered for 5 months in the drinking water of K6/ODC transgenic mice induced a small number of skin papillomas (Chen et al., 2000a). K6/ODC transgenic mice have hair follicle keratinocytes, (likely targets for skin carcinogens), which over express ornithine decarboxylase (ODC). ODC is involved in polyamine synthesis, which is needed in S phase. Over expression of ODC is sufficient to promote papilloma formation without administration of TPA, which has been demonstrated to induce ODC (O'Brien et al., 1997).

Rossman et al. (2001) administered sodium arsenite (10 ppm) in the drinking water of hairless Skh 1 mice for 26 weeks in conjunction with 1.7 kJ/m^2 solar UVR (ultraviolet radiation; considered a low, nonerythemic dose) three times weekly (duration unspecified in Rossman, 2003) demonstrated a 2.4-fold increase in the yield of skin tumors than in mice administered UVR alone. A second experiment by the same group (Burns et al, 2004), demonstrated a 5-fold increase in skin tumors using 5 mg/L arsenite with 1 kJ/m^2 solar UVR, but also observed a significant increase with 1.25 ppm arsenite. The skin tumors (mainly squamous cell carcinomas) occurred earlier, were larger, and were more invasive in mice administered arsenite. Arsenite alone did not induce skin tumors. Rossman (2003) suggests that this demonstrates arsenite enhances the onset and growth of malignant skin tumors induced by a genotoxic carcinogen in mice. Rossman (2003) also suggests that the increased tumor incidence observed by Waalkes et al. (2003) may be due to the same enhancement as C3H mice have a high background of spontaneous tumors and suggests the need for examining the transgenic effects in another strain of mice with a lower background tumorgenicity.

A critical review of the inhalation data was not conducted as part of this evaluation discussed in this report.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES - ORAL AND INHALATION

Not addressed in this draft.

4.3.1. Oral

Not addressed in this draft.

4.4. OTHER STUDIES

Possible Modes or Mechanisms of Action

As discussed above, the metabolism of inorganic Arsenic in humans occurs through alternating steps of reduction and methylation including formation of DMA^{V}, DMA^{III}, monomethylarsonic acid (MMA^{V} and MMA^{III}), and trimethanearsinic oxide (TMAO) [19]. Many of the major metabolites MMA, DMA and TMAO have been subjected to a variety of toxicological tests in vivo and in vitro. The various forms of arsenic have been shown to differ in toxicity. The trivalent species MMA^{III} and DMA^{III} have recently been identified as the most toxic and genotoxic forms in several assay systems (Thomas et al., 2001). The relative contributions of the organic metabolites, together with inorganic arsenic to the toxicity and carcinogenesis of inorganic arsenic are uncertain. Each of the arsenical metabolites exhibits its own toxicity, possibly via similar and/or separate MOAs that are responsible for inorganic arsenic toxicity and tumor formation (Kitchin, 2001).

Inorganic arsenic is unique as it is one of a small group of demonstrated individual chemicals that is carcinogenic to humans and produces tumors at multiple sites (bladder, lung, skin, liver, and possibly kidney). Rodents are generally nonresponsive to the tumorigenic effects of iAs except for a recent transplacental mouse study where arsenite gave liver, lung, ovarian, and adrenal cortical tumors. After decades of research on arsenic, we have learned a great deal about how arsenic interacts with biological systems and is affected by biological systems, but we still do not know how arsenic induces human cancer. Humans are more responsive to arsenic in terms of breadth of effects than any single rodent species. To date, we do not have enough information to explain these differences.

The biotransformation and pharmacodynamics of iAs are complex in mammalian systems with arsenite being biotransformed through a series of reduction and methylation steps in a cascade to form the final urinary metabolite, trimethylarsine oxide, and possibly its reduced form, trimethylarsine. Arsenical forms of greater instability are produced within each step, and these forms have greater reactivity toward biological and biochemical intermediates, and biological macromolecules. Each intermediate arsenical form has the potential to induce cancer (genotoxicity) or to affect the promotion and progression of cancer such as affecting signal transduction pathways and gene expression. Many of these forms have been detected in the urine of humans exposed to iAs and in rodents exposed to inorganic and organoarsencials. Moreover, the exposure of mammalian cells and organs to mixtures of these intermediates brings to the forefront potential synergistic interactions between these forms that could enhance the tumorigenesis process. To even further complicate these processes, there is a growing body of evidence that implicates arsenic-induced reactive oxygen species (ROS) and the downstream effects of arsenic-induced oxidative damage and oxidative stress in the mechanisms of cellular injury, toxicity, and

carcinogenic activity. This implies that some (if not many) of the toxicological effects of arsenic are mediated indirectly through ROS. ROS are known to induce DNA damage, lipid peroxidation, and protein oxidation. ROS themselves are not stable forms. ROS can interconvert between themselves, can react with nitric oxide to become reactive nitrogen species (RNS) which have their own spectra of biological activities, and high-energy ROS can cascade down to lower-energy forms and in that process can radicalize other biological molecules. Moreover, ROS and sequelae radicals are affected by cellular defenses that can ameliorate their activities.

Therefore, the metabolic, pharmacokinetic, pharmacodynamic, and cellular processes that are taking place within these cascades are seemingly complex and it is believed to be difficult, if not impossible, to apportion risk at this time to any one of these arsenical intermediates by any known scientifically defensible method. Inorganic arsenic represents a mixtures issue, with numerous metabolites, each of which has its own spectrum of toxicity via similar or different modes of action.

The mode of action of inorganic arsenic has been the topic of significant research effort. The literature on this topic is summarized below.

Genotoxicity

Chromosomal abnormalities have been observed in humans as well as experimental animals. Chromosomal aberrations and sister chromatid exchange have been observed in patients treated with Fowler's solution (Burgdorf et al., 1977; Nordenson et al., 1979), which contains arsenic, and in subjects occupationally exposed (Beckman et al., 1977). Although Vig et al. (1984) did not find an increase in either endpoint in the lymphocytes of subjects exposed to arsenic (109 μg/L) via drinking water, other authors have demonstrated that exposure to drinking water containing 400 μg/L caused an increase in chromosome aberrations in peripheral lymphocytes (Beckman et al., 1977; Nordenson et al., 1978; Petres et al., 1977). An increase in micronuclei in exfoliated cells also was noted in humans exposed to arsenic via the drinking water (Warner et al., 1994; Gonsebatt et al., 1997).

Liou et al. (1999) conducted a nested case-control study in the blackfoot endemic area of Taiwan to explore whether incidences of chromosome aberrations (CA) or sister chromatid exchange (SCE) could predict cancer development. Venous blood samples were taken from a cohort of 686 residents at the beginning of the study, 22 of which developed some form of cancer during the 4-year follow-up period. Cytogenetic analyses were performed on lymphocytes taken from the stored blood samples of the 22 cancer cases and 22 matched controls. It was found that chromosome-type CAs, but not chromatid-type CAs or SCEs, were significantly higher ($p<0.05$) in the cases than in the controls. The cancer risk odds ratio (OR) for subjects with > 0 chromosome-type CAs was 5.0 (95% confidence interval 1.09–22.82).

Basu et al. (2002) examined the incidences of micronuclei (MN) in a group of 45 individuals from West Bengal, India, who had cutaneous signs of arsenicism and drank water having a mean arsenic content of 368.11 μg/L. The control group consisted of 21 asymptomatic individuals with a drinking water supply having a mean arsenic concentration of only 5.49 μg/L. For the exposed and control groups, arsenic concentrations in the urine, nails and hair were 24.45, 4.88 μg/L, and 12.58 and 0.51 μg/g, 6.97 and 0.34 μg/g, respectively. In the exposed group, the frequencies of MN per 1000 cells were highly elevated over those of the control group: 5.15 vs. 0.77 in the oral mucosa, 5.74 vs. 0.56 in urothelial cells, and 6.39 vs. 0.53 in lymphocytes, respectively. This was supported by Moore et al. (1997b) who reported an increase in the prevalence of MN in Chilean males chronically exposed to 600 μg As/L. Thus, individuals exposed to high levels of arsenic via drinking water appear to be sustaining significant cytogenetic damage.

Rats orally exposed to arsenate (4 mg As/kg/day) for 2-3 weeks developed major chromosomal abnormalities in the bone marrow (Datta et al., 1986). Arsenite-induced micronuclei in the bone marrow of mice *in vivo* (Tinwell et al., 1991).

Sodium arsenate has been demonstrated to transform Syrian hamster embryo cells (Dipaolo and Casto, 1979) and to produce sister chromatid-exchange in DON cells, CHO cells, and human peripheral lymphocytes exposed *in vitro* (Larramendy et al., 1981; Ohno et al, 1982; Wan et al.,1982; Andersen, 1983; Crossen, 1983). Sodium arsenate and sodium arsenite are mutagenic at concentrations of 10-14 μg/mL and 1-2 μg/mL, respectively (Harrington-Brock et al., 1993; Moore et al., 1995). MMA and DMA were slightly less potent with 2.5-5 mg/mL and 10 mg/mL, respectively, to induce a genotoxic response. The study authors judged the mutations to be chromosomal rather than point mutations due to the small colony size.

Arsenic has generally failed as a mutagen in bacteria and has only been observed as a weak mutagen at the *hprt* locus in Chinese hamster V79 cells at toxic concentrations (Li and Rossman, 1989a). Arsenic does not appear to cause point mutations in standard assays, but instead causes large deletion mutations (Rossman, 1998). These large deletions can cause lethality when closely linked to essential genes. Therefore, the mutations are not easily observed in standard bacterial and mammalian cell mutation assays. However, even in transgenic cell lines, which were tolerant of large deletions, arsenic was still only weakly mutagenic at toxic doses (Rossman, 2003).

It has been suggested that arsenic acts as an aneugen at low doses, but as a clastogen at high doses (Rossman, 2003). This was suggested because a low-dose protocol (5 μM arsenite for 24 hours in normal human fibroblasts) results in mainly kinetochore positive (K^+) MN (usually derived from whole chromosomes), while a high-dose protocol (20 μM for 4 hours) resulted in mainly kinetochore negative (K^-) MN (derived from chromosomal fragments). Both the protocols caused the same level of arsenic accumulation and toxicity in normal human fibroblasts (Yih and Lee, 1999 as cited in Rossman, 2003). Other clastogenic agents, such as X-rays, also induce high levels of K^-

MN (Fenech et al., 1999) and other agents causing aneuploidy by interfering with spindle function, such as vinblastine, to induce K^+ MN (Eastmond and Tucker, 1989). V79 cells exposed to 10μM arsenite disrupted mitotic spindles and persistent aneuploidy (5 days after removal of arsenic) without notable chromosome aberrations in surviving cells (Sciandrello et al., 2002; Warner et al., 1994).

Zhang et al. (2003) determined that low concentrations of arsenic (<1 μM) increased telomerase activity, maintained or elongated telomere length, and promoted cell proliferation in cultured HL-60 and HaCaT cells, while high concentrations (1-40μM) of arsenic decreased telomerase activity, reduced telomere length, and induced apoptosis. Telomeres are located at the end of chromosomes and play a critical role in maintaining chromosome and genomic stability. Results of the study indicate that telomerase was involved in arsenic-induced apoptosis. Data, however, also suggest that reactive oxygen species (ROS) may be involved in the shortening of telomeres and apoptosis induced arsenic. Chou et al. (2001) report that arsenic trioxide (0.75 μM) inhibited telomerase activity in NB4 cells after 8 days of exposure with significant numbers of fusion chromosomes observed in 2-3 weeks. Chou et al. (2001) also demonstrated that the suppressed telomerase activity in NB4 cells correlated with a decrease in *h*TERT mRNA and protein. Zhang et al. (2003) propose that the increase in telomerase activity leading to promotion of cell proliferation leads to its carcinogenic effects; however, its anticarcinogenic effects are related to oxidative stress leading to telomeric DNA attrition and apoptosis.

Sodium arsenite induces DNA-strand breaks associated with DNA-protein crosslinks in cultured human fibroblasts at 1-5 mM (3 mM was the most effective), but not at 10 mM (Dong and Luo, 1993).

Injection (intraperitoneal) of DMA^V (10.6 mg/kg/day) for 5 consecutive days only weakly enhanced the frequency of *lacZ* gene mutants (at most 1.3-fold greater than control) in cells from the lung of Muta™ Mouse transgenic mouse (Noda et al., 2002), even though such treatment was known to induce DNA damage in the lung via the formation of various peroxyl radicals. Mutant frequencies were not significantly enhanced at the *lacZ* locus in the bladder or bone marrow. A marginal increase in G:C to A:T transitions occurred at the *cII* gene locus in the lung. Arsenic trioxide (7.6 mg/kg/day) also failed to enhance *lacZ* mutant frequencies in the lung, kidney, bone marrow or bladder. MN formation in peripheral blood reticulocytes was enhanced by arsenite, but not by DMA^V. The study authors concluded that the assay system may not be sensitive enough to detect DMA^V's genotoxicity.

To study the effect of methylation state on arsenic-induced genotoxicity, Yamanaka et al. (1997) exposed cultures of human alveolar epithelial type II (L-132) cells to arsenite, MMA or DMA and assayed for DNA damage using a DNA repair synthesis inhibition protocol. DNA single strand breaks, resulting from the inhibition of repair, were induced by 5–100 μM DMA, but not by arsenite or MMA, even at 100 μM concentrations.

However, DMA exposure also induced DNA repair using a bromodeoxyuridine (BrdU)-photolysis assay. Again, these results were not observed with either arsenite of MMA. However, when cells were co-exposed to MMA and the methyl-group donor SAM, cellular levels of DMA increased and DNA repair was induced, emphasizing the importance of methylation to arsenic's ability to damage DNA in these human epithelial cells.

Further exploring the impacts of methylation and valence state on the genotoxicity of arsenical, Mass et al. (2001) assessed the DNA-damaging capacities of As^{III}, As^{V}, MMA^{V} and DMA^{V} in a supercoiled .X174 RFI DNA nicking assay. The methylated trivalent arsenicals were the only arsenic compounds observed to damage naked DNA. Concentrations of up to 300 mM As^{III}, 1 M As^{V}, 3 M MMA^{V} and 300 mM DMA^{V} were without nicking activity. DMA^{III} had nicking activity at 150 μM. The compounds also were assessed in the SCG comet assay using human lymphocytes. The activity of As^{V} was only slightly greater than that of As^{III}, while MMA^{V} and DMA^{V} were without any demonstrable activity. DMA^{III} was 386 times more potent than As^{III}.

Nesnow et al. (2002) used the supercoiled ØX174 DNA nicking assay to study the involvement of ROS on MMA^{III} and DMA^{III}-induced DNA damage. MMA^{III} completely fragmented the ØX174 DNA at concentrations of 30-50 mM. Significant DNA-nicking also was observed with 37.5 μM of DMA^{III}. ROS inhibitors Tiron, melatonin, and the vitamin E analogue Trolox all inhibited the DNA-nicking activity of both MMA^{III} and DMA^{III} at low (0.01 mM) concentrations. The authors also suggest that the formation of DMPO-hydroxyl free radical adducts was dependent on time and the presence of DMA^{III}. The formation of DMPO-hydroxyl free radical adduct was completely inhibited by Trion and Trolox. Data indicate that the DNA-damaging action of DMA^{III} is an indirect genotoxic effect mediated by ROS formed concomitantly with the oxidation of DMA^{III} to DMA^{V}.

Kashiwada et al. (1998) examined the cytogenetic effects in mouse bone marrow cells after a single intraperitoneal injection of DMA^{V}. Their data suggest that DMA^{V} may cause mitotic arrest both *in vivo* and *in vitro.* The data also suggest that DMA^{V} is a significant inducer of aneuploidy (44.9 % versus 6.0 % in controls) with over 80% of the aneuploid cells hyperploids containing 1 or 2 extra chromosomes. The authors suggest that DMA^{V} aneuploid induction could be at least one of the mechanisms underlying arsenic's carcinogenicity.

Kligerman et al. (2003) exposed human peripheral blood lymphocytes, L5178Y/Tk(+/-) mouse lymphoma cells, *Salmonella*, and *E. coli* to As^{V}, As^{III}, MMA^{V}, MMA^{III}, DMA^{V}, or DDM^{III} . MMA^{III} and DMA^{III} were found to be the most potent clastogens in human lymphocytes, as well as, the most potent mutagens at the Tk (+/-) locus in mouse lymphoma cells; however, they did not induce prophage. None of the arsenic compounds, however, were potent inducers of SCE nor did they cause gene mutations in the three strains of *Salmonella* tested.

Dopp et al. (2004) found a significant ($p<0.05$) increase in the number of micronucleus (MN), chromosome aberrations (CA), and sister chromatid exchanges (SCE) in CHO cells exposed to MMA^{III} and DMA^{III}. Arsenate and arsenite induced CA and SCE, but not MN. $TMAO^{V}$, MMA^{V}, and DMA^{V} were not found to be genotoxic in the range of concentrations tested (≤ 5 mM).

Aberrant gene/protein expression

Arsenite has been demonstrated to induce gene amplification, which is a sign of gene instability, at the *dhfr* locus in human and rodent cells; however, it was unable to cause amplification of SV40 sequences in SV40-transformed human keratinocytes or Chinese hamster cells (Mure et al., 2003; Barrett et al., 1989;Rossman and Wolosin, 1992). These data suggest that arsenite feeds into checkpoint pathways common to those involving p53, whose disruption leads to cellular gene amplification, instead of signaling typical DNA-damaging agents, which tend to amplify SV40 (Livingstone et al., 1992).

DNA hypo- or hypermethylation also have been indicated as a mechanism for carcinogenesis. Cytosine methylation in the p53 promoter in human adenocarcinoma A549 cells was one of the first indications of arsenite induced methylation changes (Mass and Wang, 1997). However, both hypo- and hypermethylation of different genes after exposure to arsenite have been noted in human kidney UOK cells (Zhong and Mass, 2001).

Waalkes et al. (2004b) observed a 32% decrease in insulin-like growth factor 1 (IGF-1) expression and a 3-fold increase in the expression of IGF-1 binding protein (IGFBP-1) in arsenic-induced hepatocellular carcinomas. Scharf et al. (2001) also observed this in patients with hepatocellular carcinomas. However, Waalkes et al. (2004b) also noted a 2-fold increase in hepatic IGFBP-1 expression in nontumor tissues of arsenic-exposed animals.

Cytokeratin-8 and cytokeratin-18 were both found increased in arsenic-induced hepatocellular carcinomas (Waalkes et al., 2004b). In arsenic-exposed nontumorous tissue, cytokeratin-8 was increased and cytokeratin-18 was decreased compared to normal unexposed tissue. This is notable because cytokeratins have been suggested to play essential "guardian" role in the liver with aberrant expression associated with liver disease and hepatocellular carcinoma formation (Omary et al., 2002).

Betaine-homocystein methyltransferase was reduced by 68% in arsenic-induced hepatocellular carcinomas compared to the nontumor tissue (Waalkes et al., 2004b). However, the nontumor tissue in arsenic exposed animals had similar levels of betaine-homocystein as in spontaneously developing hepatocellular carcinomas, which was lower than normal unexposed tissue. Avila et al. (2000) also have observed a reduction in this enzyme in human liver cirrhosis and hepatocellular carcinomas. Waalkes et al (2004b) suggests that this may be associated with abnormal DNA methylation status and aberrant

gene expression, both of which have been suggested mechanisms of arsenic carcinogenesis (Huang et al., 2004; Rossman, 2003).

Possible involvement of reactive oxygen species (ROS)

Reactive oxygen species (ROS) have been postulated to be involved in both the initiation and promotional stages of carcinogenesis (Shackelford et al., 2000; Zhong, et al., 1997; Bolton, et al. 2000; Bolton et al., 1998; Chen et al., 2000b; Khan et al., 2000; and Xu et al., 1999). Arsenite does not react with DNA itself, but oxidate damage to the DNA of arsenite treated cells has been observed (Rossman, 2003). Low levels of ROS can modulate gene expression by acting as a secondary messenger, while high doses of ROS can cause oxidative injury leading to cell death (Perkins et al., 2000). ROS also has been suggested to damage cells by the following mechanisms: lipid peroxidation; DNA and protein modification, as well as causing structural alterations in DNA including base-pair mutations, rearrangements, deletions, insertions, and sequence amplifications (but not point mutations); involvement in the signaling of the cell transformation response; affecting cytoplasmic and nuclear signal transduction pathways that regulate gene expression; and increasing the expression of certain genes (e.g., MDM2 protein, a key regulator of the tumor suppression gene p53) (Rossman et al., 1980; Rossman et al., 1975; Li et al., 1998; Sen, et al., 1996; Lander, 1997; and Hamadeh et al., 1999).

ROS have been measured in cells during arsenic metabolism (Barchowsky et al., 1999). Arsenic has been demonstrated to increase oxygen consumption, superoxide ($O_2^{\bullet -}$) formation, and extracellular accumulation of hydrogen peroxide (H_2O_2). Data by Wang et al. (1996) suggest elevated intracellular peroxide levels as well. Using a confocal scanning microscope with a fluorescent probe, Liu et al. (2001) reported a 3-fold increase in intracellular oxyradical production. However, co-treatment of cultures with dimethyl sulfoxide (DMSO), an oxygen radical scavenger, caused the fluorescent intensity to be reduced to control levels indicating a reduction in oxyradical production. Increases in serum lipid peroxides have been observed in a Chinese population chronically exposed to arsenic from their drinking water (Pi et al., 2002).

Further support for the involvement of ROS in arsenic carcinogenesis, is that situations that cause a reduction of ROS also cause reductions in the genotoxic effects of arsenic, while situations that are associated with increases in ROS cause an increase in arsenic induced genotoxic response. Examples of this include, inhibition of arsenic-induced sister chromatid exchange in human cells when superoxide radical dismutase (SOD) is added to the culture medium (Rossman, 1998); protection from arsenic genotoxicity in the presence of SOD, elevated GSH, Vitamin E, catalase, and squalene (Hei et al, 1998; Lee and Ho, 1995; Nordenson and Beckmam, 1991; Huang et al., 1993; Wang and Huang, 1994; Fan et al., 1996; Kessel et al., 2002); H_2O_2-resistant CHO cells exhibiting cross-resistant to arsenite (Cantoni et al., 1994); and blockage of the mutagenicity of arsenite in A_L cells by dimethyl sulfoxide (DMSO), a free radical scavenger,(Hei et al., 1998). Depletion of GSH in cell culture increases the toxic and

clastogenic effect of arsenite (Oya-Ohta et al., 1996). GADD153, a DNA damage-inducible protein, is induced by arsenite, but this is suppressed by the antioxidant *N*-acetyl cysteine (Guyton et al., 1996). Rossman (1998, 2003) and Wang et al. (1997) also note that XRS-5 cells, which are deficient in catalase, are hypersensitive to arsenic cytotoxicity as well as arsenic-induced MN formation.

GSH depletion, increased oxidized glutathione (GSSG), and elevated malondialdehyde in the liver and brain were observed in rats administered arsenite for 12 weeks (Flora et al., 1999).

Rin et al. (1995) postulates that DMA forms a stable adduct with DNA. These adducts may be more susceptible to single strand DNA breaks by O_2^-.

Shen et al. (2003) tested the contribution of ROS to TMAO induced hepatocellular adenomas in male F344 rats administered 0, 50, or 200 ppm TMAO in their drinking water for 2 years (estimated average daily intakes of 0, 638, and 2475 mg/kg/day, respectively). Formation of 8-hydroxydeoxyguanosine (8-OHdG) was measured using high performance liquid chromatography. The 8-OHdG values were significantly ($p<0.05$) higher in the 200-ppm group compared to the controls. TMAO also was reported to increase cell proliferation in normally appearing parenchyma as measured by proliferating cell nuclear antigen index. The results indicate that TMAO possibly causes liver tumorigenicity via oxidative DNA damage and enhanced cell proliferation.

DNA repair inhibition

Arsenite appears to inhibit the DNA repair process by inhibiting both excision and ligation (Jha et al., 1992; Lee-Chen et al., 1993). DNA repair enzymes are inhibited in arsenic exposed cells (Simeonova and Luster, 2000; Maier et al., 2002). Arsenite has been demonstrated to decrease DNA ligase activity, but did not directly affect the enzyme level (Li and Rossman, 1989b; Hu et al., 1998). Hu et al. (1998) suggest that arsenite indirectly inhibits DNA ligase activity either by altering cellular redox levels or by affecting signal transduction pathways and phosphorylation of proteins related to DNA ligase activity.

L132 cells exposed to 10 mM DMA^V increased the frequency of single strand breaks in DNA, reduced DNA replication, and shortened the length of the nascent chain of DNA (Tezuka et al., 1993), suggesting that both replication and repair of DNA are affected. Kawaguchi et al. (1996) suggests an interaction between DMA and paraquat (a generator of O_2^-) in the production and persistence of DNA damage.

This inhibition of DNA repair could explain why arsenic compounds enhance the carcinogenic effect of various compounds. Arsenic has been found to enhance the mutagenicity of UV in *E. coli* (Rossman, 1981); enhance the mutagenicity and/or clastogenicity of UV, *N*-methyl-*N*-nitrosourea (MNU), diepoxybutane, X-rays, and methylmethane sulfonate in mammalian cells (Li and Rossman, 1989a; Li and Rossman,

1991; Wiencke and Yager, 1992; Lee et al., 1985; Lee et al., 1986; Yang et al., 1992; Jha et al., 1992); and enhanced skin tumors(mainly squamous cell carcinomas in hairless Skh 1 mice after exposure to 1.7 kJ/m^2 solar UVR [ultraviolet radiation; considered a low, nonerythemic dose], three times weekly with the onset earlier and tumors larger in mice exposed to arsenic, while arsenite alone did not induce tumors) (Rossman et al., 2003; Burns et al., 2004).

Signal transduction

Arsenic also has been speculated to modulate gene expression by activating signal transduction pathways (Snow, 1992; Huang et al., 1999). However, data are conflicting and generally relate to the DNA repair inhibition, gene expression, or gene amplification mentioned above. P53, a crucial tumor suppresser gene, involvement has been controversial (Huang et al., 2004; Rossman, 2003). Exposure to human cells to arsenite (0.1-100μM) for 24 hours caused a dose-dependent increase in the level of p53 protein expression, especially in the presence of cells carrying wild type p53 genes (Salazar et al., 1997). However, when immortalized human keratinocyte HaCaT cells, which normally over express p53 protein, was exposed to arsenite (0.01-1 μM) a dose- and time-dependent decrease in p53 protein levels was observed (Hamadeh et al., 1999). While yet another study demonstrated no effect of arsenite (12.5-200 μM) on p53-dependent transcription activity in p53 promoter-transfected JB6 C141 mouse cells (Huang et al., 1999).

Activation of signal transduction pathways which enhance cell proliferation, reduce antiproliferative signaling, and override checkpoint controlling cell division after genotoxic insult also have been considered as possible mechanisms of arsenic's co-carcinogenic properties (see Rossman, 2003 for review).

Other

Bladder Carcinogenesis

Drobná et al. (2002) used a UROtsa cell line, which is an SV40 immortalized cell line derived from normal human urothelium. This cell line was determined not to methylate arsenic. Therefore, it is useful in examining the effects of inorganic arsenic and methylated arsenic. Cells were incubated with inorganic (As^{III} and As^{V}) or methylated arsenicals (MMA^{V}, $MMA^{III}O$, DMA^{V}, and iododimethylarsine [$DMA^{III}I$]) for 2 or 24 hours. Because trivalent arsenic has been determined to be more toxic than pentavalent arsenic, the doses were lower for trivalent arsenic compounds (0.1-5 μM) than for pentavalent compounds (1-200 μM). Pentavalent arsenic was reported not to affect the UROtsa cells, and the data was not presented in the study report. Cell viability was only decreased with a dose of 5μM methylarsine oxide ($MMA^{III}O$) after both 2 and 24 hours. Cell proliferation also was affected to a lesser extent with 5 μM $DMAs^{III}I$. All the trivalent arsenic compounds examined increased AP-1 DNA binding activity in UROtsa cells, but the methylated compounds were much more potent activators of AP-1. The AP-1 DNA

binding activity was determined to act through the ERK-dependent induction of c-Jun phosphorylation with the possible involvement of Fra-1 phosphorylation. The order of potency of c-Jun/Fra-1 phosphorylation is as follows from most potent to least potent: $MAs^{III}O > DMAs^{III}I > As^{III}$. These results suggest that methylation of arsenic is involved in the toxicity and carcinogenicity of arsenic to urinary bladder cells.

Cohen et al. (2002) demonstrated that dimethylarsenic acid (DMA^{V}) is cytotoxic to the urinary bladder via formation of DMA^{III}. Female F344 rats (4 weeks old) were administered 100 ppm DMA^{V} via the diet with or without co-administration of 2,3-dimercaptopropane-1-sulfonic acid (DMPS), a chelator of trivalent arsenicals for 2 or 26 weeks. Controls were fed basal diets only. Bladder weights were unchanged by week 2, but were significantly greater by week 26. At 2 weeks, one of 10 rats administered DMA^{V} had simple hyperplasia, while 4 of 9 had developed simple hyperplasia by 26 weeks. None of the controls developed hyperplasia at 2 or 26 weeks. Co-administration of DMPS caused a reduction in the development of simple hyperplasia. DMA^{III} also was measured in the urine on days 1, 71, and 175. *In vitro* experiments demonstrated that arsenite compounds (i.e., arsenite, MMA^{III}, and DMA^{III}) were more cytotoxic to rat (i.e., MYP3) and human (1T1) urinary bladder cell lines than arsenate compounds (i.e., arsenate, MMA^{V}, DMA^{V}, and TMAO).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

Not addressed in this document.

4.5.2. Inhalation

A review and assessment of relevant inhalation studies is not addressed in this report.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall Weight-of-Evidence

Based upon current EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) inorganic arsenic is determined to be "carcinogenic to humans" due to convincing epidemiological evidence of a causal relationship between oral exposure of humans to inorganic arsenic and cancer.

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

There have been numerous studies examining the carcinogenic potential of inorganic arsenic via oral exposure (see NRC 2001 Table 2-1). The majority of these studies are ecological in nature and are therefore subject to the biases of ecological studies (e.g., lack of individual exposure). Ecological epidemiologic studies examine differences in disease rates among populations in relation to age, gender, race, and differences in temporal or environmental conditions. Although previous draft cancer guidelines have considered these studies insufficient to ascertain the causal agent or degree of exposure, the current guidelines (US EPA, 2005) do not provide guidance on the issue. Given the detailed information available on the drinking water exposure concentrations in the studies selected for arsenic, they are considered, for the purposes of this report, to be sufficient for both ascertaining the causal agent and determining a dose-response relationship.

The strength of the associations observed in the studies mentioned above support a relation between oral exposure to inorganic arsenic and cancer. Each study was conducted differently and contain their own biases (e.g., lack of confounding variables, possible recall bias), but the combination of all the study results provides support of an association between oral exposure to inorganic arsenic and cancer including bladder, kidney, lung, liver, and prostate. Different populations were assessed. Therefore, it is unlikely that any single attribute (e.g., nutritional habits) associated with a single population is responsible for the increased cancer rates. Although many studies did not account for confounding variables (e.g., cigarette smoking in association with lung cancer), the positive associations also were observed in those studies that did account for certain confounding variables (e.g., habits, age, socioeconomic status).

Twelve studies examined the association between arsenic concentrations in drinking water and skin cancer. All 12 had positive associations. Of the 12 studies, 9 were ecological study designs with which there was one each of cross sectional, case-control, and cohort. The major flaw in the association between oral exposure to inorganic arsenic and skin cancer was the lack of data and/or analysis of dermal contact with the water containing the inorganic arsenic through bathing.

Eighteen studies have examined arsenic in the drinking water and bladder cancer. Fifteen of the studies (9 ecological, 2 case-control, and 4 cohort studies) found a positive association. The studies that failed to observe an association between oral exposure to inorganic arsenic and bladder cancer (Bates et al., 1995; Lamm et al., 2004; Steinmaus et al., 2003; 1 ecological and 2 case-control) were those which examined low exposures. Bates et al. (1995) and Steinmaus et al. (2003), however, found an association between arsenic exposure (exposures 20 years or more) and bladder cancer in people who had ever smoked.

Nine studies (6 ecological and 3 cohort) found an association between liver cancer and arsenic concentrations in the drinking water. Smith et al. (1998) was the only study that examined liver cancer that did not find an association with arsenic. This study was

ecological in nature using mortality as an endpoint. Its major limitations were that there was limited information on smoking and the results could not be separated by age or sex.

Respiratory cancers have been associated with arsenic exposure via drinking water in 11 of 12 studies. The majority of the studies were ecological (i.e., 8), one was a case-control and 3 were cohort. One of the cohort studies demonstrated negative findings (Cuzick et al, 1992). Mortality was the endpoint in the negative study. Eight studies (7 ecological and 1 cohort) examined and found an association between arsenic in the drinking water and kidney tumors. Three studies (2 ecological and 1 cohort) found an association between arsenic concentrations in the drinking water and prostate cancer. There also have been an occasional study that has associated arsenic exposure with leukemia and lymphomas, colon cancer, and bone cancer (Chen et al., 1985, 1988; Tsai, 1999; Wu, 1989).

Lung and liver tumors also were observed in mice administered inorganic arsenic for a short duration transplacentally with possible additional exposure while nursing (pregnant dams were exposed for 10 days during gestation only). Therefore, increasing the evidence that lung and liver cancers are associated with oral exposure to inorganic arsenic.

4.6.3. Mode of Action Information

The carcinogenic mode of action for inorganic arsenic is not known and consensus in the scientific community has yet to be reached. Kitchin (2001) provides a review of nine different possible modes for arsenic carcinogenesis, including chromosome abnormalities; oxidative stress; increased transcription of mRNA and secretion of transforming growth factor-alpha (TGF-α); granulocyte macrophage-colony stimulating factor (GM-CSF) and tumor necrosis factor-alpha (TNF-α); cell proliferation, promotion and/or progression in carcinogenesis; altered DNA repair; p53 gene suppression; altered DNA methylation patterns; and gene amplification. The article suggests that the three with the most positive evidence in both animals and human cells are chromosomal abnormalities, oxidative stress, and a continuum of altered growth factors leading to increased cell proliferation which leads to the promotion of carcinogenesis. Rossman (2003), Huang et al. (2004), and Simeonova and Luster (2000) also provide reviews examining the aforementioned modes of arsenic carcinogenesis. In addition, it is noted that humans excrete more MMA than other species, and they are more prone to arsenic-induced carcinogenesis; therefore, it is likely that MMA (probably the trivalent form) is involved in the carcinogenesis of arsenic.

One possible carcinogenic process involves the metabolism of inorganic arsenic to DMA or TMAO. Wei et al. (1999; 2002) demonstrated that DMA was carcinogenic in male F344 rats. Shen et al. (2003) demonstrated the carcinogenicity of TMAO in male F344 rats. Arsenite and arsenate, however, have been negative in standard carcinogenic bioassays (NRC, 1999). Both Wei et al. (2002) and Shen et al. (2003) further established that reactive oxygen species (ROS) are likely involved in the carcinogenesis (further

description below). Wei et al. (2002) also demonstrated that multiple genes are involved at different stages of DMA-induced tumor development. The *in vitro* studies by Nesnow et al. (2002) demonstrated that MMA^{III} and DMA^{III} had DNA-damaging effects that were inhibited by chemically diverse ROS inhibitors. DMA, but not MMA or arsenite, also was demonstrated to cause DNA damage (Yamanaka et al., 1997). The DNA damage accompanied by possible increased cell proliferation (Shen et al., 2003) provide additional mechanistic insight. The association of ROS and oxidative stress with a wide variety of cancers (e.g., bladder, lung, liver, prostate, etc.) indicates either a number of different mechanisms or a mechanism generalizable over multiple organs (Klaunig and Kamendulis, 2004).

Zhang et al. (2003) determined that low concentrations of arsenic (<1 μM) increase telomerase activity, maintained or elongated telomere length, and promoted cell proliferation in cultured HL-60 and HaCaT cells, while high concentrations (1-40μM) of arsenic decreased telomerase activity, reduced telomere length and induced apoptosis. Telomeres are located at the end of chromosomes and play a critical role in maintaining chromosome and genomic stability. Results of the study indicate that telomerase was involved in arsenic-induced apoptosis. Data, however, also suggest that reactive oxygen species (ROS) may be involved in the shortening of telomeres and apoptosis induced arsenic. Chou et al. (2001) reported that arsenic trioxide (0.75 μM) inhibited telomerase activity in NB4 cells after 8 days of exposure with significant numbers of fusion chromosomes observed in 2-3 weeks. Chou et al. (2001) also demonstrated that the suppressed telomerase activity in NB4 cells correlated with a decrease in *h*TERT mRNA and protein. Zhang et al. (2003) propose that the increase in telomerase activity leading to promotion of cell proliferation leads to its carcinogenic effects, but its anticarcinogenic effects are related to oxidative stress leading to telomeric DNA attrition and apoptosis.

The following include alterations of cellular pathways that may be involved in the process of arsenic carcinogenesis. Neither a step by step process nor details of how each process is involved have been developed at this time. Data, however, implicate a role for each mode of action.

Genotoxicity

There are large amounts of information available on the *in vitro* and *in vivo* genotoxicity of arsenicals with chromosomal abnormalities observed in both humans and animals (Basu et al., 2001; Kligerman et al. 2003). Briefly, arsenicals are stronger clastogens than point mutations, and methylated trivalent arsenicals are usually much more potent genotoxins than are inorganic arsenic or pentavalent methylated arsenicals (Kligerman et al., 2003). However, there is no connection between the clastogenicity of arsenic and the carcinogenicity in various organs in humans. Some data indicates that the clastogenic effects of arsenic also are mediated via free radicals (Kitchin, 2001; Kligerman et al., 2003).

It also has been suggested that arsenic acts as an aneugen at low doses, but as a clastogen at high doses (Rossman, 2003). This was suggested because a low-dose protocol (5 μM arsenite for 24 hours in normal human fibroblasts) results in mainly kinetochore positive (K^+) MN (usually derived from whole chromosomes), while a high-dose protocol (20 μM for 4 hours) resulted in mainly kinetochore negative (K^-) MN (derived from chromosomal fragments).

Aberrant gene/protein expression

Arsenite has been demonstrated to induce gene amplification, which is a sign of gene instability at the *dhfr* locus in human and rodent cells; however, it was unable to cause amplification of SV40 sequences in SV40-transformed human keratinocytes or Chinese hamster cells (Mure et al., 2003; Barrett et al., 1989; Rossman and Wolosin, 1992). These data suggest that arsenite feeds into checkpoint pathways common to those involving p53, whose disruption leads to cellular gene amplification, instead of signaling typical DNA-damaging agents, which tend to amplify SV40 (Livingstone et al., 1992).

DNA hypo- or hypermethylation also have been indicated as a mechanism for carcinogenesis. Cytosine methylation in the p53 promoter in human adenocarcinoma A549 cells was one of the first indications of arsenite induced methylation changes (Mass and Wang, 1997). However, both hypo- and hypermethylation of different genes after exposure to arsenite have also been noted in human kidney UOK cells (Zhong and Mass, 2001).

Possible involvement of reactive oxygen species (ROS)

Reactive oxygen species (ROS) have been postulated to be involved in both the initiation and promotional stages of carcinogenesis (Shackelford et al., 2000; Zhong et al., 1997; Bolton, et al. 2000; Bolton et al., 1998; Chen et al., 2000b; Khan et al., 2000; and Xu et al., 1999). Arsenite does not react with DNA itself, but oxidate damage to the DNA of arsenite-treated cells has been observed (Rossman, 2003). Low levels of ROS can modulate gene expression by acting as a secondary messenger, while high doses of ROS can cause oxidative injury leading to cell death (Perkins et al., 2000). ROS also has been suggested to damage cells by the following mechanisms: lipid peroxidation; DNA and protein modification and causing structural alterations in DNA including base-pair mutations, rearrangements, deletions, insertions, and sequence amplifications (but not point mutations); involvement in the signaling of the cell transformation response; affecting cytoplasmic and nuclear signal transduction pathways that regulate gene expression; and increasing the expression of certain genes (e.g., MDM2 protein, a key regulator of the tumor suppression gene p53) (Rossman et al., 1980; Rossman et al., 1975; Li et al., 1998; Sen, et al., 1996; Lander, 1997; and Hamadeh et al., 1999).

DNA repair inhibition

Arsenite appears to inhibit the DNA repair process by inhibiting both excision and ligation (Jha et al., 1992; Lee-Chen et al., 1993). DNA repair enzymes are inhibited in arsenic exposed cells (Simeonova and Luster, 2000; Maier et al., 2002). Arsenite has been demonstrated to decrease DNA ligase activity, but did not directly affect the enzyme level (Li and Rossman, 1989b; Hu et al., 1998). Hu et al. (1998) suggest that arsenite indirectly inhibits DNA ligase activity either by altering cellular redox levels or by affecting signal transduction pathways and phosphorylation of proteins related to DNA ligase activity. This inhibition of DNA repair could explain why arsenic compounds enhance the carcinogenic effect of various compounds.

Signal transduction

Arsenic also has been speculated to modulate gene expression by activating signal transduction pathways (Snow, 1992; Huang et al., 1999). However, the data is conflicting and generally relates to the DNA repair inhibition, gene expression, or gene amplification mentioned above. P53, a crucial tumor suppresser gene, involvement has been controversial (Huang et al., 2004; Rossman, 2003). Activation of signal transduction pathways which enhance cell proliferation, reduce antiproliferative signaling, and override checkpoint controlling cell division after genotoxic insult also have been considered as possible mechanisms of arsenic's co-carcinogenic properties (see Rossman, 2003 for review).

Low-dose extrapolation

According to the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), a linear extrapolation to low doses is to be used when there are mode of action data to indicate that the dose-response curve is expected to have a linear component below the point of departure. Examples of such data include DNA-reactivity or direct mutagenic activity. Additionally, a linear extrapolation is used as a default approach when the available data are insufficient to establish the mode of action for a tumor site. It has been suggested, but not proven, that arsenic may be directly genotoxic. Therefore, a linear extrapolation is recommended.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

Although children are exposed to arsenic through the same sources as adults (i.e., air, water, food, and soil), their habits may cause children to have a greater exposure than adults. Children tend to eat less of a variety than adults; therefore, exposure to contaminated food or juice or infant formula prepared with contaminated water may accumulate to a significant exposure. In addition, children are more likely to ingest arsenic-contaminated soil either intentionally or via putting dirty hands in their mouths.

Accidental poisoning also is more likely to occur due to children's curiosity and aptness to taste things they find.

Although there is no data on the absorption of arsenic from the gastrointestinal tract of children to compare to adult absorption, some evidence suggests that children are less efficient at methylating (ATSDR, 2000); therefore, children may have a different tissue distribution and longer retention times which may increase their susceptibility. As noted above adults have been demonstrated to excrete 40-60% of the arsenic as DMA, 20-25% as inorganic arsenic, and 15-25% as MMA, but Concha et al. (1998b) determined that children ingesting 200 μg/L arsenic in their drinking water excreted about 49% as inorganic arsenic and 47% as DMA. Women in the same study were determined to excrete 66% of the arsenic as DMA and 32% as inorganic arsenic.

When considering infants and children, NRC (2001) addressed primarily the issues of exposure differences (due to increased water consumption rates) and the intrinsic susceptibility associated with differences in arsenic metabolism. Exposure differences are not an issue addressed in this *Toxicological Review*; however, age-related differences in arsenic metabolism are relevant. The NRC reported that previous studies indicate that young individuals may have a lower rate of methylation of inorganic arsenic than adults, but there is variation among the data and between the populations assessed. The NRC concluded that it is not known what impact such differences in methylation with age would have on arsenic toxicity. It is plausible that different or multiple modes of carcinogenic action are operative at different life-stages due to intrinsic age-related differences.

The cancer data used in the current analysis is derived from various studies of the arseniasis-endemic area of southwest Taiwan (Chen, et al., 1985. 1988, 1991; Wu, 1989). Data from this area illustrate that childhood cancer is limited (Chen, et al., 1992); this analysis used 271,530 person-years for individuals <30 years of age, and identified no cases of bladder cancer and five cases of lung cancer in this population (as well as no cases of kidney cancer and six cases of liver cancer). These numbers and the accompanying mortality rates (per 100,000) are low when compared with higher rates in older individuals.

It has been illustrated that there is an increasing dose-response relationship between increased cancer mortality and increased years of exposure to the high-arsenic artesian well water of Southwest Taiwan (Chen, et al., 1986), so it is important to consider the extent to which the exposures in assessed population included childhood exposures. The analysis of cancer potential in the same population (Chen, et al., 1992) included "only residents who had lived in the study area after birth," and assumed that the arsenic intake of each person continued from birth to the end of the follow-up period (1973-1986)[1]. No information was provided on the exposure of pregnant women in this population to the

[1] The artesian wells were introduced in 1910-1920, prior sources of fresh water included ponds, streams, or rainwater (Tseng, 1968).

artesian well water, and arsenic is believed to pass through the placenta (Hanlon and Ferm, 1977; Lindgren et al., 1984; Hood et al., 1987; Concha et al., 1998b).

Chen, et al. (1992) stated that their results on cancer potential may somewhat underestimate risk in this population because tap water (with lower arsenic concentrations) was introduced into the area in 1956, and was available for almost 75% of the residents in the 1970s. As such, the actual arsenic ingestion may be lower than estimated as residents switched away from the high-arsenic artesian wells to alternate water sources. Also, this study is based on mortality records (1973-1986) from the study region, so cancer incidence among individuals exposed during childhood and early adulthood, who then migrated from the region would not be captured. Chen, et al. (1986) reported that the 1982 migration rate for this area was 27%, with primarily the youths and young adults leaving the area to move to cities, and those 45+ years emigrated at a rate lower than 6%. There is limited migration to this region, as it has been reported than more than 90 percent of the local residents lived in the study area all their lives (Wu, et al., 1989).

The recent EPA *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA, 2005b) identifies age-dependent adjustment factors to be applied to carcinogens with a mutagenic mode of action when chemical-specific data are unavailable. As outlined above, chemical-specific early-life data are being used in the current assessment of arsenic carcinogenicity because the epidemiological data include childhood exposures of the southwest Taiwanese study population.

4.7.2. Possible Gender Differences

Some differences in methylation have been noted between men and women (Hopenhayn-Rich et al., 1996b). Men had higher MMA:DMA ratios than women (0.23 vs 0.17, respectively). In addition, Concha et al. (1998a) demonstrated that pregnant women in their third trimester excrete more than 90% DMA in plasma and urine. This indicates possible hormonal effects of arsenic methylation.

4.7.3. Other

Genetic polymorphism

Although most humans excrete 10-30% of consumed arsenic as inorganic arsenic, 10-20% as MMA, and 60-80% as DMA, some populations seem to have a somewhat different distribution. A study of urinary arsenic in a population in northern Argentina exposed to arsenic via drinking water demonstrated an average of only 2% MMA in the urine (Vahter et al, 1995; Concha et al., 1998). Studies on populations in San Pedro and Toconao in northern Chile demonstrated that differences in the ratio of MMA:DMA excretion between the two populations (Hopenhayn-Rich et al., 1996b). However, Chiou et al. (1997) found that in a population in northeastern Taiwan, 27% of the arsenic consumed was excreted as MMA. Although these variations have not been unequivocally

linked with genetic factors as opposed to environmental factors, human polymorphism has been reported for other methyltransferases (e.g., thiopurine S-methyltransferase; Yates et al., 1997).

Nutritional Status

In many of the studies listed above concerning high levels of arsenic exposure in relation to severe arsenic-related health effects (i.e., southwestern Taiwan and northern Chile) the inhabitants were of a low socioeconomic level and had poor nutritional status. Many of the studies listed above have related poor nutritional status with possible increases in adverse health effects. Mazumder et al. (1998) demonstrated that people in and around West Bengal who had body weights below 80% for their age and sex had an increase (2.1 for females and 1.5 for males) in the prevalence of keratosis. In addition, selenium has been demonstrated to reduce the teratogenic, clastogenic, and cytogenic effects of arsenic (ATSDR, 1993). Kreppel et al. (1994) demonstrated that zinc protects mice against acute arsenic toxicity.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

Not addressed in this document.

5.1.2. Methods of Analysis

Not addressed in this document.

5.1.3. RfD Derivation

Not addressed in this document.

5.1.4. Previous Oral Assessment

Not addressed in this document.

~~5.2. INHALATION REFERENCE DOSE (RfC)~~

~~Not addressed in this document.~~

5.3. CANCER ASSESSMENT (Oral Exposure)

5.3.1. Choice of Study / Data - with Rationale and Justification

As stated by NRC (2001), there are few animal carcinogenicity bioassays on arsenic with no positive animal models for dose-modeling. There is, however, a large database on human exposure to arsenic. It was concluded by NRC (2001) that the southwestern Taiwan data was the critical data set for conducting a quantitative risk assessment for exposure to arsenic in drinking water. Data provided in Chiou et al. (2001) and Ferreccio et al. (2000) were also considered, but were not used due to the large confidence intervals, which are not precise enough for quantified risk assessment.

Morales et al. (2000) established bladder, lung, and liver cancer risk for mortality in the southwestern Taiwanese population based on the same dataset that was analyzed by NRC (1999). For the purpose of this report, a re-evaluation of Morales et al. (2000) was performed. Morales et al. (2000) used the generalized linear model and the multi-stage-Weibull model on data derived from an arseniasis-endemic area of southwest Taiwan that was obtained from Chen et al. (1988), Wu et al. (1989), and Chen et al. (1992) to establish risk estimates for cancers of the bladder, liver, and lung from arsenic exposure. NRC (2001) performed a separate analysis based on the data in Morales et al. (2000). Therefore, the data presented in Morales et al. (2000), with an unexposed (assumed zero dose of arsenic in drinking water) southwest Taiwanese population as a control group, was chosen as the data for analysis.

Lung and bladder cancer have been chosen as the endpoint because they are the internal cancers most consistently seen and best characterized in epidemiology studies (NRC, 2001). The choice of this endpoint is consistent with the NRC reports (1999, 2001). It has been decided that the oral slope factor will combine the lung and bladder cancer results. Because cause of death is listed as only from one cause (e.g., either lung or bladder cancer), there is no double counting; therefore, combining the two cancers will account for deaths from both types of cancer. There are several possible modes of action that make it plausible that different types of cancers occur. Although liver cancers were also examined by Morales et al. (2000), they were not examined in the current report because liver cancers were considered to be affected by a lot of confounding factors. Skin cancer has also been noted in the Taiwan population, but skin cancer is generally an incidence instead of mortality. In addition, skin cancer may be influenced by external exposure through bathing.

Because there is a high general background level of inorganic arsenic in food, it has been suggested that effective exposures to arsenic in Taiwan are higher than represented simply by the amount of well water drunk. For this paper, the issue of intake of arsenic from food (e.g., dry rice, sweet potatoes) has been distinguished from the issue of intake of arsenic from drinking water and intake of arsenic from water used in cooking, such as water used to boil rice and potatoes. To account for background levels of arsenic in food,

U.S. EPA (2001) assumed that the inorganic arsenic consumption due to food in Taiwan was 50 μg/day, compared to 10 μg/day in the United States. NRC (1999) cited results of Schoof et al. (1998) as estimating Taiwanese daily intake from yams as 31 μg/day and rice as 19 μg/day. NRC (1999) also noted that the Li et al. (1979) study found 95% of the rice crop to contain arsenic primarily 100 to 700 μg/kg, with some up to 1.43 mg/kg. The soil had probably been treated with arsenical pesticides. The NRC (2001) found little evidence to support EPA's assumption that food contributed 50 mg/day of inorganic arsenic to the Taiwanese diet. NRC addressed the issue by determining how sensitive the calculation of ED_{01} was to the consumption rate. NRC (2001) found that changing the consumption rate from 50 μg/day to 30 μg/day did not change the calculated ED_{01} significantly (about 1% difference). This lack of sensitivity was not unexpected, since the southwestern Taiwanese population, which was used as a comparison group, had a similar dietary intake as the exposed population. It was concluded that the model should be run over a range of arsenic consumption rates to confirm that the calculated risk is insensitive to this value.

The NRC (1999, 2001) concluded that the selection of the drinking water consumption rates should consider, as possible, the uncertainty associated with trying to accurately determine the mean consumption rate of the populations, and of the variability of individuals within the populations. This document agrees that the model is highly sensitive to the term selected. Review of relevant literature suggests that the mean adult drinking water consumption rate is between 1 to 4.6 L/day. Based on an informed judgment about the most plausible values for water intake in the Taiwanese study population, a narrower range of 2 to 3.5 L/day may be appropriate.

5.3.2. Dose-response Data

Male data from Morales et al. 2000 (Table 1)

μg/L of arsenic	person-years at risk	Bladder cancer	Liver cancer	Lung cancer
<100	95,455	17	31	28
100-299	47,268	9	23	30
300-599	72,068	32	39	53
≥600	42,179	27	29	33

Female data from Morales et al. 2000 (Table 1)

μg/L of arsenic	person-years at risk	Bladder cancer	Liver cancer	Lung cancer
<100	86,975	21	12	29
100-299	43,212	11	14	19
300-599	64,903	30	13	36
≥600	38,869	28	12	38

5.3.3. Dose Conversion

Conversion was done by assuming that equivalent doses of arsenic in the US and Taiwan should correspond to the same amount in units of μg/kg/day. Drinking water concentrations of arsenic in units of μg/L (ppb) in Taiwan were adjusted for dietary intake of 0, 10, 30, or 50 μg/day by dividing the dietary intake by Taiwanese daily drinking water intake (2 or 3.5 L/day; stated in NRC, 2001 to be the estimated consumption for women and men in Taiwan, respectively) and adding it to the nominal drinking water concentration. All nonlinear optimization was calculated based upon this adjusted Taiwanese concentration. Results were then converted to μg/kg/day using Taiwanese body weight (50 kg) and drinking water (2 or 3.5 L/day) assumptions and converted back to US-relevant drinking water concentrations in μg/L using US body weight (70 kg) and drinking water (2 L/day) assumptions.

$$C_{t,adj} = C_t + (As_{food} \div V_{dw})$$

$$C_{US} = C_{t,adj} \div 50\ kg \times V_{dw} \times 70\ kg \div 2\ L/day$$

where $C_{t,adj}$ is the adjusted Taiwanese arsenic concentration, C_t is the nominal Taiwanese arsenic concentration, As_{food} is the assumed daily dietary arsenic intake for Taiwanese, V_{dw} is the assumed daily drinking water consumption for Taiwanese, and C_{US} is the U.S.-relevant arsenic concentration.

In the Morales et al. (2000) and NRC (2001) analyses, the control group was assumed to have no dietary intake of arsenic. In contrast, this analysis assumes that the control and study groups are exposed to the same level of dietary arsenic.

5.3.4. Extrapolation Method(s)

In 2001, an update of Arsenic in Drinking Water (NRC, 2001) was published, which described the statistical methodology that they used to calculate cancer risk estimates for arsenic from epidemiological data. This is a condensed discussion of the adaptation, application, and refinement of the methodology.

Analysis of human epidemiological cancer data needs to 1) adjust for effects of age on cancer incidence, and 2) adjust for differences in age-related cancer incidence between the study population (in this case, southwest Taiwanese) and the intended reference population (i.e., US population).

In order to facilitate these adjustments, a specific functional form is used to fit the data and extrapolate for low-level cancer risk. This form is the Poisson model:

$$h(x,t) = h_0(t) \times g(x)$$

where $h(x,t)$ is the risk at dose x and age t, $h_0(t)$ is the baseline risk of the reference population at age t, and $g(x)$ is the additional risk attributable to exposure at dose x. Neither $h_0(t)$ nor $g(x)$ is a single value; both are mathematical functions. When these $h_0(t)$ and/or $g(x)$ are best described by nonlinear functions, as is the case for arsenic, complex statistical programming is required to solve this equation and calculate risk.

In order to provide the best fit for the data, an additive linear model represented by the following functions were used for $h_0(t)$ and $g(x)$:

Age effect $h_0(t)$	Dose transformation x	Dose effect $g(x)$
Quadratic $\exp(\alpha_0+\alpha_1 t+\alpha_2 t^2)$	Identity x=ppb	Linear $1+\beta_1 x$

where α_0, α_1, α_2, and β are constants to be estimated, and x is the arsenic dose adjusted for dietary arsenic exposure. Adjustments for dietary exposure were made at 30 μg in food per day. The study reference population was assumed to be southwest Taiwanese. Nonlinear optimization was done using the statistical programming language R (version 1.8.0) and the optimization routine "optim." This is in contrast to the use of S-plus and "nlminb" in Morales et al. (2000) and NRC (2001). It is conceivable that differences between the two optimization routines could lead to differences in both the mean values and lower confidence limits obtained.

Once the fitted constants are calculated, the resulting benchmark doses at 1% and 5% incidence may be calculated for the southwest Taiwanese population. However, the southwest Taiwanese population is not the population of interest. In order to obtain benchmark doses relevant to an American population, the values must be adjusted based upon both an unexposed (assumed zero dose of arsenic in cooking and drinking water) southwest Taiwanese population as a control group and 1996 U.S. lifetables for males and females (Vital Statistics of the U.S., 1996). This process is described in detail in Arsenic in Drinking Water (2001). The BEIR-IV formula, corrected for two errors in NRC (2001), is

$$R(x) = \text{lifetime risk of death at dose } x = \sum_i \frac{h_i(x)}{h_i^*(x)} S_i(1-q_i) \quad (1)$$

where:

I = age group (in 5-year intervals: 20-25, ..., 85-90)
$h_i(x)$ = cancer mortality hazard, age I, dose x
$h_i^*(x)$ = total mortality hazard, age I, dose x
q_i = P(survive i-th time interval | survive (i-1)-th time interval) = $\exp(-5\, h_i^*(x))$ (2)

S_i = P(survive up to end of (i-1)-th time interval) = $\prod_{j=1}^{i-1} q_j$ (3)

Given a linear relative hazard model:

$$h_i(x) = h_i(1+\beta x) \quad (4)$$

$$h_i(x) = h_i(1+\beta x) \quad (4)$$

where $h_i = h_i(0)$ and β is the relative risk slope estimated by regression. The additional dose-related cancer hazard is $h_i \beta x$, so the total mortality hazard increases at the same time to $h_i^*(x) = h_i^* + h_i \beta x$ where again $h_i^* = h_i^*(0)$. Therefore, (1) becomes

$$R(x) = \sum_i \frac{h_i(1+\beta x)}{h_i^* + h_i \beta x} S_i(1-q_i) \quad (5)$$

The two typographical errors in NRC (2001) were:

- h_i instead of h_i^* in equation (2);
- $(1-q_i)$ instead of q_j in equation (3).

An unintended consequence ensues regarding the calculation of lower confidence limits on the effective doses (LEDs), required by the 2005 guidelines as the point of departure (POD) for cancer risk estimation. The statistical programs supplied by Morales did not calculate the lower confidence limits; therefore, this portion of the statistical programming was designed for the current analysis. This was done using the delta method (as used in EPA's BMDS software). In brief, the standard deviation (sd) of the southwest Taiwanese ED values was calculated using asymptotic assumptions. In addition to taking the mean BMD values through the ldr adjustment, the 95% lower confidence limits on those values (mean – 2 sd) also were taken through these calculations. The resulting values were taken as the estimate of the American-appropriate BMDL values.

Calculation of slope factors and unit risks was done as outlined in the 2005 Cancer guidelines. These were based upon the $BMDL_{01}$ values and were identical to the values calculated from the $BMDL_{05}$ values to 2 significant figures.

5.3.5. Oral Slope Factor

The oral slope factor presented below was calculated using combined female lung and bladder cancer because it provided the most conservative estimates (Table 5-8). The oral slope factor calculated from combined male lung and bladder cancers (5.5E-6 mg/kg-day^{-1}) resulted in an identical drinking water unit risk (1.6E-04 µg/L^{-1}). Results from the transplacental carcinogenesis studies presented in section 4.2.2 support the use of these endpoints.

Slope factors (Table 5-8) were calculated by adding the slope factors for the lung and bladder cancer. This can be done because the cause of death (e.g., lung or bladder cancer) was recorded for each subject from death certificates (Wu, 1989). Combined cancer deaths were analyzed by Morales, et. al. (2000), and this consistent with the recommendations of NRC (2001) as well as previous work by EPA (2001). There is no evidence that these two tumor types are mechanistically dependent, and the likelihood of more than one tumor type is supported by the

Oral Slope Factor[1]: 5.7E-6 (mg/kg-day) $^{-1}$
Drinking Water Unit Risk: 1.6E-04 (µg/L) $^{-1}$

Drinking Water Concentrations at Specified Risk Levels, using default assumptions of 70 kg body weight and 2 L/day water ingestion:

E-4 (1 in 10,000)	6.3E-1 µg/L
E-5 (1 in 100,000)	6.3E-2 µg/L
E-6 (1 in 1,000,000)	6.3E-3 µg/L

During its deliberations on arsenic, there were significant discussions among the various Offices about the water and food consumption parameters that should be used to calculate the slope factor(s) for arsenic. The assumption of 2 L/day water consumption was used. For inorganic arsenic exposure from the diet, three values were considered; 1) 0 µg As/day, 2) 30 µg As/day (NRC selection), and 3) 50 µg As/day (average inorganic arsenic exposure from diet of Taiwanese; Schoof et al., 1998). The National Research Council selected 2 L/day and 30 µg As/day, although no reason was given for their selection of the food value. For clarity, the slope factors for each of these permutations are given in Table 5-4 through 5-7. The Excel spreadsheet for the calculations is available at www.epa.gov/waterscience.sab. The various assumptions for dietary arsenic intake have slight effects on the derived slope factor, while the two different assumptions for drinking water consumption lead to two-fold differences in the derived slope factor. The EPA has chosen 2 L drinking water/day and 30 µg dietary As/day in derivation of the recommended oral slope factor for inorganic arsenic, as is consistent with NRC (2001).

In the Morales et al. (2000) and NRC (2001) analyses, the control group was assumed to have no dietary intake of arsenic. The effects of different assumptions of dietary intake were limited to the dosed groups. Age-dependent and nonspecified effects on cancer incidence were largely fixed by the large control population. Therefore, NRC (2001, p. 196) found: "approximately a 1% increase in ED (effective dose) estimates" when they added 30 µg/day of arsenic in diet. This analysis follows the Morales et al. (2000) assumption, with no dietary intake of arsenic for the control population.

[1] The oral slope factor was calculated using an assumption of 30µg/day for dietary intake and 2L/day for drinking water consumption

Table 5-4. Calculation for Male Lung Cancer Risk from Arsenic in the Drinking Water

dietary adjust. (µg/day)	drinking water volume (L)	ED_{01} (µg/L)	LED_{01} (µg/L)	slope factor (x 10^{-7}) $(mg/kg\text{-}day)^{-1}$	unit risk ($x10^{-5}$) $(\mu g/L)^{-1}$	ppb at 1E-4 risk
50	2	96	87	40	11	0.87
30	**2**	**90**	**82**	**43**	**12**	**0.82**
0	2	90	83	42	12	0.83

Table 5-5. Calculation for Female Lung Cancer Risk from Arsenic in the Drinking Water

dietary adjust. (µg/day)	drinking water volume (L)	ED_{01} (µg/L)	LED_{01} (µg/L)	slope factor (x 10^{-7}) $(mg/kg\text{-}day)^{-1}$	unit risk ($x10^{-5}$) $(\mu g/L)^{-1}$	ppb at 1E-4 risk
50	2	82	76	46	13	0.76
30	**2**	**80**	**72**	**49**	**14**	**0.72**
0	2	79	70	50	14	0.70

Table 5-6. Calculation for Male Bladder Cancer Risk from Arsenic in the Drinking Water

dietary adjust. (µg/day)	drinking water volume (L)	ED_{01} (µg/L)	LED_{01} (µg/L)	slope factor (x 10^{-7}) $(mg/kg\text{-}day)^{-1}$	unit risk (x10^{-5}) $(\mu g/L)^{-1}$	ppb at 1E-4 risk
50	2	320	286	12	3.5	2.86
30	**2**	**314**	**282**	**12**	**3.5**	**2.82**
0	2	304	271	13	3.7	2.71

Table 5-7. Calculation for Female Bladder Cancer Risk from Arsenic in the Drinking Water

dietary adjust. (µg/day)	drinking water volume (L)	ED_{01} (µg/L)	LED_{01} (µg/L)	slope factor (x 10^{-7}) $(mg/kg\text{-}day)^{-1}$	unit risk (x10^{-5}) $(\mu g/L)^{-1}$	ppb at 1E-4 risk
50	2	474	437	8.0	.23	4.37
30	**2**	**472**	**421**	**8.3**	**2.4**	**4.21**
0	2	465	415	8.4	2.4	4.15

Table 5-8. Combined Bladder and Lung Cancer Risk from Arsenic in Drinking Water

Sex	Site	slope factor (x 10^{-7}) $(mg/kg\text{-}day)^{-1}$	unit risk (x10^{-5}) $(\mu g/L)^{-1}$	ppb at 1E-4 risk
Female	Bladder	8.3	2.4	4.21
	Lung	49	14	0.72
	Combined	57	16	0.63
Male	Bladder	12	3.5	2.82
	Lung	43	12	0.82
	Combined	55.0	16	0.63

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

There have been numerous studies examining the carcinogenic potential of inorganic arsenic via oral exposure. The majority of these studies are ecological in nature and are therefore subject to the biases of ecological studies (e.g., lack of individual exposure). The strength of the associations observed in the studies mentioned above determine an association between oral exposure to inorganic arsenic and cancer. Each study was conducted differently and contained their own biases (e.g., lack of confounding variables, possible recall bias), but combination of

all the study results supports an association between oral exposure to inorganic arsenic and cancer including bladder, kidney, lung, liver, and prostate. Leukemia and lymphomas, colon cancer, and bone cancer have been associated with arsenic exposure in a few studies (Chen et al., 1985, 1988; Tsai, 1999; Wu, 1989). Different populations were assessed, therefore, it is unlikely that any single attribute (i.e., nutritional habits) associated with a single population is responsible for the increased cancer rates. Although many studies did not account for confounding variables (e.g., cigarette smoking in association with lung cancer), the positive associations also were observed in those studies that did account for certain confounding variables (e.g., habits, age, socioeconomic status). Details on the specific studies are provided above in Section 4.1.

The major flaw in the association between oral exposure to inorganic arsenic and skin cancer was the lack of data and/or analysis of dermal contact with the water containing the inorganic arsenic through bathing. The studies that failed to observe an association between oral exposure to inorganic arsenic and bladder cancer (Bates et al., 1995; Lamm et al., 2004; Steinmaus et al., 2003) examined low exposures. However, Bates et al. (1995) and Steinmaus et al. (2003) did find an association between arsenic exposure (exposures 20 years or more) and bladder cancer in people who had ever smoked. Lung and liver tumors also were observed in mice administered inorganic arsenic for a short duration transplacentally with possible additional exposure while nursing (pregnant dams were exposed for 10 days during gestation only) (Waalkes et al., 2003, 2004a). Therefore, increasing the evidence that lung and liver cancers are associated with oral exposure to inorganic arsenic.

The confidence in the data is strong because the assessment is based on lung cancer mortality in humans (females). The data demonstrates a definite dose-related effect in humans. Uncertainty lies in exact exposure by subjects primarily due to the fact that this is an ecological study. The calculations did integrate the possibility of arsenic exposure from other sources (i.e., food; assumption of 30 µg/day). There is also uncertainty in the amount of water consumed/day by Taiwanese females (2.0 or 3.5 L).

6.2. DOSE RESPONSE

Cancer has been selected as the most sensitive endpoint The oral slope factor varies depending on assumptions of the volume of water consumed over the course of a day and the amount of arsenic consumed through the diet. Tables 5-4 through 5-7 provide estimates for assumptions of 2 L/day of water consumed with 0, 30 or 50 µg/day of arsenic consumed via the diet, with 2 L/day and 30 µg/day being the assumptions recommended by the NAS (NRC, 2001). Changes in these assumptions may change the oral slope factors (this is spelled out in more detail in the cancer section), and would lead to changes in the concentration of arsenic in the drinking water associated with an increased risk of cancer. Cancer assessment via inhalation exposures was not assessed in this report.

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APPENDIX A. Summary of External Peer Review and Public Comments and Disposition

To Be Added

APPENDIX B:Quantitative Issues in the Cancer Risk Assessment for Inorganic Arsenic

1. Description of the data set used for modeling

The data set chosen for analysis is the southwest Taiwanese study by Chen et al. (1988), which had previously been analyzed by Morales et al. (2000) and by NRC (2001). Briefly, mortality due to lung and bladder cancer was recorded in a set of Taiwanese villages with measured arsenic concentrations in their well water. This information was stratified by village, age, and sex. A set of southwest Taiwanese villages without measurable well water arsenic was used as a background population.

2. Choice of model

The model selected for this analysis is an additive (linear) Poisson model with a linear term for dose and quadratic adjustment for age. The basic formula for a Poisson model is:

$$h(x,t) = h(t)^* \times g(x)$$

where:

$h(x,t)$ = Cancer hazard function

$h(t)^*$ = a function describing the baseline risk in the absence of exposure to the chemical in question. Although the baseline function can incorporate a number of factors (e.g., age, gender, smoking/non-smoking, etc.), the Taiwanese data used by Morales et al. only contained gender and age (t) information.

$g(x)$ = a function describing the effect of exposure to the chemical. The term x is the chemical dose.

The use of a Poisson model provides great flexibility regarding the inclusion of covariates (such as age) without the introduction of restrictive assumptions about the data. Previous analyses had used either additive (linear) or multiplicative (exponential) Poisson models; linear, logarithmic, or square-root terms for dose; and linear, quadratic, or spline adjustments for age (Table B-1) (Morales et al., 2000).

Table B-1. Poisson modeling options. (Modified from Morales et al., 2000)

Age effect $h(t)^*$	Dose transformation	Dose effect $g(x)$
Linear	Linear	Linear
$\exp(\alpha_0 + \alpha_1 t)$	$x = \text{ppb}^a$	$1+\beta_1 x$
Quadratic	Logarithmic	Quadratic
$\exp(\alpha_0 + \alpha_1 t + \alpha_2 t^2)$	$x = \log(1 + \text{ppb})$	$\beta_1 x + \beta_2 x^2$

Regression spline	Square root	Exponential linear
$\exp[\alpha_0 + \alpha_1 ns(t)]^b$	$x = \sqrt{\text{ppb}}$	$\exp(\beta_1 x)$
		Exponential quadratic
		$\exp(\beta_1 x + \beta_2 x^2)$

[a] Represents exposure concentration in parts per billion, which is equivalent to micrograms per liter.
[b] $ns(t)$ represents a natural spline applied to t.

The previous EPA draft assessment for arsenic (USEPA, 2001) used a multiplicative (exponential) model with a linear term for dose and a quadratic age effect. The multiplicative model was chosen based upon lack of necessity for an external control population, which EPA believed at that time would skew the results in a non-linear fashion. The linear term for dose and quadratic age effect were selected as representing the best fit to data by Akaike information criterion (AIC). NRC (2001) recommended the use of an additive (linear) model with a southwest Taiwanese background population. The rationale for this choice was that no objective criteria could be found for excluding the use of the additive model with background, and the additive model was a better fit to the data by AIC. The current analysis accepts the NRC recommendation.

3. Low-dose extrapolation method

According to the Guidelines for Carcinogen Risk Assessment (USEPA, 2005):

> Linear extrapolation should be used in two distinct circumstances: (1) when there are data to indicate that the dose-response curve has a linear component below the POD, or (2) as a default for a tumor site where the mode of action is not established (see Section 3.3.1). For linear extrapolation, a line should be drawn from the POD to the origin, corrected for background. This implies a proportional (linear) relationship between risk and dose at low doses. (Note that the dose-response curve generally is not linear at higher doses.)
>
> The slope of this line, known as the *slope factor*, is an upper-bound estimate of risk per increment of dose that can be used to estimate risk probabilities for different exposure levels. The slope factor is equal to $0.01/LED_{01}$ if the LED_{01} is used as the POD.

As the mode of action for the carcinogenicity of inorganic arsenic is not established, a linear low-dose extrapolation from the LED01 is used for this analysis.

4. Selection of background population

For this analysis, a background population from southwest Taiwanese villages without detected arsenic in their drinking water supply was used. The

previous EPA draft assessment for arsenic (USEPA, 2001) did not use any comparison population. This was due to concerns regarding the tendency of large background populations to skew dose-response curves in a non-linear fashion. NRC (2001) recommended the use of a southwest Taiwanese background population. The rationale for this choice was that no objective criteria could be found for excluding the use of this background population, that the presence of a background population was not a unique determinant of the linearity or non-linearity of the dose-response, and that the absence of a background population could easily lead to an underestimate of the slope of the dose-response. The current analysis accepts the NRC recommendation.

5. Adjustment for background cancer risks

The hazard (or relative risk) of cancer due to arsenic exposure has been estimated based on Taiwanese data, including the Taiwanese background rate. The results are then used to calculate lifetime risk for the U.S. population, using the BEIR IV formula (NRC, 2001) and the U.S. background cancer rates. The BIER-IV formula, corrected for two errors in NRC (2001), is

$R(x)$ = lifetime risk of death at dose x

$$= \sum_i \frac{h_i(x)}{h_i^*(x)} S_i(1-q_i) \qquad (1)$$

where:

i = age group

$h_i(x)$ = cancer mortality hazard, age i, dose x

$h_i^*(x)$ = total mortality hazard, age i, dose x

q_i = P(survive i-th time interval | survive (i-1)-th time interval)

$$= \exp(-5\, h_i^*(x)) \qquad (2)$$

S_i = P(survive up to end of (i-1)-th time interval)

$$= \prod_{j=1}^{i-1} q_j \qquad (3)$$

The q_i's and S_i's also depend on x through equations (2) and (3), but the dependence is not shown, and in fact is sometimes ignored when h_i is small relative to h_i^*, so that $h_i^*(x) \approx h_i^*(0)$ for x of interest.

NRC (2001, p. 186) printed two errors related to (1):

- they wrote h_i in place of h_i^* in equation (2);
- they wrote $(1-q_i)$ in place of q_j in equation (3).

Now we use a linear relative hazard model

$$h_i(x) = h_i(1+\beta x)$$

where $h_i = h_i(0)$ and β is the relative risk slope that we estimate by regression. The additional dose-related cancer hazard is $h_i \beta x$, so the total mortality hazard

increases at the same time to $h_i^*(x) = h_i^* + h_i \beta x$ where again $h_i^* = h_i^*(0)$. Therefore, (1) becomes

$$R(x) = \sum_i \frac{h_i(1+\beta x)}{h_i^* + h_i \beta x} S_i(1-q_i) \quad (4)$$

and again the q_i's and S_i's also depend on x.

The previous EPA draft assessment for arsenic (USEPA, 2001) did not adjust the Taiwanese results to the U.S. population. NRC (2001) recommended the use of the BEIR IV formula for this adjustment. The current analysis accepts the NRC recommendation, with corrections.

6. Adjustment for dietary arsenic intake

In addition to arsenic intake from drinking and cooking water, measurable amounts of dietary arsenic intake have been suggested to occur in southwest Taiwan, with estimates in the range of 50 µg/day (Schoof et al. 1998). For this analysis, arsenic concentrations for the exposed (but not background) southwest Taiwanese populations have been adjusted by adding either 0, 30, or 50 µg/day as follows:

$$conc_{adj} = conc_{dw} \times \frac{amt_{diet}}{vol_{dw} + vol_{cw}}$$

where:

$conc_{adj}$ = the diet-adjusted concentration of arsenic

$conc_{dw}$ = the actual well-water arsenic concentration

amt_{diet} = the daily dietary arsenic intake

vol_{dw} = the daily drinking water intake

vol_{cw} = the daily cooking water intake

As is evident in Table B-2, the different assumptions as to dietary arsenic intake have no significant effect upon the calculation of cancer slope factors to 2 significant figures. The previous EPA draft assessment for arsenic (USEPA, 2001) used a value of 50 µg/day for dietary arsenic intake. Although NRC (2001) found little or no support for that assumption, they stated that the results would be insensitive to the assumption. The current analysis supports the NRC assertion and uses their recommended value of 30 µg/day for dietary arsenic intake.

Table B-2. Sensitivity of cancer slope factor to dietary arsenic assumptions

site	sex	dietary arsenic µg/day	ED_{01} µg/mL	LED_{01} µg/mL	slope $(\mu g/mL)^{-1}$
lung	female	0	79	67	1.5E-04
lung	female	30	77	69	1.4E-04
lung	female	50	72	65	1.5E-04
lung	male	0	92	82	1.2E-04
lung	male	30	91	84	1.2E-04
lung	male	50	100	82	1.2E-04
bladder	female	0	364	332	3.0E-05
bladder	female	30	369	346	2.9E-05
bladder	female	50	375	340	2.9E-05
bladder	male	0	260	233	4.3E-05
bladder	male	30	255	240	4.2E-05
bladder	male	50	260	237	4.2E-05

7. Adjustment for drinking water volumes

The calculated risk associated with arsenic exposure through drinking water is, as expected, inversely linearly related to the assumed volumes of drinking water for the study population. For this analysis, the total drinking water volume (including cooking water) is assumed to be 2 L/day for Taiwan and 1 L/day for the US. When combined with the differences in mean body weights between the Taiwanese and US populations (50 kg vs.70 kg), the resulting dose adjustment factor is 3. This approach is different from that of the previous EPA draft assessment for arsenic (USEPA, 2001), which used a Monte Carlo analysis to generate a distribution of exposures. NRC (2001) noted the broad interindividual variability in water intake and the lack of data specific to the southwest Taiwanese population. The current analysis does not directly address this issue.

8. Adjustment for cooking water

In addition to arsenic intake from drinking water and dietary sources, measurable amounts of arsenic intake from cooking water have been suggested to occur in southwest Taiwan. Estimates of the volume of water consumed by this route are estimated at 1 L/day (USEPA, 1989). For this analysis, 1 L/day is added to the Taiwanese drinking water volume to account for cooking water. This is in accordance with what was done in the previous EPA draft assessment for arsenic (USEPA, 2001). NRC (2001) agreed with EPA's method for accounting for the extra water consumption due to use of drinking water in cooking food, although they noted that the rationale for using 1 L/day was not documented.

9. Adjustment for mortality

The hazard (or relative risk) of cancer due to arsenic exposure has been estimated based on Taiwanese mortality data, as opposed to incidence data. The results are then used to calculate lifetime risk for the U.S. population, using the

BEIR IV formula (NRC, 2001) and the U.S. background cancer mortality rates. The BEIR IV formula adjusts for US background mortality rates, resulting in a relative risk. Therefore, no additional adjustment is needed between incidence and mortality. The previous EPA draft assessment for arsenic (USEPA, 2001) did not adjust the Taiwanese results to the U.S. population, and therefore required an adjustment between mortality and incidence. NRC (2001) recommended the use of the BEIR IV formula for this adjustment. The current analysis accepts the NRC recommendation.

10. Model Implementation Details

The additive Poisson models for each of the endpoints (lung and bladder cancer in males and females) and their related BEIR IV calculations are implemented using two Microsoft Excel 2002 SP3 spreadsheets: MCcancerfit.xls and BEIR.xls. A listing of the contents of these spreadsheets is in Table B-3. The process for running the calculations is a multi-step algorithm:

1) In MCcancerfit.xls, for each of flung (female lung cancer), mlung (male lung cancer), fblad (female bladder cancer), and mblad (male bladder cancer), run the Solver add-in to maximize sum (cell N2) by adjusting a1, a2, a3, and b (cells G1:G4). This provides starting values for the Bayesian regression.

2) In MCcancerfit.xls, for each of MC flung, MC mlung, MC fblad, and MC mblad, paste the starting values into a1.start, a2.start, a3.start, and b.start (cells N1:N4). Force a recalculation (key F9 on Windows PCs). Random values for a1, a2, a3, and b are calculated and placed into cells E:H.

4) In MCcancerfit.xls, for each of MC flung, MC mlung, MC fblad, and MC mblad, run the respective macro MCflung, MCmlung, MCfblad, or MCmblad. These macros calculate the log-likelihood of the data given the parameter values by placing the random parameter values (cells E:H) in the model calculation sheets (flung, mlung, fblad, or mblad) in cells G1:G4 and paste the resulting log-likelihood (cell N2) back into the respective MC sheet (column I). Likelihoods (scaled by the maximum likelihood to avoid numeric instability) are then calculated. The mean and standard deviation values for beta, weighted by the likelihood function, are calculated and placed in cells N8 and N9, respectively. Upper and lower bounds (95% confidence limits) on beta are calculated by adding or subtracting 2 × the weighted standard deviation to the weighted mean (cells N10 and N11).

5) In BEIR.xls, for each of flung, mlung, fblad, and mblad, the beta values (mean, upper, or lower) calculated in MCcancerfit.xls are placed in b (cell T10). Risks at given arsenic concentrations are calculated by adjusting x (cell U10) and reading the total relative risk at sum(er) (cell T15). Concentrations corresponding to a given risk level can be calculated using either Solver or Goal Seek to seek out the value of x (cell U10) that gives a particular value to sum(er) (cell T15).

There are several user issues involving implementation of these spreadsheets:

1) These spreadsheets use macros. Security levels on machines used to run these spreadsheets must be adjusted to allow signed macros to run.

2) Macro run time is expected to be several minutes on a 3 gigahertz Pentium 4 PC with 512 KB RAM running Windows XP SP2 and Microsoft Excel 2002 SP3. Other computers may take more or less time. These spreadsheets have not been tested for compatibility with other CPUs, operating systems, or versions of Microsoft Excel.

3) The Solver add-in is required for setting initial parameter values in MCcancerfit.xls. Solver is not used in the calculation of final parameter values. Solver may be used in BEIR.xls calculations, but it is not required.

4) Automatic spreadsheet calculation is switched off in MCcancerfit.xls to prevent unintended recalculation of random parameter values.

The intent of these spreadsheets is to make the calculations underlying the arsenic cancer risk assessment as transparent as possible, thereby facilitating peer review and subsequent modification. This is not intended to be the most rapid, computationally efficient implementation possible. Nor is it intended to run on the platforms of choice of most statisticians (SAS, S-PLUS, R, etc.). By programming these calculations in Microsoft Excel, they are available, accessible, understandable, and modifiable by the largest possible audience of peer reviewers and stakeholders.