TOXICOLOGICAL REVIEW

OF

INORGANIC ARSENIC

(CAS No. 7440-38-2)

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

March 2009

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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.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey 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 (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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List of Abbreviations

~	approximately (if before a listing of concentrations, it applies to all)
293 cells	cell line derived from adenovirus-transformed human embryonic kidney epithelial cells
2-AAAF	2-acetoxyacetylaminofluorene
8-OHdG	8-hydroxydeoxyguanosine
AG06 cells	SV40-transformed human keratinocytes
AIC	Akaike information criterion
AGT	Average generation time
AMI	acute myocardial infraction
AP	activator protein or activating protein
APE	apurinic/apyrimidinic endonuclease
As	arsenic
As^{III}	arsenite
As^{V}	arsenate
AS3MT	arsenic(+3 oxidation state) methyltransferase
AQP	Aquaglycoporins
ATG	arsenic triglutathione
ATO	arsenic trioxide
ATSDR	Agency for Toxic Substances and Disease Registry
B[*a*]P	benzo[*a*]pyrene
BBDR	biologically based quantitative dose response
BCC	basal cell carcinoma
BER	base excision repair
BFD	blackfoot disease
BMI	body mass index
BPDE	benzo[*a*]pyrene diol epoxide, an active metabolite of B[*a*]P
BrdU	bromodeoxyuridine
BSO	L-buthionine-S,R- sulphoximine (depletes GSH, γ-GCS inhibitor)
BW or bw	body weight
CA	chromosome aberrations
Caco-2	Human intestinal cell line
CAE	Cumulative arsenic exposure
CASRN	CAS registry number
CAT	catalase (decomposes H2O2)
CCA	chromate copper arsenate
CCRIS	Chemical Carcinogenesis Research Information System
cDNA	complementary DNA
cen+	centromere positive
cen-	centromere negative
Chang cells	human cell line thought to be derived from HeLa cells
CHO	Chinese hamster ovary
CI	confidence interval

c-Jun or c-jun	an AP-1 protein
CL3 cells	human lung adenocarcinoma cells (established from a non-small-cell lung carcinoma)
COPD	chronic obstructive pulmonary disease
DEB	diepoxybutane (DNA cross linking agent)
DES	diethylstilbestrol
dhfr gene	dihydrofolate reductase gene
DHLP	dihydrolipoic acid
DI-I or II or III	iodothyronine deiodinase-I or II or III (are 3 forms of this selenoenzyme)
dL	deciliter
DMA^{III}	dimethylarsenous acid
DMA^{V}	dimethylarsinic acid
DMA	dimethyl arsenic (used when the oxidative state is unknown or not specified)
DMAG	dimethylarsinic glutathione
$DMMTA^{III}$	dimethylmonothioarsinic acid
$DMMTA^{V}$	dimethylmonothioarsonic acid
DMPS	2,3-dimercaptopropane-1-sulfonic acid
DMSA	dimercaptosuccinic acid or meso 2,3- dimercaptosuccinic acid
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
DTT	Dithiothreitol
DW	drinking water
E. coli	Escherichia coli
ED	effective dose
EGFR-ECD	extracellular domain of the epidermal growth factor receptor
EPA	Environmental Protection Agency
ERCC1	excision repair cross-complement 1 component
ERCC2	excision repair cross-complementing rodent repair deficiency, complementation group 2 (also known as xeroderma pigmentosum group D or XPD)
ERK	extracellular signal-regulated kinase
ER-α	estrogen receptor-alpha
FAK	focal adhesion kinase
FPG	formamidopyrimidine-DNA glycosylase (digestion of DNA)
G6PDH	glucose-6-phosphate dehydrogenase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GI	gastrointestinal
GLM	Generalized linear model
GM04312C cells	SV-40 transformed XPA human fibroblast NER-deficient cell line
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPx	glutathione peroxidase
GSH	Glutathione

GST	glutathione-*S*-transferase
GSTO1	glutathione S-transferase omega 1
GSTP1-1	glutathione S-transferase P1-1
H69AR	A multi-drug resistant human cancer cell line
H9c2 cells	immortalized myoblast cell line derived from fetal rat hearts
HAC	Highest arsenic concentration
HCC	hepatocellular carcinoma
HEALS	Health Effects of Arsenic Longitudinal Study
HELF cells	Human embryo lung fibroblast cell line
HepG2 cells	human hepatocellular liver carcinoma cell line (Caucasian)
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
hGST-O1	Human glutathione-S-transferase omega 1
HMOX-1	heme oxygenase 1
hOGG1	human 8-oxoguanine DNA glycosylase
HPBM	human peripheral blood monocytes
HSDB	Hazardous Substance Database
HXT	hexose permease transporters
IC_{50}	concentration that is needed to cause 50% inhibition
IFN-γ	Interferon-gamma
IL	interleukin
ILK	integrin-linked kinase
IRIS	Integrated Risk Information System
IRR	incidence rate ratio
iv	Intravenous
JAK	Janus kinase
LED	lowest effective dose
LI	Labeling index
LOEC	lowest observed effective concentration
LOEL	lowest observed effective level
MADG	monomethylarsonic diglutathione
MAP	mitogen-activated protein
MCF-7 cells	human breast carcinoma cell line
M-CSF	Macrophage colony-stimulating factor
MDA	Malondialdehyde
mdm2	murine double minute 2 proto-oncogene
MEK	MAP/ERK kinase
MI	Mitotic index
MLE	maximum likelihood
MMA^{III}	monomethylarsonous acid
MMA^{V}	monomethyl arsonic acid
MMA	monomethyl arsenic (used when oxidative state is unknown or not specified)
MMS	methyl methanesulfonate
MN	Micronuclei

MNU	N-methyl-N-nitrosourea
MOA	mode(s) of action
MPR2/cMOAT	Multi-drug resistance associated protein 2 transporter
MRP	multidrug resistance protein
mRNA	messenger ribonucleic acid
MTHFR	methylene trihydrofolate reductase
NAC	*n*-acetyl-cysteine
NAD	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NER	nucleotide excision repair
NHEK cells	primary normal human epidermal keratinocytes
NK	Natural killer
NO	nitric oxide
NRC	National Research Council
OATP-C	Organic anion transporting polypeptide-C
ODC	ornithine decarboxylase
OGG1	8-oxoguanine DNA glycosylase
OPP	Office of Pesticide Programs
OR	odds ratio
PARP	poly(adenosine diphosphate–ribose) polymerase
PBPK model	physiologically based pharmacokinetic model
PBMC	peripheral blood mononuclear cells
PCNA	Proliferating cell nuclear antigen
PCR	polymerase chain reaction
PGK	Phosphoglyerate kinase
PHA	Phytohemagglutinin
PMI	primary methylation indices
PNP	purine nucleoside phosphorylase
POD	Point of departure
ppb	parts per billion
ppm	parts per million
PTEN	phosphatase and tensin homolog
PYR	person years at risk
RAGE	receptor for advanced glycation end products
RBCs	red blood cells
RED	Reregistration Eligibility Decision
RfC	inhalation reference concentration
RfD	oral reference dose
RI	Replication index
RNS	reactive nitrogen species
ROS	reactive oxygen species
RR	Relative risk

RT	Real time
SAB	science advisory board
SAM	S-adenosylmethionine
SBET	simplified bioaccessibility extraction test
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCGE	single cell gel electrophoresis
Se	selenium
SEER	surveillance epidemiology and end result
SHE cells	Syrian hamster ovary cells
SIRs	standard incidence ratios
SMI	secondary methylation indices
SMR	standard mortality ratio
SOD	superoxide radical dismutase
STAT	signal transducer and activator of transcription
SV-HUC-1 cells	SV40 large T-transformed human urothelial cell line
T_3	thyroid hormone triiodothyronine
T_4	thyroid hormone thyroxine
TAT	Tyrosine aminotransferase
TCEP	Tris(2-carboxylethyl)phospine
Tg.AC	strain of transgenic mice that contains the fetal beta-globin promoter fused to the v-Ha-ras structural gene (with mutations at codons 12 and 59) and linked to a simian virus 40 polyadenylation/splice sequence
TGF-α	transforming growth factor alpha
TMA^{III}	Trimethyl arsine
TMA^{V}	trimethylarsinic acid
TMAO	trimethylarsine oxide
TNF-α	tumor necrosis factor alpha
TPA	12-*O*-tetradecanoyl phorbol-13-acetate
Trx	thioredoxin
TrxR	thioredoxin reductase
TWA	Time-weighted average
UCL	Upper confidence limits
UROtsa	an SV40-immortalized human urothelium cell line
UV	ultraviolet radiation
V79 cells	cell line derived from lung fibroblasts of a male Chinese hamster
VEGF	Vascular endothelial cell growth factor
XRCC1	X-ray repair cross-complimentary group 1

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of inorganic arsenic. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The document is based on the EPA-sponsored reviews of the reports, "Arsenic in Drinking Water" and "Arsenic in Drinking Water, 2001 Update" published by the National Research Council (NRC) in 1999 and 2001, respectively. The NRC arsenic committee that wrote the reports 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 NRC. This IRIS document based on reviews of those reports has undergone evaluation by EPA health scientists from several program offices and regional offices and external peer review by the Science Advisory Board (SAB).

Compared to the Toxicological Review submitted to the SAB in 2005, this assessment is expanded to provide a detailed review of epidemiological studies, and mode of action (MOA) studies, as well as the revisions in dose-response analysis to address the recommendations suggested by the SAB in 2007 (SAB, 2007). Specifically, additional sensitivity analysis on effects of modeling assumptions on estimated cancer risk is included in this revised Toxicological Review for cancer.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a non-linear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as 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 effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. 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 may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

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 (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment 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), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988a), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through December, 2007; however, a few references from 2008 have also been included.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

2.1 PROPERTIES

Arsenic (As) is a metalloid that can exist in the -3, 0, +3, and +5 oxidation states[1]. The arsenite (As^{III}; +3) and arsenate (As^{V} ; +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
Oxidation State	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

[1] Oxidation states for arsenic have been abbreviated differently by different organizations or authors. For example arsenite can be abbreviated by either As(III) or As^{III}. Both refer to trivalent inorganic arsenic compounds. This document uses the superscript abbreviation.

2.2 USES

The metalloid, arsenic, is used for hardening copper and lead alloys (HSDB, 2005). It also is used in glass manufacturing as a decolorizing and refining agent, as a component of electrical devices, in the semiconductor industry, and as a catalyst in the production of ethylene oxide. Arsenic compounds are used as a mordant in the textile industry, for preserving hides, as medicinals, pesticides, pigments, and wood preservatives. Approximately 90% of the domestic consumption of arsenic is currently used with production of chromate copper arsenate (CCA), a wood preservative (ATSDR, 2007), the production of which is currently being phased out.

2.3 OCCURRENCE

Arsenic naturally comprises ~ 3.4 parts per million (ppm) of the Earth's crust, where it is the twentieth most abundant element (ATSDR, 2007; Merck Index, 1989). Arsenic leaches from natural weathering of soil and rock into water and low concentrations of arsenic are found in water, food, soil, and air. However, industrial activities such as coal combustion and smelting operations release higher concentrations of arsenic to the environment (Adams et al., 1994). The highest background arsenic levels found in the environment are in soils, with concentrations ranging from 1 to 40 ppm (ATSDR, 2007). Food typically contains arsenic concentrations of 20 to 140 parts per billion (ppb) (ATSDR, 2007). The majority of surface and ground waters contain less than 10 ppb (although levels of 1000–3400 ppb have been reported, especially in areas of the western United States). Average arsenic content in drinking water in the U.S. is 2 ppb with 12% of water supplies from surface water in central portions of the U.S. and 12% of groundwater sources in western portions of the U.S. exceeding 20 ppb (ATSDR, 2007). Mean arsenic concentrations in ambient air have generally been found to range from 1 to 2000 ng/m^3 (ATSDR, 2007).

2.4 ENVIRONMENTAL FATE

Arsenic as a free element (0 oxidation state) is rarely encountered in the environment (HSDB, 2005). Under normal conditions in water, arsenic is present as soluble inorganic As^V because it is more thermodynamically stable in water than As^{III}. In soil there are many biotic and abiotic processes controlling arsenic's overall fate and environmental impact. Arsenic in soil exists in various oxidation states and chemical species, depending upon soil pH and oxidation-reduction potential (ATSDR, 2007). Arsenic is largely immobile in agricultural soils, and tends to remain in upper soil layers (ATSDR, 2007). However, reducing conditions form soluble mobile forms of arsenic and leaching is greater in sandy soil than in clay loam (ATSDR, 2007). The most influential parameter affecting arsenic mobility is the iron content of the soil.

3. TOXICOKINETICS

Arsenic exposures discussed in this toxicology review are from oral waterborne arsenic exposure. Although inhalation exposures also are common, they are not addressed specifically in this report. Dermal exposure and exposure from food consumption, however, can potentially be significant and may be confounding variables in epidemiological studies. Therefore,

toxicokinetic information will focus mainly on oral exposure from water sources, but absorption issues from dermal exposure and arsenic in food also will be briefly addressed.

The behavior of arsenic in the body is very complex. After absorption, inorganic arsenic can undergo a complicated series of enzymatic and non-enzymatic oxidation, reduction, and conjugation reactions. Although all these reactions may occur throughout the body, the rate at which the reactions occur varies greatly from organ to organ. In addition, there are important differences in arsenic metabolism across animal species, and these variations make it difficult to identify suitable animal models for predicting human metabolic patterns.

Each metabolic transformation affects the subsequent biokinetic behavior (transport, persistence, elimination) and toxicokinetics of the arsenic species. Thus, absorption, transport, and metabolic processes are highly interdependent and cannot easily be discussed separately. The general pattern that will be seen in this chapter involves the gastrointestinal (GI) absorption of inorganic arsenic species, followed by a cascade of oxidation-reduction reactions and methylation steps, resulting in the partial transformation of the inorganic species into mono- or dimethylated species (collectively referred to as MMA and DMA, recognizing that there is often ambiguity in characterizing the oxidation state of the methylarsenic compounds). Conjugated arsenic species either methylated or not (e.g., glutathione conjugates or other sulfur-containing derivatives) also may be produced.

As discussed in Section 3.3, several metabolic schemes have been proposed that describe the general pathway that converts inorganic arsenic to its primary metabolites MMA and DMA. These pathways involve numerous enzymes and cofactors. Some of the proposed metabolic pathways involve the cycling of arsenic species back and forth between the +3 (trivalent) and +5 (pentavalent) oxidation states, and there is good evidence that key metabolic processes may be saturable, so that metabolic patterns differ with exposure levels. MMA, DMA, and inorganic As levels in tissues, blood, and urine are the most easily and frequently measured metabolites; the relative levels of these compounds in blood or urine are often the primary evidence in support of one or another metabolic pathway. Genomic tools are being increasingly employed to better characterize human arsenic metabolism and to identify individuals at higher risk from arsenic exposures.

3.1 ABSORPTION

Water soluble forms of inorganic As (both trivalent and pentavalent) are readily absorbed from the GI tract of experimental animal models (about 80-90% 0.62 mg/kg of sodium arsenate; Freeman et al., 1995) as well as humans (Pomroy et al., 1980, who recovered 62% of a 0.06 ng dose of arsenic in 7 days). Monomethyl arsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) also appear to be well absorbed (75–85%) in humans and experimental animals (Stevens et al., 1977; Buchet et al., 1981; Yamauchi and Yamamura, 1984; and Hughes et al., 2005). Juhasz et al. (2006), however, using an *in vivo* swine test determined that MMA (oxidation state not specified) and DMA (oxidation state not specified) were poorly absorbed with only 16.7 and 33.3%, respectively, bioavailable.

Laparra et al. (2006) used a Caco-2 permeability model, which measured transport through a monolayer of human intestinal cells, to examine the intestinal permeability of As^{III}. A decrease in the apical to basolateral permeability with increasing dose was found, indicating the presence of a saturable intestinal transport system. The data also indicated that Caco-2 cells have a secretory system for As^{III}. In an earlier study, Laparra et al. (2005a) demonstrated that the retention and transport of As^{III} in Caco-2 cells was more efficient than that of As^{V}. However, this could have been due to the presence of phosphate in the culture medium, which would compete with arsenate for transport across the membrane.

Gastrointestinal absorption of low-solubility arsenic compounds such as arsenic trisulfide, lead arsenate, arsenic selenide, gallium arsenide (Mappes, 1977; Webb et al., 1984; and Yamauchi et al., 1986), and arsenic-contaminated soil (Freeman et al., 1995) is much less efficient than that of soluble inorganic arsenic compounds. The degree of absorption of arsenic from soil was found to be dependent on the arsenic species present in the soil and on the type of soil. Juhasz et al. (2007) performed *in vivo* bioavailability studies in swine and determined that the bioavailability of total arsenic in soils was highly variable with a range of 6.9% to 74.7% depending on the soil type. They also determined that a Simplified Bioaccessibility Extraction test (SBET; a rapid *in vitro* chemical extraction method) had results highly correlated with the *in vivo* results. Therefore, they concluded that the less expensive *in vitro* test was just as effective for determining bioavailability.

There is little information concerning the bioavailability of inorganic arsenic from various types of food (NRC, 1999, 2001). However, there have been recent studies examining the bioaccessibility of arsenic from rice (Laparra et al., 2005b and Juhasz et al., 2006). Laparra et al. (2005b) determined that while cooking rice (tested several types, but didn't specify the types) in deionized water caused no change in arsenic content compared to the raw form, cooking in water contaminated with 0.5 µg/mL of As^{V} increased the inorganic arsenic content 5-17 fold over the raw rice. Rice samples (10 grams) were then subjected to an *in vitro* simulated digestion process. Levels of soluble arsenic were measured to determine bioaccessibility. The results demonstrated that large amounts of the arsenic (i.e., 63–99%), mainly in the pentavalent form, were bioaccessible for intestinal absorption. Ackerman et al. (2005) also found 89-105% bioaccessible arsenic in different samples of white and brown rice cooked in water containing As^{V}.

Juhasz et al. (2006) examined the bioavailability of arsenic from rice (mainly white rice samples) using an *in vivo* swine assay. Quest rice was grown in arsenic contaminated water and cooked in arsenic-free water. This caused the rice to contain arsenic mainly in the form of DMA. Administration of the cooked rice to swine demonstrated a bioavailability similar to that observed after a single oral administration of DMA in water (i.e., 33.3%). Basmati white rice cooked in water contaminated with 1000 ppb of As^{V}, which contained entirely inorganic As as a result of the arsenate in the cooking water, had a bioavailability of 89.4%.

Although there have been no studies performed on the rate of inorganic arsenic absorption through intact human skin, systemic toxicity due to high dermal occupational exposure to aqueous inorganic arsenic solutions indicates that the skin is possibly a significant exposure route (Hostynek et al., 1993). The systemic absorption via the skin from less

concentrated solutions, however, appears to be low (NRC, 1999). An *in vivo* study by Wester et al. (1993) demonstrated that 2% to 6% of radiolabeled arsenate (as a water solution) was absorbed by rhesus monkey skin over a 24-hour period. Results demonstrated that the lower dose (0.000024 μg/cm^2) was absorbed at a greater rate (6%) than the higher arsenic exposure (2.1 μg/cm^2; 2%), but the difference didn't reach statistical significance. Wester et al. (2004) performed another *in vivo* dermal absorption study using female Rhesus monkeys. Using the levels excreted in the urine and the applied dose, they calculated 0.6% to 4.4% was absorbed in the three monkeys tested, which was similar to their previous results. *In vitro* results on human skin (from donors) demonstrated a 24-hour absorption of 1.9% (Wester et al., 1993). Mouse dorsal skin was demonstrated to absorb 30% to 60% of applied arsenic (Rahman et al., 1994) using similar *in vitro* testing, with 60 to 90% of the absorbed arsenic being retained in the skin. NRC (1999) suggests this indicates that inorganic arsenic binds significantly to skin and hair. Lowney et al. (2007) found that dermal absorption of arsenic from soils was negligible in an *in vivo* study in rhesus monkeys.

Harrington et al. (1978) compared arsenic metabolite levels in the urine from a group of people in Fairbanks, Alaska who had arsenic-contaminated water (345 ppb) in their home, but drank only bottled water, with the levels seen in a group of people who drank home water containing less than 50 ppb. The results demonstrated that the group with high arsenic in their water had close to the same average concentration of total arsenic metabolites in their urine (i.e., 43 μg/L) as the group who drank home water with less than 50 ppb arsenic (i.e., 38 μg/L in urine), indicating possible dermal absorption via bathing or other exposure sources. Levels of arsenic in the bottled water, however, were not measured. Possible exposure through using contaminated water for cooking also was not examined.

3.2 DISTRIBUTION

The retention and distribution patterns of arsenic species are strongly dependent on their chemical properties. While both As^{III} and As^{V} bind to sulfhydryl groups, As^{III} has approximately a 5- to 10-fold greater affinity for sulfhydryl groups than As^{V} (Jacobson-Kram and Montalbano, 1985). Cellular uptake rates and resulting tissue concentrations are substantially lower for the pentavalent than for the trivalent forms of arsenic. DMA (an important metabolite of inorganic arsenic) appears to be more readily excreted than MMA (NRC, 2001). Liu et al. (2002) found arsenite to be transported into cells by aquaglycoporins (AQP7 and AQP9), whose usual substrates are water and glycerol. Liu et al. (2006a) also detected transport of monomethylarsonous acid (MMA^{III}) by AQP9. MMA^{III} was transported at a rate nearly 3 times faster than As^{III}. A hydrophobic residue at position 64 was required for the transport of both species, suggesting that both species are transported by AQP9 using the same translocation pathway. As^{V}, however, has been suggested to be transported by the phosphate transporter (Huang and Lee, 1996). Retention of arsenic can vary not only with its form, but also with tissue (Thomas et al., 2001). Other factors that affect the retention and distribution of arsenic include the chemical species, dose level, methylation capacity, valence state, and route of administration.

3.2.1 Transport in Blood

Once arsenic is absorbed, it is transported in the blood throughout the body. In the blood, inorganic arsenic species are 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; and Delnomdedieu et al., 1994a,b). Because of the different binding and transport characteristics of various arsenic compounds, the persistence in the blood varies across species. Inorganic As elimination in humans has been observed to be triphasic, with first-order half-lives for elimination of 1 hour, 30 hours, and 200 hours (Mealey et al., 1959 used As^{III}; Pomroy et al., 1980 used As^{V}). A single intravenous (iv) dose of 5.8 μg As/kg body weight (in the form of $^{73}As^{V}$) administered to 2 male chimpanzees had a half-life plasma elimination rate of 1.2 hour and a half-life elimination rate from red blood cells (RBCs) of about 5 hours (Vahter et al., 1995a).

Rats retain arsenic in the blood considerably longer than other species because dimethylarsenous acid (DMA^{III}) and DMA^{V} accumulate in RBCs, apparently bound to hemoglobin (Odanaka et al., 1980; Lerman and Clarkson, 1983; Vahter, 1983; and Vahter et al., 1984). Naranmandura et al. (2007) found that 75% of an oral dose of arsenite accumulated in rat RBCs mainly in the form of DMA^{III}; however, less than 0.8% of the same dose to hamsters was found in their RBCs. Rats maintained this level in their RBCs for at least 7 days whereas the treated hamsters had levels equivalent to those in controls by 3 days after the administered dose. Stevens et al. (1977) calculated an elimination half-life for inorganic As of 90 days in rat whole blood 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 the retained arsenic was in the form of DMA.

The relative concentration of arsenic in human plasma and RBCs apparently differs depending on exposure levels and the health status of the exposed individuals. Heydorn (1970) reported that healthy people in Denmark with low arsenic exposures had similar arsenic concentrations in their plasma and RBCs (2.4 μg/L and 2.7 μg/L, respectively; the RBC:plasma ratio was 1.1). However, normal healthy Taiwanese exposed to arsenic-contaminated water had plasma levels of 15.4 μg/L and RBCs levels of 32.7 μg/L (RBC:plasma ratio 2.1). Blackfoot disease (BFD) patients and their unaffected family members had 38.1 μg/L and 93 μg/L of arsenic species in their plasma and RBCs, respectively (RBC:plasma ratio 2.4). These results indicate a different distribution between the RBCs and the plasma depending on exposure levels. However, examining the BFD patients and their families, who have presumably the same exposure levels, demonstrates a different distribution, possibly due to disease state. BFD patients had a ratio of 3.3 (106 μg/L in RBCs and 32.3 μg/L in plasma) compared to 1.8 (81 μg/L in RBCs and 45.2 μg/L in plasma) in family members without BFD. This indicates that accumulation of arsenic in the RBCs is greater as exposure increases and possibly even greater when health is compromised. The ratio between plasma and RBC arsenic concentrations may also depend on the exposure form of arsenic (NRC, 1999).

3.2.2 Tissue Distribution

Once arsenic compounds enter the blood, they are transported and taken up by other tissues and organs, with a large proportion of ingested material being subject to "first pass" processing through the liver. Uptake varies with arsenic species, dose, and organ. The observed uptake of inorganic arsenic (mainly As^{III}) in the skin, hair, oral mucosa, and esophagus is most likely due to the binding of inorganic arsenic species with sulfhydryl groups of keratin in these organs. In studies using rabbits and mice, where the transfer of methyl groups from S-adenosylmethionine (SAM; a proposed major reaction during arsenic metabolism; see below) was chemically inhibited, the concentration of arsenic in most tissues (especially the skin) was found to be increased (Marafante and Vahter, 1984). The important role of chemical binding of arsenic species also is supported by the observed tissue distribution in the marmoset monkey, which does not methylate inorganic arsenic (Vahter et al., 1982).

Human subjects also have demonstrated high concentrations of arsenic in tissues containing a high content of cysteine-containing proteins, including the hair, nails, skin, and lungs. Total As concentrations in these tissues of human subjects exposed to background levels of As ranged from 0.01 to 1.0 mg/kg of dry weight (Liebscher and Smith, 1968 and Cross et al., 1979). Benign and malignant skin lesions from 14 patients, with a minimum of 4 years exposure to inorganic arsenical medication, had higher As levels (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 average As concentration in the drinking water ranges from 193–737 ppb, As concentrations in the skin, hair, and nails were 1.6–5.5, 3.6–9.6, and 6.1–22.9 mg/kg dry weight, respectively (Das et al., 1995). Mandal et al. (2004) measured different As species in the hair and fingernails of 41 subjects in West Bengal, India who were drinking As contaminated water and in blood from 25 individuals who had stopped drinking contaminated water 2-years earlier. Results were: fingernail contained As^{III} (62.4%), As^{V} (20.2%), MMA^{V} (5.7%), DMA^{III} (8.9%), and DMA^{V} (2.8%); hair contained As^{III} (58.9%), As^{V} (34.8%), MMA^{V} (2.9%), and DMA^{V} (3.4%); RBCs contained arsenobetaine (22.5%) and DMA^{V} (77.5%); and blood plasma contained arsenobetaine (16.7%), As^{III} (21.1%), MMA^{V} (27.1%), and DMA^{V} (35.1). However, the amount of As in these tissues resulting from other exposure pathways (e.g., dermal exposure) was not determined.

The longest retention of inorganic As in mammalian tissues during experimental studies has been observed in the skin (Marafante and Vahter, 1984), hair, squamous epithelium of the upper GI tract (oral cavity, tongue, esophagus, and stomach wall), epididymis, thyroid, skeleton, and the lens of the eye (Lindgren et al., 1982). Although the study authors measured radioactive arsenic (^{74}As) in the various tissues, they did not differentiate between the different species of arsenic and could not determine if accumulation was due to the originally administered compound or metabolites. As levels in all these tissues, with the exception of the skeleton, were greater in mice administered As^{III} than in mice administered As^{V}. This could indicate that As^{III} is taken up more efficiently than As^{V} and that less was found in the tissues of As^{V} treated mice due to the initial reduction to As^{III}. The calcified areas of the skeleton in mice administered As^{V} accumulated and retained more As than mice administered As^{III}, most likely due to the similarities between As^{V} and phosphate, causing a substitution of phosphate by As^{V} in the apatite crystals in bone. Marmoset monkeys were found not to accumulate As in the ocular lens or the

thyroid (Vahter et al., 1982); however, intravenous administration of ^{74}As-labelled DMA to mice resulted in accumulation of DMA in the ocular lens and the thyroid. Because marmoset monkeys do not methylate arsenic and DMA was found to accumulate in the ocular lens and thyroid, it would suggest that only the methylated species are retained in these organs. Mouse tissues with the largest retention of DMA were the lens of the eyes, thyroid, lungs, and intestinal mucosa (Vahter et al., 1984). Methylated arsenic species (DMA), in general have a shorter tissue retention time in mice than rats (i.e., more than 99% of the administered dose was eliminated in mice within 3 days as compared to 50% in rats due to accumulation in blood), (Vahter et al., 1984).

Hughes et al. (2003) estimated that a steady-state, whole-body arsenic balance was established after 9 repeated oral daily doses of 0.5 mg As/kg as radioactive As^V in adult female B6C3F1 mice. Twenty-four hours after the last dose, the whole-body burden of As was about twice that observed after a single dose. The rate of elimination was slower following repeated doses. Accumulation of radioactivity was highest in the bladder, kidney, and skin, while the loss of radioactivity was greatest from the lungs and slowest from the skin. Atomic absorption spectrometry was used to characterize the organ distribution of arsenic species. MMA was detected in all tissues except the bladder. DMA was found at the highest levels in the bladder and lung after a single oral exposure, with increases after repeated exposures. Inorganic As was predominantly found in the kidney. After a single oral exposure of As^V (0.5 mg As/kg), DMA was the predominant form of arsenic in the liver, but after nine repeat exposures, the proportion of DMA decreased while the proportion of inorganic As increased (this could indicate metabolic saturation or GSH depletion; see below for more details). A trimethylated form of As also was detected in the liver.

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

In a follow-up study by Kenyon et al., (2008), adult female C57Bl/6 mice were administered 0, 0.5, 2, 10, or 50 ppm of arsenic as sodium arsenate in the drinking water for 12 weeks. The average daily intakes were estimated to be 0, 0.083, 0.35, 1.89, and 7.02 mg As/kg/day, respectively. After 12 weeks of exposure, the tissue distributions were as follows: kidney>lung>urinary bladder>skin>blood>liver. In the kidney, MMA was the predominant form measure, while DMA was more prominent in the lungs and blood. The skin and urinary

bladder had nearly equal levels of both inorganic arsenic and DMA and the liver had equal proportions of all three species.

Naranmandura et al. (2007) characterized the tissue distribution in rats and hamsters administered a single oral dose of As^{III} [5.0 mg As/kg body weight (BW)]. In rats, the highest concentrations were found in RBCs. Because hamsters did not accumulate arsenic species in their RBCs, they exhibited a more uniform tissue distribution. While the quantity of arsenic in the liver and kidneys of the hamster were significantly greater than those observed in the rat, arsenic accumulated more and was retained longer in the kidneys compared to the liver in both species. The hamster had greater levels of MMA^{III} bound to protein in the kidney than rats.

As^{III} and As^{V}, as well as methylated metabolites, cross the placenta at all stages of gestation in mice, marmoset monkeys, and hamsters (Hanlon and Ferm, 1977; Lindgren et al., 1984; Hood et al., 1987; and Jin et al., 2006a), with tissue distribution of arsenic similar between the mother and the fetus in late gestation. Jin et al. (2006a) found increased levels of inorganic As and DMA in the livers and brains of newborn mice from dams administered either As^{III} or As^{V} in their drinking water throughout gestation and lactation. The levels of total As in the mothers' livers increased in a dose-dependent manner and were greater than that observed in the mothers' brains or in the newborns' brains or livers. The level of total As in the livers and brains of newborn mice, however, were greater than those observed in the mothers' brains, suggesting easier passage through the placenta than through a mature blood-brain barrier. Because the levels of inorganic As in the newborn livers and brains were nearly identical, it appears that there was no difficulty in passing through an immature blood-brain barrier. In addition, the nearly 2:1 ratio of DMA in the brains compared to the livers of newborns indicates either a preferential distribution of DMA in the newborns' brains or an increased distribution of inorganic As to the brain that is subsequently metabolized. The marmoset monkey (known to not methylate arsenic) displayed somewhat less placental transfer after administration of As^{III} than was seen in mice (Lindgren et al., 1984).

The As concentration in the cord blood (11 μg/L) 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 concentration in the drinking water was approximately 200 ppb (Concha et al., 1998a). Hall et al. (2007) also found a strong association between maternal (11.9 μg/L) and cord blood levels (15.7 μg/L) in Matlab, Bangladesh (arsenic exposure ranged from 0.1 ppb to 661 ppb in drinking water). They also measured arsenic metabolite levels and found that the association also was observed for the metabolites MMA and DMA. Elevated As concentrations also were noted in pregnant women living in cities with low dust fall (i.e., low As inhalation exposures), where an average of 3 μg/L was measured in the maternal blood and 2 μg/L in cord blood (Kagey et al., 1977). Women living near smelters also have been observed to have an increased concentration of placental arsenic (Tabacova et al., 1994). Although the human fetus is exposed to arsenic, it may be more in the form of DMA (at least in late gestation) because 90% or more of the arsenic in the urine and plasma of newborns and mothers (at time of delivery) was DMA.

3.2.3 Cellular Uptake, Distribution, and Transport

Cellular uptake of inorganic arsenic compounds also is dependent on oxidation state, with As^{III} generally being taken up at a much greater rate than arsenate (Cohen et al., 2006). In Chinese hamster ovary (CHO) cells, the rate of uptake was $DMA^{III} > MMA^{III} > As^{III}$ (Dopp et al., 2004), with the pentavalent forms being taken up much more slowly than the trivalent forms. Delnomdedieu et al. (1995) demonstrated that As^{III} is taken up more readily than As^{V}, MMA^{V}, or DMA^{V} by RBCs in rabbits. Drobná et al. (2005) found that MMA^{III} and DMA^{III} were taken up by modified UROtsa cells expressing arsenic methyltransferase (this is a human urothelial cell line that normally does not methylate inorganic arsenic) at an order of magnitude faster than As^{III}. Because arsenate uptake is inhibited in a dose-dependent manner by phosphate (Huang and Lee, 1996), it has been suggested that a common transport system is responsible for the cellular uptake for both compounds. As^{III} uptake, however, is not affected by phosphate; therefore, Huang and Lee (1996) suggested that cellular uptake of As^{III} occurs through simple diffusion. Liu et al. (2002, 2006a), however, suggested that transport of As^{III} and MMA^{III} across the cellular membrane may be mediated by AQP7 and AQP9 with MMA^{III} transported at a higher rate. Lu et al. (2006) found that inorganic As (both pentavalent and trivalent oxidation states) can be transported by organic anion transporting polypeptide-C (OATP-C; which was transfected into cells of a human embryonic kidney cell line), but not MMA^{V} or DMA^{V}. In a cell line resistant to arsenic (R15), Lee et al. (2006a) found little AQP7 or AQP9 messenger RNA (mRNA) and only half the AQP3 mRNA expression compared to the parental cell line (CL3, a human lung adenocarcinoma cell line). Suppressing the AQP3 expression in CL3 cells caused less As to accumulate in these cells. Over-expression of AQP3 in a 293 cell line (a human embryonic kidney cell line) resulted in an increase in arsenic accumulation in the cells. Hexose permease transporters (HXT) also have been suggested as another influx pathway for As^{III} (Thomas, 2007).

Shiobara et al. (2001) demonstrated that the uptake of DMA in RBCs was dependent on not only the chemical form (or oxidation state), but by animal species. DMA^{III} and DMA^{V} were incubated with rat, hamster, mouse, and human RBCs. DMA^{V} was only minimally absorbed by RBCs, and the cellular 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. Mouse RBCs were less efficient at the uptake of DMA^{III} than any of the other species. Rat RBCs retained the DMA^{III} throughout the 4 hours of the experiment, but hamster RBCs were found to excrete the arsenic absorbed as DMA^{III} in the form of DMA^{V}. Human RBCs also excreted DMA^{III} as DMA^{V}, though the rate of uptake of DMA^{III} and efflux of DMA^{V} was much slower than in hamster RBCs.

Cellular excretion of As species also is dependent on oxidation state and the degree of methylation. Leslie et al. (2004) using membrane vesicles from a multi-drug resistant human lung cancer cell line (H69AR) found that a multi-drug resistance protein (MRP) 1 transports As^{III} in the presence of GSH but did not transport As^{V} under any conditions, suggesting that As^{V} must be reduced to As^{III} prior to its being excreted from the cell. Further, the MRP1 transport was more efficient with arsenic triglutathione (ATG) as the substrate. This finding, along with the observation that As^{III} transport is more efficient at neutral or low pH where ATG is more readily formed and more stable, suggests that ATG is formed prior to transport. Leslie et al. (2004) also suggest that the formation of the conjugate is catalyzed by the glutathione S-transferase P1-1 (GSTP1-1) enzyme. MRP2 may also be involved in the efflux of arsenic species from cells

(Thomas, 2007). MRP2 expression was found to be five-fold higher in arsenic-resistant (R15) cells compared to the parent cell line (CL3). However, expression levels of MRP1 and MRP3 were similar to parent cells (Lee et al., 2006a). Suppressing the multi-drug resistant transporters reduced the efflux of arsenic from R15 cells.

In a study of rabbits and mice exposed to radio-labeled arsenic (as As^{III}), the majority of the arsenic was found in the nuclear and soluble fractions of liver, kidney, and lung cells (Marafante et al., 1981; and Marafante and Vahter, 1984). The marmoset monkey had a different intracellular distribution, with approximately 50% of the arsenic dose found in the microsomal fraction in the liver (Vahter et al., 1982 and Vahter and Marafante, 1985). Chemical inhibition of arsenic methylation in rabbits did not alter the intracellular distribution of arsenic (Marafante and Vahter, 1984 and Marafante et al., 1985).

Increases in tissue As concentration (especially in the liver) have been found to be associated with increased As concentrations in the microsomal fraction of the liver in rabbits fed diets containing low concentrations of methionine, choline, or proteins, which leads to decreased arsenic methylation (Vahter and Marafante, 1987). The levels of As in the microsomal fraction of the liver in these rabbits were similar to those observed in the marmoset monkey (Vahter et al., 1982), indicating nutritional factors may play a role in determining the subcellular distribution of arsenic.

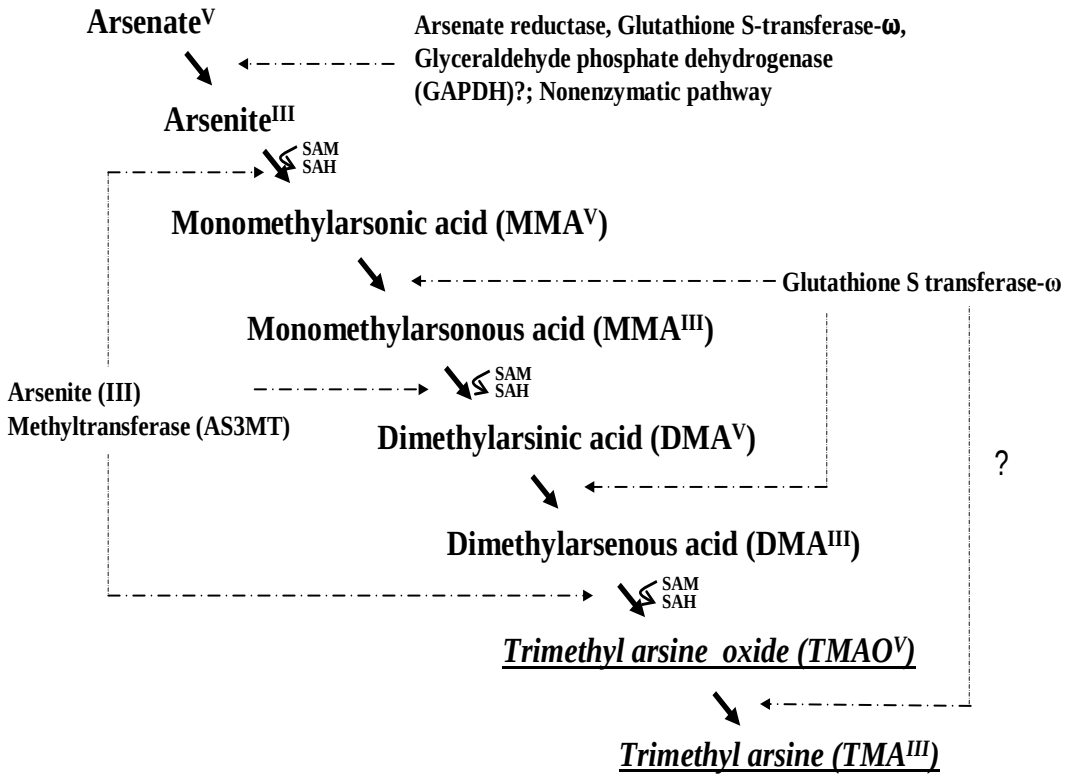
3.3 METABOLISM

After entering the body, As^{V} can be reduced to As^{III}, which can then proceed through a series of methylation and conjugation reactions, some of which involve re-oxidation of arsenic to As^{V}. The traditional metabolic pathways proposed for arsenic are shown in Figure 3-1 below. In this metabolic scheme, less toxic species (i.e., As^{V}, MMA^{V}, and DMA^{V}) can be converted to more toxic species (i.e., As^{III}, MMA^{III}, and DMA^{III}). The trivalent species have been found to be more cytotoxic, genotoxic, and more potent inhibitors of enzyme activity (Thomas et al., 2001). While the final metabolite in humans is predominantly DMA^{V}, as this is the form most highly excreted, some animal species further metabolize DMA^{V} through DMA^{III} to trimethylarsine oxide (TMAO).

Hayakawa et al. (2005) suggested a possible alternate metabolic pathway for inorganic arsenic (Figure 3-2). As in the previously described model, the first step involves reduction of As^{V} to As^{III}. A major difference, however, is that Hayakawa et al. (2005) suggest that arsenic-glutathione complexes are important intermediates in the metabolism of arsenic and are the primary substrates for arsenic methyltransferases. The proposed model was based on the observation that more DMA^{V} is produced from As^{III} than from MMA^{V}. This should not be the case if the reactions depicted in Figure 3-1 are the primary arsenic metabolic pathways. Their data suggest that arsenite, in the presence of GSH, non-enzymatically reacts to form ATG. In support of this mechanism, they observed a dose-dependent increase in concentration of ATG with increasing doses of GSH, up to 4 mM. Monomethyl and dimethyl arsenic species were generated by the transfer of a methyl group from SAM in the presence of human recombinant arsenic (+3 oxidation state) methyltransferase (AS3MT), and only occurred when ATG or monomethylarsonic diglutathione (MADG) was present. At concentrations of glutathione of 2.0

mM or greater, there was a dose-dependent increase in DMA^V levels, accompanied by a dose-dependent decrease in As^V.

Figure 3–1. Traditional Metabolic Pathway for Inorganic Arsenic in Humans

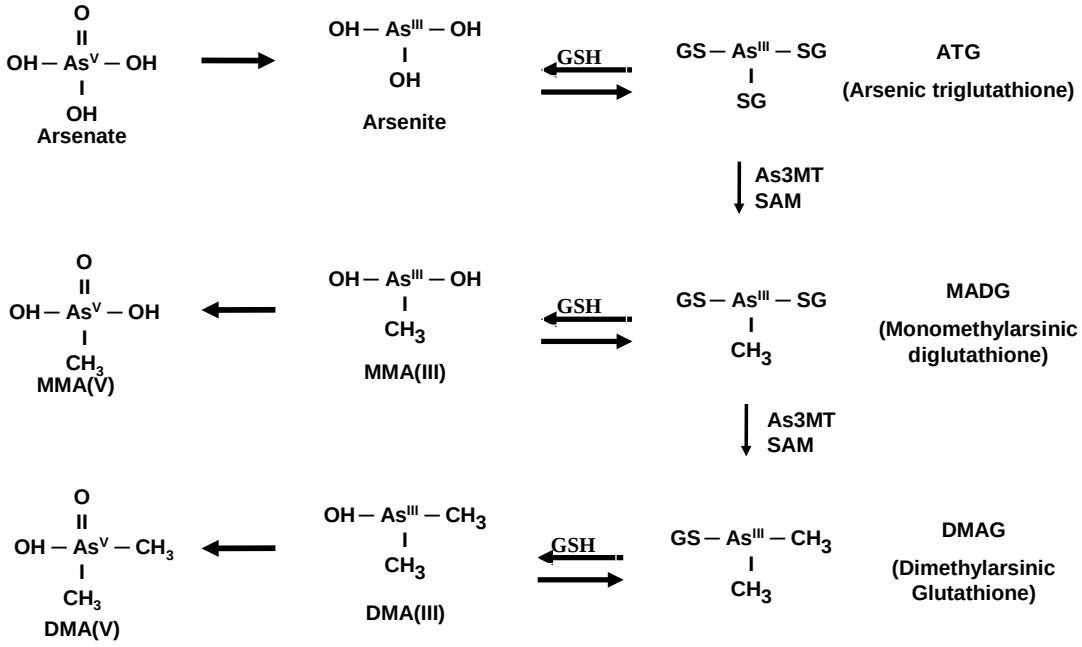


In summary, the proposed metabolic model of Hayakawa et al. (2005) suggests that As^V is first reduced to As^{III}, which then reacts (non-enzymatically) with GSH (producing ATG). In the presence of AS3MT (specified as cyt19 in their article)[2], ATG is methylated to MADG if the GSH concentration is sufficient, which then comes to equilibrium with MMA^{III} (GSH concentrations lower than 1 mM caused MADG to be unstable in solution and was readily hydrolyzed and oxidized to MMA^V). While some of the MMA^{III} is oxidized to MMA^V, some of the MADG is methylated by AS3MT to dimethylarsinic glutathione (DMAG), which, like MADG, is in equilibrium with its trivalent form and can be oxidized to its pentavalent form.

[2] Arsenic (+3 oxidative state) methyltransferase (AS3MT) has been referred to by many investigators as cyt19 in their references. According to Thomas et al. (2007), the Human Genome Nomenclature Committee (http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl) recommends that this protein be systematically named AS3MT. Therefore, in this document when an author refers to cyt19 it has been changed to AS3MT to avoid confusion and for uniform consistency.

This more recently proposed pathway leads to higher proportions of less toxic final species than the original proposed metabolic pathway (Figure 3-1).

Figure 3–2. Alternative Metabolic Pathway for Inorganic Arsenic in Humans Proposed by Hayakawa et al. (2005)



Hayakawa et al. 2005

Arsenic 3 methyl transferase (As3MT); SAM -S-adenosyl methionine; GSH -Glutathione

Results reported by Hughes et al. (2005) may provide support for the Hayakawa et al. (2005) revised pathway. B6C3F1 mice administered MMA^V *per os* demonstrated its rapid absorption, distribution, and excretion, with 80% of the dose eliminated within 8 hours. Very little of the absorbed dose, however, was methylated to DMA and/or TMAO. Less than 10% of the dose excreted in urine and 25% or less of the dose measured in the tissues were in the form of DMA. In contrast, in MMA^{III}-treated mice, greater than 90% of the excreted dose and more than 75% of the arsenic measured in the tissues was identified as DMA. This discrepancy between the two forms of MMA is not expected if the generally accepted metabolic pathway (Figure 3-1) is followed. However, if MMA^{III} is the form methylated to DMA while MMA^V is an end product as is suggested by Hayakawa et al. (2005), then it would be expected that a greater proportion of MMA^{III} would be methylated to DMA than MMA^V. There are, however, factors that may limit the *in vivo* methylation of MMA^V that are unrelated to the metabolic pathway proposed by Hayakawa et al. (2005). First MMA^V does not appear to be taken up well by the liver (Hughes et al., 2005), a major site of inorganic As metabolism (Thomas et al., 2001). In fact, pentavalent species of arsenic are not taken up by cells as readily as trivalent arsenicals

(Dopp et al., 2004). In addition to this in the generally accepted metabolic pathway (Figure 3-1), MMA^V needs to be reduced to MMA^{III} in order to be methylated. Therefore, if very little is taken up into cells, very little can be methylated.

Aposhian and Aposhian (2006) suggest that it is too early to accept AS3MT as the primary methyltransferase responsible for arsenic methylation in humans because it has only been observed in experiments involving deoxyribonucleic acid (DNA) recombinant technology and because there is no indication that the enzyme is expressed in human liver. Although AS3MT has been detected in human liver cell lines (Zakharyan et al., 1999), it has not been isolated from surgically removed liver tissue. Thomas et al. (2007) also state that the evidence supports the conclusion that arsenic methylation catalyzed by AS3MT is not strictly dependent on the presence of GSH, which would suggest that other pathways may be involved in addition to those included in Hayakawa et al.'s (2005) model. GSH depletion would likely occur at high arsenic exposures under Hayakawa et al.'s (2005) proposed pathway. Therefore, it is possible both pathways work in conjunction, or one is predominant over the other depending on the concentration of arsenic. Hayakawa et al. (2005) found that levels of MMA^V were not dependent on GSH level (from 2–5 mM), suggesting that this indicated possible further methylation to DMA^V. Since this is not part of the proposed Hayakawa et al. (2005) pathway, it would suggest that at least some of the MMA^V is methylated through the classic pathway.

3.3.1 Reduction

A substantial fraction of absorbed As^V is rapidly reduced to As^{III} in most species studied; the reduction apparently occurs mainly in the blood of mice, rabbits, and marmoset monkeys (Vahter and Envall, 1983; Vahter and Marafante, 1985; and Marafante et al., 1985). Reduction also may occur in the stomach or intestines prior to absorption, but quantitative experimental data are not available to determine the importance of this GI reduction. In addition to the reduction of inorganic As^V, as shown in Figure 3-1, methylated As^V species also may be reduced, apparently by different enzymes.

GSH may play a role in the reduction of As^V, but apparently is not the only cofactor, as cysteine and dithiothreitol (DTT) also have been found to reduce As^V to As^{III} *in vitro* (Zakharyan et al., 1995; NRC, 1999; and Németi and Gregus, 2002). Inorganic phosphate inhibits the formation of As^{III} from As^V in intact RBCs (Németi and Gregus, 2004), probably by competing with the phosphate transporter for the uptake into cells.

Arsenate reductase enzymes have been detected in the human liver (Radabaugh and Aposhian, 2000). At least one of these enzymes has been characterized as a purine nucleoside phosphorylase (PNP) (Gregus and Németi, 2002 and 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 the most active naturally occurring thiol in mammalian systems and appears to be required for the enzymatic reduction of As^V to As^{III}. PNP, however, did not catalyze the reduction of MMA^V to MMA^{III}. An MMA^V reductase 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 humans, this reductase is human glutathione-S-transferase ω

(hGST-O1), which is a member of the glutathione-*S*-transferase (GST) superfamily (Aposhian and Aposhian, 2006).

Although PNP has been determined to reduce As^{V} to As^{III}, Németi et al. (2003) observed this reduction only *in vitro*. PNP did not appear to be a major player in the reduction of As^{V} to As^{III} in either human erythrocytes or in rats *in vivo*. Németi and Gregus (2004, 2005) further demonstrated that human erythrocytes exhibit a PNP-independent As^{V}-reducing pathway that requires GSH, nicotinamide adenine dinucleotide (NAD), and a substrate for either one or both of the following enzymes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or phosphoglycerate kinase (PGK). This mechanism of reduction also was demonstrated in rat liver cytosol (Németi and Gregus, 2005). In addition, another unidentified enzyme in the liver cytosol had the capacity to reduce As^{V}. A further study (Gregus and Németi, 2005) demonstrated that GAPDH exhibited As^{V} reductase activity, but that PGK served as an auxiliary enzyme when 3-phosphoglycerate was the glycolic substrate.

The reduction of pentavalent arsenicals also has been observed to be catalyzed by AS3MT (Waters et al., 2004a). According to Waters et al. (2004b), AS3MT may possess both As^{III} methyltransferase and As^{V} reductase activities. In the presence of an exogenous or physiological reductant, AS3MT was found to catalyze the entire sequence converting arsenite to all of its methylated metabolites through both methylation and reduction steps (Figure 3-1). Thomas et al. (2007) also suggest that thioredoxin [Trx, isolated from *Escherichia coli* (*E. coli*)] is necessary, possibly reducing some critical cysteine residue in AS3MT as a step in the methyltransferase reaction. Cohen et al. (2006) suggest that Trx, thioredoxin reductase (TrxR), and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) are the primary reducing agents involved in the conversion of MMA^{V} to DMA^{V}, but they are orders of magnitude less effective than the arsenic methyltransferase isolated from rabbit liver (i.e., AS3MT). Zakharyan and Aposhian (1999) found that MMA^{V}-reductase was the rate-limiting enzyme in arsenic biotransformation in rabbit livers. Jin et al. (2006a) also suggest that As^{V} reduction is possibly a rate-limiting step in arsenic metabolism at low concentrations. At higher concentrations, saturation or methylation inhibition may cause other reactions to become rate-limiting.

3.3.2 Arsenic Methylation

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

Although the kinetics of arsenic methylation *in vivo* is not fully understood, it is believed that the liver may be the primary site of arsenic methylation. However, the testes, kidney, and lung also have been observed to have a high methylating capacity (Cohen et al., 2006). Marafante et al. (1985) found that DMA appeared in the liver prior to any other tissue in rabbits exposed to inorganic As. It also has been demonstrated that oral administration of inorganic As favors methylation more than either subcutaneous or intravenous administration (Charbonneau et al., 1979; Vahter, 1981; and Buchet et al., 1984), presumably because the arsenic will pass through the liver first after oral administration. However, liver disease (i.e., alcoholic, post-necrotic or biliary cirrhosis, chronic hepatitis, hemochromatosis, and steatosis) can be associated with increased ratios of DMA to MMA in the urine following a single injection of sodium arsenite (Buchet et al., 1984 and Geubel et al., 1988). This appears to indicate that efficient methylation of arsenic continues in the presence of liver damage, possibly indicating a different organ is responsible for methylation under these circumstances. 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 from As^{V} than As^{III} (Lerman and Clarkson, 1983). Therefore, it is likely that any As^{V} not initially reduced can be efficiently methylated in the kidney for subsequent urinary excretion.

Identifying the main organs responsible for methylation of arsenic *in vivo* has not been straightforward because *in vitro* results do not necessarily reflect *in vivo* methylation patterns (NRC, 1999). Buchet and Lauwerys (1985) identified the rat liver as the main organ for methylation, with the methylating capacities in the RBCs, brain, lung, intestine, and kidneys being insignificant in comparison. Assays of arsenite methyltransferases from mouse tissues demonstrated that the testes had the highest methylating activity, followed by the kidney, lung, and liver (Healy et al., 1998). Aposhian (1997) determined that the amount of methyltransferases vary in the liver of different animal species. Arsenite bound to components of tissue can be methylated and released (Marafante et al., 1981 and Vahter and Marafante, 1983). This may explain the initial rapid phase (immediate methylation and excretion) followed by a slow elimination phase (continuous release of bound arsenite through methylation) (NRC, 1999), as described below in Section 3.4.

It has been demonstrated that inhibition of arsenic methylation results in increased tissue concentrations of arsenic (Marafante and Vahter, 1984 and Marafante et al., 1985). Loffredo et al. (2003) suggest that the second methylation step is inducible and that the inducibility is possibly polymorphic (i.e., more than one enzyme or enzyme form may be involved, depending on the individual). This suggestion is based on observations that human urinary DMA concentrations in high-exposure groups were higher and more variable than urinary MMA levels, and because urinary DMA levels appeared to have a bimodal distribution in a population from Mexico, regardless of exposure status. Others have suggested that the second methylation step may be saturable, which would be consistent with the decreasing excretion of DMA with increasing arsenic exposures (Ahsan et al., 2007). Cysteine, GSH, and DTT have been shown to increase the activity of arsenite methyltransferase and MMA methyltransferase (both later identified as AS3MT, Lin et al. 2002) in purified rabbit liver enzyme preparations (Zakharyan et al., 1995). Dithiols (e.g., reduced lipoic acid) have also been found to enhance arsenite methylation by MMA^{III} methyltransferase (Zakharyan et al., 1999). Glutathione-S-transferase

omega 1 (GSTO1) has also been associated with arsenic biotransformation (Meza et al., 2007). Although humans have been observed to methylate arsenic, no arsenic methyltransferase has yet been isolated from human tissues (Aposhian and Aposhian, 2006).

In vitro studies using rat liver preparations indicate that the methylating activity is localized in the cytosol, with SAM being the main methyl donor for As^{III} methylation (Marafante and Vahter, 1984; Buchet and Lauwerys, 1985; Marafante et al., 1985; Styblo et al., 1995, 1996; and Zakharyan et al., 1995). AS3MT catalyzes the transfer of the methyl group from SAM to the arsenic substrates (Lin et al., 2002 and Thomas, 2007). Expressing AS3MT in UROtsa (human urothelial cells that do not normally methylate inorganic arsenic) caused the cells to effectively methylate arsenite (Drobná et al., 2005). High concentrations of As^{III} or MMA^{III} in the culture caused an inhibition in the formation of DMA, but had little effect on the formation of MMA. The inhibition of DMA production resulted in MMA accumulation in cells. Drobná et al. (2006) demonstrated that AS3MT was the major enzyme for arsenic methylation in human hepatocellular carcinoma (HepG2) cells, but reducing it by 88% (protein levels) only accounted for a 70% reduction in methylation capacity, suggesting that there is another methylation process that is independent of AS3MT.

The addition of GSH has been found to increase the yield of mono- and dimethylated arsenicals but suppressed the production of TMAO in the presence of rat AS3MT (Waters et al., 2004a), indicating that GSH suppresses the third methylation reaction but not the first two (Thomas et al., 2007). 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. It has subsequently been concluded that this methyltransferase was the same as AS3MT.

GSH alone does not support recombinant rat AS3MT catalytic function, but when added to a reaction mixture containing other reductants, the rate of arsenic methylation increases (Waters et al., 2004b). GSH alone (5mM) does not support the catalytic activity of AS3MT, but stimulates the methylation rate in the presence of the reductant tris(2-carboxylethyl)phosphine (TCEP; 1 mM) (Thomas et al., 2007). GSH (5 mM) did not have any affect on DTT (1 mM)-induced arsenic methylation. Drobná et al. (2004) linked the genetic polymorphism of AS3MT, with other cellular factors and to the interindividual variability in the capacity of primary human hepatocytes to retain and metabolize As^{III} (see Section 4.7).

The main products of arsenic methylation in humans 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 studies do not differentiate the valence state of mono- or dimethylated arsenic species detected in urine or tissue samples. Le et al. (2000a,b) and 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. Le et al. (2000b) found 43 to 227 μg/L of MMA^{III} in the urine of populations from Inner Mongolia, China who were exposed to 510-660 ppb (0.46 μM) of arsenic via the drinking water.

A small percent of DMA^{III} may further be methylated to TMAO in mice and hamsters (see Kenyon and Hughes, 2001 for a review). A single human volunteer ingesting DMA,

excreted 3.5% of the dose as TMAO (Kenyon and Hughes, 2001). TMAO can be detected in 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 orally exposed to inorganic As. This may be due to rapid clearance of DMA and MMA from cells (Styblo et al., 1999b); however, most analytical methods are not optimized for the detection of TMAO that could have been present but not detected.

3.3.3 Species Differences in the Methylation of Arsenic

There is considerable variation in the patterns of inorganic arsenic methylation among mammalian species (NRC, 1999). Humans, rats, mice, dogs, rabbits, and hamsters have been shown to efficiently methylate inorganic arsenic to MMA and/or DMA. Rats and hamsters appear to methylate administered DMA into TMAO more efficiently than other species (NRC, 1999 and Yamauchi and Yamamura, 1984). About 40% of urinary As was present as TMAO 1 week after exposure to DMA in the drinking water while 24% was present as TMAO after 7 months of exposure (100 mg/L) in 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% to 30% of the arsenic in its inorganic form, 10% to 20% as MMA, and 55% to 75% as DMA (see Vahter, 1999a for a review). In contrast, a study of urinary arsenic metabolites in a population from northern Argentina exposed to arsenic via drinking water demonstrated an average of only 2% MMA in the urine (Vahter et al., 1995b and Concha et al., 1998b). This may indicate variations in methylation activity depending on the route of exposure, level of exposure, and possible nutritional or genetic factors. Although humans are considered efficient at arsenic methylation, they are less efficient than many animal models, as indicated by the larger proportion of MMA^V excreted in the urine (Vahter, 1999a). This is important because it may explain why humans are more susceptible to cancer from arsenic exposures, and why no adult animal model for inorganic-arsenic induced cancers has yet been identified (Tseng et al., 2005).

The rabbit (Marafante et al., 1981; Vahter and Marafante, 1983; and Maiorino and Aposhian, 1985) and hamster (Charbonneau et al., 1980; Yamauchi and Yamamura, 1984; and Marafante and Vahter, 1987) appear to be more comparable to humans with respect to arsenic methylation than other experimental animals (NRC, 1999). However, rabbits and hamsters, in general, excrete more DMA and less MMA than humans. In contrast, Flemish giant rabbits (De Kimpe et al., 1996), excrete MMA in amounts similar to humans. Mice and dogs, efficient methylators of arsenic, excrete more than 80% of a single arsenic dose administered as DMA within a few days (Charbonneau et al., 1979 and Vahter, 1981). Guinea pigs (Healy et al., 1997), marmoset monkeys (Vahter et al., 1982 and Vahter and Marafante, 1985), and chimpanzees (Vahter et al., 1995a), on the other hand, do not appear to appreciably methylate inorganic arsenic. In addition, no methyltransferase activity was detected in these species (Zakharyan et al., 1995, 1996; Healy et al., 1997; and Vahter, 1999a). Li et al. (2005) identified a frameshift mutation in the chimpanzee AS3MT gene that resulted in the production of an inactive truncated protein, possibly explaining the lack of methylation activity in that species.

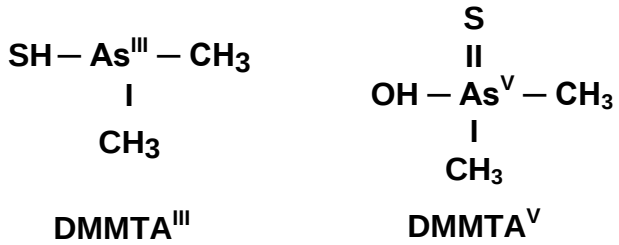
AS3MT homolog proteins with five fully conserved cysteine residues have been observed in the genome of numerous species (Thomas et al., 2007). Chimpanzees were found to differ from other species studied in that their AS3MT protein was shorter and lacked the 5th cysteine (Thomas et al., 2007). Healy et al. (1999) identified marked variations in the activity of methyltransferases, while Vahter (1999b) characterized differences in methylation efficiency among different human populations. The observed 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, 1999a and NRC, 2001).

Although arsenic methylation is generally believed to take place in order to enhance excretion, there are several species (i.e., guinea pigs, marmoset monkeys, and chimpanzees) that do not methylate arsenic, but still efficiently excrete it. In fact, these animals do not retain arsenic any longer than species that methylate arsenic (Cohen et al., 2006), indicating that factors other than methylation also affect arsenic excretion rates. In support of this is also the fact that inorganic arsenic is found in the urine of even the most efficient methylators (Vahter, 1994).

3.3.4 Thioarsenical Metabolites

In 2004, Hansen et al. reported the detection of unusual arsenic-containing metabolites in the urine of sheep exposed to arsenic-contaminated vegetation. Hansen et al. (2004) tentatively identified the metabolite as dimethylmonothioarsinic acid ($DMMTA^{III}$), a sulfur containing derivative of DMA^{III}. Because the exposed sheep consumed algae known to contain arsenosugars, some of which contain sulfur, the relevance of this finding to human exposures was not initially clear. Subsequently, Raml et al. (2006) detected the presence of $DMMTA^{III}$ in the urine of Japanese men, but again, consumption of arsenosugars was suspected as a source of the observed arsenic containing species.

Figure 3-3 Thioarsenical Structures



In experiments addressing this issue, Adair et al. (2007) and Naramandura et al. (2007) found substantial concentrations of thioarsenical metabolites in arsenic-exposed experimental animals. Adair et al. (2007) administered drinking water containing 100 ppm As^V or up to 200 ppm DMA^{III} to female Fisher 344 rats for 14 days. During analysis of the urine (collected during the last 24 hours of exposure) for metabolites, they found high levels of $DMMTA^{III}$ and trimethylarsine sulfide (another sulfur-containing metabolite) in the urine of rats treated with DMA^{III}. Lower levels of the sulfur-containing metabolites were detected in the urine of

arsenate-treated animals. They proposed a mechanism whereby the reaction of DMA^{III} and DMA^{V} with hydrogen sulfide resulted in the observed metabolites.

Naranmandura et al. (2007) administered single doses of 5.0 mg/kg As^{III} to Syrian hamsters and Wistar rats by gavage and measured the levels of sulfur-containing arsenic metabolites in urine. Both $DMMTA^{III}$ and dimethylmonothioarsonic acid ($DMMTA^{V}$) were found at appreciable levels in urine from hamsters, but only the latter metabolite was found in rat urine. A previously uncharacterized metabolite, monomethylmonothioarsonic acid, was also found in urine from both species.

The above studies suggest that the generation of sulfur-containing arsenic metabolites does not depend on exposures to arsenosugars, at least in rodents, but can occur during the metabolism of inorganic arsenic compounds. In 2007, Raml et al. presented evidence that this pathway was also significant in humans. $DMMTA^{III}$ was detected in the urine of 44% (33 of 75) women exposed to inorganic arsenic-contaminated drinking water in Bangladesh. The metabolite was present in urine samples at concentrations between "trace" amounts and 24 µg/L, with total arsenic concentrations ranging from 8 to 1034 µg/L. It was suggested that thioarsenical metabolites may have been present in urine from other epidemiological studies of arsenic-exposed populations, but may have not been detected due to analytical difficulties.

3.4 ELIMINATION

The major route of excretion for most arsenic compounds by humans is via the urine (Yamauchi and Yamamura 1979; Tam et al., 1979; Pomroy et al., 1980; and Buchet et al., 1981). Six human subjects who ingested 0.01 µg of radio-labeled $^{74}As^{V}$ excreted an average of 38% of the administered dose in the urine within 48 hours and 58% within 5 days (Tam et al., 1979). Inorganic As elimination in humans has been observed to be triphasic, with first-order half-lives for elimination of 1 hour, 30 hours, and 200 hours (Mealey et al., 1959 used As^{III}; Pomroy et al., 1980 used As^{V}).

As mentioned in the preceding section, MMA and DMA are important metabolites generated after exposure to inorganic As. These methylated metabolites (oxidative states are generally not measured and/or specified) are excreted in the urine faster than the inorganic As. In humans orally exposed to MMA or DMA in aqueous solution, 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 excretion was found to be about 2 hours following iv administration (Hughes and Kenyon, 1998).

Kenyon et al., (2008) administered 0, 0.5, 2, 10, or 50 ppm of arsenic as sodium arsenate to adult C57Bl/6 female mice in the drinking water for 12 weeks. The average daily intakes were estimated to be 0, 0.083, 0.35, 1.89, and 7.02 mg As/kg/day, respectively. Levels of MMA^{III}, DMA^{III}, DMA^{V}, and TMAO in the urine collected at the end of treatment increased in a linear manner with dose, but As^{V} and MMA^{V} did not.

Rats excrete DMA slowly compared to other species (Vahter et al., 1984), even though they are efficient at methylating inorganic As to DMA. The slow excretion is believed to be

associated with retention of a significant portion of the DMA in erythrocytes (Odanaka et al., 1980; Lerman and Clarkson, 1983; Vahter, 1983; and Vahter et al., 1984). The biliary excretion of inorganic As by rats is about 800 times greater than observed in dogs and 37 times that of rabbits, as proportion of administered dose. Hughes et al. (2005) found that in mice the level of MMA^V excreted in the urine compared to the bile was related to dose, with fecal excretion increasing at higher doses. Cui et al. (2004a) also found that rat biliary excretion rates varied with dose, but found it was also related to route of administration and chemical form. After oral administration of inorganic arsenic (either form) to male Sprague-Dawley rats, MADG and DMA^V (likely present due to dissociation of DMAG) were the predominant forms in the bile. MADG was found at a higher level after a higher (i.e., 100 ppm) dose, while DMA^V was more prevalent at the lower dose (i.e., 10 ppm). Kala et al. (2000) found that the secretion of arsenic into the bile of rats was dependent on the multi-drug resistance-associated protein 2 transporter (MPR2/cMOAT) and that GSH is necessary for the transport, as arsenic-glutathione complexes accounted for the majority of arsenic found in the bile.

Although absorbed arsenic is removed from the body mainly via the urine, small amounts of arsenic are excreted through other routes (e.g., skin, sweat, hair, breast milk). While arsenic has been detected at low levels in the breast milk of women in northwestern Argentina (i.e., 2 μg/kg), breastfeeding was associated with lower concentrations of arsenic in the urine of newborn children (Concha et al., 1998c) than formula feeding, owing to the use of arsenic-contaminated water in formula preparation. Parr et al. (1991) measured arsenic (as well as other elements) in the breast milk from 3 groups of mothers from four countries (i.e., Guatemala, Hungary, Nigeria, and the Philippines), and 1-2 groups from Sweden and Zaire. The breast milk was collected 3 months after birth. Levels of arsenic in the breast milk from women in the Philippines were higher than other regions with levels about 19 μg/kg. Women from Nigeria had levels similar to those observed by Concha et al. (1998c). Women from all the other areas measured had levels of 0.24 to 0.55 μg/kg.

The average concentration of arsenic in sweat induced in a hot and humid environment was 1.5 μg/L, with an hourly loss rate of 2.1 μg (Vellar, 1969). Based on 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 to 0.2 μg in males with no known exposure to arsenic.

3.5 PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Physiologically based pharmacokinetic (PBPK) models for inorganic arsenic are important for developing a biologically based quantitative dose-response (BBDR) assessment. The development of useful BBDR models has proved to be challenging because inorganic As appears to mediate its toxicity through a range of metabolites, and their roles with regard to specific adverse effects are not clear (Clewell et al., 2007).

A PBPK model for exposure to inorganic As (orally, intravenously, and intratracheally) was developed in hamsters and rabbits by Mann et al. (1996a). The model includes tissue compartments for lung (nasopharynx, tracheobronchial, pulmonary), plasma, RBCs, liver, GI tract, skin, kidney, keratin, and combined other tissues. Oral absorption of As^{III}, As^V, and DMA (pooled III and V oxidation states) was modeled as a first-order transport process directly from

the GI contents into the liver. Distribution to tissues was diffusion-limited, with transfer rates estimated based upon literature values for capillary thickness and pore sizes for each tissue. Reductive metabolism of As^{V} to As^{III} was modeled as a first-order process occurring in the plasma. Oxidative metabolism of As^{III} to As^{V} was 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 were modeled as saturable Michaelis-Menten processes taking place in the liver. Urinary, biliary, and fecal excretion of As^{III}, As^{V}, MMA, and DMA also 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 (Charbonneau et al., 1980; Yamauchi and Yamamura, 1984; and Marafante et al., 1985, 1987). The model was found to accurately simulate the excretion of arsenic metabolites in the urine of rabbits and hamsters and to produce reasonable fits to liver, kidney, and skin concentrations in rabbits and hamsters (Yamauchi and Yamamura, 1984; Marafante et al., 1985; and Marafante and Vahter, 1987).

Mann et al. (1996b) extended their PBPK model for use in humans by adjusting physiological parameters (organ weights, blood flows) and re-estimating absorption and metabolic rate constants. The model was fit to literature data on the urinary excretion of total arsenic following a single oral dose of As^{III} or As^{V} in human volunteers (Tam et al., 1979 and Buchet et al., 1981). The extended human model was further tested against empirical data on the urinary excretion of the different metabolites of inorganic arsenic following oral intake of As^{III}, intake of inorganic As via drinking water, and occupational exposure to arsenic trioxide (ATO) (Harrington et al., 1978; Valentine et al., 1979; Buchet et al., 1981; and Vahter et al., 1986). The model predicted a slight decrease (i.e., about 10%) 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 predicted that adults' drinking water containing 50 ppb would excrete more arsenic in urine than an occupational inhalation exposure of 10 $\mu g/m^3$ (Mann et al., 1996b).

Yu (1999a,b) also developed a PBPK model for arsenic in humans that includes tissue compartments for lung, skin, fat, muscle, combined kidney and richly perfused tissues, liver, intestine, GI and stomach contents, and bile. Oral absorption of As^{III}, As^{V}, and DMA (pooled III and V oxidation states) was modeled as first-order transport from the GI contents into the intestinal tissue. Distribution to tissues was modeled as perfusion-limited. Reductive metabolism of As^{V} to As^{III} was modeled as a first-order, GSH-dependent process taking place in the intestinal tissue, skin, liver, and kidney/rich tissues. Oxidative metabolism of As^{III} to As^{V} was not modeled. Methylation of inorganic As species to MMA (pooled III and V oxidation states) and then to DMA were modeled as saturable Michaelis-Menten processes occurring in the liver, and kidney. Urinary, biliary, and fecal excretion of As^{III}, As^{V}, MMA, and DMA were 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 and Pomroy et al., 1980). The model was not tested further 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 and perfusion rates). The absorption, partition, and metabolic rate constants were re-estimated 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 mice (Kenyon et al., 1997 and Hughes et al., 1999). Additionally, the description of methylation in the model was refined to include 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 a 26-week drinking water exposure of As^{III} to C57Black mice (Moser et al., 2000). This data was found to adequately fit the model without further parameter adjustment. Ng et al. (1999) had found arsenic-induced tumors in C57Bl/6J mice, while numerous other mouse strains (Swiss CR:NIH[S], C57Bl/6p53[+/-], C57Bl/6p53[+/+], and Swiss CD-1) have not resulted in a significant increase in arsenic-induced tumors. The Gentry et al. (2004) model was unable to explain the different outcomes in the mouse bioassay on the basis of predicted target organ doses.

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 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.

Clewell et al. (2007) noted that the then-available PBPK models did not incorporate the most recent available information related to arsenic methylation kinetics and suggested several steps for improving the PBPK models. El-Masri and Kenyon (2008; available online in October, 2007) have developed a PBPK model incorporating some of the improvements suggested by Clewell et al. (2007) (although not the simulation of changes in gene expression). The model predicts the levels of inorganic As and its metabolites in human tissues and urine following oral exposure of As^{V}, As^{III}, and for oral exposure to organoarsenical pesticides. The model consists of interconnecting submodels for inorganic arsenic (As^{III} and As^{V}), MMA^{V}, and DMA^{V}. Reduction of MMA^{V} and DMA^{V} to their trivalent forms also is modeled. The submodels include the GI tract (lumen and tissue), lung, liver, kidney, muscle, skin, heart, and brain, with reduction of MMA^{V} and DMA^{V} to their trivalent forms modeled as occurring in the lung, liver, and kidney. The model also incorporates the inhibitory effects of As^{III} on the methylation of MMA^{III} to DMA and MMA^{III} on the methylation of As^{III} to MMA into consideration, modeled as noncompetitive inhibition. This model differs from the other models described above because it provides an updated description of metabolism using recent biochemical data on the mechanism of arsenic methylation. In addition, it uses *in vitro* studies to estimate most of the model parameters (statistically optimizing those that are sensitive to urinary excretion levels to avoid problems with parameter identifiability), and the model can predict the formation and excretion of trivalent methylated arsenicals. The partition coefficients estimated in the model are comparable to those developed by Yu (1999a). The performance of the model was tested against limited human data on urinary excretion and evaluation of the model for predicting the tissue and urinary concentrations of arsenicals in large number of subjects is needed. This model is an

improvement over previous models because it can quantitatively assess impacts of parameter variability arising from genetic polymorphism.

4. HAZARD IDENTIFICATION

4.1 STUDIES IN HUMANS

Numerous epidemiologic investigations have examined the association between waterborne arsenic exposure and cancer outcome. These epidemiologic investigations used many different study designs, each with their inherent limitations. Regardless of the study type, the majority of these investigations found some level of association between arsenic exposure and cancer outcome. This association is not new since arsenic exposure has been linked with cancer as far back as 1887 when Hutchinson reported an unusual number of skin tumors in patients treated with arsenicals. Since 1887, the association between skin cancer and arsenic has been reported in a number of studies (Tseng et al., 1968; Tseng, 1977; Chen et al., 1985; Chen et al., 1988a,b; Wu et al., 1989; Hinwood et al., 1999; NRC, 1999; Tsai et al., 1999; Karagas et al., 2001; Knobeloch et al., 2006; and Lamm et al., 2007).

The SAB Arsenic Review Panel provided comments on key scientific issues associated with arsenicals on cancer risk estimation in July of 2007 (SAB, 2007). It was concluded that the Taiwanese database is still the most appropriate source for estimating bladder and lung cancer risk among humans (specifics provided in Section 5) because of 1) the size and statistical stability of the database relative to other studies; 2) the reliability of the population and mortality counts; 3) the stability of residential patterns; and 4) the inclusion of long-term exposures. However, SAB also noted considerable limitations within this data set (EPA-SAB-07-008, http://www.epa.gov/sab). The panel suggested that one way to mitigate the limitations of the Taiwanese database would be to include other relevant epidemiological studies from various countries. For example, SAB referenced other databases that contained studies of populations also exposed to high levels of arsenic (e.g., Argentina and Chile), and recommended that these alternate sources of data be used to compare the unit risks at the higher exposure levels that have emerged from the Taiwan data. The SAB also suggested that, along with the Taiwan data, published epidemiology studies from the United States and other countries where the population is chronically exposed to low levels of arsenic in drinking water (0.5 to 160 ppb) be critically evaluated, using a uniform set of criteria presented in a narrative and tabular format. The relative strengths and weaknesses of each study should be described in relation to each criterion. The caveats and assumptions used should be presented so that they are apparent to anyone who uses these data. The risk assessment background document should be a complete and transparent treatment of variability within and among studies and how it affects risk estimates. Additionally, SAB (2007) recommended that the following issues be considered when reviewing "low-level" and "high level" studies: a) estimates of the level of exposure misclassification; b) temporal variability in assigning past arsenic levels from recent measurements; c) the extent of reliance on imputed exposure levels; d) the number of persons exposed at various estimated levels of waterborne arsenic; e) study response/participation rates; f) estimates of exposure variability; g) control selection methods in case-control studies; and h) the resulting influence of these factors on the magnitude and statistical stability of cancer risk estimates.

In order to address these issues, this Toxicological Review provides a comprehensive review of the significant epidemiologic investigations in the literature from 1968 to 2007 with the focus on the more recent publications. The report includes data from all populations that have been examined in regards to cancer from arsenic exposure via drinking water. Earlier publications were reviewed and are included as needed to facilitate the understanding of results from certain study populations. As recommended by the SAB, studies were presented in both a narrative (below) and tabular (Appendix B) format. Each publication was evaluated using a uniform set of criteria, including the study type, the size of the study population and control population, and the relative strengths and weaknesses of the study. While the information in the tables mirrors the information in the narrative, the narrative may provide additional important information concerning the investigation. The studies are presented by country of origin, then in chronological order by publication year. In order to facilitate comparisons across the epidemiological studies, the arsenic concentrations pertaining to water exposure levels have been converted from milligrams (mg) per liter (or ppm) to parts per billion (ppb). This was not applied when discussing animal or *in vitro* MOA studies due to a wide range of concentrations employed and converting the arsenic levels or doses into ppb would not be reader friendly.

TAIWAN

More than 80 years ago (between 1910 and 1920), parts of southwestern Taiwan began using artesian (ground water) wells to increase water supplies and decrease the salt content of their drinking water. Some of these artesian wells were discovered to be contaminated with naturally occurring arsenic, thus resulting in widespread arsenic exposure. As a result, the Taiwanese population has been extensively studied. Due to the high arsenic content in the artesian wells, water was piped into certain areas in Taiwan from the reservoir of the Chia-Nan irrigation system in 1956. This water was reported to contain 10 ppb (Tseng, 1977). Almost 75% of the residence had tap water by the 1970s, however, a survey in 1988 noted that artesian well water was still used for drinking, aquaculture, and agriculture in 1988, especially during the dry season (Wu et al., 1989).

Tseng et al. (1968) conducted a general survey using an ecologic study design of 40,421 inhabitants (21,152 females, 19,269 males) from the southwest coast of Taiwan in order to determine the potential relationship between skin cancer and chronic arsenicism. For this investigation 293 water samples were collected from the arseniasis endemic area from both deep artesian (n=134) and shallow (n=89) wells. The arsenic content was measured in 142 samples from 114 wells (110 artesian and 4 shallow) and ranged from 10 to 1820 ppb. The authors noted, however, that the arsenic content varied considerably over a two-year period when measurements were taken. For example, in one well measurements were 528 ppb in July, 1962; 530 ppb in June, 1963; and 1190 ppb in February, 1964. These variations made dose-response relationships difficult to determine. Study subjects were categorized by arsenic exposure into three groups (low: 0-290 ppb, medium: 300-590 ppb, and high: 600 ppb or greater). The overall prevalence rate for skin cancer was 10.6 per 1000. The male-to-female ratio was 2.9:1 for skin cancer. The prevalence rate increased steadily with age (recorded in 10-year increments), except for declining cancer prevalence rates for females older than 69 years. Age-specific (plotted in 20-year intervals) and sex-specific prevalence rates for skin cancer increased with arsenic concentration. The most common type of lesion was intra-epidermal carcinoma (51.7%), and the

body areas most frequently involved were unexposed surfaces (74.5%). In addition, an extremely high percentage of cases with multiple skin cancer (99.5%) were observed. The association between BFD and skin cancer was significantly higher than expected. Strengths of the Tseng et al. (1968) study include the large number of participants and the inclusion of dose response information. Weaknesses, however, include the lack of individual exposure data (ecologic study design) and the potential for recall bias among study participants in determining the age of cancer onset and the length of residence in the area. In addition, changes in water supply over time were not noted, information on smoking history was not obtained, and the arsenic concentration from individual wells varied over time.

Tseng (1977) also used the general ecologic survey design discussed in Tseng et al. (1968) to report skin cancer incidence among the 40,421 individuals and to follow-up on 1108 patients with BFD (identified between 1958-1975). By the end of the follow-up period, almost half (i.e., 528) of the BFD patients had died. Tseng (1977) identified 428 cases (prevalence of 10.6/1000) of skin cancer and 370 cases (prevalence of 9.0/1000) of BFD, and analyzed the relationship between the two. Skin cancer and BFD occurred in 61 cases (1.51/1000), but only 4 cases (0.09/1000) were expected. The observed/expected ratio was 16.77. Tseng (1977) determined that the patients with BFD consumed artesian water before the onset of the disease, and none of the residents who had consumed only surface water or water from shallow wells developed BFD. This finding is important as it illustrates that no cases were found among the inhabitants who were born after the tap water supply was introduced, and supports the close association between the consumption of arsenic contaminated water and the development of BFD. In addition, the study found that patients with skin cancer or BFD had a greater incidence of death due to cancers of various sites (i.e., 28 and 19%, respectively) when compared to the general population of the endemic area (i.e., 13%) or to the entire population of Taiwan (i.e., 8%).

Using the same arsenic exposure categories (low <300 ppb, medium 300-600 ppb, and high >600 ppb) from the Tseng et al. (1968) investigation, the skin cancer and the BFD prevalence rates (cases/1000) showed an ascending gradient from low to high arsenic exposure for both sexes (Tseng, 1977). Skin cancer prevalence rates by age and arsenic exposure group were as follows: 20-39 years (high - 11.5, medium - 2.2, and low - 1.3); 40-59 years (high - 72.0, medium - 32.6, and low - 4.9); and 60+ years (high - 192.0, medium - 106.2, and low - 27.1). BFD prevalence rates by age and arsenic exposure group were as follows: 20-39 years (high - 14.2, medium - 13.2, and low - 4.5); 40-59 years (high - 46.9, medium - 32.0, and low - 10.5); 60+ years (high - 61.4, medium - 32.2, and low - 20.3). The common cause of death in the patients with skin cancer and BFD was carcinoma of various sites, including lung, bladder, liver, and kidney. The Tseng (1977) investigation observed that the prevalence of skin cancer increased steadily with age. It was difficult to obtain the age at onset of arsenical cancer from patient interviews, as most of the patients were unable to name a date. Strengths and weaknesses of this study are the same as Tseng et al. (1968); however, this study also included adjusted analysis for age and gender.

The objective of the Chen et al. (1985) ecological study was to evaluate the possible association between exposure to elevated levels of arsenic from artesian well water and cancer in the BFD-endemic area of southwestern Taiwan (i.e., Peimen, Hsuechia, Putai, and Ichu

townships). The population of the BFD-endemic area in 1982 was 120,607 and consisted primarily of individuals engaged in farming, fishing, and salt production operations. The educational and socioeconomic status of the BFD-endemic area was below average compared to the remainder of Taiwan. Chen et al. (1985) cited arsenic results from 83,565 wells from all of Taiwan measured by Lo et al. (1977), which showed that 29.1% of the wells in the study areas had concentrations greater than 50 ppb (with the highest concentrations measuring 2500 ppb), while only 5.7% of wells in other areas of Taiwan exceeded 50 ppb. A previous study by Chen et al. (1962) demonstrated a range of 350 to 1140 ppb with a median of 780 ppb arsenic content in Taiwanese artesian wells in BFD-endemic areas. As compared with the general population in Taiwan, both the standardized mortality ratio (SMR) and cumulative mortality rate were significantly higher in BFD-endemic areas. SMRs for males were significant for bladder [11.00, 95% confidence interval (CI): 9.33-12.87], kidney (7.72, 95% CI: 5.37-10.07), skin (5.34, 95% CI: 3.79-8.89), lung (3.20, 95% CI: 2.86-3.54), liver (1.70, 95% CI: 1.51-1.89), and colon (1.80, 95% CI: 1.17-2.03) cancers. SMRs for females also were significantly increased for bladder (20.09, 95% CI: 17.02-23.16), kidney (11.19, 95% CI: 8.38-14.00), skin (6.52, 95% CI: 4.69-8.35), lung (4.13, 3.60-4.66), liver (2.29, 95% CI: 1.92-2.66), and colon (1.68, 95% 1.26-2.10) cancers. Cancer SMRs were greater in villages where only artesian wells were used as the drinking water source, as compared to villages that used both artesian and shallow wells. Villages and townships using only shallow wells generally had the lowest SMRs. Strengths of the investigation include the use of general population of Taiwan and world population for determining SMRs and potential confounders of age and gender were controlled for in the analysis. Weaknesses were that arsenic measurements were not linked to cancer mortality, death certificates list the main cause of death (Yang et al., 2005) rather than all causes, and SMRs were only presented by township and by well type.

To evaluate the association between high arsenic exposure from artesian well water and cancer mortality in the BFD-endemic area of the southwest coast of Taiwan (i.e., the Peimen, Hsuechia, Putai, and Ichu townships), Chen et al. (1986) used a case-control study design to evaluate 69 bladder cancer, 76 lung cancer and 65 liver cancer deceased cases and 368 alive community controls matched on age and gender. This study area was the same as examined by Chen et al. in 1985. Cases were selected from the Republic of China National Health Department between January 1980 and December 1982. The age distribution for cases was significantly lower than the controls. Similar gender distributions were observed for bladder and lung cancer cases and controls; however, there was a slightly higher proportion of male in liver cancer cases compared to controls. Other sociodemographic factors (marital status, educational, occupation and resident years) were comparable between cases and controls. Age and gender differences were adjusted for in the analysis. The artesian well water arsenic content from the BFD-endemic area ranged from 350 to 1140 ppb (median - 780 ppb), and the shallow well water arsenic concentration ranged from below detection limits to 300 ppb (median – 40 ppb). A positive dose-response relationship was observed between the exposure to artesian well water and cancers of bladder, lung, and liver. The age-gender-adjusted odds ratios (ORs) of bladder, lung, and liver cancers for those who had used artesian well water for 40 or more years were 3.90, 3.39, and 2.67, respectively, when compared with those who never used artesian well water. Regression analyses examined the associations between exposure to artesian well water and bladder, lung, and liver cancers after adjusting for other variables including age, gender, and cigarette smoking. Results showed a statistically significant association between exposure to

artesian well water and bladder and lung cancers ($p<0.01$) when other variable were controlled, but the association between the exposure to artesian well water and liver cancer was not statistically significant ($p<0.05$). (The text of the article specifies that liver cancers are not significantly associated with arsenic, but the table that the text refers to illustrates a significant association.) Strengths of the Chen et al. (1986) study include that most cases were confirmed using histology or cytology findings, cancer cases and controls were from the same BFD community, and that potential confounders were adjusted for in the analysis (i.e., age, gender, smoking, tea consumption, vegetable consumption, and fermented bean consumption). Weaknesses include selection bias (control selection) and not controlling recall bias for the following confounders: lifestyle, diet, daily water consumption, and source of water.

In a cohort study conducted by Chen et al. (1988a), cancer mortality associated with BFD was analyzed in area residents (i.e., Peimen, Hsuechia, Putai, and Ichu townships, Taiwan) from 1973 to 1986. Arsenic levels in drinking water were measured between 1962 and 1964; these levels were used to divide the study population into three groups: <300 ppb; 300-599 ppb; and >600 ppb. Sociodemographic characteristics including life style, diet, and living conditions were comparable among study participants. Between 1974 and 1976, water from more than 83,000 wells in 313 villages throughout Taiwan was reanalyzed for arsenic content. The levels of arsenic in the drinking water were consistent between the two measurement periods. Death certificates (n=1031) were obtained from Taiwanese health care registration offices. Age-adjusted cancer mortality rates were calculated using the 1976 world population as the standard. Significantly elevated dose-response cancer mortality was observed among residents of the BFD area (<300 ppb, SMR female=118.8, male=154.0; 300-599 ppb SMR female=182.6, male=258.9; and >600 ppb SMR female=369.1, male=434.7) as compared to the general population of Taiwan (SMR female=85.5, male=128.1). For both genders, significantly elevated dose-response mortality also was observed for cancers of the liver, lung, skin, bladder, and kidney in comparison to the general population of Taiwan. A strength of the study is that data from arsenic monitoring conducted in 1962-64 and 1974-76 revealed similar results. A weakness of the study is that arsenic exposure levels are not individualized.

The objective of the Chen et al. (1988b) cohort (nested case-control) study was to examine multiple risk factors and their correlation to malignant neoplasms related to BFD. A total of 241 BFD cases, including 169 with spontaneous or surgical amputations of affected extremities and 759 age-sex-residence-matched healthy community controls, were identified in the Peimen, Hsuechia, Putai, and Ichu townships of southwest Taiwan and studied. Multiple logistic regression analysis showed that artesian well water consumption, arsenic poisoning, familial history of BFD, and undernourishment were significantly associated with the development of BFD. In a nonconcurrent cohort, cancer mortality of 789 BFD patients followed for 15 years also was examined using a life table. Results showed a significantly higher mortality from cancers of the bladder (SMR=38.80, $p<0.001$), skin (SMR=28.46, $p<0.01$), lung (SMR=10.49, $p<0.001$), liver (SMR=4.66, $p<0.001$), and colon (SMR=3.81, $p<0.05$) as compared with the general population in Taiwan. When non-BFD residents in the BFD-endemic area were used as controls, significant differences in mortality rates were found for cancers of the bladder (SMR=2.55, $p<0.01$), skin (SMR=4.51, $p<0.05$), lung (SMR=2.84, $p<0.01$), and liver (SMR=2.48, $p<0.01$). The results strongly suggest carcinogenic effects of the artesian well water in the BFD-endemic area. Study strengths are recall bias was minimized through

interview techniques; interviews identified the education, hours of occupational sunshine exposure, artesian well use, family medical history, history of smoking and alcohol use, and frequency of categories of food consumption; SMRs were calculated using both the national Taiwanese population and the local endemic area population; and BFD cases were matched to healthy community controls for age, sex and residence. The study weakness was not providing the individual arsenic dose levels.

Chiang et al. (1988) conducted a case-control prevalence study of bladder cancer in the BFD-endemic and surrounding areas of the southwestern coast of Taiwan. Four groups (cases: 246 BFD patients; controls: 444 residents of the BFD-endemic area, 286 residents of the region neighboring the endemic area, and 731 residents of the non-endemic area) were screened using a detailed questionnaire and urinalysis. Three hundred and four cases received a urinary cytology examination. The study revealed no difference in the prevalence of bladder cancer between the BFD-patients and non-BFD controls in the BFD-endemic area, indicating that individuals in the BFD-endemic area were equally affected by a high prevalence of bladder cancer. A high prevalence of bladder cancer in the BFD-endemic area was noted when compared with the neighboring region and residents of the non-endemic area. However, sporadic cases of bladder cancer were noted in the region neighboring the endemic area. This study also found that the non-BFD-endemic areas, which had a high arsenic content in the well water, did not have a high prevalence of bladder cancer, indicating possible other environmental factors. The histological confirmation of bladder cancer diagnoses is a strength of the study, however, the lack of individual arsenic exposure data is a significant weakness of this study.

Wu et al. (1989) analyzed age-adjusted mortality rates using an ecologic study design to determine whether a dose-response relationship exits between ingested arsenic levels and the risk of cancer among residents in the BFD endemic area. The study population consisted of a cohort of individuals from the southwestern coast of Taiwan (27 villages from the townships of Peimen, Hsuechia, Putai, and Ichu and 15 villages from the townships of Yensui and Hsiaying). The arsenic levels in well water for the 42 villages were determined from 1964 to 1966, while mortality and population data were obtained for the years of 1973 to 1986 from the local registration offices and from the Taiwan Provincial Department of Health. Age-adjusted mortality rates from various cancers by gender were calculated using the 1976 world population as the standard population. A significant dose-response relationship was observed between arsenic levels in well water and bladder, kidney, skin, and lung cancers in both males and females. A similar relationship was observed for prostate and liver cancers in males. There was no association for leukemia or cancers of the nasopharynx, esophagus, stomach, colon, and uterine cervix. Strengths of the study include the fact that adjustments were made for age and gender, and that life-style, access to medical care, and socioeconomic status were similar among the study groups. The use of mortality data can be considered as a weakness of the study, since death certificates may not list all cancers. Additionally, associations observed at the local level may not be accurate at the individual level.

The Chen and Wang (1990) ecological study was carried out to examine correlations between the arsenic level in well water and mortality from various malignant neoplasms in 314 precincts and townships of Taiwan. The arsenic content of water from 83,656 wells was available from measurements taken in 1974 through 1976. Mortality rates from 1972 to 1983

were derived from residents in study precincts and townships who displayed one or more of the 21 examined malignant neoplasms. Arsenic content in the water was available at the precinct or township level. A statistically significant association with the arsenic level in well water was observed for cancers of the liver, nasal cavity, lung, skin, bladder, and kidney in both males and females, as well as for prostate cancer in males. These associations remained significant after adjusting for indices of urbanization and industrialization through multiple regression analyses. No significant association was identified for the other 14 cancers examined. The multivariate-adjusted regression coefficient showed an increase in age-adjusted mortality for cancers in males and females for every 100 ppb increase in arsenic level in well water. Coefficients for males and females, respectively, were as follows: 6.8 and 2.0 (liver), 0.7 and 0.4 (nasal cavity), 5.3 and 5.3 (lung), 0.9 and 1.0 (skin), 3.9 and 4.2 (bladder), and 1.1 and 1.7 (kidney). Results were unchanged when 170 southwestern townships were included. Strengths of the study were that potential confounders were controlled for including socioeconomic differences (i.e., urbanization and industrialization), ecological correlations reported between arsenic content in well water and mortality from various cancers, and that cancer rates in endemic BFD townships were compared with cancer rates in non-endemic townships of Taiwan. Potential confounders not controlled for were gender, other potential well water exposure contaminants, and individual arsenic exposures that were not available.

Chen et al. (1992) using an ecologic investigation showed a comparable excess risk of cancer of liver, lung, bladder, and kidney cancers induced by arsenic in drinking water. The study area and population were previously described by Wu et al. (1989). In order to compare the risk of developing various cancers as the result of ingesting inorganic As and to assess the differences in risk between males and females, cancer potency indices were calculated with the Armitage-Doll multistage model using mortality rates among residents of 42 villages in six townships (i.e., Peimen, Hsuechia, Putai, Ichu, Yensui, and Hsiaying) located on the southwest coast of Taiwan. Locations selected were considered to be chronic arsenicism endemic areas. Arsenic exposure levels of drinking water from these villages were categorized into four groups: <100 ppb (13 villages), 100-299 ppb (8 villages), 300-599 ppb (15 villages), and 600 ppb or greater (6 villages). Based on a total of 898,806 person-years during the study period from 1973 through 1986, a significant dose-response relationship was observed between the arsenic level in drinking water and cancer mortality of the liver, lung, bladder, and kidney. The lifetime risk (determined using the Armitage-Doll model) of developing cancer of the liver, lung, bladder and kidney due to an intake of 10 μg/kg-day of arsenic was estimated to be 4.3×10^{-3}, 1.2×10^{-2}, 1.2×10^{-2}, and 4.2×10^{-3} for males and 3.6×10^{-3}, 1.3×10^{-2}, 1.7×10^{-2}, and 4.8×10^{-3} for females, respectively. Strengths include that potential confounders including age, gender, access to medical care, socioeconomic status, and life-style were all controlled for during the analysis, and that villages shared similar socioeconomic status, living environments, lifestyles, dietary patterns, and medical facilities. A weakness of the study is the assumption that an individual's arsenic intake remained constant from birth to the end of the follow-up period; this flaw possibly led to the underestimation of risk. Additional weaknesses included that the Armitage-Doll model constrains risk estimates to be monotonically increasing function of age, that dietary sources of arsenic were not quantified, and that age stratification was for under 30, over 70, and 20-year strata.

To determine whether a dose-response relationship exists between ingested inorganic As

and cancer, Chiou et al. (1995) used a cohort study with a total of 263 BFD patients and 2293 healthy residents in the arseniasis endemic area of southwestern coast of Taiwan (i.e., Peimen, Hsuechia, Putai, and Ichu townships). Participants were followed for an average of 4.97 years (range: 0.05 to 7.69 years). Data concerning the consumption of artesian well water containing high levels of arsenic, sociodemographic characteristics, life-style and dietary habits, and cancer histories were obtained by means of a standardized interview. Internal cancers were determined via health examinations, personal interviews, household registration data checks, and Taiwan's national death certification and cancer registry databases. Concentrations used in the assessment were ≤ 50 ppb, 50-70 ppb, 71+ ppb, and unknown. Disregarding the unknown category, a dose-response relationship was observed between the long-term arsenic exposure from drinking artesian well water and the incidence of lung cancer, bladder cancer, and cancers of all sites combined after adjusting for age, sex, and cigarette smoking through a Cox's proportional hazards regression analysis. BFD patients had a significantly increased incidence of bladder cancer and for all sites combined after adjusting for age, gender, smoking history, and cumulative arsenic exposure (CAE). Strengths include that the analysis adjusted for BFD status, age, gender, and smoking; incidence data were reported; and the results of the study showed a significant dose-response relationship. A weakness of the study is that well water artesian arsenic concentrations were unknown for some study subjects; consequently, this was a significant confounder.

To further evaluate the association between arsenic exposure in drinking water and urinary cancers of various cell types, Guo et al. (1997) conducted an ecologic study encompassing 243 townships using Taiwanese National Cancer Registry data of patients diagnosed with cancer between 1980 and 1987. Wells with known arsenic concentrations in each township were used to separate people into the following exposures: <50 ppb, 50-80 ppb, 90-160 ppb, 170-320 ppb, 330-640 ppb and >640 ppb. The effects of urbanization and smoking were evaluated by an urbanization index based on 19 socioeconomic factors shown to be good indicators of urbanization and the number of cigarettes sold per capita. For both genders, Guo et al. observed associations between high arsenic levels in drinking water and transitional cell carcinomas (bladder, kidney, ureter, and all urethral cancers combined). Positive associations between the proportion of wells with arsenic levels above 640 ppb and the incidence of transitional cell carcinomas of the bladder, kidney, ureter, and all urethral cancers combined in both genders were identified after the model was adjusted for urbanization and age. Arsenic exposure in males was associated with adenocarcinomas of the bladder, but not in squamous cell carcinomas of the bladder or renal cell carcinomas or nephroblastomas of the kidney. Males also exhibited a positive association between the urbanization index and transitional cell carcinomas of the ureter. The results provide evidence to support the case that the carcinogenicity of arsenic may be cell-type specific. Analyses were adjusted for age, gender, urbanization, and smoking; however, the study's ecologic design was a limitation.

Tsai et al. (1999) conducted a cross-sectional study in BFD-endemic areas located in the southwest coast of Taiwan (i.e., Peimen, Hsuechia, Putai, and Ichu townships) to analyze mortality from neglected cancers related to artesian well water containing high levels of arsenic. The median artesian well water arsenic content was 780 ppb (range=250-1140 ppb). Local endemic area residents' daily ingestion of arsenic was estimated to be ≤1 mg. SMRs were calculated for cancer diseases, by gender, during the period from 1971 to 1994. These SMRs

were compared to the local reference group (Chiayi-Tainan County population) and a national reference group (Taiwanese population). The comparisons revealed significant differences between SMRs of the three groups. Mortality increases ($p<0.05$) were found in males and females, respectively, for all cancers (SMR=2.19, 95% CI: 2.11-2.28; SMR=2.40, 95% CI: 2.30-2.51) when compared to the local reference population. Additionally, the following other cancers showed mortality increases in males and females, respectively, when compared to the local reference population: bladder (SMR=8.92, 95% CI: 7.96-9.96; SMR=14.07, 95% CI: 12.51-15.78); kidney (SMR=6.76, 95% CI: 5.46-8.27; SMR=8.89, 95% CI: 7.42-10.57); skin, lung, nasal-cavity, bone, and liver (SMR=1.83, 95% CI: 1.69-1.98; SMR=1.88, 95% CI: 1.64-2.14); and larynx, stomach, colon, intestine, rectum, lymphoma, and prostate cancer in males only (SMR=2.52, 95% CI: 1.86-3.34). When compared to the national reference population, significantly increased ($p<0.05$) mortality was found in males and females, respectively, for all cancers (SMR=1.94, 95% CI: 1.87-2.01; SMR=2.05, 95% CI: 1.96-2.14) and for the other following cancers: bladder (SMR=10.50, 95% CI: 9.37-11.73; SMR=17.65, 95% CI: 5.70-19.79); and lung (SMR=2.64, 95% CI: 2.45-2.84; SMR=3.50, 95% CI: 3.19-3.84). The results of the Tsai et al. (1999) investigation indicate that the hazardous effect of arsenic may be systemic. Key strengths of the study are that the exposed group and local reference group had similar lifestyle factors; all cancers were pathologically confirmed; and the analysis controlled for gender. Weaknesses of the study are that only one underlying cause of death (not multiple causes) was indicated on death certificate, resulting in possible distortion of association between exposure and disease; individual exposure data was not provided; and certain potential confounders were not controlled for (age, smoking history, alcohol consumption, and occupational exposures).

The Morales et al. (2000) ecological investigation re-analyzed data originally reported by Chen et al. (1988a, 1992) and Wu et al. (1989) from 42 villages in the arseniasis-region of southwestern Taiwan by considering the number of liver, lung, and bladder cancer deaths. Morales et al. (2000) used a generalized linear model (i.e., Poisson distribution) and the multistage-Weibull models to determine lifetime cancer risk estimates. Liver, lung, and bladder cancer mortality data were collected from death certificates of residents in 42 villages during 1973 through 1986. Drinking water samples had been collected from wells in the 42 villages between 1964 and 1966. SMRs were used to summarize the observed patterns of mortality in the collected data. Morales et al. (2000) selected two comparison populations (the Taiwanese population as a whole, and a population from a southwestern region of Taiwan) to account for urban versus non-urban populations differences. Although a non-significant trend was observed in the combined cancer analyses with respect to age, there was no observed tendency in liver, lung, or bladder SMRs with respect to age. This suggests that there is no age dependency on the risk ratio. Liver cancer mortality was higher than expected, although there was no strong exposure-response relationship found. The Morales et al. (2000) investigation results showed that exposure-response assessments were highly dependent on the choice of the analysis model, and whether or not a comparison population is used in the analysis. One possible explanation for this observation is the inherent uncertainty associated with the limitations of an ecologic study design. Depending on the model used and the comparison population used in the analysis, the 1% excess risk dose (ED_{01}) estimates ranged from 21 to 633 ppb for male bladder cancer, and from 17 to 365 ppb for female bladder cancer. The lung cancer risk for males was found to be slightly higher than the bladder cancer risk, with ED_{01} estimates ranging from 10 to 364 ppb.

The risk for female cancer tended to be higher than that of males for each cancer type. For lung cancer, female ED_{01} estimates ranged from 8 to 396 ppb.

In summary, the Morales et al. (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 ppb, the drinking water standard for arsenic in the U.S. at the time of publication. The following are considered strengths of the study: person years at risk (PYR) were stratified by 5-year age groups, gender, and median arsenic level for each village. Weaknesses include the ecologic study design (i.e., there was no individual monitoring data and individual exposures were not available), and potential confounders such as smoking, dietary arsenic, and the use of bottled water (U.S. population) were not controlled for in the analysis.

Between 1991-1994, Chiou et al. (2001) recruited a cohort of 8102 residents aged 40 years or older from 4 townships (18 villages) in northeastern Taiwan (4 villages in Chiaohsi, 7 in Chuangwei, 3 in Wuchih, and 4 in Tungshan) and followed it until the end of 1996. The study examined the risk of transitional cell carcinoma in relation to ingested arsenic. The Chiou et al. (2001) findings were consistent with previously reported findings from the arsenic-endemic area of southwestern Taiwan. Based on the arsenic concentration in well water, estimation of each study subject's individual exposure to inorganic As was determined. Information concerning the duration of consumption of the well water was obtained through standardized questionnaire interviews. Urinary tract cancers were identified by follow-up interviews, community hospital records, the Taiwanese national death certification profile, and the cancer registry profile. A significantly increased incidence of urinary tract cancers for the study cohort was observed (Standardized Incidence Ratio [SIR]=2.05; 95% CI: 1.22-3.24) when compared to the general population in Taiwan. In addition, a dose-response relation was observed between the risk of cancers of the urinary organs, especially transitional cell carcinoma, and indices of arsenic exposure after adjusting for age, sex, and cigarette smoking. The relative risks (RR) of developing transitional cell carcinoma were 1.9, 8.2, and 15.3 for arsenic concentrations of 10.1-50.0 ppb, 50.1-100.0 ppb, and >100.0 ppb, respectively, compared with the referent level of ≤10.0 ppb. No association was observed for the duration of well water drinking (<40 years compared to ≥40 years). The findings of this study suggest that arsenic ingestion may increase the risk of urinary tract cancer at levels around 50 ppb. Strengths are adjustments for potential confounders (age, gender, smoking history), individual arsenic exposure estimates, and a dose-response relationship even with the low levels of arsenic. Weaknesses include possible diagnostic bias as the result of medical data collection from various community hospitals and recall bias from self-reported information. The short duration of follow-up also is a limitation because it impacted 1) the number of person-years of observation and 2) only a few cases were recorded. This study also has an apparent supralinear curve, which is likely due to dose misclassification in the low-dose individuals. If food arsenic concentrations (estimated in NRC, 2001 to be approximately 50 µg/day) were included the curve might not be supralinear.

Guo et al. (2001) conducted an ecological investigation of the 243 townships from their 1997 publication; however, this investigation focused on arsenic exposure through drinking water and the potential association with skin cancers. Data regarding arsenic levels in drinking water was available from the previous investigation, and cases of skin cancer were identified using the Taiwanese National Cancer Registry. Data were analyzed with regression models

using multiple variables to describe exposures, including arsenic. To adjust for potential confounding variables, an urbanization index based on 19 socioeconomic factors shown to be good indicators of urbanization was developed. A total of 2369 individuals with skin cancer (954 females and 1415 males) were registered with the Cancer Registry between January 1980 and December 1989. After age and urbanization adjustment, arsenic levels above 640 ppb showed a statistically significant ($p<0.01$) association with the incidence of basal cell carcinoma (BCC) in males. Exposed females also exhibited an increased incidence in skin cancer rates; however, this increase did not reach statistical significance ($p=0.20$). For squamous cell carcinomas (SCC), a significant ($p<0.01$), positive association was found for males exposed to 170-320 ppb and >640 ppb. However, a statistically significant ($p<0.01$), negative association was found for males exposed to 330-640 ppb. For females, a similar statistically significant ($p<0.01$), positive association was observed at >640 ppb, while a statistically significant ($p<0.05$), negative association was observed in 330-640-ppb females. For melanomas, no significant associations were identified in females or males at any exposure. The results of the investigation suggest that skin cancers are cell-type specific, as previously was demonstrated for urinary tract cancers (Guo et al. 1997). Strengths of the study include that cases were identified from a government operated National Cancer Registration Program, pathological classifications were determined by board certified pathologists, and potential confounders (gender and age) were adjusted in the analysis. A limitation of the study is the ecologic study design.

Studies on cancers of the urinary system and skin showed that arsenic's carcinogenic effect was cell-type specific (Guo et al. 1997, 2001). Guo (2003) conducted an ecological investigation in 243 townships in Taiwan, previously used in the Guo et al. (1997, 2001) investigations for urinary and skin cancers, to determine if a similar relationship could be identified for liver cancer. Many previous epidemiologic studies did not provide data on pathological diagnoses; therefore, there was no information to support the hypothesis that hepatocellular carcinoma (HCC) or cholangiocarcinoma of the liver were not associated with arsenic ingestion. Liver cancers were identified through the Taiwanese National Cancer Registry. The distribution of cancer cell-types between an arseniasis-endemic area and a township outside the arseniasis area were compared. Between January 1980 and December 1999, 32,034 men and 8798 women living in the study townships were diagnosed with liver cancer. The distribution of two cancer cell-types (HCC and cholangiocarcinoma) did not appear to be different between the arseniasis-endemic and non-arseniasis-endemic areas, and an association between HCC and arsenic ingestion was not observed. The remainder of the cell-types did not have enough cases to provide stable estimates. Identified strengths of the study include the following: cases were identified from the government operated National Cancer Registration Program; pathological classifications were determined by board certified pathologists; and analyses were adjusted for gender and age. Weaknesses include the limitations of ecological study design (no monitoring data were presented).

A cohort investigation of residents from two arsenic endemic areas were followed for 8 years by Chen et al. (2004a) to investigate the dose-response relationship between arsenic exposure and lung cancer, as well as how cigarette smoking influenced the relationship between arsenic and lung cancer. Arsenic endemic areas included the southwestern coast (Peimen, Hsuechia, Putai, and Ichu; n=2503) and the northeastern coast (Tungshan, Chuangwei, Chiaohsi, and Wuchieh; n=8088) of Taiwan. The amount of arsenic in well water from these areas ranged

from less than 0.15 ppb to more than 3000 ppb. The Taiwanese National Cancer Registry was used to identify new cases of lung cancer diagnosed between January 1, 1985 and December 31, 2000. For each participant, follow-up person-years were calculated using the time from date of the initial interview date to the date of diagnosis, death, or December 31, 2000, whichever came first. Arsenic concentration was arbitrarily divided into five categories: <10 ppb (referent), 10-99.9 ppb, 100-299.9 ppb, 300-699.9 ppb, and ≥700 ppb. Smoking histories were obtained from interviews. Cox proportional hazards regression models were used to estimate RR and 95% CI. The final model was adjusted for age, gender, years of schooling, study cohort (BFD cases and matched controls of the southwestern coast, residents along the arseniasis-hyperendemic southwestern coast villages, and residents living in the northeastern coast Lanyang Basin), smoking status, and alcohol consumption. During the study follow-up period, there were 139 lung cancers diagnosed, resulting in an incidence rate of 165.9 per 100,000 person-years. When the highest level of arsenic exposure was compared to the lowest, the RR was 3.29 (95% CI: 1.60-6.78). The risk of lung cancer was four times higher for past and current smokers when compared to non-smokers. A synergistic effect of ingested arsenic and cigarette smoking on lung cancer was noted, with synergy indices ranging from 1.62-2.52. Strengths of the study include controlling for confounders (age, gender, education, smoking history and alcohol consumption), having a long follow-up period, using a national computerized cancer case registry, and pathologically confirming all lung cancer cases. Weaknesses include the lack of historical monitoring data, and possible misclassification bias (exposure measurements were based on one survey).

Chiu et al. (2004), using a cohort study design, examined whether liver cancer mortality rates were altered after the consumption of high arsenic artesian well water ceased. SMRs for liver cancer were calculated for the BFD-endemic area of the southwest coast of Taiwan (i.e., Peimen, Hsuechia, Putai, and Ichu townships) for the years 1971-2000. Median well water arsenic concentrations in the early 1960s were 780 ppb. Temporal changes in the SMRs were monitored using cumulative-sum techniques and were reported for 3 year intervals between 1971 and 2000. Study results showed that female mortality from liver cancer started declining 9 years after consumption of high-arsenic artesian well water stopped. The SMR for liver cancer in females was 2.041 during the1983-1985 period (peak) and was 1.137 during 1998-2000. Data in males, however, showed fluctuations in liver cancer mortality rates. The SMR for liver cancer in males from 1989-1991 was 1.868 and 1.242 during 1998-2000. Based on analyses by Chiu et al. (2004), it was determined that the relationship between arsenic exposure and liver cancer mortality was possibly causal in females, but not in males. Strengths of the study are: 1) residents in the study area were similar in terms of socioeconomic status, living environments, lifestyles, dietary patterns, and availability of health service facilities; and 2) the study used an accurate death registration system. Weaknesses include the limitations of the mortality data.

To obtain data on the potential dose-response relationship between lung cancer and the level of arsenic in drinking water, Guo (2004) conducted an ecological investigation in ten townships (138 villages) in Taiwan. Measurements of arsenic levels in drinking water were available for the 138 villages from a census survey conducted by the Taiwanese government. Death certificates dated between January 1, 1971 and December 31, 1990 were reviewed, and 673 males and 405 females were identified as dying from lung cancer. Multivariate regression models were applied to assess the relationship between arsenic levels in drinking water and lung

cancer mortality. After adjusting for age, arsenic levels above 640 ppb were associated with a significant increase in lung cancer mortality for both genders; however, no significant effect was observed at lower arsenic exposure levels. Regression analyses and stratified analyses confirmed a dose-response relationship at >640 ppb. Guo (2004) noted that the results of this investigation show a carcinogenic effect of high arsenic levels in drinking water on the lung. Guo (2004), however, recommended that further studies with exposure data on individuals were warranted to confirm these findings. As a result of the study's ecologic design, the association observed on an aggregate level may not necessarily represent the association that exists at an individual level. In addition, the study design may have contributed to biases introduced by the effects of population mobility. Strengths of the study include that analyses adjusted for gender and age, and cases were ascertained using information from household registry offices in each township. Weaknesses of the investigation include the inherent limitations of ecological studies and the fact that smoking was not controlled for in the analysis.

In a cross-sectional study, Yang et al. (2004) examined whether kidney cancer mortality decreased in the southwest coast of Taiwan (i.e., Peimen, Hsuechia, Puta, and Ichu townships) after the elimination of arsenic exposure in the 1970s. SMRs for kidney cancer were calculated for the BFD-endemic area for the years 1971-2000. There were 308 kidney cancer deaths (135 men and 173 women) in the BFD-endemic area between 1971 and 2000. The means of the 3-year SMRs for female and male kidney cancer were significantly higher than for Taiwan as a whole. Time series plots for male SMRs showed decreasing mortality rates. The estimated slope for male SMRs (rate of decrease per year) in the linear time trend analysis was a -15.13 ($p<0.01$). The time series plot for female SMRs also showed decreasing mortality rates. Kidney cancer mortality rates among residents in the BFD-endemic area decreased after removal of the arsenic source through tap water implementation. SMRs decreased each year, on average, from 1971 to 2000 ($p<0.01$). Study strengths include the adjustment of potential confounders (gender and age); mandatory registering all births, deaths, marriages, divorces, and migration issues to the Household Registration Office in Taiwan, making it an accurate data source; a comparable study population (i.e., residents probably had similar socioeconomic status, living environments, lifestyles, dietary patterns; worked in farming, fisheries, or salt production); and had comparable access to medical care (i.e., all kidney cancer cases probably had similar access to medical care). Possible weaknesses of the study include cross-sectional mortality limitations and not adequately controlling for smoking histories

Tsai et al. (2005) used a cross-sectional study to compare primary urethral carcinomas from the BFD-endemic area of Taiwan with those in the U.S. and explore the potential influence of chronic arsenic exposure. Cases were identified by the only medical center near the BFD area. There were 21 pathologically proven primary urethral carcinomas diagnosed (7 females and 14 males) between 1988 and 2001. Seven of 14 male patients had reported an average of 23 years of chronic arsenic exposure from drinking water. Tsai et al. (2005) compared these cases to cases identified in three U.S. cancer centers (MD Anderson, Memorial Sloan-Kettering, and Barbara Ann Karmanos; n=79 females, n=80 males), and analyzed for a relationship with chronic arsenic exposure. In comparison to the three U.S. cancer centers, there was a higher frequency of bulbomembranous adenocarcinoma (43% vs. 18, 2, and 0%, respectively, $p<0.0001$). In those with chronic arsenic exposure there was an even greater association with bulbomembranous adenocarcinoma compared to those without chronic arsenic exposure (73%

vs. 14%, p=0.031). Based on these results, Tsai et al. (2005) concluded that the BFD-endemic area in Taiwan had a high frequency of bulbomembranous urethral adenocarcinoma, which may be associated with chronic arsenic exposure. A strength of the study is that cases were pathologically confirmed. The small number of cases and the lack of arsenic exposure information are study weaknesses.

The objective of the Yang et al. (2005) cross-sectional study was to determine whether bladder cancer mortality decreased after the implementation of the tap water system and the subsequent elimination of arsenic exposure. SMRs for bladder cancer were calculated for the BFD-endemic area for the years 1971–2000. The study showed that bladder cancer mortality decreased gradually after the instillation of the tap water system, thereby eliminating exposure to arsenic through artesian well water, (1971, male SMR=10.25, female SMR=14.89; 2000, male SMR=2.15, female SMR=7.63). Strengths include similar access to medical care for bladder cancer, the adjustment for age and gender, and the mandatory registering of all births, deaths, marriages, divorces, and migration issues to the Household Registration Office in Taiwan, making it an accurate data source. Limitations of the study include the cross-sectional mortality study design and smoking history confounding.

JAPAN

Tsuda et al. (1995) used a cohort study to investigate the long-term effect of ingesting arsenic in drinking water contaminated for estimated exposure period of 5 years (1955 and 1959). Four hundred and fifty four residents identified in 1959 as living in an arsenic-polluted area of Niigata Prefecture, Japan were followed until 1992. The mortality of these residents between October 1, 1959 and February 29, 1992, was examined using death certificates. These individuals used arsenic contaminated well water, and none worked at a nearby factory that was the source of the water contamination. Death certificates for the people who died between 1959 and 1992 were examined and a total of 113 of the 454 residents were estimated to have drunk well water containing a high concentration of arsenic (≥1000 ppb). The SMRs of these 113 residents were 15.69 for lung cancer (95% CI: 7.38-31.02) and 31.18 for urinary tract cancer (95% CI: 8.62-91.75). Cox's proportional hazard analyses demonstrated that the hazard ratios of the highest exposure level group (≥1000 ppb) versus the background exposure level group (1.0 ppb) were 1.74 (95% CI: 1.10-2.74) for all deaths, 1972.16 (95%CI: 4.34-895,385.11) for lung cancer, and 4.82 (95% CI: 2.09-11.14) for all cancers. The study also analyzed skin signs of chronic arsenicism, and results indicated that they were useful risk indicators for subsequent cancer development. These results indicate a relationship between well water arsenic exposure and lung and urinary tract cancers. The study also showed that arsenic-induced cancer could develop years following the end of arsenic exposure. For lung cancer, there was evidence of synergistic effects between arsenic exposure and smoking history. Strengths of this study include data on smoking history, age and gender; and an examination of the cohort by 3 arsenic exposure categories. Weaknesses, however, include the lack of detailed arsenic intake information, a small study population, as well as possible misclassification and recall bias pertaining to smoking history.

SOUTH AMERICA

Hopenhayn-Rich et al. (1996a) used an ecologic study design to investigate bladder cancer mortality for the years 1986-1991 in the province of Cordoba, Argentina using rates for all of Argentina as the standard for comparison. The study compiled arsenic measurements from a major water survey performed more than 50 years earlier. Using these earlier arsenic data, a crude estimate of exposure was made. The data were matched to the population listings from the national census bureau. This study grouped counties into three defined arsenic exposure categories of low, medium, and high (groups were defined based on the location of counties and the concentrations were only provided for the high group, which had a mean arsenic level of 178 ppb). In the absence of smoking data for each county, mortality from chronic obstructive pulmonary disease (COPD) was used as a surrogate. SMRs for bladder cancer were higher in counties with known elevated levels of arsenic exposure through drinking water. The SMRs (95% CI) for corresponding arsenic exposure categories were 0.80 (0.66-0.96), 1.42 (1.14-1.74), and 2.14 (1.78-2.53) for males, and 1.21 (0.85-1.64), 1.58 (1.01-2.35), and l.82 (1.19-2.64) for females, respectively. Significant trends were noted in both males and females.

Results of this study showed a dose-response relationship existed between arsenic exposure from drinking water and bladder cancer in spite of the limitations inherent from the ecologic design. Argentina has one of the world's highest rates of per capita beef consumption. The high-arsenic region of Cordoba is an important agricultural and beef-producing area, and animal protein is considered to be one of the basic foods of the population. This is important because protein deficiency in the Taiwanese population has been suggested to diminish their capacity to detoxify arsenic. The similar findings between the two populations, regardless of genetic and dietary differences, strengthens the link between arsenic exposure and bladder cancer. Strengths of the study include the adjustment for age and gender, the use of stomach cancer as a non-arsenic-induced comparison, and that the analysis was restricted to rural counties to limit confounders. The lack of individual smoking history (mortality from COPD was used as a surrogate for smoking), the lack of arsenic measurements in low and medium groups, and the lack of individual arsenic exposure data (ecological study) are important potential weaknesses of this study.

To investigate dose-response relationships between arsenic exposure from drinking water and cancer mortality, Hopenhayn-Rich et al. (1998) conducted an ecologic study in Cordoba, Argentina. Cancer mortality from the lung, kidney, liver and skin during the period 1986-1991 in 26 counties of Cordoba were studied. This investigation expanded the analysis of their previous study (Hopenhayn-Rich et al., 1996a), which only examined bladder cancer in Cordoba, Argentina. Counties were grouped into low, medium and high arsenic exposure categories based on arsenic exposure data taken from Hopenhayn-Rich et al. (1996a). In the absence of smoking data for each county, mortality from COPD was used as a surrogate. SMRs were calculated using all of Argentina as the reference population. Hopenhayn-Rich et al. (1998) found increasing trends for kidney and lung cancer mortality with increasing arsenic exposure (i.e., low, medium, high) as follows: male kidney cancer SMRs=0.87 (95% CI: 0.66-1.10), 1.33 (95% CI: 1.02-1.68), and 1.57 (95% CI:1.17-2.04); female kidney cancer SMRs=1.00 (95% CI: 0.71-1.37), 1.36 (95% CI: 0.94-1.89), and 1.81 (95% CI: 1.19-2.64); male lung cancer SMRs=0.92 (95% CI: 0.85-0.98), 1.54 (95% CI: 1.44-1.64), and 1.77 (95% CI: 1.63-1.90); and female lung cancer SMRs=1.24 (95% CI: 1.06-1.42), 1.34 (95% CI: 1.12-1.58), and 2.16 (95% CI: 1.83-2.52), respectively ($P < 0.001$ in trend test). These findings were similar to the previously

reported bladder cancer results. Additionally, the Hopenhayn-Rich et al. (1998) study showed a weakly positive trend for liver cancer, with SMRs being significantly increased even in the lowest exposure category. Skin cancer mortality was elevated only for females in the highest arsenic exposure group, while males showed an increase in mortality only in the lowest exposure group. The results add to the evidence that arsenic ingestion through drinking water increases the risk of lung and kidney cancers. The association between arsenic and mortality from liver and skin cancers was not as clear. Risk analyses were restricted to rural Cordoba counties to limit confounders and to account for cancer diagnosis and detection bias. Strengths and weaknesses are the same as those observed for Hopenhayn-Rich et al. (1996a).

Smith et al. (1998), using an ecological design, studied cancer mortality in a population of approximately 400,000 people exposed to high arsenic levels in drinking water in past years in Region II of northern Chile. Arsenic concentrations in drinking water from 1950 to 1996 were available. The population-weighted average arsenic levels reached 570 ppb between 1955 and 1969, but decreased to less than 100 ppb by 1980. SMRs were calculated for the years 1989 to 1993, and increased SMRs were identified for bladder, kidney, lung, and skin cancers. Bladder cancer mortality was the most elevated (female SMR=8.2, 95% CI: 6.3-10.5; male SMR=6.0, 95% CI: 4.8-7.4). Lung cancer mortality was likewise significantly elevated (female SMR=3.1, 95% CI: 2.7-3.7; male SMR=3.8, 95% CI: 3.5-4.1). Smoking survey data and mortality rates from COPD provided evidence that smoking did not contribute to the increased mortality from these cancers. These results provide additional evidence that ingestion of inorganic As in drinking water can lead to increases in cancers of the bladder and lung. Smith et al. (1998) estimated that approximately 7% of all deaths in individuals more than 30 years old might be attributable to arsenic exposure. Strengths of the study are the large size of the study population, the adjustment of SMRs by age and gender, and the use of Chilean national data for comparison. Weaknesses include that arsenic levels were not available at the individual source level, dose response information was not provided, and only limited individual smoking history information was available (i.e., participants were asked if they had smoked cigarettes over a one-month period in 1990).

In a case-control study, Ferreccio et al. (2000) investigated the association between lung cancer and arsenic in drinking water by comparing patients diagnosed with lung cancer (1994-1996; 152 cases) with frequency-matched hospital controls (419 controls). Using a full-logistic regression model, a clear trend in lung cancer ORs was observed with increasing concentration of arsenic in drinking water: 10-29 ppb arsenic, OR: 1.6 (95% CI: 0.5–5.3), 30-49 ppb arsenic, OR: 3.9 (95% CI:1.2–12.3), 50-199 ppb arsenic, OR: 5.2 (95% CI: 2.3–11.7), and 200-400 ppb, OR: 8.9 (95% CI: 4.0-19.6). Evidence of synergistic effects between arsenic in drinking water and cigarette smoking history was much greater than expected, as the OR for lung cancer was 32.0 (95% CI: 7.2–198.0) among smokers exposed to more than 200 ppb. In comparison, an OR of 8.0 was observed for those who never smoked but were in the highest arsenic category, and an OR of 6.1 was observed for smokers in the lowest arsenic category. Based on these results, the effect was considered synergistic because an OR of 13.1 was expected if the effect was additive. This study provided strong evidence that ingestion of inorganic As through drinking water is associated with lung cancer. ORs for the full-analysis model were adjusted for age, gender, cumulative lifetime cigarette smoking, working in copper smelting, and socioeconomic status; this is considered a study strength. The fact that more controls were obtained from Antofagasta

than from the lower exposure cities of Arica and Iquique, which could lead to an improper (lower) estimation of risk, is considered a study limitation.

Bates et al. (2004) recognized that epidemiologic studies had found an association between increased bladder cancer risk and high levels of arsenic in drinking water; however, little information was found concerning cancer risks at lower concentrations. It also was recognized that ecologic studies in Argentina had found increased bladder cancer mortality in Cordoba Province, where some wells were contaminated with moderate arsenic concentrations. Therefore, Bates et al. (2004) decided to use a population-based bladder cancer case-control study during 1996-2000 in two Cordoba, Argentina counties and recruited 114 case-control pairs, matched by age, sex, and county of residence over the past 40 years. Three arsenic exposure metrics based on questionnaire and water sampling data were used: average arsenic concentration in domestic water, arsenic concentration adjusted to fluid intake, and reported years of well water consumption. Statistical analyses showed no evidence of an association of bladder cancer with arsenic exposure estimates based on arsenic concentrations in drinking water. Additional time-trend analyses, however, did suggest that the use of arsenic contaminated well water at least 50 years prior to the study was associated with increased bladder cancer risk. This positive association was limited to ever smokers (OR=2.5, 95% CI: 1.1-5.5 for the time period 51-70 years before the study interview). Bates et al. (2004) suggested that it could not be excluded that these associations were based on chance.

The results of this study suggest a decreased bladder cancer risk for arsenic exposure than had been predicted from other studies. The results of the Bates et al. (2004) study did add to the evidence that the latency for arsenic-induced bladder cancers may be longer than previously thought and that increased lengths of follow-up for studies may be required to accurately measure the induced risk. Strengths include that potential confounders (age, gender, smoking history, and residence county) were controlled for in the analysis. However, weaknesses related to the lack of a cancer registry, arsenic exposure misclassification, and recall and selection bias exist. Selection bias may have occurred as the controls had a significantly reduced rate of participation than cases. Additional selection bias may have occurred with the selection of cases from the tumor registry. An additional weakness is that other harmful exposures (including arsenic exposure through food) were not measured.

Using a cohort study design, Smith et al. (2006) investigated lung cancer, bronchiectasis, and COPD mortality rates in Antofagasta, Chile in the period 1989-2000 and compared these rates to the rest of Chile. Study subjects (30-49 years old at time of death) were selected primarily from those born during or just prior to the peak in the arsenic exposure period. Results show a lung cancer SMR of 7.0 (95% CI: 5.4-8.9, $p<0.001$) for the cohort born just before the peak exposure period (i.e., from 1950 through 1957), and, therefore, were exposed to arsenic during their childhood. For those cases born between 1958 and 1971 (i.e., the high-exposure period), a lung cancer SMR of 6.1 (95% CI: 3.5-9.9, $p<0.001$) was estimated; this group was probable exposed to arsenic *in utero* and early childhood. These findings suggest that exposure to arsenic in drinking water during early childhood or *in utero* has pronounced pulmonary effects greatly increasing subsequent mortality in young adults from malignant lung disease. The study concluded that the observed effects are most probably due to arsenic in water, even though possible effect-dilution occurred as the result of in-migration of those from other regions of

Chile. A strength of the study was the extensive documentation of drinking water arsenic levels in the Antofagasta water system. Weaknesses include that place of residence was determined from the death certificates, which relates to residence at the time of death, and the reliance on death certificates (potential diagnostic bias). Smoking, although considered unlikely by Smith et al. (2006), is a potential confounder for this study.

Marshall et al. (2007) conducted an ecologic study to investigate lung and bladder cancer mortality from 1950-2000 in a region of Chile where drinking water was contaminated with arsenic (Region II), and in another region of Chile where arsenic was not an issue (Region V). Elevated arsenic exposure through drinking water began in Region II in 1958 and continued into the early 1970s. Mortality data tapes and mortality data from death certificates for the two regions for 1950-1970 identified 307,541 deaths from the two regions for 1971-2000. Poisson regression models were used to compare Region II with Region V by identifying time trends in rate ratios of mortality from lung and bladder cancers. Lung and bladder cancer mortality rate ratios for Region II compared with Region V began to increase approximately 10 years after high arsenic exposures commenced and continued to rise until peaking between 1986 and 1997. The peak lung cancer mortality rate ratios for women and men were 3.26 (95% CI: 2.50 to 4.23) and 3.61 (95% CI: 3.13-4.16), respectively. The peak bladder cancer rate ratios for women and men were 13.8 (95% CI: 7.74-24.5) and 6.10 (95% CI: 3.97 to 9.39), respectively. Together, lung and bladder cancer mortality rates in Region II were highest during the period 1992-1994, with mortality rates of 50/100,000 for women and 153/100,000 for men compared with 19/100,000 and 54/100,000, respectively, in Region V. The long latency for lung and bladder cancer mortality continued to have a residual effect through the late 1990s, even though there was a significant decrease in arsenic exposure through drinking water more than 25 years earlier. Strengths of the investigation include the large study population, the availability of past exposure data, and that potential confounders of age, gender, and smoking history were controlled for in the analysis. However, weaknesses include the inability to account for migration, the ecologic design (i.e., lack of individual exposure data) and lack of information concerning occupation.

Yuan et al. (2007) investigated mortality from 1950 to 2000 using an ecological study design in the arsenic-exposed Region II of Chile and the unexposed population from Region V. Before 1958, the drinking water in Region II contained approximately 90 ppb of arsenic. In 1958, it became necessary to supplement the Region II water supply using rivers that had an average arsenic concentration of 870 ppb. After the installation of an improved water treatment operation in the early 1970s, the arsenic concentrations in the Region II water supply dropped sharply (<10 ppb). While acute myocardial infraction (AMI) mortality was the predominant cause of excess deaths during and immediately after the high-exposure period, due to the longer latency of cancer, excess deaths from lung and bladder cancer became predominated years later. Yuan et al. (2007) concluded that after a 15-20 year lag period following initial exposure to significantly elevated levels of arsenic from drinking water (1958-1970), mortality from bladder and lung cancer surpassed other causes of mortality. Strengths of the study included known arsenic concentrations and the large study population. In addition, to ensure appropriate selection of a control population, preliminary investigations were conducted to compare regional income, smoking history, and availability and quality of death certificate information. The major weakness of the study was its ecological study design (i.e., lack of individual arsenic exposure). In addition, potential confounders (i.e., smoking histories, diet, and exercise) were not examined

on an individual basis, but were compared on a regional basis.

NORTH AMERICA (UNITED STATES and MEXICO)

Bates et al. (1995), in a case-control study, used data obtained from Utah respondents for the 1978 National Bladder Cancer Study to examine the potential relationship between bladder cancer in a U.S. population exposed to measurable levels of arsenic in drinking water. Arsenic levels in drinking water were lower than those in Asian and South American studies. A total of 117 cases and 266 controls were selected as participants for this study. Restricting subjects to those who had lived in study areas for at least half of their lives, the number of subjects still eligible was 71 cases and 160 controls. Arsenic exposures were in the range 0.5-160 ppb (mean, 5.0 ppb). Two measurements of arsenic exposure were used. One measure used was the total CAE and the other was the arsenic concentration ingested adjusted for individual water consumption. Bates et al. (1995) found no association between bladder cancer and either arsenic exposure measure. However, among smokers, positive trends in cancer risk were found for arsenic exposures between 30 to 39 years prior to cancer diagnosis. The risk estimates were stronger for the drinking water measure that estimated the ingested arsenic concentration than the CAE. The risk estimates obtained, however, were higher than predicted based on the results of the Taiwanese studies, which raised concerns by Bates et al. (1995) regarding confounders, bias, and chance.

The data from this study raised the potential that smoking contributes to the increased effect of arsenic on the risk of bladder cancer. Potential confounders included in the logistic models were gender, age, smoking status, years of exposure to chlorinated water, history of bladder infection, and the highest educational level attained. Strengths of the Bates et al. (1995) investigation are that these confounders were controlled for as well as occupation, population size of geographic area, and urbanization were addressed in the analysis, and cases were histologically confirmed. Potential weaknesses of the study are the small size of the study population, the subjects were mostly male and the data on females were inadequate, and that arsenic exposure levels were based on measurements close to the time that cases were diagnosed. Due to the low concentration in the water, the lack of measurement of arsenic in the food was a limitation of this study. Although the purpose of the Bates et al. (1995) study was to compare low-level arsenic exposure and bladder cancer with the results from the Taiwanese population, the results cannot be interpreted without consideration of potential confounders and bias resulting from the retrospective study design.

Employing a retrospective cohort mortality investigation of residents from Millard County, Utah, Lewis et al. (1999) examined the relationship between arsenic exposure from drinking water and mortality outcome. Median drinking water arsenic concentrations for selected study areas ranged from 14 to 166 ppb. Drinking water samples were obtained from public and private sources and were collected and analyzed under supervision of the State of Utah Department of Environmental Quality, Division of Drinking Water. Cohort members were assembled using historical documents made available by the Church of Jesus Christ of Latter-day Saints. Residential histories and median drinking water arsenic concentration were used to construct a matrix for CAE. Previous drinking water arsenic concentrations (from 1964 forward) were obtained from historical records of arsenic measurements maintained by the state of Utah.

Without regard to specific exposure levels, statistically significant increases in mortality from prostate cancer (SMR=1.45, 95% CI: 1.07-1.91) among cohort males was observed. Non-significant increases in mortality for males were observed in cancer of the kidney (SMR=1.75, 95% CI: 0.80-3.32). There was no increase risk for cancer of the bladder and other urinary organs (SMR=0.42, 95% CI: 0.08-1.22) in males. Among cohort females, there was no statistically significant increase in mortality observed. Females did, however, exhibit non-significant increases in mortality from kidney cancer (SMR=1.60, 95% CI: 0.44-4.11) and melanoma of the skin (SMR=1.82, 95% CI: 0.50-4.66). Female cancer of the bladder and other urinary organs (SMR=0.81, 95% CI: 0.10-2.93) was not increased. Risk analysis using low-, medium-, and high-arsenic exposure groups did not provide any clear indication of a dose response for prostate cancer. Confounding was not considered to be a significant concern by Lewis et al. (1999). Exposure to other arsenic sources (food or air-borne), however, may have contributed to the total exposure potential of this population. Strengths of the study included the cohort study design. In this design type, the exposure precedes the effect being measured so a variety of effects from a single type of exposure can be considered. A study weakness was that the study population was mostly rural and Morman (low tobacco and alcohol use). In addition, NRC (2001) identified that Lewis et al.'s (1999) was not powerful enough to estimate risk.

To address the association between skin cancer and arsenic exposure in drinking water, Karagas et al. (2001) used data collected on 587 basal cell and 284 squamous cell skin cancer cases and 524 controls. Cases and controls were interviewed as part of a case-control study conducted in New Hampshire (and bordering regions) between 1993 and 1996. Arsenic exposure levels were determined using toenail clippings. The ORs for SCC (range 0.93-1.10) and BCC (range 0.72-1.06) were not significant and near unity (1.0) in all but the highest category (0.345-0.81 µg/g). For cases with significantly elevated toenail arsenic concentrations, the adjusted ORs were 2.07 (95% CI 0.92-4.66) for SCC and 1.44 (95% CI: 0.74- 2.81) for BCC, compared with those with concentrations at or below the median. Since the risks of SCC and/or BCC were not elevated in the range of toenail arsenic concentrations detected in most study subjects, the authors did not exclude the possibility of a dose-related increase at the highest levels of exposure. Strengths include evaluating the effects of potential confounders such as, age, gender, race, educational attainment, smoking status, skin reaction to first exposure to the sun, and history of radio-therapy. Toenail arsenic concentrations can be considered a strength and a weakness. They are a strength because they individualize the dose and could account for arsenic exposure from other sources (e.g., food), but toenail arsenic concentrations also could be considered a weakness because toenail arsenic is a biomarker of recent past exposure (covering a period of about one year according to Cantor and Lubin, 2007). Some confounding variables were not controlled for and may have influenced the results. The latency of arsenic-induced skin cancer is unknown and, as a result, the follow-up period for this study may have been inadequate.

The identification of a potential leukemia cluster in Churchill County, Nevada, where arsenic levels in water supplies are relatively high, prompted a study by Moore et al. (2002). Using an ecological study design, Moore et al. (2002) examined the incidence of childhood cancer between 1979 and 1999 in all 17 Nevada counties. For analysis, arsenic exposures were grouped into low (<10 ppb), medium (10-25 ppb), and high (35-90 ppb) population-weighted arsenic levels based on the levels obtained from public drinking water. SIRs for all childhood

cancers combined were 1.00 (95% CI: 0.94-1.06) for low-exposure, 0.72 (95% CI: 0.43-1.12) for medium, and 1.25 (95% CI: 0.91-1.69) for high-exposure counties. Moore et al. (2002) found no apparent relationship between the three arsenic levels and childhood leukemia with SIRs of 1.02 (95% CI: 0.90-1.15), 0.61 (95% CI: 0.12-1.79), and 0.86 (95% CI: 0.37-1.70) in the low, medium, and high exposure categories, respectively. No association was found for all childhood cancers, excluding leukemia, with SIRs of 0.99 (95% CI: 0.92-1.07), 0.82 (95% CI: 0.47-1.33), and 1.37 (95% CI: 0.96-1.91), respectively. There was, however, an excess for bone cancers in 5 to 9 year olds and 10 to 14 year olds and an excess in cancer (primarily lymphomas) in 15 to 19 year old young adults in the high-exposure group. The findings in this study showed no increase in leukemia risk at the concentrations of arsenic identified and categorized in the water. Although the results did not eliminate the possibility for increased risks for non-leukemia childhood cancers, there is no reason to suspect that the exposures to low levels of arsenic in the small study group is responsible. Strengths of the study are that the analysis of the data was stratified by age, the study was a low-level arsenic exposure study, and the findings were reported at different arsenic concentrations. Weaknesses of the study include the small study size, the potential for exposure misclassification, and the limitations of the ecologic study design.

Steinmaus et al. (2003) used a case-control study to evaluate the effects of arsenic ingestion on bladder cancer risk in seven counties in the western United States. These counties contain the largest populations historically exposed to arsenic via drinking water at levels of approximately 100 ppb. These populations provided Steinmaus et al. (2003) the opportunity to critically evaluate the effects of relatively low-level arsenic exposure on bladder cancer incidence. Incident bladder cancer cases diagnosed between 1994 and 2000 were recruited based on information obtained from the Nevada Cancer Registry and the Cancer Registry of Central California. Arsenic measurements for community-supplies drinking water within the study were provided by the Nevada State Health Division and the California Department of Health Services. Over 7000 arsenic measurements were obtained. Individuals' data on water sources, water consumption patterns, smoking history, and other sociodemographic factors were obtained for 181 bladder cancer cases and 328 matched controls. There were no observed increased risk for bladder cancer associated with intakes greater than 80 µg/day (OR=0.94, 95% CI: 0.56-1.57; linear trend, p=0.48). This observed OR was below the risk predicted based on higher arsenic concentrations in drinking water studies from Taiwan. However, when the analysis focused solely on previous smokers who had arsenic exposures greater than 80 µg/day (median 177 µg/day) for more than 40 years, the risk was significantly increased (OR=3.67, 95% CI: 1.43-9.42; linear trend, $p< 0.01$). These data provide evidence that smoking and ingesting arsenic at elevated concentrations (i.e., greater than 100 µg/day) may result in an increased risk of bladder cancer. A strength of the Steinmaus et al. (2003) study is the use of individual exposure level data to examine low-dose drinking water arsenic exposure; however, the lack of arsenic exposure from food is a study weakness due to the low levels of exposure through drinking water. In addition, the use of cancer registries allowed for improved case identification. Potential confounders adjusted for in the analysis included gender, age, smoking history, education, occupation associated with elevated rates of bladder cancer, and income. However, bias as the result of next-of-kin interviews may have influenced the exposure assessment. Arsenic exposures from outside the study area also may have influenced the exposure assessment. In the arsenic exposed areas, the percentage of non-participants was 5% higher among cases than controls. This difference probably means that more exposed cases were missed in analyses of

recent exposure, biasing the odds ratio towards the null.

There has been little research investigating the link between arsenic and cutaneous melanoma, although arsenic has been associated with increased risk of non-melanoma skin cancer. Beane-Freeman et al. (2004) performed a case-control study to examine the potential relationship between melanoma and environmental arsenic exposure in a cohort from Iowa. Study participants included 368 cutaneous melanoma cases (selected from 645 eligible cases) and 373 colorectal cancer controls (selected from 732 eligible controls) diagnosed in 1999 or 2000, frequency-matched on gender and age. Participants completed a mailed survey and submitted toenail clippings (obtained from 355 cases and 353 controls) for analysis of arsenic content. The authors identified an increased risk of melanoma in study cases with elevated toenail arsenic concentrations (OR= 2.1, 95% CI: 1.4-3.3; p-trend = 0.001) and an increased risk of melanoma with previous diagnosis of skin cancer and elevated toenail arsenic concentrations (OR=6.6, 95% CI: 2.0-21.9). There was a greater association between the toenail arsenic and melanoma when subjects reported a previous diagnosis of melanoma. Strengths of this investigation include the potential confounders (age, gender, skin color/skin type, prior history of sunburn, education, and occupational exposure) were controlled for in the analysis. Ascertainment of cases and controls was accomplished by using the Iowa Cancer Registry, a Surveillance, Epidemiology, and End Results Program registry. This allowed newly diagnosed melanoma cases to be identified for a specific period and assured a greater degree of certainty regarding the accuracy of diagnosis. Another strength is that toenail arsenic concentrations individualize the exposure and accounts for arsenic exposure from other sources. A limitation of this study was that toenail samples were collected 2-3 years after diagnosis and therefore does not measure arsenic concentrations prior to diagnosis, resulting in possible exposure misclassification.

Karagas et al. (2004) used a case-control study design to examine the effects of low-level arsenic exposure on the incidence of bladder cancer in New Hampshire (and bordering regions), where levels above 10 ppb are commonly found in private wells. The authors studied 383 cases of transitional cell carcinoma of the bladder, diagnosed between July 1, 1994 and June 30, 1998, and 641 general population controls. Individual exposure to arsenic was determined through the use of toenail clippings. Karagas et al. (2004) found arsenic concentrations ranged from 0.014-2.484 µg/g among bladder cancer cases and 0.009-1.077 µg/g among controls. When stratified by smoking history, toenail arsenic concentrations were not associated with the risk of bladder cancer. However, among smokers in the uppermost category of arsenic exposure, an elevated OR for bladder cancer was observed (OR: 2.17, 95% CI: 0.92–5.11 for >0.330 µg/g compared to < 0.06 µg/g). When Karagas et al. (2004) stratified their analysis by duration of current water system usage (<15 years and ≥15 years), an increased bladder cancer OR for ever smokers with the highest category of arsenic exposure with less than 15 years of use was identified (<15 years, OR=3.09, 95% CI: 0.80-1.19; ≥15 years, OR=1.86, 95% CI: 0.57-6.03). These data suggest that ingestion of low to moderate arsenic levels may affect bladder cancer incidence and that cigarette smoking may act as a co-carcinogen. Strengths of the study include using a stratified analysis to evaluate the potential that an extended latency period was required for bladder cancer development and minimizing misclassification by using biomarkers. The following potential confounders were adjusted for: age, gender, race, educational attainment, smoking status, family history of bladder cancer, study period and average number of glasses of tap water consumed per

day. Toenail clippings were used in an attempt to minimize misclassification. This however, is a limitation because it only measures recent past exposures. Limitations of the study were that misclassification at the lower exposures was possible and that
lifetime exposure could not be calculated since data from previous residences could not be determined. In addition, there was limited data at extreme ends of exposure.

The Lamm et al. (2004) ecologic study investigated the association between arsenic exposure from drinking water and bladder cancer mortality in 133 counties in the United States. Caucasian male county-specific bladder cancer mortality data between 1950 and 1979 and county-specific groundwater arsenic concentration data were obtained for counties solely dependent on groundwater for their public drinking water supply. Arsenic exposure was based on measurements for at least 5 wells for each county. No arsenic-related increase in bladder cancer mortality (SMR=0.94, 95% CI: 0.90-0.98) was identified (arsenic exposure range: 3-60 ppb) using stratified analysis and regression analyses. These findings are consistent with other previously published U.S. studies. Strengths of the study include the large nationwide study population, which included more than 75 million person-years of observation. Weaknesses, however, are the lack of available individual exposure data, the assumption that study participants consumed only local drinking water, the assumption that available data was representative of actual arsenic content in the water, arsenic contribution from food sources were not analyzed, and the analysis did not directly adjust for smoking, urbanization, or industrialization.

The Wisconsin Division of Public Health in July 2000 through January 2002 conducted a cross-sectional study in 19 rural Wisconsin townships concerning private drinking-water wells and arsenic exposure (Knobeloch et al., 2006). Residents in these townships were asked to collect well-water samples and complete a questionnaire regarding residential history, consumption of drinking-water, and family health. In Wisconsin, skin cancer is not reportable; therefore, no skin cancer registry data were available. During the study, 2233 private wells were tested, and 6669 residents provided information on water consumption and health. Water arsenic levels ranged from less than 1.0 to 3100 ppb. The median arsenic level was 2.0 ppb. Eighty percent of the wells had arsenic levels below 10 ppb, but 11% had an arsenic level of above 20 ppb. Age-, gender-, and smoking-adjusted ORs of residents 35 years of age and older who had consumed water with arsenic levels greater than 1.0 ppb for at least 10 years showed a significant increase in individuals who reported skin cancer compared to those whose water arsenic levels were less than 1.0 ppb (arsenic 1.0-9.9 ppb OR=1.81, 95% CI: 1.10-3.14). Similarly, adults whose well-water reportedly contained arsenic concentrations greater than 10 ppb were significantly more likely to report skin cancer than those whose water arsenic levels were less than 1.0 ppb (OR=1.92, 95% CI: 1.01-3.68). Tobacco use also was associated with higher rates of skin cancer and may synergistically with arsenic exposure affect the development of skin cancer. Strengths of the study include: 1) the large sample size; 2) a history of individual tobacco use; 3) arsenic well water analysis for each household; 4) an exposure duration of at least 10 years in participants who consumed water from the tested wells; and 5) the analysis controlled for age, gender, and tobacco use. Weaknesses include: 1) skin cancers were self-reported and not confirmed by a medical records review; 2) few people could provide information about specific types of cancer; 3) potential bias could have resulted from the participating families being concerned about arsenic exposure; 4) sun exposure and occupation

were not controlled for in the analysis; and 5) food sources of arsenic were not considered.

Meliker et al. (2007) performed an ecologic study in a contiguous six county study area of southeastern Michigan to investigate the relationship between moderate arsenic levels (10-100 ppb) and selected disease outcomes. This region of southeastern Michigan was chosen because it had moderately high arsenic concentrations in the ground water and low rates of migration. The six counties had a population-weighted mean arsenic concentration of 11.00 ppb and a population-weighted median of 7.58 ppb. In comparison, the remainder of Michigan has a population-weighted mean of 2.98 ppb with a median of 1.27 ppb. SMRs for cancers were not significantly different from the age- and race-adjusted expected values for males or females for the state of Michigan (SMR skin melanoma female=0.97, 95% CI: 0.73-1.27, melanoma male=0.99, 95% CI: 0.79-1.22; SMR bladder female=0.98, 95% CI: 0.80-1.19, bladder male=0.94, 0.82-1.08; SMR kidney female=1.00, 95% CI: 0.80-1.20, kidney male=1.06, 95% CI: 0.91-1.22; SMR trachea, lung, bronchus female=1.02, 95% CI: 0.96-1.07, trachea, lung, bronchus male=1.02, 95% CI: 0.98-1.06). The only exception was cancer of the female reproductive organs (SMR=1.11, 95% CI: 1.03-1.19). The potential explanations for the lack of significant cancer findings were the relatively low level of arsenic in the groundwater of southeastern Michigan, which may be below the threshold for cancer induction and other moderating factors that were not considered by this study (i.e., food as a source of arsenic exposure). Strengths include that mortality rates, which were gathered from Michigan Resident Death Files for a 20 year period, were stratified by gender, age, and race. Weaknesses include that the ecologic study design did not provide individual arsenic exposure data and may not permit the detection of significant risk; possible differences in reporting and classification of underlying causes of death; case migration occurred; preferential sampling was conducted based on home owners' request; arsenic contribution from food was not measured; and there was a lack of information concerning smoking history and obesity.

CHINA

Using an ecologic study design, Lamm et al. (2007) conducted dermatological examinations for 3179 of the 3228 (98.5%) residents of three villages (i.e., Zhi Ji Liang, Tie Men Geng, and Hei He) in Huhhot, Inner Mongolia with well water arsenic levels that ranged from undetectable (<10 ppb) to 2000 ppb. Individual water consumption histories were obtained for this population, and arsenic levels were measured for 184 wells. Arsenic exposures were summarized as the highest arsenic concentration (HAC) and CAE. Thirty-five percent of the study population had HAC of less than 50 ppb, 86% had HAC less than 150 ppb, and only 1% of the participants had HAC greater than 500 ppb. The proportion of females to males was similar in each of the three villages (female range 49-50% and male range 50-51%), and almost all study subjects identified themselves as being of Chinese (99.8%) rather than Mongolian (0.2%) origin. The median age for all participants was 29 years; however, participants from Hei He tended to be older than those from the other two villages (>30 years, Hei He-55.0%; Zhi Ji Liang and Tie Men Geng-42.4%). Participants (female or male) who reported occupations listed student or farmer. None of the examinations revealed any evidence of BFD. Analyses included frequency-weighted, simple linear regression, and most likely estimate models. Eight people were found to have skin cancer. In addition to skin cancer, these 8 cases also had both hyperkeratoses and dyspigmentation. Skin cancer cases were only identified in those participants with HAC

exposures >150 ppb or whose CAE was less than 1000 ppb-years. The models showed a threshold of 122-150 ppb. Lamm et al. (2007) identified a general exposure-prevalence pattern (higher prevalence for HAC exposure group) for skin disorders (hyperkeratosis, dyspigmentation, and skin cancers). Duration of water usage (arsenic exposure), age, latency, and misclassification did not appear to markedly affect the analysis. Strengths of the study include the large study population, the HAC and CAE were used in the analyses, and that arsenic concentrations were measured in 184 wells. Confounders that were controlled for included age and differences in cumulative arsenic dose, and duration of exposure. A confounder not adjusted for in the analysis was sun exposure. An additional weakness of the investigation is the ecological study design, and the potential for recall or misclassification bias resulting from the collection of arsenic exposure histories through interviews.

FINLAND

In a case-cohort study, Kurttio et al. (1999) examined the levels of arsenic in Finnish water wells and its relationship to the risk of bladder and kidney cancers. The study population consisted of 61 bladder cancer cases and 49 kidney cancer cases diagnosed between 1981 and 1995, and a randomly selected age- and gender-adjusted reference cohort of 275 subjects. Arsenic exposure for cancer cases and for the reference cohort for two periods was estimated. The first period was from the third to ninth calendar years (the shorter latency period) prior to either the cancer diagnosis or the respective year for referent cohort, while the other was from the tenth or earlier calendar years (the longer latency period). Water specimens were obtained from the wells used by the study cohort from 1967-1980. The arsenic concentrations in the wells of the control population were low with approximately 1% exceeding 10 ppb. Bladder cancer was associated with arsenic concentration and daily dose during the third to ninth calendar years prior to the cancer diagnosis. The risk ratios for arsenic exposure concentration categories 0.1-0.5 and >0.5 ppb relative to the category with <0.1 ppb were 1.53 (95% CI: 0.75-3.09) and 2.44 (95% CI: 1.11-5.37), respectively. In spite of low levels of arsenic exposure, Kurttio et al. (1999) found evidence of a relationship between exposure to arsenic at the higher exposure level and bladder cancer risk. No association, however, was observed between arsenic exposure level and kidney cancer risk. Strengths include the accessibility of Finnish Cancer Registry records; the 1985 Population Census file of the Statistics Finland was used to identify areas in which less than 10% of the population used the municipal water supply; and that age, gender and smoking histories were accounted for in the risk ratio calculations. Possible weaknesses include misclassification and recall bias resulting from the study choosing to use water consumption from the 1970s. In addition, because of the low arsenic concentrations, arsenic exposure from other sources (e.g., food) could bias the results.

Michaud et al. (2004) used a cohort (nested case-control) study design to investigate the relationship between arsenic levels in toenail and bladder cancer risk among Finnish male smokers aged 50–69 years who were participating in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Data for 280 incident bladder cancer cases, identified between 1985–1988 and April, 1999, were available for analysis. Controls (n=293) were matched to each case on the basis of age, toenail collection date, intervention group, and duration of smoking. Logistic regression analyses were performed to estimate ORs. Arsenic toenail concentrations in this Finnish study (cases and controls) ranged between 0.01 and 2.11 µg/g, with one control

outlier at 17.5 µg/g. Arsenic toenail concentrations were similar to those reported in the United States (range: 0.02-17.7 µg/g). Men were categorized into quartiles based on the distribution of arsenic among the controls (<0.050, 0.050-0.105, 0.106-0.161, and >0.161). The study observed no significant relationship between arsenic concentration and bladder cancer risk (OR=1.13, 95% CI: 0.70-1.81 for the highest vs. lowest quartile). Strengths of the Michaud et al. (2004) study were that they excluded toenail samples with non-detectable arsenic levels greater than 0.09 µg/g, in an attempt to avoid potential misclassification of samples with high detection limits, and potential confounders were controlled for in the analysis (i.e., smoking history, beverage intake, place of residence, toenail weight, smoking cessation, smoking inhalation, educational level, beverage intake, and place of residence). Cases and controls were matched according to age, toenail collection date, intervention group (alpha tocopherol and beta carotene), and smoking duration. Toenail arsenic concentrations are a strength because they individualize the dose and could account for arsenic exposure from other sources, but toenail arsenic concentrations also could be considered a weakness because toenail arsenic is a biomarker of recent past exposure (covering about one year according to Cantor and Lubin, 2007). The weakness of the study was that water consumption was not included in the total beverage intake variable.

DENMARK

The Basstrup et al. (2008) cohort study was designed to determine whether exposure to low levels of arsenic in drinking-water in Denmark is associated with an increased risk for cancer. The study population was selected from participants of the prospective Danish cohort Diet, Cancer and Heath. A cohort of 56,378 people (39,378 from Copenhagen and 17,000 from Aarhus) accepted an invitation to participate in the study. Cancer cases were identified in the Danish Cancer Registry, and the Danish civil registration system was used to trace residential addresses of the cohort members. The study used a geographical information system to link residential addresses with water supply areas and using this information estimated arsenic exposure by addresses. The average arsenic exposure for the cohort ranged between 0.05 and 25.3 ppb (mean=1.2 ppb) and was based on 4954 measurements reported between 1987 and 2004 (the majority between 2002 and 2004). The exposure was generally higher among Aarhus participants than those enrolled in the Copenhagen area (Aarhus mean=2.3 ppb, min=0.09 ppb and max=25.3 ppb; Copenhagen mean=0.7 ppb, min=0.05 ppb, and max=15.8 ppb). Regression models were used to analyze possible relationships between arsenic and cancer. The study found no significant association between arsenic exposure and risk for cancers of the lung, bladder, liver, kidney, prostate, colon, or melanoma skin cancer. The incidence rate ratio (IRR) for non-melanoma skin cancer (0.88, 95% CI: 0.84-0.94) decreased with per ppb increases in the time-weighted average exposure to arsenic. The study did identify a significant increased risk for breast cancer in association with time-weighted average exposure to arsenic (IRR=1.05, 95% CI: 1.01-1.10). Strengths of the study include the large study population, the socioeconomic/demographic similarities of the cohort, and the adjustment for potential confounders (smoking, alcohol consumption, education, body mass index [BMI], daily intake of fruits/vegetables, red meat, fat and dietary fiber, skin reaction to the sun, hormone replacement therapy use, reproduction, occupation, and enrollment area). Weaknesses of the study include the low arsenic levels in Danish drinking water, the lack of information on other sources of arsenic exposure, and the inability to assess arsenic exposures before 1970, all resulting in

possible misclassification bias.

AUSTRALIA

Hinwood et al. (1999) conducted an ecologic study that investigated areas of Victoria Australia with elevated environmental arsenic concentrations, areas with arsenic concentrations in the soil of more than 100 mg/kg and/or drinking water arsenic concentrations greater than 10 ppb, and the relationship with cancer incidence. SIRs for cancer were generated for 22 areas between 1982 and 1991 using cancer registry data. In addition, SIRs for combined areas according to environmental exposure (high soil and/or high water arsenic concentrations, etc.) were generated. The SIRs (females and males together) for the combined 22 areas were significantly elevated for all cancers (1.06, 95% CI: 1.03-1.09), melanoma (1.36, 95% CI: 1.24-1.48), chronic myeloid leukemia (1.54, 95% CI 1.13-2.10), breast cancer in females (1.10, 95% CI: 1.03-1.18) and prostate cancer in males (1.14, 95% CI: 1.05-1.23). The SIR for kidney cancer (females and males combined) was 1.16 (95% CI: 0.98-1.37), and although elevated was not statistically significant. When stratified by exposure category, the SIR for prostate cancer was significant at 1.20 (95% CI: 1.06-1.36) for the high soil/high water category only. This result was likely confounded by misclassification (level of population exposure) and limited by low statistical power. There was no significant dose-response relationship observed between drinking water and any individual cancer. Strengths of the study include that water and soil arsenic levels were provided and a large area was examined. Hinwood et al. (1999) recognized that the results of this study were potentially confounded by a number of factors including the ecologic study design, socioeconomic status, race, occupation, and urban versus rural status. Due to the low concentrations in the drinking water, the lack of arsenic exposure from food could cause exposure misclassification.

4.2 PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL

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 DMA^V (a major metabolite of inorganic arsenic) in their drinking water for 104 weeks had no effect 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 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 generally 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 species.

4.2.2.1 Mice—Transplacental

Timed pregnant female C3H mice (10/group) were administered 0 (control), 42.5, or 85 ppm As^{III} 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 HCC (12.5% in the control group versus 38.1% in the 42.5-ppm group and 60.9% in the 85-ppm group) and adrenal cortical tumors (37.5% in the control group versus 66.6% in the 42.5-ppm group and 91.3% in the 85-ppm group), 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 HCC (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 were 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 the control group versus 26% in the 42.5-ppm group and 37.5% in the 85-ppm group). 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 occurring at both doses. Females also had a dose-related increase in hyperplasia of the oviduct. The number of both tumor-bearing and malignant tumor-bearing males was significantly increased in both dose groups and followed a significant ($p=0.0006$ and 0.0001, respectively), dose-related trend. Female animals had a slight increase in the number of tumors, 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/or multiplicity of hepatocellular adenomas and carcinomas in treated males, both in the absence and presence of TPA. In the absence of TPA, the incidence of adenomas was 41.7, 52.2, and 90.5% for the 0-, 42.5-, and 85-ppm exposure groups, respectively; the incidence of carcinomas was 12.5, 34.8, and 47.6%, respectively; total incidence was 50, 60.9, and 90.5%, respectively; and multiplicity was 0.75, 1.87, and 2.14, respectively. In the presence of TPA, the incidence of adenomas was 34.8, 52.2, and 76.2% for the 0-, 42.5-, and 85-ppm exposure groups, respectively; the incidence of carcinomas was 8.7, 26.0, and 33.3%, respectively; total incidence was 39.1, 65.2, and 85.7%, respectively; and multiplicity was 0.61, 1.44, and 2.14, respectively. A statistically significant increase was noted at 85 ppm. 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.%, 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.

Waalkes et al. (2006a) used female CD1 mice, which have a low rate of spontaneous tumors. Thirty-five percent (12/34) of female offspring receiving 85 ppm of As^{III} via the dams' drinking water on gestational days 8 to 18 developed urogenital tumors with 9% being malignant compared to 0% in the controls.

4.2.2.2 Rat—Oral

Soffritti et al. (2006) administered male and female Sprague-Dawley rats 0, 50, 100, or 200 mg/L (i.e., ppm) of sodium arsenite via the drinking water for 104 weeks. There was a consistent dose-dependent decrease in water and food consumption accompanied by a dose-related decrease in body weight (there was no difference in body weight in females administered 50 mg/L). There was only a slight decrease in survival in male rats administered 100 or 200 mg/L beginning at 40 weeks of age. Females only had a decrease in survival rate after 104 weeks of age. Males and females administered 100 mg/L had an increase in the number of tumor bearing animals and in the number of tumors. Although there is no dose-related trends in tumors, there were sporadic benign and malignant tumors of the lung, kidney, and bladder observed in treated rats that are extremely rare in the authors' extensive historical controls. These tumors consisted of adenomas and carcinomas of the lung, adenomas and carcinomas of the kidney, papillomas and one carcinoma of the renal pelvis transitional cell epithelium, and one carcinoma of the bladder transitional cell epithelium.

Wei et al. (1999 and 2002) demonstrated that 10-week-old male F344/DuCrj rats (36/group) administered 50 or 200 ppm DMA^V 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 bromodeoxyuridine (BrdU) labeling of morphologically normal epithelium of the bladder in the 50- and 200-ppm groups (Wei et al., 2002). There was no significant increase in any other tumor type related to DMA^V 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^V-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 As, to male F344 rats for 2 years via their drinking water at concentrations of 0, 50, or 200 ppm. Total 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.

4.2.2.3 Other

Transgenic models also have been developed to examine arsenic carcinogenesis. Arsenic exposure (200 ppm sodium arsenite in drinking water for 4 weeks) in Tg.AC transgenic mice containing activated H-ras did not induce skin tumors alone; however, the group of mice that were administered arsenic and a subsequent skin painting with TPA showed an increase in the number of papillomas compared to mice treated with TPA alone. Thus, it was suggested that arsenite may be a "tumor enhancer" in skin carcinogenesis (Germolec et al., 1997 and 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. Mice were also administered 1.7 kJ/m^2 solar ultraviolet radiation (UV), which is considered a low, nonerythemic dose, 3 times weekly, either with or without sodium arsenite exposure. Results demonstrated a 2.4-fold increase in the yield of skin tumors for mice exposed to both sodium arsenite and UV than in mice administered UV alone. A second experiment by the same group (Burns et al, 2004), demonstrated a 5-fold increase in skin tumors using 5 mg/L As^{III} with 1 kJ/m^2 solar UV, but also observed a significant increase with 1.25 mg/L As^{III} with 1 kJ/m^2 solar UV. The skin tumors (mainly SCCs) occurred earlier, were larger, and more invasive in mice administered As^{III}. Arsenite alone did not induce skin tumors. Rossman (2003) concluded that this demonstrates that arsenite enhances the onset and growth of malignant skin tumors induced by a genotoxic carcinogen in mice. Rossman (2003) also suggested 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

Not addressed in this draft.

4.4 OTHER STUDIES

4.4.1 Possible Modes of Action (MOAs) and Key Events of Possible Importance

As discussed in Section 3.3, the metabolism of inorganic As in humans occurs through alternating steps of reduction and oxidative methylation mostly to DMA^V. Many of the metabolites have been subjected to a variety of toxicological tests *in vivo* and *in vitro*, and they often differ considerably in their toxicological responses. The relative contributions of the many different forms of arsenic to the toxicity and carcinogenicity of inorganic As are uncertain. Each of the arsenical metabolites exhibits its own pattern of toxicity, possibly via similar and/or separate MOAs that together are responsible for inorganic arsenic toxicity and tumor formation (Kitchin, 2001).

The biotransformation and pharmacodynamics of inorganic As are complex in mammals, with inorganic As being biotransformed through a complex cycle of reduction, oxidation, and methylation steps to form the trimethylated TMAO metabolite, and possibly its reduced form, trimethylarsine, which may not be of consequence in humans. Arsenical forms of greater instability (i.e., trivalent forms) are produced within each step, and those forms have greater reactivity toward biological and biochemical intermediates and biological macromolecules. The trivalent species MMA^{III} and DMA^{III} have been identified as the most toxic and genotoxic forms in several assay systems (Thomas et al., 2001). Each intermediate arsenical form, however, has the potential to induce cancer or to affect the promotion and progression of cancer, such as by disrupting signal transduction pathways and gene expression. Many of these forms have been detected in the urine of humans exposed to inorganic As and in rodents exposed to inorganic and organoarsenicals. Through the process of metabolizing arsenic, cells and organs are exposed to mixtures of these intermediates, which bring to the forefront potential synergistic interactions between them that could enhance the tumorigenesis process.

Inorganic As has been demonstrated to cause tumors in humans at multiple sites (bladder, lung, skin, liver, and possibly kidney). Rodents are generally much less sensitive to the tumorigenic effects of inorganic arsenic, except for a few recent transplacental mouse studies where As^{III} caused liver, lung, ovarian, and/or adrenal cortical tumors (Waalkes et al., 2003, 2004a, and 2006a). Currently, there is insufficient information to fully explain the differences between human and rodent sensitivity to arsenic carcinogenicity.

Based on its extensive review of health consequences of inorganic As in drinking water, NRC (1999) concluded that,

- "The mode of action for arsenic carcinogenicity has not been established. Inorganic arsenic and its metabolites have been shown to induce deletion mutations and chromosomal alterations (aberrations, aneuploidy, and SCE [sister chromatid exchange]), but not point mutations. Other genotoxic responses that can be pertinent to the mode of action for arsenic carcinogenicity are co-mutagenicity, DNA methylation, oxidative stress, and cell proliferation; however, data on those genotoxic responses are insufficient to draw firm conclusions. The most plausible and generalized mode of action for arsenic carcinogenicity is that it induces structural and numerical chromosomal abnormalities without acting directly with DNA."

- "For arsenic carcinogenicity, the mode of action has not been established, but the

several modes of action that are considered most plausible (namely, indirect mechanisms of mutagenicity) lead to a sublinear dose-response at some point below the level at which a significant increase in tumors is observed. However, because a specific mode (or modes) of action has not been identified at this time, it is prudent not to rule out the possibility of a linear response."

Several of their other concluding statements drew attention to the possible importance of ROS to several health effects caused by arsenic and suggested that, "intracellular production of ROS might play an initiating role in the carcinogenic process by producing DNA damage." (NRC, 1999). At the time of the NRC (1999), report the prevailing view was that metabolism of inorganic As through several methylated forms represented a detoxification pathway. One of the fundamental changes in thinking about the effects of inorganic As since the NRC (1999) report has been the growing awareness that some of those metabolites (specifically, MMA^{III} and DMA^{III}) can have especially high levels of toxicity. Thus, metabolism also represents a toxification pathway. Regardless, when there is a steady influx of inorganic As into the body as through continual exposure from drinking water, metabolism is essential to eliminate that arsenic, including the highly reactive As^{III}, from the body.

In 2001, NRC produced an update to its major review on inorganic As in drinking water. It summarized, in tabular format, the mechanistic studies completed since 1998 and included a discussion of them. It focused on experiments that appeared to induce biochemical effects at moderate to relatively low concentrations of arsenic *in vitro* (e.g., less than 10 μM); however, some studies that used higher concentrations were included for comparative purposes. The focus was on moderate to relatively low-dose studies because it was felt that studies that required arsenic concentrations greater than 10 μM to produce a biological response *in vitro* would be less likely to be relevant to the health effects related to chronic ingestion of arsenic in drinking water. NRC (2001) concluded that: "The mechanistic studies reviewed herein and those reviewed previously in the 1999 NRC report suggest that trivalent arsenic species (primarily As^{III}, MMA^{III}, and, possibly, DMA^{III}) are the forms of arsenic of greatest toxicological concern." They estimated concentrations of arsenic that could be expected in human urine from the known human experience and concluded that, "Arsenite concentrations in excess of 10 μM generally exceed concentrations that can occur in the urine of individuals chronically exposed to arsenic in drinking water and have less direct relevance to understanding the modes of action responsible for human cancer induced by this route of exposure." They also stated that,

- "Experiments in animals and *in vitro* have demonstrated that arsenic has many biochemical and cytotoxic effects at low doses and concentrations that are potentially attainable in human tissues following ingestion of arsenic in drinking water. Those effects include induction of oxidative damage to DNA; altered DNA methylation and gene expression; changes in intracellular levels of murine double minute 2 proto-oncogene (mdm2) protein and p53 protein; inhibition of thioredoxin reductase (TrxR; MMA^{III} but not As^{III}); inhibition of pyruvate dehydrogenase; altered colony-forming efficiency; induction of protein-DNA cross-links; induction of apoptosis; altered regulation of DNA-repair genes, thioredoxin, glutathione reductase, and other stress-response pathways; stimulation or inhibition of normal human keratinocyte cell proliferation,

depending on the concentration; and altered function of the glucocorticoid receptor."

Despite the extensive research on MOA up to that time, they stated that, "the experimental evidence does not allow confidence in distinguishing between various shapes (sublinear, linear, or supralinear) of the dose-response curve for tumorigenesis at low doses."

The present review uses the terms "*mode of action*" and "*key event*" as they are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). According to EPA, "'*mode of action*' is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. A '*key event*' is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element. Mode of action is contrasted with '*mechanism of action*', which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose, but are not part of the mode of action as the term is used here. There are many examples of hypothesized modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression."

Tables were compiled in order to make a large amount of information on the biological effects of inorganic As readily available. Appendix C contains Tables that deal with (1) *in vivo* human studies (Table C-1), (2) *in vivo* experiments on laboratory animals (Table C-2), and (3) *in vitro* studies (Table C-3). An attempt was made to include in these tables as many experiments published from 2005 through August of 2007 as possible. Numerous earlier experiments have been included as well, based on various selection criteria, which included: being mentioned in the SAB Arsenic Review Panel comments of July of 2007 (SAB, 2007) or in NRC (NRC, 2001); or inclusion in an earlier draft that lacked tables (U.S. EPA, 2005c). The tables provide information on the (a) arsenic species tested, (b) the cell types, tissues, or species tested, (c) all concentrations or doses tested, (d) all durations of exposure, (e) estimates of the LOEC or LOEL (i.e., lowest observed effect concentration or level), (f) a summary of the most important results of each study, and (g) the citations. The 22 categories into which the hypothesized key events are grouped in those tables are listed in column 1 of Table 4-1, and the number of data rows under each category provide an estimate of the amount of available data pertaining to each category topic. Data from a single publication are sometimes entered under multiple event categories. For example, the results in Wang et al. (1996) are summarized in rows under the categories for Apoptosis, Cytotoxicity, and Effects Related to Oxidative Stress (ROS).

When judging the possible relevance of *in vitro* experiments or *in vivo* laboratory animal experiments on human health, it is useful to keep in mind that the total concentration of As^{III} and As^{V} in drinking water pumped from tube wells in Bangladesh (as an example of one country with high exposures to inorganic As in drinking water) ranges from 20 to over 2000 ppb As (i.e., 0.3 to 27 µM). In people exposed at those high levels, total blood arsenic levels range from 0.5 to 1.2 µM (Snow et al., 2005), and total arsenic concentrations in urine would probably not exceed 10 µM (NRC, 2001).

Table 4-1. Summary of Number of Rows Derived from Peer-reviewed Publications for Different Hypothesized Key Events*

Hypothesized Key Events	Number of Rows in Tables		
	In Vivo Human Studies (Table C-1)	*In Vivo* Experiments Using Laboratory Animals (Table C-2)	*In Vitro* Experiments (Table C-3)
Aberrant Gene or Protein Expression[a]	6	32	124
Apoptosis	1	6	78
Cancer Promotion	0	3	3
Cell Cycle Arrest or Reduced Proliferation	0	1	29
Cell Proliferation Stimulation	0	18	21
Chromosomal Aberrations and/or Genetic Instability	13	3	83
Co-carcinogenesis	0	2	3
Co-mutagenesis	0	1	21
Cytotoxicity	0	2	118
DNA Damage	5	6	35
DNA Repair Inhibition or Stimulation	2	0	11
Effects Related to Oxidative Stress (ROS)	2	30	69
Enzyme Activity Inhibition	0	0	5
Gene Amplification	0	0	5
Gene Mutations	1	2	7
Hypermethylation of DNA	2	1	2
Hypomethylation of DNA	1	2	7
Immune System Response	1	0	46
Inhibition of Differentiation	0	0	13
Interference with Hormone Function	0	1	7
Malignant Transformation or Morphological Transformation	0	0	13
Signal Transduction	1	2	51

* Details of the studies are presented in Appendix C.

[a]Some hypothesized key events are shown in boldface to emphasize that in at least one of the tables they contain much more data than the other categories.

4.4.1.1 *In Vivo* Human Studies

Table C-1 summarizes *in vivo* human studies. Here and elsewhere in the consideration of human studies there was particular interest in the subset of people who develop skin lesions (usually keratoses, which are often considered premalignant, or hyperpigmentation) following long-term exposure to inorganic As in drinking water. Indeed four of the six studies related to the hypothesized key events of Aberrant Gene or Protein Expression compared groups of people with and without arsenic-related skin lesions following similar exposures to high levels of inorganic As in drinking water, and in three cases, they also compared them to groups of people with much lower inorganic As exposure levels. The genomics study by Argos et al. (2006) showed that there were 312 additional genes down-regulated in the group with skin lesions than in the inorganic As-exposed group without such lesions. No genes were shown to be up-regulated. Other studies showed increased levels of the EGFR-ECD protein (i.e., extracellular domain of the epidermal growth factor receptor) in serum (Li et al., 2007), increased levels of

transforming growth factor alpha (TGF-α) protein in bladder urothelial cells (Valenzuela et al., 2007), and decreased levels of 3 integrins in and around skin lesions following exposures to inorganic As in drinking water (Lee et al., 2006b). Integrins are important in the control of differentiation and proliferation of the epidermis. Many skin diseases, including arsenical keratosis, show altered patterns of integrin distribution and expression. In the first two instances, there were bigger increases in the group with skin lesions. The study on integrins only made comparisons to a control group. One of the other studies showed a decrease in the concentration of the receptor for advanced glycation end products (RAGE) protein in sputum when there was a higher concentration of inorganic As in the urine (Lantz et al., 2007). Changes in that biomarker are related to several chronic inflammatory diseases in the lung, including lung cancer. The remaining study showed that two oncogenes were up-regulated in tumor tissues in patients with As-related urothelial cancer, but not in those from patients with non-As-related urothelial cancer (Hour et al., 2006).

The category of Chromosomal Aberrations and/or Genetic Instability has the most entries in the table on human studies. Although some of the studies found no effects (usually on SCE induction) in people exposed to inorganic As, most of the studies included in the table showed clear increases of chromosomal aberrations (CA) in lymphocytes, micronuclei (MN; in various cell types), or both CA and MN in people who had been exposed to high levels of inorganic As in drinking water or to Fowler's solution (i.e., a solution containing 1% arsenic that was commonly used as a medicine in the 1800s and early 1900s). Arsenic was shown to increase the incidence of MN specifically in bladder cells (Warner et al., 1994; Moore et al., 1996; and Moore et al., 1997b). There also was suggestive evidence that some arsenic-induced MN (a minority of them) result from aneuploidy (Moore et al., 1996). There was some evidence for induction of SCE. Three of the papers showed that those persons with arsenic-induced skin lesions had higher frequencies of induced chromosomal damage seen either as CA or MN than those without lesions (Gonsebatt et al., 1997; Ghosh et al., 2006; and Banerjee et al., 2007). It is intriguing that one of the studies demonstrated an apparent predisposition to both skin lesions and CA that was correlated with (and was thus perhaps caused by) a single polymorphism of the ERCC2 (excision repair cross-complementing rodent repair deficiency gene, complementation group 2) gene, which plays a key role in the nucleotide excision repair (NER) pathway. The polymorphism resulted from an A→C mutation at codon 751 that caused a change from lysine to glutamine, and the allele conferring the higher predisposition in homozygotes had the remarkably high gene frequency of 0.40 in that population (Banerjee et al., 2007). Although only some of the homozygotes heavily exposed to inorganic As in drinking water developed skin lesions or had chromosomal aberrations, those that were affected had both endpoints.

Table C-1 also provides data showing that oral inorganic As exposure increases DNA damage. Two papers reported oxidative damage to DNA revealed by increases in the concentration of 8-hydroxydeoxyguanosine (8-OHdG) in the urine. Both studies were in Japan, with the first showing a positive correlation between urinary concentrations of arsenic and 8-OHdG after analyzing samples from 248 people in the general population (Kimura et al., 2006). The other study (Yamauchi et al., 2004) involved clinical examination of 52 patients following an incident in which 63 people (4 of whom died within about 12 hours after being poisoned) were poisoned by eating food contaminated with ATO. Those 52 patients were followed up for various effects including levels of 8-OHdG in urine. Maximal levels of ~150% compared to

normal Japanese levels were reached 30 days after the exposure, and by 180 days the levels had returned to normal. The same paper reported that people in Inner Mongolia, China, who drank water contaminated with about 130 ppb As had a significant increase in urinary 8-OHdG, which returned to normal after they drank "low-arsenic" water for one year.

Table C-1 includes data that demonstrate DNA damage (i.e., single-strand breaks) detected by the single cell gel electrophoresis (SCGE) comet assay. One of those studies, in which the high-exposure group drank water containing about 247 ppb As, also included a comet assay combined with formamidopyrimidine-DNA glycosylase (FPG) digestion and thereby showed that arsenic also induced oxidative base damage. (Digestion with the FPG enzyme breaks the DNA at the sites of oxidative damage so that those sites are seen in this modified comet assay.) Besides looking at baseline DNA damage, the other comet study investigated the capacity of the lymphocytes of subjects who used drinking water containing 13–93 ppb As to repair damage induced by an *in vitro* challenge with the mutagen 2-acetoxyacetylaminofluorene (2-AAAF). Adducts formed following treatment with 2-AAAF are primarily repaired through the NER pathway and lymphocytes from arsenic-exposed individuals had more adducts. The lymphocytes from the people with high-arsenic exposure had reduced NER ability (Basu et al., 2005). The remaining DNA damage study (Mo et al., 2006) used 8-oxoguanine DNA glycosylase (OGG1) expression as an indicator of oxidative-induced DNA damage. The OGG1 gene codes for an enzyme involved in base excision repair (BER) of residues that result from oxidative damage to DNA. OGG1 expression was found to be closely linked to the levels of arsenic in drinking water and in toenails, thereby indicating a link between ROS damage to DNA and inorganic As exposure. An inverse relationship between OGG1 expression and selenium (Se) levels in toenails was found, which suggests a possible protective effect of Se against arsenic-induced oxidative stress. As was often the case when populating the MOA tables in Appendix C, some studies could equally well be placed into one or another hypothesized key event category, and clearly some studies listed here under DNA Damage also relate to the hypothesized key events of DNA Repair Inhibition or Stimulation and Effects Related to Oxidative Stress (ROS).

In another polymorphism study, homozygotes for two different alleles of the p53 gene were shown to be at higher risk (than those carrying other alleles) of developing arsenic-induced keratosis among individuals who used drinking water that contained roughly 180 ppb arsenic (De Chaudhuri et al., 2006). Because that gene is so important in controlling apoptosis, that study was listed under the key event of Apoptosis. It is unclear, however, why mutations at that gene would predispose those who consume high levels of arsenic to develop skin lesions. Two studies described under the heading DNA Repair Inhibition or Stimulation demonstrated reduced expression of three nucleotide excision repair (NER) genes in a population that used drinking water that contained 10–75 ppb arsenic (Andrew et al., 2003 and 2006). Still more evidence that arsenic causes Effects Related to Oxidative Stress (ROS) comes from school children in Taiwan who showed a positive correlation between urinary concentrations of arsenic and 8-OHdG; no information was provided regarding the level of arsenic in their drinking water (Wong et al., 2005). Subjects with arsenic-related skin lesions from a population in Inner Mongolia, China, that used drinking water with a mean of 158 ppb arsenic showed a statistically significant positive correlation between 8-OHdG adducts in their urine and individual urinary concentrations of inorganic As, MMA, and DMA. In contrast, those without skin lesions

showed no correlation (Fujino et al, 2005).

Evidence is presented under the category Hypermethylation of DNA that arsenic exposure causes hypermethylation of the promoter sequence in the DNA for four tumor suppressor genes. For two of the genes, p53 and p16, there was a positive dose response between arsenic contamination of drinking water and the level of effect; however, this was only seen in individuals with skin lesions (Chanda et al., 2006). For the other two genes, RASSF1A and PRSS3, the association was demonstrated with regard to the level of arsenic consumption estimated from toenail clippings (Marsit et al., 2006). Because the Marsit et al. (2006) study was done on bladder cancer patients, it provides a potential link between arsenic exposure and epigenetic alterations in patients with bladder cancer. The Chanda et al. (2006) study also demonstrated hypomethylation in a few individuals, but it was found only in persons having prolonged arsenic exposure at high doses.

Regarding the hypothesized key event category Immune System Response, there was suggestive evidence of an association between changes in sensitive markers of lung inflammation (i.e., metalloproteinase concentrations in induced sputum) and levels of only about 20 ppb of arsenic in drinking water. The initial comparison between the high- and low-level exposure towns showed no difference with regard to these biomarkers, but a significant association appeared when the analysis was adjusted for possible confounding factors (Josyula et al., 2006). Islam et al. (2007) found that IgG and IgE levels were significantly elevated in arsenic-exposed individual with skin lesions. More details about that experiment, including clinical findings possibly related to inflammatory reactions, are found in Appendix D. Appendix D discusses several other studies (including *in vitro* experiments and experiments on laboratory animals) related to immunotoxicity, including some that are not included in any of the tables in Appendix C.

The only study listed under Gene Mutations gave no more than a hint of an effect (Ostrosky-Wegman et al., 1991). Regarding Signal Transduction, a study in Taiwan showed that both the levels of plasma TGF-α and the proportion of individuals with TGF-α over-expression were significantly higher in the high CAE group than in the control group (Hsu et al., 2006).

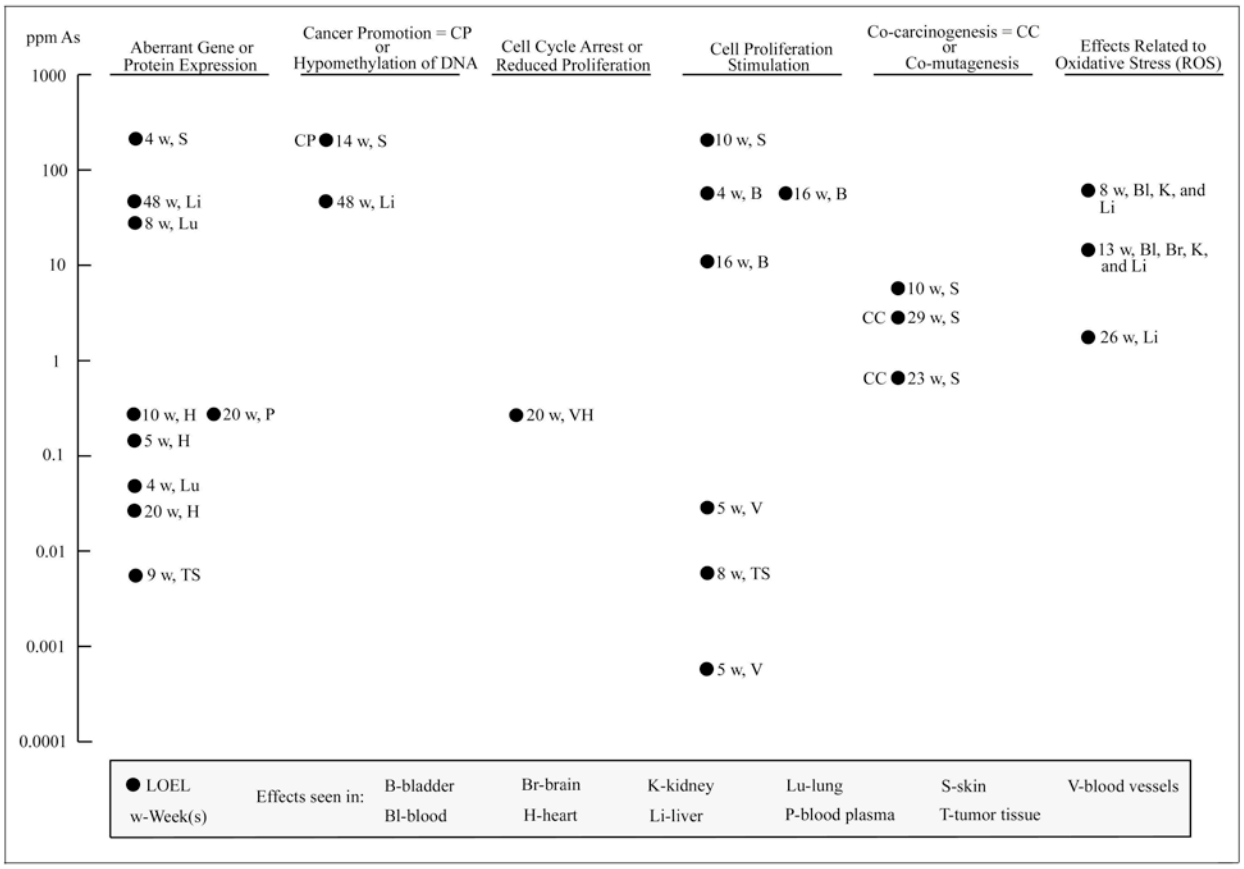
Only limited information from the cited experiments has been included in this discussion. Much more detail on these studies can be found in Table C-1 of Appendix C as well as in Table C-2 for *in vivo* experiments using laboratory animals and Table C-3 for *in vitro* experiments. Brief discussions of the information in Table C-2 and C-3 are found in Sections 4.4.1.2 and 4.4.1.3, respectively, below.

4.4.1.2 *In Vivo* Experiments Using Laboratory Animals

Table C-2 summarizes *in vivo* experiments using laboratory animals. All doses given in this section are stated in terms of the amount of arsenic in the dose. Twenty-four of the 112 row in Table C-2 involve studies of nine key event categories in mice that drank water containing arsenic for several to many weeks. Results are of particular interest because they involved most of the lowest dose levels tested, and As^{III} is the most toxic oxidation state of inorganic As. Figure 4-1 summarizes the results according to key events by showing for each endpoint: the

concentration of arsenic in the water that was the LOEL; the period of treatment; and the organ or tissue in which the effect was seen. Because the result for Gene Mutations was a negative finding, it is not shown in the figure. Sometimes more than one entry in Table C-2 corresponds to a single item in the figure, and sometimes a single entry in the table deals with separate groups of animals. Consequently, there may be multiple LOELs shown in the figure. It should also be kept in mind that sometimes only one dose was tested in an experiment, and, of course, if an effect was found, that dose became the LOEL (even though a much lower dose might have been effective). One benefit of the detailed descriptions found in Table C-2 is that all doses tested are listed. As Figure 4-1 shows, roughly half of the dose levels used exceed a couple thousand ppb and are thus much higher than levels ever found in drinking water used for human consumption. While all of the experiments summarized in Table C-2 are useful in terms of showing their effects in mice, more attention will be given in this discussion to doses that overlap higher exposure levels to humans from drinking water. A better understanding of the pharmacokinetic characteristics in different species may aid in determining the relevance of the high-dose animal studies to human subjects exposed to arsenic in drinking water at lower concentrations for a longer period.

Figure 4-1. Level of Significant Exposure of Adult Mice to Sodium Arsenite in Drinking Water in ppm As



The aberrant gene or protein expression effects seen at those lower levels included

increases in levels of several proteins and in mRNA levels of a few genes that are important in angiogenesis and remodeling. For example, vascular endothelial cell growth factor [VEGF] and its receptors VEGFR1 and VEGFR2 were measured in hearts, and increases were sometimes restricted to areas around blood vessels (Kamat et al., 2005 and Soucy et al., 2005). However, increases in dose (up to 0.288 ppm in drinking water) and duration (up to 20 weeks) actually caused decreases in the protein and mRNA levels for VEGFR1 and VEGFR2, suggesting that chronic exposure at these higher levels was toxic to the cardiac vasculature in mice. Consistent with the decreased mRNA levels seen for VEGFR1 and VEGFR2 following 20-week chronic exposures to 0.288 ppm, the same treatment regimen produced evidence of reduced cell proliferation, which was represented as a decrease in the density of microvessels of less than 12 μm in the heart (Soucy et al., 2005). These data thus provide an interesting example of the concentration and time-dependent effects of arsenic exposure that might be important in the etiology of some of the diseases that it causes. In contrast, stimulation of cell proliferation at low-dose levels involved increases in (a) blood vessel number in Matrigel implants (Soucy et al., 2005), (b) tumor growth rates after implantation of tumor cells (Kamat et al., 2005), and (c) number of metastases to the lungs after implantation of those tumor cells (Kamat et al., 2005).

Proteomic analysis of bronchoalveolar lavage fluid from lungs of mice that drank 0.05 ppm (i.e., 50 ppb) As in water for 4 weeks showed an increase in peroxiredoxin-6 and enolase 1 levels and a decrease in GSTO1, RAGE, contraspin, and apolipoproteins A-I and A-IV (Lantz et al., 2007). That same paper had demonstrated a decrease in the level of RAGE protein in human sputum that was associated with arsenic exposure. Two microarray experiments at much higher dose levels of 28.8 and 45 ppm showed changes in expression of dozens of genes (Chen et al., 2004b; Lantz and Hays, 2006). In each experiment, the LOEL was the only dose tested, which leaves open the possibility that such high doses might not have been necessary to obtain these changes.

Mice that were exposed for 23 weeks to 0.7–5.8 ppm As in drinking water developed no skin tumors; however, when they were also exposed to UV thrice weekly for most of that time, they showed a strong dose-related increase up through 2.9 ppm As, thus providing strong evidence of co-carcinogenesis (Burns et al., 2004). Another part of the same study (reported in Uddin et al., 2005) demonstrated that at 2.9 ppm there was oxidative DNA damage caused by the co-treatment. Effects Related to Oxidative Stress (ROS) following 26 weeks of exposure at 1.8 ppm included decreases in GSH content, and in the activities of glucose-6-phosphate dehydrogenase (G6PDH), glutathione peroxidase (GPx), and plasma membrane Na^+/K^+ ATPase. Additional changes suggestive of such damage, such as an increase in the concentration of malondialdehyde (MDA), were apparent after 9, 12, or 15 months at the same dose level (Mazumder, 2005).

Eighteen of the 112 rows in Table C-2 involved rats that drank water containing sodium arsenite for several to many weeks, but those studies are distributed among only two key event categories and do not extend down to nearly as many effects at low exposure levels. Most experiments cited in the 18 rows involved drinking water containing 57.7 ppm As for several to many weeks and showed findings of numerous changes indicative of oxidative damage in several organs. A few experiments show differing levels of oxidative damage in different regions of the brain (Samuel et al., 2005 and Shila et al., 2005a,b). By far the lowest dose tested among these

experiments was 0.03 ppm As, and it was found to be effective in decreasing the GSH level and superoxide radical dismutase (SOD) activity in the liver. The other two dose levels tested, 1.4 and 2.9 ppm, caused bigger changes in these two variables, as well as additional changes indicative of oxidative stress. It is of interest that the changes per unit dose were much higher for GSH and SOD at 0.03 ppb than they were at the two much higher doses tested (Bashir et al., 2006a). In experiments using 5.8 ppm As, which rats drank for 4, 8, or 12 weeks, activities of catalase (CAT) and SOD in kidney, liver, and RBCs were found to be elevated at 4 weeks, but they decreased to baseline levels or lower by 12 weeks; MDA levels were always elevated (Nandi et al., 2006). Consumption of water containing 1.4 ppm As for 60 days led to a demonstrable increase in apoptosis in liver cells (Bashir et al., 2006a).

Twenty-six of the 112 rows in Table C-2 involve rats or mice that consumed pentavalent arsenicals (As^V, MMA^V, DMA^V, or TMA^V) for several to many weeks, and in all but 3 rows they were delivered in drinking water instead of food. As would be expected for these less potent forms of arsenic, LOELs were typically high and usually above 50 ppm. Only a few results occurred at much lower concentrations, and are mentioned in this discussion. After rats were exposed for 28 days to 0.35 ppm As in drinking water in the form of DMA^V, microarray analysis demonstrated significant effects on the expression of 503 genes (i.e., 11% of the genes tested with that microarray) in urothelial cells. Even more genes were affected at the three higher doses tested (i.e., 1.4, 14, and 35 ppm As). Most of the effected genes related to the functional categories of apoptosis, cell cycle regulation, adhesion, signal transduction, stress response, or growth factor and hormone receptors. There was a change in the types of genes affected at the different doses, particularly when comparing the higher 2 doses (both cytotoxic) with the 2 non-cytotoxic doses (Sen et al., 2005). When rats were exposed to 0.24 ppm As^V for 1 or 4 months in drinking water, changes in signal transduction were increased expression of integrin-linked kinase (ILK) and decreased expression of phosphatase and tensin homolog (PTEN) in the liver. At higher doses, the expression of these genes and additional cancer-related genes was affected (Cui et al., 2004b).

DNA damage (both fragmentation and oxidative) was demonstrated in peripheral blood leukocytes of mice using the comet assay following exposure of 50, 200 or 500 ppb As in drinking water in the form of As^V for 3 months with and without a low-Se diet. Arsenic caused increased DNA fragmentation only in mice consuming the low-Se diet, and induced oxidative damage only in mice consuming the normal-Se diet. Neither case showed a positive dose response (Palus et al., 2006). In lung adenocarcinomas from mice exposed for 18 months to 0.24, 2.4, or 24 ppm As^V in drinking water, there was an increase in the extent of hypermethylation of promoter regions of tumor suppressor genes $p16^{INK4a}$ and RASSF1A (genes frequently found inactivated in many types of cancer including lung cancer), based on methylation-specific polymerase chain reaction (PCR). All doses had an effect, and there was a positive dose response. Reduced expression or lack of expression of these 2 genes was correlated with the extent of hypermethylation. Mice without tumors, whether control or As-treated, had normal (i.e., not reduced or eliminated) expression of these genes in their lungs. The authors concluded that epigenetic changes of tumor suppressor genes are involved in inorganic As-induced lung carcinogenesis (Cui et al., 2006).

Of the experiments described in Table C-2 in which arsenic exposure occurred through

consumption of arsenic in drinking water or food, the only group not yet discussed consists of the series of experiments in which pregnant female mice drank water containing 42.5 or 85 ppm As in the form of sodium arsenite for 10 days on gestation days 8 to 18. These studies follow-up on the interesting observation that arsenic seems to be a complete carcinogen in mice following such a treatment. The offspring were observed for effects (sometimes only after they had grown to be adults), and results are categorized in Table C-2 under Aberrant Gene or Protein Expression, Cell Proliferation or Stimulation, Hypomethylation of DNA, and Signal Transduction. Some of the more noteworthy findings were as follows. Numerous microchip analyses were conducted, often with some of the findings confirmed by real-time (RT) PCR. Microarrays containing from 588 to 22,000 genes were used. It was not unusual to find changes in the expression of scores of genes (sometimes even of thousands) in the different studies. Changes (often many fold) included both increases and decreases of expression occurring at both dose levels. Some of the many types of genes often altered included oncogenes, HCC biomarkers, cell proliferation-related genes, stress proteins, insulin-like growth factors, estrogen-linked genes, and genes involved in cell-cell communication. Tissues in which gene expression changes were found in offspring that had been exposed to arsenic *in utero* included: (a) arsenic-induced HCC tumors that developed in adult males; (b) normal appearing cells in livers of adult males; (c) fetal livers of males right at the end of treatment; (d) livers of newborn males; (5) fetal lungs of females right at the end of treatment and (6) arsenic-induced adenomas and adenocarcinomas that developed in lungs of adult females.

The expression of three estrogen-related genes was shown to increase synergistically in the uteri of females (at 11 days of age) that had been exposed *in utero* to arsenic and also subcutaneously injected with diethylstilbestrol (DES) on the first 5 days after birth. These and other results showed that inorganic As acts with estrogens to enhance production of urogenital cancers in female mice (Waalkes et al., 2006a). Females that had been exposed to arsenic *in utero* and then received a 21-week post-weaning treatment with TPA showed changes in gene expression that were similar to those seen in liver samples from males that had received only the arsenic treatment *in utero*. This is interesting because it parallels another situation in which TPA-treated females showed a response similar to males without TPA treatment. Specifically, female mice exposed *in utero* to arsenic develop HCC only after TPA treatment (Liu et al., 2006b); however, male mice exposed *in utero* to arsenic develop those tumors without receiving any TPA treatment. Observed changes in estrogen-related genes sometimes seemed especially important in the interpretation of results, and fetal lungs of females exposed to arsenic *in utero* showed a large increase in estrogen receptor-alpha (ER-α), as well as several other estrogen-related genes and numerous other genes, including some associated with lung cancer. There also was a large increase in nuclear ER-α in adenomas and adenocarcinomas that developed in the lungs of adult females that had been exposed to arsenic *in utero* (Shen et al., 2007).

Stimulation of cell proliferation during treatment of males while *in utero* at 85 ppm induced kidney cystic tubular hyperplasia in 23% of the animals, and although males did not develop bladder hyperplasia from the arsenic treatment alone, they often did if treated in conjunction with DES or tamoxifen on the first 5 days after birth because of a synergistic interaction that occurred with those chemicals. Although females exposed while *in utero* showed bladder hyperplasia similar to the males, arsenic exposure *in utero* alone caused no hyperplasia in their kidneys (Waalkes et al., 2006a,b). Global hypomethylation of GC-rich

regions was demonstrated in livers of newborn males that received 85 ppm *in utero* (Xie et al., 2007).

Almost all remaining experiments summarized in Table C-2 involved treatments of mice or rats by gavage, and those results are summarized under the categories Aberrant Gene or Protein Expression, Apoptosis, Chromosomal Aberrations and/or Genetic Instability, Effects Related to Oxidative Stress (ROS), and Interference with Hormone Function. In all rows where As^{III} was administered, it was usually as sodium arsenite, but sometimes as arsenic trioxide (ATO). One study also included treatment with pentavalent arsenicals. By using gavage, the amount of the arsenical administered to each animal was controlled precisely, and it was given as a certain weight of arsenic per animal, often with adjustment to the individual weight of each animal (i.e., µg/animal or mg/kg bw, respectively). Most treatments were administered repeatedly, with treatment regimens in one case lasting an entire year. As in all other studies on experimental animals, there was an attempt here to state all doses in terms of the amount of arsenic. Because it was unclear from the reporting of a few experiments whether doses were expressed as As compound or as As, Table C-2 always makes it clear whether or not such a correction was made. In a gavage study with one of the smallest amounts of arsenic per dose (equivalent to 36 µg/mouse if a mouse weighed 25 g), Patra et al. (2005) found induction of chromosomal aberrations (and probably also of polyploidy) in mice that received 1.44 mg As/kg bw given as sodium arsenite by gavage once-per-week for 4 weeks. Induction of chromosomal aberrations also was seen after 5 and 6 treatments; however, 7 and 8 treatments were lethal to the mice. A 25 g mouse in that study would have received the same amount of arsenic in that one day if it had drunk water that contained 6 ppm As (assuming that it drank 6 mL of water, which would be a reasonable amount for a mouse).

In the only gavage study with *in utero* treatments, 9 daily treatments of 4.35 mg As/kg bw was shown to increase the activity of the selenoprotein iodothyronine deiodinase-II (DI-II) in fetal brains and to decrease the activity of the selenoprotein TrxR in fetal livers. In both cases, these results were observed only if the mice were on a Se-deficient diet (Miyazaki et al., 2005). In a gavage study lasting a full year (Das et al., 2005), mice were administered 50, 100, or 150 µg/mouse, 6 days a week for 3, 6, 9, or 12 months; it took 9 months before substantial increases were seen in the activities of tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 at any dose, but by then all doses had an effect and there was a positive dose response. Three months later both effects had increased substantially at all doses, still with a positive dose response. A similar response was seen for the concentration of total collagen, although increases were not as large in comparison to the control group. That same study examined six components of the antioxidant defense system and found numerous interesting changes over time. While all of the affected components had a LOEL of 50 µg at the 3, 9, and 12 month test periods, all 5 affected components had a LOEL of 100 µg at 6 months. GSH levels and activities of GPx and CAT increased by 3 months, but decreased by 9 and 12 months. In another experiment with single, large doses of As^{III} or As^{V} given to mice by gavage, there were large increases in heme oxygenase 1 (HMOX-1) activity within 6 hours in liver and kidney but not in the brain. The effect was somewhat higher with As^{III}, but DMA^{V} had no effect. This study also tested some much smaller doses, and a dose as high as 2.25 mg/kg bw had no effect on this endpoint in kidneys (Kenyon et al., 2005b).

Various biochemical indicators of apoptosis were seen in brain and liver 24 hours after giving rats a single high dose of sodium arsenite by gavage (Bashir et al., 2006b). The same paper showed that single, large doses of sodium arsenite given to rats by gavage affected many biochemical indicators of oxidative stress in liver and brain 24 hours after treatment. Some studies on Effects Related to Oxidative Stress (ROS) included co-treatments with antioxidants that were shown to reduce the level of effects seen (Modi et al., 2006; Sohini and Rana, 2007). With regard to Interference with Hormone Function, rats given 30.3 mg As^{III}/kg bw as ATO by gavage every other day for 30 days were shown to have a large increase in the levels of thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) in their blood serum (Rana and Allen, 2006).

4.4.1.3 *In Vitro* Experiments

Table C-3 summarizes a large number of *in vitro* experiments; and some highlights are discussed below. The potencies of many arsenicals, including both trivalent and pentavalent forms, have been compared in several series of experiments, with the obvious conclusion that the pentavalent forms almost always have much higher LOECs (e.g., Moore et al., 1997a; Sakurai et al., 1998; Petrick et al., 2000; Drobná et al., 2002; and Kligerman et al., 2003). Consequently, the discussion below does not focus on the studies that analyzed pentavalent arsenicals.

Three chemical properties of arsenic likely to account for its biological activity are: (a) the soft acid/soft base principle (which is related to trivalent arsenicals and sulfhydryl binding); (b) the nucleophilicity of trivalent arsenicals; and (3) the formation of free radicals, ROS, or both by arsenicals (Kitchin et al., 2003). As noted by Kitchen et al. (2003),

- "If trivalent arsenicals acting as soft acids are causally important, then the likely modes of action of arsenic carcinogenesis may include altered DNA repair, altered growth factors, cell proliferation, altered DNA methylation patterns and promotion of carcinogenesis."

Arsenic is readily absorbed from the GI tract in humans and is primarily transported in the blood bound to sulfhydryl groups in proteins and low-molecular-weight compounds, such as amino acids and peptides (NRC, 1999). At any given time, about 99% of absorbed As^{III} is bound to tissue sulfhydryls, mostly to monothiol sites (Kitchin and Wallace, 2006). Based on the results of their peptide binding studies, Kitchen and Wallace (2006) suggested that dithiol- and trithiol-binding sites would be "the most likely causal triggers of biological effects because of their stronger affinity and because the bi- and tri-dentate complexes last so much longer than the rapidly dissociating and reforming binding of arsenite to monothiol sites." While the As^{III} attachment to the monothiol-binding sites are short lived, a substantial part of the total As^{III} attaches to those sites because of their great abundance in mammals. Because the functional group of the amino acid cysteine in a protein or peptide is a thiol group, any proteins that contain cysteine are of importance for interactions with As^{III}. Although Table C-3 includes large amounts of data under the heading Effects Related to Oxidative Stress (ROS), arsenic's action as a soft acid and its nucleophilicity are not included as key events. It is obvious, nonetheless, that those chemical properties play important roles in the interactions of inorganic As with organisms at early stages in multiple key event(s) leading to tumor development.

Much data is summarized in Table C-3 under the hypothesized key event category Aberrant Gene or Protein Expression. Abundant evidence is presented showing that changes can easily occur at concentrations of As^{III} (as either sodium arsenite or arsenic trioxide) of less than 10 µM and often with durations of exposure of 24 hours or less. Results from 10 microarray analyses are found in this category, and they all demonstrated changes in expression of large numbers of genes, often numbering in the hundreds. Two studies with longer exposures to especially low concentrations are of special interest. In one study, NB4 cells were exposed to 0.5 µM ATO for periods up to 72 hours for transcriptome analysis and up to 48 hours for proteomic analysis. The regulation of 487 genes was affected at the transcriptome level; however, at the proteome level, 982 protein spots were affected. The finding of more significant changes at the proteomic level, in comparison with the relatively minor changes found at many of the corresponding genes at the transcriptome level, suggests that ATO particularly enhances mechanisms of post-transcriptional/translational modification (Zheng et al., 2005). In the second experiment, which was a cDNA (complementary DNA) microarray analysis of about 2000 genes, the LOECs for SV40 large T-transformed human urothelial cells (SV-HUC-1) exposed to As^{III}, MMA^{III} or DMA^{III} for 25 passages (with subculturing twice weekly) were found to be 0.5, 0.05, and 0.2 µM, respectively. DMA^{III} was shown to have a substantially different gene profile from the other two arsenicals. Most genes were down-regulated by these arsenicals, and evidence suggested that the suppression of two of these genes resulted from epigenetic hypermethylation (Su et al., 2006). Since each finding is presented only one time in Table C-3, subjectivity was often involved in the placement of data into the different key event categories. As a result, the densities of data in the different categories presented in Table 4-1 are only approximate estimates. This situation was especially common with regard to the key event categories of Aberrant Gene or Protein Expression, Signal Transduction, and Effects Related to Oxidative Stress (ROS), all of which have large densities of data.

Table C-3 also presents details as to the genes and proteins affected and changes related to dose and time. The possible significance of such changes is also provided when available. A few examples are as follows. When primary normal human epidermal keratinocytes (NHEK) cells were exposed to 1 µM sodium arsenite for 24, 48, and 72 hours, there was an increase in focal adhesion kinase (FAK) protein at 24 hours followed by a decrease to below the background level at later times, with almost none being present at 72 hours (Lee, et al., 2006b). The concentration of some enzymes increased after exposures to 0.5 µM for 24 hours, but the concentrations decreased at higher levels of exposure up to 25 µM (Snow et al., 2001). DuMond and Singh (2007) demonstrated the same relationship for proliferating cell nuclear antigen (PCNA) with exposures to sodium arsenite lasting 70 days. The expression of PCNA increased at 0.008 µM, but decreased at 0.77 and 7.7 µM. Similar results also have been observed for telomerase activity (Zhang et al., 2003). Numerous studies investigated effects of various modulators or inhibitors or of different genetic conditions (e.g., knockout mutations or transfections). Cell type can have a major influence on the effect of arsenic on protein expression, as was shown for p53 expression, with some cells having no response to 50 µM sodium arsenite for 24 hours while other cells showed an increase after exposure to only 1 µM sodium arsenite (Salazar et al., 1997). Clearly, small levels of arsenic exposure can have large effects on many genes and proteins, and the relationships regarding time and dose can be complicated and subject to many influences.

Results found in the Apoptosis category show that ATO and sodium arsenite can often induce apoptosis in cells with exposures to less than 10 µM (often much less) for a few days or less. Zhang et al. (2003) demonstrated a large difference in the sensitivity of cell lines to arsenic-induced apoptosis. A positive association between telomerase activity in cell lines and their susceptibility to induction of apoptosis by exposure to sodium arsenite was found. Exposure to extremely low concentrations of sodium arsenite (i.e., 0.1-1 µM in HaCaT cells and 0.1-0.5 µM in HL-60 cells) for 5 days increased telomerase activity, maintained or elongated telomere length, and promoted cell proliferation. At concentrations higher than those stated, exposure of these cell lines to sodium arsenite for 5 days decreased telomerase activity, decreased telomere length, and induced apoptosis. The positive association noted earlier means that cell lines that innately have more telomerase activity are more likely to be affected by sodium arsenite in inducing apoptosis. Many experiments tested effects of modulators on the arsenic-induced apoptosis. For example, Chen et al. (2006) demonstrated that co-treatment with L-buthionine-S,R- sulphoximine (BSO) markedly increased induction of apoptosis presumably because of its effect in decreasing GSH levels. Other experiments looked at the effects of inhibitors of various proteins involved in signal transduction pathways. For example, Lunghi et al. (2005) showed that use of MAP/ERK kinase (MEK) 1 inhibitors greatly increased ATO-induced apoptosis. Other studies showed that different genetic conditions established using knockout mutations or transfections could markedly affect the extent of arsenic-induced apoptosis (e.g., Bustamante et al., 2005; Poonepalli et al., 2005; and Ouyang et al., 2007). Many of the experiments related to apoptosis were motivated by the desire to improve methods for using ATO in cancer therapy, but in the process they have provided much additional information about the complex pathways by which arsenic can affect apoptosis.

In the hypothesized key event category Cancer Promotion, Tsuchiya et al. (2005) tested sodium arsenite and three pentavalent arsenicals in a two-stage transformation assay in BALB/c 3T3 A31-1-1 cells. Sodium arsenite caused cancer promotion at a LOEC of 0.5 µM when the initiating treatment was exposure to 0.2 µg/mL 20-methylcholanthrene for 3 days before the 18-day post treatment with sodium arsenite. Sodium arsenite caused promotion at a LOEC of 1 µM when the initiating treatment was exposure to 10 µM sodium arsenite for 3 days before the 18-day post treatment with sodium arsenite. When As^{V} was tested in the same way with the same initiating treatments, it was somewhat less potent, with LOECs of 1 and 5 µM respectively. The two methylated arsenicals had little or no effect. Paralleling their cancer promotion effects, the same study demonstrated LOECs for As^{III} and As^{V} of 0.7 and 5 µM, respectively, for inhibition of gap-junctional intercellular communication, which is a mechanism linked to many tumor promoters.

The category of Cell Cycle Arrest or Reduced Proliferation includes many experiments that showed that levels of exposure to ATO and sodium arsenite of less than 10 µM (often much less) for a few days or less can often increase the numbers of cells in mitosis and otherwise disrupt mitosis, so as to reduce cell proliferation. In the Drobná et al. (2002) experiment, the LOECs for reduced cell proliferation were 1, 1, and 5 µM for 24-hour exposures to As^{III}, MMA^{III}, and DMA^{III}, respectively; no effects were seen following exposures to the pentavalent forms of these arsenicals at 200 µM. By testing cells enriched in different phases of the cell cycle using centrifugal elutriation, McCollum et al. (2005) showed that As^{III} slowed cell growth in every phase of the cell cycle. Cell passage from any cell cycle phase to the next was inhibited

by 5 µM sodium arsenite. By looking at caspase activity, they showed that As^{III} induced apoptosis specifically in cell populations delayed in the G2/M phase. Tests with knockout mutations showed that poly(adenosine diphosphate–ribose) polymerase-1 (PARP-1) (Poonepalli et al., 2005) and securin (Chao et al., 2006a) protect against arsenic-induced cell cycle disruption. Yih et al. (2005) provided evidence that 1 µM sodium arsenite appears to inhibit activation of the G2 DNA damage checkpoint and thereby allows cells with damaged DNA to proceed from G2 into mitosis.

Extremely small concentrations of As^{III} can stimulate cell proliferation. For example, 0.005 µM sodium arsenite exposure for 24 hours stimulated cell proliferation in NHEK; however, concentrations of 0.05 µM or higher inhibited it (Vega et al., 2001). In other studies, stimulation occurred at much higher concentrations. Mudipalli et al. (2005) exposed NHEK cells to many exposure levels of As^{III}, MMA^{III}, and DMA^{III} for 24 hours. The LOECs were 2, 0.5, and 0.6 µM, respectively. There was increased stimulation of cell proliferation up to doses of 6, 0.8, and 0.6 µM, respectively, and in all cases significant cytotoxicity was observed at higher doses. Proliferation was often stimulated to a considerable extent. Yang et al. (2007) showed that human embryo lung fibroblast (HELF) cells exposed to 0.5 µM sodium arsenite for 24 hours had 175% of the cell proliferation efficiency of control cells. When the concentration of As^{III} was increased to 5 µM, however, the cell proliferation efficiency decreased to 60% that of the control. The increased proliferation rates can extend over long periods, as shown by Bredfeldt et al. (2006), who exposed UROtsa cells to 0.05 µM MMA^{III} for 12, 24, or 52 weeks. Cell population doubling times were 27, 25, and 21 hours, respectively, in comparison to the 42 hours observed in the control.

Mutations can play an important part in initiating carcinogenesis or in the development of cancers, and they range from gene mutations that involve a single base-pair change to chromosomal aberrations (CAs). Much evidence is presented in Table C-3 under the category Chromosomal Aberrations and/or Genetic Instability to show that inorganic As can induce CAs, SCEs, MN, multilocus deletions, and several other endpoints such as changes in the length of telomeres. Arsenic appears to be ineffective in inducing gene (point) mutations, but mutations at some genes tend to be deletions that are so large that they extend over several genes (termed multilocus deletions). These multilocus deletions have been grouped with CA in Table C-3. CD59 mutations (Liu et al., 2005) and gpt mutations (Klein et al., 2007) provide examples of such mutations. Numerous experiments are summarized in Table C-3 that show that CAs can be induced by exposure to 10 µM or less of sodium arsenite for periods of 24 hours or less. Following exposures of human primary peripheral blood lymphocytes for 24 hours, LOECs for As^{III}, MMA^{III}, and DMA^{III} were 2.5, 0.6, and 1.35 µM, respectively (Kligerman et al., 2003). Examination of data shown in the table for the few other experiments on MMA^{III} and DMA^{III} are consistent with this experiment in suggesting that both of those methylated arsenicals tend to be more effective in inducing CAs than As^{III}. The table includes estimates of about 15 LOECs for induction of SCEs and about 20 LOECs for induction of MN following exposure to As^{III}, and it appears that CAs, SCEs, and MN are all induced to roughly the same extent by As^{III}. Some experiments fail to show a dose response, which makes them difficult to interpret.

Several of the experiments on CAs provided evidence of arsenic induced changes in chromosome number (e.g., Barret et al., 1989 and Ochi et al., 2004). In the Ochi et al. (2004)

experiment, DMA^{III} was much more potent than As^{III}, and it induced mitotic spindle, centrosome, and microtubule elongation abnormalities. Experiments on induction of MN were conducted in such a way as to distinguish between MN caused by aneuploidy and those caused by chromosomal breakage; these experiments provided evidence that both mechanisms may be important (e.g., Colognato et al., 2007 and Ramírez et al., 2007). Chou et al. (2001) showed that exposure to 0.25 µM ATO for 4 weeks caused a decrease in telomere length. Mouse embryo fibroblasts that are homozygous for the PARP knockout mutation were shown to be much more sensitive to both arsenite-induced telomere attrition and induction of MN by As^{III} (Poonepalli et al., 2005). Many experiments investigated the effects of various modulators on induction of arsenic-induced chromosomal damage. For example, Jan et al. (2006) found that co-treatment with low concentrations of dimercaptosuccinic acid, meso 2,3- dimercaptosuccinic acid (DMSA), or 2,3-dimercaptopropane-1-sulfonic acid (DMPS) markedly increased the induction of MN by sodium arsenite, ATO, MMA^{III}, and DMA^{III}, while co-treatment with high concentrations of the same chemicals decreased the ability of arsenic to induce MN. Although the authors stated that the reasons are obscure why these dithiol compounds effectively enhanced the toxic effects of arsenic when they were at micromolar concentrations, they speculated that the observed results might be related to the influence of dithiols on retention of arsenite in cells, with low concentrations of dithiols increasing arsenite levels and high concentrations of dithiol decreasing them. Ramírez et al. (2007) also showed that co-treatment with SAM blocked As^{III} induction of centromere positive (cen+) MN without having any effect on its induction of centromere negative (cen-) MN. The authors suggested that the reason for this might be that SAM in some way influences some components (probably microtubules) of the mitotic spindle. As the main methyl group donor, SAM plays a major role in chromatin methylation and condensation, and it might stop the lagging of chromosomes by in some way correcting the cell's methylation status. Alternatively, they suggested that SAM might interfere with the effects of ROS in causing aneuploidy. Whatever SAM does to block induction of cen+ MN, it does not appear to affect induction of double strand DNA breaks that would lead to cen- MN.

The results from the Co-carcinogenesis category all relate to promotion of benzo[*a*]pyrene (B[*a*]P)-mediated carcinogenesis via exposure to 1.5 µM sodium arsenite for 12 weeks. Transformation (i.e., anchorage-independent growth in soft agar) of a rat lung epithelial cell line occurred because of the arsenite treatment alone, and the transformed cells were shown by proteomic analysis to have changes in the amounts present of many proteins. When the arsenite treatment was preceded by exposure to 100 nM B[*a*]P for 24 hours, there was a synergistic interaction. Results indicate that the transformation rate increased more than 500 and 200 times when compared to arsenite and B[*a*]P treatments alone, respectively. The findings in the proteomic analysis also showed synergistic interactions (Lau and Chiu, 2006). BPDE (benzo[*a*]pyrene diol epoxide) is an active metabolite of B[*a*]P. Shen et al. (2006) showed that a 24-hour pretreatment of GM04312C cells, a SV-40 transformed XPA human fibroblast NER-deficient cell line, with 10 or 50 µM As^{III} markedly increased the cellular uptake of BPDE in a dose-dependent manner.

The results found under the category Co-mutagenesis showed that As^{III} affected the induction of mutations (using different assays) when there was also a treatment with UV, diepoxybutane (DEB), methyl methanesulfonate (MMS), X-radiation, gamma-radiation or N-methyl-N-nitrosourea (MNU). Many of the types of mutations affected were gene mutations

(i.e., point mutations and numerous other changes in the DNA of single genes, such as small deficiencies), which are not normally induced by arsenic alone. Arsenic treatment also caused co-mutagenesis regarding CAs and MN. Sometimes the timing of the As^{III} treatment relative to the treatment with the other agent was of importance to the result observed. For example, a 24-hour pretreatment with 10 µM sodium arsenite reduced the frequency of induction of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) mutations by MMS, but a 24-hour post-treatment with the same concentration of sodium arsenite caused a synergistic interaction with MMS in induction of HGPRT gene mutations (Lee et al., 1986).

The data found in Table C-3 in the category Cytotoxicity are sometimes important to help determine the possible relevance to human health of findings related to other key events. For example, a large arsenic-induced increase in the expression of some protein that is important in signal transduction is much more likely to have such relevance if it occurs at concentrations having little or no cytotoxicity than if it occurs only when most cells are dying. Table C-3 shows that large differences in LOECs for cytotoxicity can result from a change in any of the following variables: species of arsenic, duration of treatment, cell line, and particular assay used. As another example, LOECs of As^{III} were 0.1 and 50 µM after 24-hour exposures in Jurkat cells and HeLa cells, respectively (Salazar et al., 1997). Petrick et al. (2000) showed that 3 different cytotoxicity assays yielded substantially different 24-hour LC_{50}s for each of 5 different arsenic species. Sometimes the different assays yield more similar results when treatments last at least 48 hours (Komissarova et al., 2005). Overall it appears that in comparison to As^{III}, MMA^{III} has substantially higher cytotoxicity, DMA^{III} has higher cytotoxicity, and As^{V} has substantially lower cytotoxicity.

Effects of modulators on arsenic-induced cytotoxicity were tested in many experiments. Snow et al. (1999) showed that pretreatment with BSO, to decrease GSH levels, markedly increased cytotoxicity of sodium arsenite following a 48-hour exposure. Jan et al. (2006) found that co-treatment with low concentrations of DMSA or DMPS (dithiols that are currently used to treat arsenic poisoning) markedly increased the cytotoxicity of ATO, while co-treatment with high concentrations of DMSA or DMPS had the opposite effect. Probably the most important observation related to cytotoxicity from perusal of Table C-3 is that exposure of a large number of different cell lines to trivalent arsenicals results in significant cytotoxicity at molarities smaller than what would be found in urine, or even in the blood streams, of individuals exposed to high levels of inorganic As in drinking water in places like Bangladesh. In some cell lines, even the pentavalent arsenicals destroyed more than 50% of the cells following a 7-day exposure with concentrations such as those observed in Bangladesh; As^{III} and MMA^{III} would do the same at concentrations far below such levels (Wang et al., 2007). Also from the numerous dose-response curves published in those papers, it is apparent that cytotoxicity generally has a threshold below which there is no apparent effect.

DNA Damage is another key event category for which much experimental data is summarized in Table C-3. Evidence showed induction of oxidative DNA damage, DNA single-strand breaks, and DNA-protein crosslinks by exposures at 10 µM (and often much less) of As^{III} for periods of often much less than one day. MMA^{III} is especially effective in inducing damage detected by the comet assay (Gómez et al., 2005). Much more DNA damage was detected in the comet assay by using enzyme treatments to reveal oxidative DNA adducts and DNA protein

crosslinks, and DNA damage was induced at levels of sodium arsenite that caused no cytotoxicity in 2 different cell types (Wang et al., 2001). In a third cell type, no DNA damage was observed up to the maximum concentration tested (2 µM), even though in each of the other two cell types the LOEC was 0.25 µM. Jan et al. (2006) found that co-treatment with low concentrations of DMSA or DMPS markedly increased the DNA damage detected by the comet assay following treatment with ATO, while co-treatment with high concentrations of DMSA or DMPS had the opposite effect. Several experiments looked at induction of 8-OHdG formation as a measure of oxidative DNA damage. In one such experiment, sodium arsenite was shown to be effective. However, MMA^{III} was shown to be about 200 times more effective than As^{III} (with an LOEC of 0.05 µM) following a one-hour treatment (Eblin et al., 2006). Pre-incubation with SOD or catalase to reduce effects of ROS almost completely blocked induction of 8-OHdG formation by a 24-hour treatment with sodium arsenite (Ding et al., 2005). Tests with a cell line containing a knockout mutation of the PARP-1 gene showed that the PARP-1 protein protects against arsenic-induced DNA damage detected by the comet assay at pH >13 in the version of the assay that does not include further digestion to detect additional types of DNA damage (Poonepalli et al., 2005).

The category of DNA Repair Inhibition or Stimulation includes rather few experiments in Table C-3. A microarray experiment that showed decreased expression of DNA repair genes involved exposure to only 0.77 µM of sodium arsenite for 70 days (DuMond and Singh, 2007). Arsenic does not always have the effect of decreasing repair. Snow et al. (2005) found that W138 cells exposed to 0.1 µM sodium arsenite for 24 hours showed increased DNA ligase activity. Increasing the As^{III} concentration to 1 µM further increased the activity, but 5 µM decreased DNA ligase activity to below normal levels. The same paper demonstrated a rather similar reversal-of-direction effect for DNA polymerase β. In another experiment, when CHO K1 cells were treated with MMS followed by 5 µM sodium arsenite for 6 hours, there was a decrease in repair of MMS induced single-strand breaks in DNA (Lee-Chen et al., 1993). Andrew et al. (2006) demonstrated that in Jurkat cells the LOEC for sodium arsenite was 0.01 µM for reduction of expression of NER gene ERCC1 (excision repair cross-complement 1 component). The decrease in expression was 45% at that concentration and 60% at concentrations of 0.1 and 1 µM. The functional effect of this decrease in expression was shown by reduced repair following a challenge with the mutagen 2-AAAF immediately after the sodium arsenite treatment. Clearly exposure to inorganic arsenic at low concentrations can modify the level of DNA repair.

The category Effects Related to Oxidative Stress (ROS) in Table C-3 includes many experiments in which antioxidants or radical scavengers were used as modulators. When a reduction in the effects was seen, it was taken as evidence that oxidative stress was the cause of the original effects observed, as, for example, in the study by Sasaki et al. (2007). Results from a series of experiments by Lynn et al. (2000) led to the conclusion that As^{III} activates NADH oxidase to produce superoxide, which then causes oxidative damage to DNA. Experiments by Liu et al. (2005) dealt with the effects of various modulators on induction of $CD59^{-}$ mutations and lead to the conclusion that peroxynitrites, which are formed as a result of ROS and reactive nitrogen species, have an important role in the induction by As^{III} of such mutations. Wang et al. (2007) measured formation of oxidative damage to lipids, proteins, and DNA (comet assay) by three trivalent arsenicals and three pentavalent arsenicals in two different cell lines. For As^{III},

As^{V}, MMA^{III}, and DMA^{III}, the LOECs were all 0.2 µM for a 24-hour exposure for all three types of damage. The order of effectiveness of the different arsenicals differed in the two cells lines used and for the different types of damage. Consistent with these effects, increased levels of nitric oxide, superoxide ions, hydrogen peroxide, and the cellular free iron pool were consistently detected in both cell lines after treatments by all three trivalent arsenicals. A microarray analysis in which genes were identified for which the response to ATO and hydrogen peroxide was reversed by *n*-acetyl-cysteine (NAC) suggested that 26% of the genes significantly responsive to ATO were directly altered by ROS (Chou et al., 2005). Further evidence that ROS is likely involved in arsenite-induced DNA damage comes from comet assays done on splenic lymphocytes from SOD knockout mice (Kligerman and Tennant, 2007). Results showed homozygotes exhibiting a large decrease in splenic SOD levels and a large increase in arsenite-induced DNA damage, while heterozygotes had intermediate changes in SOD levels and DNA damage.

Little information is included in Table C-3 regarding Enzyme Activity Inhibition. Hu et al. (1998) and Snow et al. (1999) tested the effect of sodium arsenite on the activity of several purified enzymes *in vitro*, including enzymes required for DNA repair and some related to GSH metabolism. The purpose of the study was to examine whether As^{III} binding to sulfhydryls caused protein denaturation and inhibited enzyme activity. In almost all cases, the purified enzymes were not inhibited by physiologically relevant concentration of As^{III}. The concentrations that are needed to cause 50% inhibition (IC_{50}s) for the rate of the reaction (over 6 minutes for many of those enzymes) ranged from 6.3 to 381 mM. The one exception was purified pyruvate dehydrogenase for which the IC_{50} was 5.6 µM. Table C-3 also lists IC_{50}s for GSH peroxidase and ligase when tested in extracts of AG06 (SV40-transformed human keratinocyte) cells that were pretreated for 24 hours with an unspecified concentration of sodium arsenite; these IC_{50}s were both low, i.e., 2.0 and 14.5 µM, respectively.

Table C-3 under Gene Amplification shows that As^{III} caused amplification of dihydrofolate reductase (dhfr) genes in three different experiments with LOECs ranging from 0.0125 to 6 µM (Barrett et al., 1989; Rossman and Wolosin, 1992; and Mure et al., 2003). Takahashi et al. (2002) showed that several neoplastic transformed cell lines produced by 48-hour treatments with either $\leq$ 8 µM As^{III} or $\leq$ 150 µM As^{V} contained gene amplification of either the c-Ha-ras or the c-myc oncogene. Almost all of the data in Table C-3 for Gene Mutations show no induction of mutations by arsenic.

Hypermethylation of DNA was demonstrated in a number of specific DNA sequences in two human kidney carcinoma cell lines and in one human lung carcinoma cell line. In the lung cell line, the LOEC for As^{III} was 0.08 µM for a 7-day exposure, and there was a positive dose response extending over the two higher doses tested (0.4 and 2.0 µM). Hypermethylation in this cell line was demonstrated within a 341-base-pair fragment of the promoter region of *p53* (Mass and Wang, 1997 and Zhong and Mass, 2001).

Hypomethylation of DNA has been demonstrated globally and for a number of specific DNA sequences. In one instance, exposure of HaCaT cells to 0.2 µM sodium arsenite for 10 serial passages in folic-acid depleted media caused genomic hypomethylation. Sodium arsenite repressed the expression of the DNA methyltransferase (DNMT) genes DNMT1 and DNMT3A

and caused depletion of SAM, the main cellular methyl donor. It is thought that long-term exposure to sodium arsenite may have resulted in DNA hypomethylation as a consequence of those two complementary mechanisms (Reichard et al., 2007). Singh and DuMond (2007) demonstrated methylation changes in DNA at 18 genetic loci in TM3 cells, with some showing hypomethylation and others hypermethylation, following sodium arsenite exposures ranging from 0.008–7.7 µM that lasted for either 25 or 75 days. The LOEC was the lowest dose. Some loci were affected only after 25 days of exposure, while others were affected after 75 days of exposure. In one of several other demonstrations of hypomethylation, a 19-week exposure of TRL 1215 cells to 0.125 µM sodium arsenite was sufficient to cause global hypomethylation (Zhao et al., 1997).

The category Immune System Response in Table C-3 describes a wide-range of effects on the immune system. This discussion provides highlights from that table and Appendix D, which is devoted entirely to the immunotoxicity of inorganic arsenic. Appendix D provides a much more detailed discussion of some aspects of the immunotoxicity of inorganic arsenic, including more emphasis on human studies and *in vivo* experiments on laboratory animals, as well as on some older *in vitro* studies. It overlaps very little with data found in Table C-3. Effects thought to be related to Immune System Response were grouped under that heading in Table C-3 even if they dealt mainly with other key events. For example, several findings related to Apoptosis, Cytotoxicity, or Signal Transduction are included in this section of Table C-3.

Exposures to low concentrations of As^{III} over 1–2 weeks inhibited maturation of human peripheral blood monocytes (HPBMs) into the following types of cells: M-type and GM-type macrophages, immature dendritic cells, and multinucleated giant cells (Sakurai et al., 2006). The IC_{50}s for this inhibition ranged from 0.06–0.70 µM. Lemarie et al. (2006a) showed that ATO inhibited macrophage differentiation of peripheral blood mononuclear cells (PBMCs) and that concentrations as low as 0.125 µM over 6 days induced apoptosis and necrosis in PBMCs co-treated with granulocyte-macrophage colony-stimulating factor (GM-CSF) or macrophage colony-stimulating factor (M-CSF). Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days were exposed to 0.25 µM ATO for 6 days. The ATO treatment caused major alterations in morphology, adhesion, and actin organization, giving the impression that the ATO "de-differentiated" the macrophages back into monocytic cells (Lemarie et al, 2006b). The same series of experiments showed that macrophages exposed to 1 µM ATO for 6 days also caused a reduction in several surface markers, markedly decreased endocytosis and phagocytosis, and increased the secretion of inflammatory cytokines in response to a co-treatment with lipopolysaccharide.

Exposure of PBMCs that had been stimulated with phytohemagglutinin (PHA) after exposure to 1–5 µM sodium arsenite for 120 hours caused a marked dose-related decrease in both cell proliferation and the percentage of divided cells (Tenorio and Saavedra, 2005). Even at the higher doses, most of the cells were viable but unable to divide. The treatments also modified the expression of CD4 and CD8 molecules. Judging from evaluation of blast transformation, $CD4^+$ and $CD8^+$ T cells appear to have different sensitivities to As^{III}. As the concentration of the sodium arsenite increased from 1 to 5 µM in the 120-hour treatment, there was an accumulation of resting $CD8^+$ cells with a positive dose response, but there was not an accumulation of $CD4^+$ cells. The Janus kinase (JAK)-signal transducer and activator of

transcription (STAT) pathway is an essential cascade for mediating normal functions of different cytokines in the development of the hematopoietic and immune systems. Huang et al. (2007a) showed that exposure of SV-HUC-1 cells to sodium arsenite for 48 hours caused changes in levels of proteins that are part of that cascade, and the LOEC was 2 µM. Sometimes there was a dose response, and sometimes the direction of the change reversed. Cheng et al. (2004) showed that a 48-hour pretreatment of HepG2 cells with 4 µM sodium arsenite was sufficient to block induction of STAT3 activity by an IL-6 treatment. Other experiments showed that As^{III} acted directly on the JAK1 protein to cause JAK-STAT inactivation. Di Gioacchino et al. (2007) studied the effects of several arsenicals on PBMC proliferation and cytokine release. At a concentration of 100 µM, sodium arsenite was effective in decreasing PHA-induced cell proliferation and in reducing interferon-gamma (IFN-γ) and TNF-α release. However, at a concentration of 0.1 µM, As^{III} significantly increased cell proliferation. More details about that experiment are found in Appendix D.

Regarding Inhibition of Differentiation, in experiments done on spontaneously immortalized human keratinocytes and on normal human epidermal cells derived from foreskin, sodium arsenite was shown to delay differentiation and preserve the proliferative potential of keratinocytes (Patterson et al., 2005 and Patterson and Rice, 2007). A concentration of sodium arsenite as low as 0.1 µM over 4 days had a noticeable effect, but most experiments were done using 2 µM sodium arsenite over 4-14 days, which yielded a much larger effect. Treatment of C3H 10T1/2 cells with 6 µM sodium arsenite for 8 weeks completely inhibited their differentiation into adipocytes following dexamethasone/insulin treatment, and treatment with 3 µM sodium arsenite for only 48 hrs was the LOEC for that effect (Trouba et al., 2000).

Interference with Hormone Function was demonstrated in experiments by Bodwell et al. (2004, 2006). Some effects were observed at approximately 0.09 µM of sodium arsenite; however, the increases found in glucocorticoid-receptor-mediated gene transcription of reporter genes that contained tyrosine aminotransferase (TAT) response elements were highly dependent on, and inversely related to, the amount of activated steroid receptor within cells. More detailed information on interference with hormone function can be found in Table C-3.

Under the category Malignant Transformation or Morphological Transformation, Table C-3 shows that concentrations of less than 1 µM of As^{III}, MMA^{III}, or DMA^{III} are capable of causing transformation. HaCaT cells exposed to 0.5 µM As^{III} for 20 passages caused the cells to become tumorigenic, as shown by production of tumors 2 months after injection into Balb/c nude mice (Chien et al., 2004). Zhao et al. (1997) found similar results with another cell line after 18 weeks of exposure to 0.25 µM As^{III}. UROtsa cells exposed to 0.05 µM MMA^{III} for 52 weeks caused anchorage-independent growth as detected by colony formation in soft agar, and cells from those colonies showed enhanced tumorigenicity in SCID mouse xenographs (Bredfeldt et al, 2006). After 26 weeks, this experiment showed that there was much anchorage-independent growth but not yet enhanced tumorigenicity. Syrian hamster ovary (SHE) cells exposed to DMA^{III} for 48 hours showed morphological transformation at a concentration of only 0.1 µM, and at the highest dose tested of 1.0 µM, 3.35% of the surviving colonies had become transformed (Ochi et al., 2004). In contrast, at a dose of 10 µM after the same exposure duration of 48 hours, As^{III} had only transformed 0.48% of the surviving cells.

Many findings related to the category Signal Transduction are summarized in Table C-3 even though considerable data found under Aberrant Gene or Protein Expression could alternatively have been placed into this category. Most of the data in this category are for sodium arsenite or ATO. In addition, there are numerous LOECs smaller than 10 µM (often much less), and they are often for treatments that lasted much less than one day. Drobná et al. (2002) evaluated phosphorylation of extracellular signal-regulated kinase (ERK)-2, activator protein (AP)-1 binding activity, and phosphorylation of c-Jun (an AP-1 protein) by six arsenicals in treatments lasting up to 2 hours. As^{V}, MMA^{V}, and DMA^{V} were all tested at concentrations up to 100 µM and had no effect. As^{III}, MMA^{III}, and DMA^{III} each had an LOEC of 0.1 for at least one endpoint. Details presented in Table C-3 show that the responses of those three arsenicals were different and that, in some cases, the direction of the response reversed direction as the concentration increased. In some cases a reduction from an increase was observed, which is interesting because various responses for some endpoints described above showed a reversal in which the lowest doses caused a bigger effect. Another experiment showing a reversal in response (from a decrease to an increase) was for phosphorylation of Akt Thr308 in JB6 C141 cells (P+ mouse epidermal cell line) (Ouyang et al., 2006). Following 1-hour exposures to sodium arsenite, there was slight decrease at 0.1 µM, a larger decrease at 0.5 µM, increases above the control level at 1 and 5 µM, and a much larger increase at 10 µM. Additionally, several experiments in this category related to different ways in which arsenic affects signal transduction to either increase or decrease apoptosis. For example, MCF-7 cells exposed to 2 µM ATO for one hour activated the pro-survival MEK/ERK pathway (Ye et al., 2005). By decreasing apoptosis, such an effect might permit the survival of cells containing damage that could eventually lead to a cancer. Yancy et al. (2005) did a series of experiments on H9c2 cells (an immortalized myoblast cell line derived from fetal rat hearts) and concluded that sodium arsenite exposure decreases cell migration through an effect on focal adhesions and by disrupting cell interactions with the extra-cellular matrix. Focal adhesions are involved in integrin signaling. Florea et al. (2007) showed that ATO triggered three different kinds of Ca^{2+} signals (i.e., steady state increases, transient elevations, and calcium spikes). The Ca^{2+} concentration in cells was substantially increased (and by rather similar amounts) by exposure to either 0.1 or 1 µM ATO for about 1 hour in two different cell lines (i.e., the human neuroblastoma cell line SY-5Y and the human embryonic kidney cell line HEK 293).

4.5 SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

Not addressed in this document.

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, 2005a), 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. Arsenic is a multisite carcinogen with numerous studies finding arsenic associated with increased incidences of a number of different types of cancers. The carcinogenic

effect of arsenic has been reported for populations in many different countries. While the studies detailed in this report provide evidence for cancer after oral exposure to arsenic, the fact that arsenic also has been associated with cancer after inhalation exposure (U.S. EPA, 1994) provides additional support.

4.6.2 Synthesis of Human, Animal, and Other Supporting Evidence

Numerous epidemiologic investigations, each conducted differently and containing its own biases (e.g., lack of confounding variables, possible recall bias, etc.), provide support for an association between oral exposure to inorganic arsenic and cancer including skin, bladder, kidney, lung, liver, and prostate. The most extensively studied population is from southwest Taiwan. This is because between 1910 and 1920, water supplies were changed from shallow surface water wells to artesian wells, which were subsequently found to contain high levels of arsenic in various regions. Studies in these endemic regions of Taiwan have found increases in all of the aforementioned cancer types. The link between these cancers and arsenic exposure in drinking water also have been observed in other parts of the world, including Japan, Chile, and Argentina. Therefore, it is unlikely that any single environmental factor (e.g., nutritional habits) associated with a single population is entirely 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 between arsenic intake and cancer risk were still observed in studies that did account for confounding variables (e.g., life style habits, age, and socioeconomic status).

Most of the epidemiology studies examining the relationship between arsenic exposure from drinking water and cancers are ecological in nature and are therefore subject to the limitations inherent in such studies (e.g., lack of measured individual exposure). For a number of reasons, the southwest Taiwanese database remains the most appropriate source for estimating bladder and lung cancer risk among humans (NRC, 1999, 2001; SAB, 2000, 2007), despite lacking individual water consumption and non-water arsenic intake. Strengths of the data include the size of the population, the reliability of the population and mortality counts, the stability of residential patterns, the homogenous lifestyle as confirmed by surveys, the long-term exposures, the extensive follow-up (almost 900,000 person years), the large number of exposed villages (42), and the large number of cancer deaths (1152 recorded from 1973-1986). Population records in Taiwan have been well kept since 1905, and death certificates include all primary cancers. In addition, cancer cases were pathologically confirmed in some of the Taiwanese studies.

Although there have been dose-response relationships observed for the majority of cancers noted in areas with high-levels of arsenic in their drinking water, results for low-level arsenic epidemiologic investigations (primarily from the United States and Europe) have been equivocal with regard to the relationship between these cancers and arsenic exposure. This could be due to the fact that none of the studies accounted for arsenic exposure through food sources. Kile et al. (2007) found that as the level of arsenic in the water decreased for women in Bangladesh, the contribution of arsenic from dietary sources became of greater importance. Uchino et al. (2006) found that with concentrations of 50 ppb or less of arsenic in the drinking water in a population in West Begal, India the contribution of arsenic from food was the main

source of arsenic exposure (i.e., contribution from water with less than 50 ppb was less than 27% of the total arsenic consumed). Therefore, as the exposure of arsenic from drinking water decreases and the relative contribution from food increases, misclassification of exposure groups can become significant. Average estimate of inorganic As consumption in food ranges from 1.34 µg/day in infants to a high of 18 µg/day in adults for a total arsenic average of 62 µg/day for people in the United States (NRC, 1999). At the lower concentrations, dietary intake could easily create total arsenic intake levels to be similar between the referent group and what is considered the exposure group.

Cantor and Lubin (2007) also conclude that misclassification occurs because exposure is not necessarily assessed during disease-relevant exposure periods. In regards to cancer, there is a long latency period, which appears to vary depending on the type of cancer and exposure. This means that exposure to arsenic sources during the decades prior to cancer outcome is necessary. Therefore, studies with low levels of exposure that are ecological in nature (no individual exposure) are more prone to misclassification, which means they are biased towards the null hypothesis. In addition, studies that attempted to individualize exposure by examining toenail arsenic levels are looking at only the prior year of exposure (Cantor and Lubin, 2007) and may miss the important exposure period. Despite all these numerous limitations in low-level exposure studies, significant associations have been observed for cancers of the prostate (Hinwood et al., 1999; Lewis et al., 1999), skin (Hinwood et al., 1999; Karagas et al, 2001; Beane-Freeman et al., 2004; Knobeloch et al., 2006), and bladder (Kuritto et al., 1999; Steinmaus et al., 2003; Karagas et al, 2004). In most cases, however, there is no dose-response with increases observed at the highest concentrations only and in many cases significant results occurred in smokers only.

There is very little animal data demonstrating arsenic's carcinogenic potential. This is likely due to the fact that rodents, which are the most likely animal model, are better methylators of arsenic than humans (Vahter, 1999a). Since it has been noted that humans that are better methylators are at lower risk (Yu et al., 2000; Chen et al., 2005a; Steinmaus et al, 2005; Valenzuela et al., 2005; Ahsan et al., 2007; Huang et al., 2007b; and McCarthy et al., 2007a), it is not surprising that animals that are better methylators are at even lower risk. As stated before, arsenic has been associated with cancers of the skin, lung, kidney, bladder, and liver. Below is a look at these different types of cancers and their association with arsenic exposure in drinking water.

4.6.2.1 Skin Cancer

Epidemiologic investigations of populations in the arseniasis-endemic areas of Taiwan have shown that exposure to arsenic from drinking water is associated with skin cancer (Tseng et al, 1968; Tseng, 1977; Chen et al, 1985, 1988a,b; Wu et al., 1989; Chen and Wang, 1990; and Tsai et al., 1999). The prevalence rate for skin cancer showed an increasing gradient according to the arsenic content of the well water. Guo et al. (2001) found significant increases in SCCs at the highest dose only (>640 ppb) with results at lower doses variable, suggesting that skin cancers may be cell-type specific. Contrastingly, Karagas et al. (2001) found increases in both SCC and BCC in the highest toenail arsenic concentration in a population in the United States. Beane-Freeman et al. (2004) also found an increase in the risk of melanoma with elevated toenail

arsenic concentrations. Therefore, these results demonstrate that skin cancers may not be cell-type specific. Although Taiwan has been the area most associated with skin cancers in relation to arsenic exposure, the association has been made in other populations as well. Arsenic has also been associated with skin cancers in Argentina where signs of arsenicism also have been observed (Smith, 1998). Hopenhayn-Rich et al. (1998), however, found a significant association in women in the highest category and surprisingly in males in the lowest category only. Skin cancer has also been found in China with drinking water concentrations of 150 ppb or greater (Lamm et al., 2007). Skin cancer was not found associated with arsenic in Denmark (Baastrup et al., 2008) or in the United States (Meliker et al., 2007), but these studies were at lower concentrations of arsenic.

Skin tumors have only been induced in transgenic mice or with subsequent TPA or UV exposure (indicating co- carcinogenesis) in mice. Because co-carcinogenesis has been demonstrated in animal models it is possible that the same occurs in humans. Sun exposure would likely be high and the use of sun block is less likely in the areas where skin cancer has been noted (i.e., Taiwan and Argentina). Therefore, a possible co-carcinogenic effect also may be contributing to the association.

4.6.2.2 Lung Cancer

Lung cancer has been associated with arsenic in populations that were exposed to exceedingly high arsenic levels in Taiwan, Chile, and Argentina. Studies of populations with lower arsenic exposure, especially <50 ppb, have not conclusively found an association between arsenic and lung cancer. Lung cancer was not associated with arsenic exposure in the United States (Lewis et al., 1999 and Meliker et al., 2007), Denmark (Baastrup et al., 2008), or Australia (Hinwood et al., 1999). Yang et al. (2004) found that lung cancer incidence in endemic areas of Taiwan remained elevated even after the use of the arsenic-containing well water ceased. Yuan et al. (2007) also found that mortality from lung cancers exceeded that observed in regions with consistently low arsenic exposure even after a 10-20 year lag period after removal of the arsenic source. These were likely due to the long latency for cancer. Many of the studies have not controlled for smoking history, which is a potential confounder for lung cancer.

4.6.2.3 Kidney, Bladder, and Liver Cancer

Significant increases in mortality rates for cancers of the kidney, bladder, and liver have been identified in populations from Taiwan, Argentina, and Chile. These three regions all have elevated levels of arsenic exposure through drinking water. Yang et al. (2004) found that arsenic was associated with kidney cancers in Taiwan. Unlike lung cancer, the mortality associated with kidney cancer decreased after reducing arsenic exposure. Yang et al. (2005) also found a reduction in bladder cancer after removal of arsenic exposure (through tap water instillation), but the decline was gradual. In Chile, supplementation of drinking water with water from rivers caused exposure to high levels of arsenic, but after the installation of improved water treatment in the early 1970s, arsenic exposure dropped dramatically. Yuan et al. (2007), however, found that even after a 10-20 year lag period after removal of the arsenic source, mortality from bladder cancers still exceeded that observed in regions with consistently low arsenic exposure.

While high levels of arsenic have been found to be related to bladder, kidney, and liver cancers, low-dose exposures from the United States, Europe, and Australia have been less clear. Lewis et al. (1999) observed increased SMRs in kidney cancer for both males (SMR=1.75) and females (SMR=1.60), but the results were not significant. Because the highest concentration in this population was 166 ppb, the results are still noteworthy. Kurttio et al. (1999) found that despite the low levels of arsenic (median=0.1 ppb; max=64 ppb) there was evidence of a relationship between exposure to arsenic at levels above 0.5 ppb and bladder cancer risk. No association was observed for kidney cancer risk. Hinwood et al. (1999), Meliker et al. (2007), and Baastrup et al. (2008) did not find associations between these cancers and the low levels of exposure in Australia, U.S., and Denmark.

Although inorganic As exposure in rodents has not been observed to cause increases in cancer, long-term (104 weeks) exposure to DMA^V in rats has been found to increase bladder tumors with doses of 50 ppm or greater. These concentrations are quite high in comparison to the amount of inorganic arsenic exposure in humans.

4.6.2.4 *In Utero* Exposure

There is no adult animal model available to study the relationship between arsenic exposure via drinking water and cancer outcome; however, lung and liver tumors have been induced by inorganic As in mice when exposed during gestation. Pregnant dams were exposed for 10 days during gestation only; thereby, increasing the evidence that lung and liver cancers are associated with oral exposure to inorganic arsenic. Reproductive and adrenal tumors also have been observed with transplacental exposure in mice.

There is very little epidemiology information specifically linking *in utero* arsenic exposure to cancer outcome. Although the available epidemiological studies conducted in Taiwan and other countries included women of reproductive age, the cancer outcomes from adult exposures were not differentiated from *in utero* exposures. Recently, Smith et al. (2006), examined lung cancer rates (and other respiratory diseases) in cohorts born just before the peak exposure period in Antofagasta, Chile (meaning that they were not exposed *in utero* to high levels of arsenic, but were exposed during childhood) and cohorts born during the high-exposure period (indicating likely *in utero* exposure). Results demonstrated that exposure during either period of development caused increased risk of lung cancer; however, the results from early childhood exposures and/or *in utero* exposures were not compared to exposures during adulthood to determine the possible cancer sensitivity effects in humans.

Because both *in utero* studies in mice and a study in humans by Smith et al. (2006) indicate that lung cancer development may be associated with transplacental arsenic exposure, it provides an opportunity to examine the similarities in mechanistic effects mediating lung cancers between the two species. Several PBPK models exist for humans (Yu, 1999a,b and El-Masri and Kenyon, 2008) and in mice (Gentry et al., 2004). However, these studies are inadequate in interpreting the findings from the *in utero* studies in mice and relating them to human exposure concentrations.

4.6.3 Mode of Action Information

4.6.3.1 General Comments on MOAs

The carcinogenic MOA for inorganic arsenic is unknown. Multiple MOAs for inorganic As seem likely in view of the numerous ways in which arsenic acts upon living organisms and the several metabolites produced before it is excreted from the body. While this review focuses on inorganic As, the methylated species produced during its metabolism, especially the highly reactive MMA^{III} and DMA^{III}, probably play an important role in the carcinogenesis of inorganic As consumed in drinking water. Each successive product in the metabolic pathway has its own toxicity and carcinogenic potential, with possible differential transport into and out of different organs. In comparison to laboratory animals, humans excrete more MMA in urine and are more prone to arsenic-induced carcinogenesis. These findings suggest that MMA (probably in the trivalent form) may be of special importance to arsenic-induced carcinogenesis in humans. The finding of numerous different tumor types associated with arsenic exposure both in humans and transplacental animal models also supports the view that multiple MOAs are likely. Due to the complexities of the available data related to MOA, including the range of possible toxicities of the different arsenic species, the different levels of each arsenic compound in target tissues, multiple hypothesized key events, and multiple tissue tumor effects in humans, there is a need for improved PBPK models to assist in understanding the MOA. Although there are several PBPK models available (see Section 3.5), none have sufficiently addressed the complex nature of the kinetics associated with arsenic carcinogenesis; therefore, this is an ongoing effort along with BBDR modeling.

It seems useful to describe a few MOAs for cancer to use as a frame of reference when considering arsenic specifically. Although inorganic arsenic and its metabolites have not been found to induce gene (point) mutations, the key events involved in mutagenesis, i.e., (1) exposure of target or stem cells (2) reaction with DNA to produce DNA damage, (3) misreplication of a damaged DNA template or misrepair of DNA damage leading to a mutation in a critical gene in the replicating target cell, (4) replication forming a clone of mutated cells, (5) DNA replication possibly leading to additional mutations in critical genes, (6) unbalanced and uncontrolled clonal growth of mutant cells possibly leading to pre-neoplastic lesions, (7) progression of pre-neoplastic cells in those lesions resulting in emergence of overt neoplasms, solid tumors (which require neoangiogenesis), or leukemia, (8) additional mutations in critical genes occurring as a result of uncontrolled cell division, and (9) cancer occurring due to malignant behavior (adapted from Preston and Williams, 2005) may contribute to one or more arsenic mediated MOA(s) for carcinogenesis. A mutagen with the above MOA would likely be thought to have a linear dose response. It is unclear what the shape of the dose response curve is for any specific key event that might be involved in the MOA for arsenic and its metabolites. Therefore, a linear dose response is the prudent choice unless a full understanding of the dose response of the identified key events mediating the carcinogenesis is known.

A second example of a MOA is the one hypothesized for arsenical-induced urinary bladder carcinogenesis as follows: after the requisite arsenical ingestion, absorption, and metabolism, (1) DMA^{III} is excreted into urine above a critical concentration, (2) it reacts with urothelial critical sulfhydryl groups, (3) urothelial cytotoxicity and necrosis results, (4) urothelial

regenerative cell proliferation (hyperplasia) results, and (5) urothelial cancer develops; oxidative damage might possibly stimulate both steps 3 and 4 (adapted from Cohen et. al., 2007). Obviously this MOA directly relates to the topic of this review, and any combination of factors in which consumption of inorganic As would lead to more than the critical (threshold) concentration of DMA^{III} for a particular individual for a sufficient time could result in bladder cancer.

Section 4.4.1 provided abundant evidence that many potential key events can occur at levels of exposure that would be encountered in populations exposed to high levels of inorganic arsenic in drinking water. It seems possible that those key events could fit together in many ways to result in a MOA for carcinogenesis. For example, some known mutagen and/or carcinogen commonly encountered in the environment might cause the initiation step, and then various arsenic-induced key events would provide the later steps necessary to result in a cancer. Alternatively, oxidative damage to DNA (or other types of DNA damage caused by arsenic) would make the DNA more prone to be acted upon by some other agent to produce a mutation that fulfils the initiation step. Although arsenic exposure does not induce gene mutations, evidence from all three tables in Appendix C shows that chromosomal aberrations can be induced, and if a chromosome happened to break, for example, in a tumor suppressor gene, that mutation might provide an important step in a MOA. After the steps in a MOA resulted in cell proliferation and genomic instability, cancer would result when changes occurred that provided evasion of apoptosis, self sufficiency of growth signals and insensitivity to anti-growth signals, and limitless replicative potential (Hanahan and Weinberg, 2000). Vascularization would also be needed to help the tumors grow larger.

Many detailed reviews in the past decade have discussed possible MOAs for arsenic carcinogenesis Numerous ideas expressed in these reviews agree that exposure to inorganic As may be able to cause cancer by many alternative MOAs. For example, Kitchin (2001) discussed nine possible MOAs for arsenic carcinogenesis suggesting 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, and then the promotion of carcinogenesis. Florea et al. (2005) suggested that genomic damage, apoptosis, and changes in gene expression associated with arsenic exposure are related to arsenic-induced intracellular calcium disruption. Rossman (2003), Huang et al. (2004), and Simeonova and Luster (2000) also provided noteworthy reviews related to MOAs of arsenic carcinogenesis. Snow et al. (2005) reviewed effects of arsenic at low concentrations and suggested that hormesis (i.e., a biphasic response) occurs in regard to cell proliferation and/or viability, base excision DNA repair, and telomerase activity. While some low-dose effects (e.g., increased DNA repair) may be protective of carcinogenesis, other effects (e.g., cell proliferation or telomerase activation) may be protective and thus permit mutant cells to survive by preventing cellular senescence and death and may thereby be involved in arsenic's cancer promoting capacity.

Kitchin and Ahmad (2003) provided an in-depth review on oxidative stress. They did not reach a definitive conclusion on the role of oxidative stress in arsenic carcinogenesis, but rather stated,

- " …it may eventually be found that many arsenic species act through several modes of carcinogenic action at many stages of multistage carcinogenesis and that the concept of a single cause of arsenic carcinogenesis simply does not fit the existing facts."

Oxidative stress seems particularly attractive as an important early step for some of the following reasons. Some ROS species can interconvert between themselves or react with nitric oxide (NO) to become reactive nitrogen species (RNS). RNS have their own spectra of biological reactivity. High-energy ROS can convert to lower-energy forms and in the process can damage biological molecules. ROS and related species can be inactivated by cellular defenses. Extended, high-level exposure to reactive arsenic species might result in the depletion of generalized cellular defense mechanisms against oxidative damage. ROS have been postulated to be involved in both the initiation and promotional stages of carcinogenesis (Zhong et al., 1997; Bolton et al., 1998; Shackelford et al., 2000; Bolton, et al. 2000; and Chen et al., 2000b). 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). It has also been demonstrated or suggested that ROS can (or does) damage cells by the following mechanisms: lipid peroxidation; DNA and protein-modification; 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) (Li et al., 1998; Sen and Parker, 1996; and Lander, 1997). Activation of signal transduction pathways that 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 (Rossman, 2003). Luster and Simeonova (2004) cited the results of *in vitro* studies suggesting that arsenic stimulates cell proliferation through specific signal transduction pathways that are similar to other classic tumor promotors. There has been much research in the last few years on the effectiveness of As^{III}, especially ATO, on apoptosis, with much of it aimed at improving cancer therapy. Those results reveal the extreme complexity of the signal transduction cascades involved in controlling apoptosis. Regarding causation of cancer, any effects that inorganic arsenic ingestion might have on signal transduction pathways that inhibit apoptosis could result in proliferation of damaged cells and thereby lead to cancer.

The few animal studies that suggest inorganic As is a complete carcinogen are those of Waalkes and his group that involved treatments *in utero*. Doses received by the pregnant dams were large compared to human exposures, but tissue levels in the fetuses were reported as being comparable to levels sometimes seen in humans. Almost all of the categories of key events discussed in this review can be caused by inorganic arsenic at exposure levels comparable to, or smaller than, those that would be present in large population groups presently. The experiments also indicate that typically when a treatment is extended over a longer period of time, the concentration of inorganic As necessary to cause an effect decreases. This indicates that the impact in humans suggested by the *in vitro* findings might be substantially greater than might be expected by just comparing the concentrations found in humans and in those used in experiments. Due to the complexities of the possible MOAs of inorganic arsenic mediated

carcinogenesis, various scientific tools (e.g., genomic tools, human pharmacokinetic and biologically based dose response models) may be needed in order to interpret the data for the hypothesized key events qualitatively and quantitatively in a meaningful way.

4.6.3.2 Low-Dose Extrapolation

According to the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a linear extrapolation to low doses is to be used either when there are MOA data to indicate that the dose-response curve is expected to have a linear component below the point of departure (e.g., DNA-reactivity or direct mutagenic activity) or when the available data are insufficient to establish the MOA for a tumor site. Since inorganic arsenic's MOA is unknown, a linear low-dose extrapolation was applied as a default option.

4.7 SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Several studies (Yu et al., 2000; Chen et al., 2005a; Steinmaus et al, 2005; Valenzuela et al., 2005; Ahsan et al., 2007; Huang et al., 2007b; and McCarthy et al., 2007a) have observed a correlation between increased disease risk and low urinary DMA and/or high urinary MMA, indicating a slower secondary methylation. Valenzuela et al. (2005) measured the levels of MMA^{III} in the urine of the residents of the Zimapan region of central Mexico. They found that individuals exposed chronically to arsenic who also had arsenic-related skin lesions had significantly greater concentrations and proportions of MMA^{III} in their urine than exposed individuals without skin lesions. These findings support the hypothesis that any factor (e.g., genetic variability in metabolic enzymes) associated with reduced secondary methylation (i.e., the conversion of MMA to DMA) may also be correlated with increase susceptibility to arsenic-induced disease. In the following sections, factors affecting DMA and/or MMA ratios and level in the urine or secondary methylation will be evaluated with regard to how they may affect individual susceptibility.

4.7.1 Possible Childhood Susceptibility

Although children are exposed to arsenic through generally the same sources as adults (i.e., air, water, food, and soil), their behaviors and physiology may result in them receiving higher absorbed doses in relation to their body weight than adults for a given set of exposure conditions. Because children tend to eat less varied foods than adults, exposure to contaminated food, juice, or infant formula prepared with contaminated water may result in higher doses than adults. In addition, children are more likely to ingest arsenic-contaminated soil, either intentionally or by putting dirty hands in their mouths.

There are few data on the relative efficiency of absorption of arsenic from the gastrointestinal tract of children compared to adults, but measurement of urinary arsenic levels in children indicate that absorption does occur. ATSDR (2007) suggests that there is some evidence that children may be less efficient at methylating arsenic. A decreased methylation capacity could lead to different tissue distribution and longer retention times that might possibly increase their susceptibility relative to adults. Adults have been demonstrated to excrete 40 to 60% of the arsenic as DMA, 20 to 25% as inorganic As, and 15 to25% as MMA. Concha et al.

(1998b), however, determined children ingesting 200 ppb (μg/L) arsenic in their drinking water excreted about 49% as inorganic As and 47% as DMA. Women in the same study were found to excrete 66% of the arsenic as DMA and 32% as inorganic arsenic. In contrast, others (Chowdhury et al., 2003; Meza et al., 2005, 2007; and Sun et al., 2007) have found that children have a higher urinary DMA:MMA ratio than adults, suggesting increased capacity for secondary methylation. Lindberg et al. (2008) also concluded that children and adolescents (i.e., <20 years of age) are more efficient methylators than adults (i.e., >20 years of age). Studying a population in Bangladesh exposed to high levels of arsenic in drinking water, Sun et al. (2007) found increased secondary methylation indices (SMI) in children exposed to 90 or 160 ppb of arsenic in drinking water, but not in controls. Chowdhury et al. (2003) also found that the increased methylation in children was only observed in exposed individuals (average concentration in drinking water 382 ppb) and not in the controls (<3 ppb in drinking water). This could indicate a lower saturation point for secondary methylation in adults than in children. Primary methylation indices (PMI) were not age-dependent in any case.

Epidemiological studies provide only limited data on whether childhood exposures to arsenic may result in increased cancer risk later in life. Because a significant, dose-response relationship has been found between cancer mortality and increased years of exposure to the high-arsenic artesian well water of southwestern Taiwan (Chen et al., 1986), it is important to consider the extent to which childhood exposures contributed to lifetime arsenic intake. The analysis of cancer risks 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 to 1986)[3]. No information was provided on the exposure of pregnant women in this population to the artesian well water. Arsenic has been found to pass through the placenta (Hanlon and Ferm, 1977; Lindgren et al., 1984; Hood et al., 1987; Concha et al., 1998a; and Jin et al., 2006a).

Chen et al. (1992) stated that their cancer study results may somewhat underestimate arsenic-related risks in this population because tap water with lower arsenic concentrations was introduced into the study area in 1956 and was available to almost 75% of the residents in the 1970s. Thus, the actual lifetime arsenic ingestion may be lower than estimated as residents switched from the high-arsenic artesian wells to alternate water sources. Also, because this study is based on mortality records (1973 to 1986) from the study region, it would not capture cancer incidence among individuals exposed during childhood and early adulthood who then migrated from the region. 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 less than 6%. There is limited migration into this region, and it has been reported that more than 90% of the local residents lived in the study area all their lives (Wu et al., 1989).

There is very little epidemiology information specifically linking *in utero* arsenic exposure to cancer outcome. Although the available epidemiological studies conducted in

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

Taiwan and other countries included women of reproductive age, the cancer outcomes from adult exposures were not differentiated from *in utero* exposures. Recently, Smith et al. (2006), examined lung cancer rates (and other respiratory diseases) in cohorts born just before the peak exposure period in Antofagasta, Chile (meaning that they were not exposed *in utero* to high levels of arsenic, but were exposed during childhood) and cohorts born during the high-exposure period (indicating likely *in utero* exposure). Results demonstrated that exposure during either period of development caused increased risk of lung cancer; however, the results from early childhood exposures and/or *in utero* exposures were not compared to exposures during adulthood to determine the possible cancer sensitivity effects in humans.

Although there is no adult animal model available for arsenic carcinogenesis, administering inorganic As to mice for 10 days during gestation has been found to increase the incidence of lung, liver, reproductive and adrenal tumors (Waalkes et al., 2003, 2004a, and 2006a). Thus demonstrating that, at least in animals, embryos are more sensitive to the carcinogenic effects of arsenic.

The *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) indicates that age-dependent adjustment factors should be applied to the slope factor and combined with early-life exposure estimates when estimating cancer risks from exposures to carcinogens with a mutagenic MOA. A mutagenic MOA for inorganic arsenic has not been determined, therefore, the application of age-dependent adjustment factors is not recommended.

4.7.2 Possible Gender Differences

Differences in methylation patterns have been noted between men and women in a number of studies. Higher MMA:DMA ratios have been observed in men compared to women in a variety of populations tested, including in the U.S. (Hopenhayn-Rich et al., 1996b and Steinmaus et al., 2005, 2006, 2007), Taiwan (Tseng et al., 2005) and Bangladesh (Ahsan et al., 2007). In contrast, Loffredo et al. (2003) found that gender differences in arsenic methylation varied across populations studied in Mexico, China, and Chile, and sometime by exposure level. Based on mean urinary metabolites levels, they found no difference in the MMA:DMA ratio between males and females in China in the group with the highest arsenic levels in their drinking water (i.e., 405 ppb). Low-exposure Chinese males (i.e., 18 ppb in drinking water) had MMA:DMA ratios similar to both the high-dose males and females (0.31 to 0.32), but low-dose females had a much lower (i.e., 0.22) MMA:DMA ratio. In Mexico, there was a difference between the sexes at high concentrations (408 ppb in the drinking water) of arsenic (i.e., the MMA:DMA ratio was 0.23 in males vs. 0.18 in females), but there was no differences in the MMA:DMA ratio (0.11) at low concentrations (i.e., 30 ppb in the drinking water). In Chile, a completely different pattern was observed, with females exposed to high concentrations (600 ppb in the drinking water) demonstrating a higher MMA:DMA ratio (0.27) than males (0.20), while the opposite pattern was seen at low concentrations (30 ppb in the drinking water; 0.18 in males vs. 0.13 in females). Studying a population in Bangladesh exposed to high levels of arsenic in drinking water, Heck et al. (2007) found a higher percentage of urinary MMA in men and a higher proportion of urinary DMA in women.

Age and reproductive status also may affect the male-female differences in arsenic methylation patterns. Concha et al. (1998a) demonstrated that pregnant women in their third trimester excrete approximately 90% of arsenic as DMA. Engström et al. (2007) also found pregnant women to have an increased proportion of DMA in their urine compared to non-pregnant women in the same population, with increases occurring with gestational age. This indicates possible hormonal effects on arsenic methylation. Lindberg et al. (2007) also found possible hormonal effect on arsenic methylation, noting that females younger than 60 (i.e., likely pre-menopausal) generally had a more efficient methylation than men of the same age, while the difference narrowed considerably in males and females over 60. Lindberg et al. (2008) found that although females of all ages generally were better at methylating arsenic than males, the greatest disparity between the sexes occurred between the ages of 20 and 55 (childbearing age in women). Lindberg et al. (2007) also found that selenium, BMI, and AS3MT polymorphism affected the observed proportions of methylated urinary arsenic metabolites in males only. The pattern of arsenic methylation was also altered in males with mutations in one allele of the methylenetetrahydrofolate reductase (MTHFR) gene, but in females variants in both alleles were required.

Brenton et al. (2006) used a case-control study with 900-case-control pairs to examine the effect of hemoglobin levels on skin lesion prevalence in Pabna, Bangladesh. A 1.0 g/dL increase in hemoglobin was found to be associated with a 21% decrease in the odds for having skin lesions even after adjusting for toenail arsenic levels, BMI, education, biri or cigarette smoking, chewing tobacco, and betel nut chewing. However, when the data was examined further, it was discovered that the hemoglobin levels were correlated with decreased skin lesion prevalence only in males (40% reduction), but not in females. Females, however, were more likely to have anemia than males (18.2% vs. 8.2%; $p<0.0001$). A subsequent cohort study (Brenton et al., 2006) found that hemoglobin levels were not associated with changes in urinary arsenic levels or MMA/DMA ratios.

4.7.3 Other

4.7.3.1 Genetic Polymorphism

Despite the observed differences in methylation related to age and sex, data from Bangladesh analyzed by Lindberg et al. (2008) suggest that genetic polymorphism is the most important factor affecting the methylation of inorganic arsenic, with only 30% of variation in methylation patterns attributable to level of arsenic exposure, gender, and age. Most humans excrete 10 to 30% of absorbed inorganic arsenic as unchanged in urine, 10 to 20% as MMA, and 60 to 80% as DMA. Excretion patterns vary across populations, however. 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., 1995b and Concha et al., 1998b). Studies on populations in San Pedro and Toconao in northern Chile demonstrated differences in the ratio of MMA:DMA excretion between the two populations (Hopenhayn-Rich et al., 1996b). 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 or nutritional factors, human genetic

polymorphism has been reported for methyltransferases believed to be involved in arsenic metabolism (e.g., thiopurine S-methyltransferase; Yates et al., 1997).

Chung et al. (2002) studied the association of familial relationships with urinary arsenic methylation patterns in eleven families (father, mother, and 2 children studied from each family) from Chile where drinking water concentrations were 735-762 ppb. Their results indicate that 13-52% of the variation in methylation patterns could be explained by being a member of a specific family. There was a high and significant correlation in the methylation patterns between siblings and a much lower correlation between parent and child, which could be attributed to inherent differences in methylation patterns between children and adults. Adjusting for nutritional factors (blood levels of methionine, homocysteine, folate, vitamin B_6, selenium, and vitamin B_{12}) did not notably alter the correlation. As might be expected, the correlation between father and mother was relatively low, even when adjusted for age and gender. However, the correlation became stronger when adjusting for homocysteine levels as well.

Meza et al. (2005) found a strong association between the variations in the DNA sequence of AS3MT and urinary DMA:MMA ratios in native populations in Yaqui Valley in Sonora, Mexico. Three polymorphic sites were found to be associated with increased DMA:MMA levels in the study population, but site 30585 was most strongly associated with urinary arsenic metabolite patterns. Using a stepwise linear regression model with DMA:MMA as the dependent variable and 30585 genotype, age, sex, and log-converted daily arsenic dose as independent variables, only the 30585 genotype and age were found to have a highly significant association with DMA:MMA levels. Further investigation determined that there was no significant genetic association observed in adults, but there was a highly significant effect in children aged 7 to11 years. There was no difference in the allele frequencies at the 23 sites examined between the adults and children.

Engström et al. (2007) also found a strong association between the presence of three intronic single nucleotide polymorphisms in AS3MT (i.e., G12390C, C14215T, and A35991G) and increased DMA levels. The study population consisted of adult women living in San Antonio de los Cobres (a village in the northern Argentinean Andes) who were exposed to approximately 200 ppb of arsenic in their drinking water. This group provided a rather uniform genetic background against which to examine the impact of polymorphism alone as a variant. Subjects who were homozygous for one or more of the variant alleles had lower MMA and higher DMA levels than heterozygotes, which in turn had lower MMA:DMA ratios than individuals lacking the alleles. Because the proportion of ingested inorganic As that was excreted was relatively constant across the groups, the effects of the variants were attributed primarily to increased secondary methylation. Individuals homogenous for all three variant alleles were found to have the lowest proportions of urinary MMA and the highest proportions of DMA among all the groups studied.

A case-referent study in Bangladesh evaluated arsenic metabolite patterns in 594 individuals with arsenic-related skin lesions compared to 1041 controls (Ahsan et al., 2007). A correlation was found between increased arsenic concentrations in the drinking water, increased proportions of MMA in the urine, and the risk of skin lesions, suggesting that variations in secondary methylation could increase the risk of developing such lesions. Individuals with

variants in MTHFR (677TT/1298AA and 677CT/1298AA diplotypes) also had slightly increased skin lesion risk (OR 1.66 and 1.77, respectively). However, the risk for developing skin lesions in relation to all at-risk alleles for the GSTO1 diplotype was 3.91. Additivity of effect was observed when the genotypes were analyzed jointly with water arsenic concentrations and proportion of urinary MMA.

Steinmaus et al. (2007) examined the association between genetic polymorphisms in MTHFR and GST and urinary arsenic metabolites in 170 subjects from Argentina. Subjects with the TT/AA variant of MTHFR 677/1298 were found to have higher urinary proportions of inorganic arsenic and MMA (not statistically significant), and lower levels of DMA, with the results being more pronounced in males. A null genotype of GSTM1 in woman was significantly associated with lower proportions of urinary MMA and higher proportions of urinary DMA compared to women with the active genotype. While the same trend was observed in males, it was weaker and did not achieve statistical significance. Polymorphism in the GSTT1 gene was not associated with differences in arsenic methylation. Lindberg et al. (2007) also found that carriers of the variant allele of the M287T (C→T) polymorphism of the AS3MT gene or the A222V (C→T) polymorphism in the MTHFR gene had higher proportions of urinary MMA.

McCarthy et al. (2007a,b) examined the effect of GST polymorphisms on skin lesion risk in a case-control (600 pairs) study in Pabna, Bangladesh. In one study (2007a), they found that a 10-fold increase in MMA/inorganic As ratio was associated with a 1.5-fold increase in risk of skin lesions. There was a significant interactive affect between GSTT1 wildtype and secondary methylation on skin lesions, but no interactive affects with the GSTM1 or GSTP1 genotypes or any of the genotypes with primary methylation. In their second study (2007b), however, they found a greater risk for skin lesions in GSTT1 wildtype (OR=1.56, 95% CI 1.10-2.19) compared to GSTT1 null status (referent group). The presence of the GSTP1 GG genotype was associated with a 1.86-fold increase (95% CI: 1.15-3.00) in risk of skin lesions over the AA genotype. However, none of the polymorphisms examined (i.e., GSTT1, GSTM1, and GSTP1) were found to modify the association between arsenic exposure and skin lesion risk.

Banerjee et al. (2007) also found a significant correlation between genetic polymorphism and skin lesions in a population in West Bengal, India. This population was selected because even though over 6 million people are exposed to high arsenic levels, only 15 to 20% developed skin lesions. Polymorphisms in ERCC2, which is a NER pathway gene, was examined. Specifically, the relationship between the ERCC2 codon 751 A→C polymorphism (lysine to glutamine) and skin lesion risk. Subjects exposed to arsenic-contaminated drinking water with hyperkeratosis (n=165) were compared to those without skin lesions (n=153). Occurrence of hyperkeratosis was strongly associated with the Lys/Lys genotype in the ERCC2 codon 751, with an OR of 4.77 (95% CI: 2.75–8.23). A significant increase in chromosomal aberrations in individuals with the AA genotype compared to either the AC or CC genotypes combined was also observed.

Brenton et al. (2007a) observed a positive association between total urinary arsenic and oxidative stress (as measured by 8-OHdG) in healthy women (only woman were studied) from Pabna, Bangladesh with the GSTM1 null genotype. No such association was found in GSTM1

positive women. APE1 (apurinic/apyrimidinic endonuclease) was found to be a predictor of 8-OHdG levels with the variant allele associated with a decrease in 8-OHdG. Other factors that also were predictive of 8-OHdG levels included creatinine, betel nut chewing, presence of environmental tobacco smoke in the home (even though none of the women reportedly smoked themselves), and education.

In a case-control study with 792 pairs with and without skin lesions in Pabna, Bangladesh, Brenton et al. (2007b) studied the association between genetic polymorphisms in the base excision DNA repair pathway and arsenic-induced skin lesions. Four common base excision repair (BER) genetic polymorphisms (X-ray repair cross-complimentary group 1 [XRCC1] Arg399Gln, XRCC1 Arg194Trp, human 8-oxoguanine DNA glycosylase [hOGG1] Ser326Cys, and APE1 Asp148Glu) were examined. APE1 148 Glu/Glu individuals were twice as likely to have skin lesions compared to APE1 148 Asp/Asp individuals, even after adjusting for toenail arsenic concentration, BMI, education, smoking, and betel nut use. Presence of the Glu/Glu variant of APE1 Asp148 Glu was associated with a 2-2.5 fold increased OR for skin lesions compared to the Asp/Asp variant, in the low and middle tertiles, but no increase was observed in risk at the highest tertile of exposure. XRCC1 Arg194 Trp genotypes, however, were not associated with skin lesion risk in the low and middle tertiles, but were associated with 3-fold difference in the highest exposure tertile (i.e., OR of 2.9 for Trp/Trp compared to 8.4 for Arg/Arg where Arg/Arg at the lowest tertile is the referent group). No association was observed between skin lesions and genetic polymorphisms in XRCC1 Arg399Gln or hOGG1 Ser326Cys alleles.

4.7.3.2 Nutritional Status

In many of the epidemiological studies discussed above (e.g., southwestern Taiwan and Bangladesh), the study subjects were relatively poor and had poor nutritional status. 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 increased RR (2.1 for females and 1.5 for males) in the prevalence of arsenic-associated keratosis. Lindberg et al. (2008), however, found that women in Bangladesh were better at methylating arsenic compared to men even though they were less likely to eat nutritious food (e.g., meat and fresh vegetables) than men, indicating that gender was a better predictor of methylation capacity than nutritional status in this group.

Selenium (Se) has been demonstrated to reduce the teratogenic, clastogenic, and cytogenic effects of arsenic (ATSDR, 1993). Chen et al. (2007) found individuals in the Health Effects of Arsenic Longitudinal Study (HEALS; population from Araihazar, Bangladesh) with low selenium intake were at a greater risk for developing pre-malignant skin lesions than those with adequate intake. Christian et al. (2006) found in 93 pregnant women from Antofagasta that increases in urinary selenium levels were associated with increased urinary arsenic excretion, and with a greater percent excreted as DMA and less excreted as inorganic arsenic. The proportion of urinary MMA was fairly consistent in the study population. Using 4 quartiles of increasing urinary selenium levels, results showed that the total arsenic excretion increased steadily across quartiles of selenium intake. The proportion of DMA excreted increased, and the proportion of inorganic As excreted decreased with increasing selenium intake, but only in the

first two quartiles. Although different gestational stages of pregnancy have been associated with differences in urinary arsenic excretion patterns, this was controlled for in the analysis.

Gamble et al. (2005) suggest that adequate folate is necessary for both primary and secondary arsenic methylation and that adequate folate intake is associated with increased urinary DMA. Gamble et al. (2006) found that providing folate supplements to individuals from Araihazar, Bangladesh with a diet low in folate caused a significant increase in the proportion of arsenic excreted as DMA in the urine. Heck et al. (2007), however, found that levels of folate consumption (measured by levels in the food) were directly related to percentages of urinary MMA, but not to changes in urinary DMA in a population from Bangladesh (participants of the HEALS study) exposed to arsenic in drinking water. In Heck et al.'s (2007) study, they found no correlation between intake of folate-related nutrients and urinary DMA levels, but found that increases in methionine, vitamin B_{12}, calcium, protein, and riboflavin were associated with decreases in the proportion of urinary inorganic arsenic and increases in the percent of urinary MMA. Niacin and choline were found to be the better predictors of secondary methylation (as measured by DMA/MMA). Although high levels of plasma homocysteine were not associated with urinary MMA levels, they were associated with a decrease in DMA levels (Gamble et al. 2005).

Mitra et al. (2004) studied whether nutritional deficiencies increased the susceptibility of individuals to arsenic-related health effects as measured by skin lesions. In West Bengal, India, where exposures were <500 ppb, nutritional assessments were based on a 24-hour recall for major dietary constituents and a 1-week recall for less common constituents. Increases in risk were associated with low intake of animal protein (OR=1.94, 95% CI: 1.05–3.59), calcium (OR=1.89, 95% CI: 1.04–3.43), fiber (OR=2.20, 95% CI: 1.15–4.21), and folate (OR=1.67, 95% CI: 0.87–3.2). Nutrient intake was not related to arsenic exposure. The authors concluded that the potential protective effects of these nutrients were small in comparison to eliminating the exposure to arsenic.

Steinmaus et al. (2005) found an association between low dietary protein, iron, zinc, and niacin, and decreased production of urinary DMA accompanied by increased levels of urinary MMA in arsenic-exposed individuals from a U.S. population. An associations between arsenic methylation patterns and dietary intake of thiamine, vitamin B_6, lutein, and α-carotene were found, but the links were not as clear when adjusted for confounding variables (i.e., age, sex, smoking, and total urinary arsenic levels). The authors suggest, however, that the effect of specific nutrient intake levels on methylation patterns were small in comparison with the known magnitude of interindividual variability associated with genetic polymorphisms. Kreppel et al. (1994) found that dietary zinc protects mice against acute arsenic toxicity.

4.7.3.3 Cigarette Smokers

Cigarette smokers (current or former) were found to have a decreased secondary methylation capacity, resulting in increased urinary MMA and decreased DMA concentrations (Huang et al., 2007b). Tseng et al. (2005) reported a decrease in secondary metabolism in cigarette smokers exposed to arsenic contaminated drinking water, resulting in a significant increase in the secreted MMA as a fraction of total metabolites. Steinmaus et al. (2005) found

that current smokers in a U.S. population had lower proportion of arsenic excreted as DMA than either former or never-smokers (although the difference was not statistically significant). Steinmaus et al. (2006) found that in a population in Argentina the proportion of excreted MMA was associated with bladder cancer risk in former-smokers, but not in individuals who had never smoked. Subjects who had ever smoked and had proportions of MMA in the upper tertile had a two-fold elevated risk of bladder cancer compared to subjects with proportions of MMA in the lower two tertiles. Therefore, it was concluded that individuals who smoke had an increased susceptibility to arsenic toxicity. Steinmaus et al. (2006) also studied a population in the U.S. Although the results indicated increased MMA was associated with increased cancer risk, the number of cases was too small to estimate separate odds ratios for never-smokers and ever-smokers.

5. DOSE-RESPONSE ASSESSMENTS

5.1 ORAL REFERENCE DOSE (RfD)

An RfD was developed for inorganic arsenic and posted on the IRIS database in 1991. An oral noncancer dose-response estimation is not addressed in this document. However, the Agency is currently reviewing the literature and will develop an updated RfD at a later date.

5.2 INHALATION REFERENCE CONCENTRATION (RfC)

An inhalation noncancer dose-response estimation is not addressed in this document. An RfC is not developed for inorganic arsenic, nor does a current value exist on the IRIS database.

5.3 CANCER ASSESSMENT (Oral Exposure)

5.3.1 Background: History of Cancer Risk Assessments for Arsenic

This assessment is unusual in that it builds on a long history of previous efforts by EPA and others to evaluate potential risks from oral exposure to arsenic via drinking water. Table 5-1 summarizes previous assessments and expert reviews of arsenic carcinogenicity.

The table starts (chronologically) with EPA's 1988 risk assessment for skin cancer (U.S. EPA, 1988b). The scope of the 1988 assessment was to review the applicability of EPA's 1984 assessment (U.S. EPA, 1984) on skin cancer risk from the Taiwanese population to the U.S. population. The skin cancer risk from oral exposure was estimated based on two studies (Tseng et al. 1968; Tseng, 1977) of age-specific prevalence rates for skin cancer in a large cohort of Taiwanese (40,241 subjects in 37 villages) in an "arseniasis endemic" area, where arsenic concentrations in water supply wells ranged from less than 10 µg/L (ppb) to 1820 µg/L. The occurrence of skin cancer was estimated in a survey lasting approximately two years (U.S. EPA, 1988b). Preliminary data from the same cohort suggested that risks of internal cancers (lung, liver, and bladder) were also elevated, but U.S. EPA (1988b) concluded that insufficient data were available to support a dose-response assessment for these effects.

The second entry in the table is the National Research Council's 1999 review (NRC, 1999) of EPA's 1988 risk assessment. EPA commissioned NRC to review the U.S. EPA (1988b) assessment and also the qualitative and quantitative evidence on arsenic and health effects for reassessment of human health risks from arsenic in drinking water. One of the major recommendations of NRC's 1999 review was that studies from the arsenic endemic area of Taiwan (Wu 1989; Chen et al., 1988a, 1992) provide the best available empirical human data for assessing the risks of arsenic-induced cancer. The report explored quantitative modeling approaches for the male bladder cancer data, but did not provide a formal risk assessment; additional modeling analyses were recommended. NRC 1999 applied absolute Taiwan risks to the U.S. populations.

NRC (1999) published the arsenic concentration in village wells, person years of males and females by village and the village-specific lung, bladder, and liver deaths for the Wu et al. (1989) and Chen et al. (1992) studies. Additional raw data were obtained from study authors by Morales and Ryan during reanalysis and these data were subsequently provided to EPA (personal communications). All of the succeeding assessments summarized in Table 5-1 derive dose-response estimates based on the internal cancer data.

In the first of these efforts, Morales et al. (2000) gathered data on lung, bladder, and liver cancer, as well as detailed exposure data (well arsenic concentrations) from the three epidemiological studies (Wu 1989; Chen et al., 1988a, 1992), and evaluated a range of statistical models for estimating potential arsenic related cancer risks in the Taiwanese population, and for extrapolating these risks to the U.S. population. In promulgating the *Primary Drinking Water Standard for Arsenic*, U.S. EPA (2001) adopted one of Morales et al.'s models, with adjustments of some exposure assumptions, for estimating the health benefits of regulatory alternatives. The Office of Pesticide Programs (OPP) also recently applied cancer slope factors based on the U.S. EPA (2001) assessment in their Reregistration Eligibility Decision (RED) Documents for organic arsenic pesticides (U.S. EPA, 2006c) and for Inorganic Arsenicals and/or Chromium Based Wood Preservatives (U.S. EPA, 2008).

In response to continued public concern over arsenic-related cancer risks, EPA requested the NRC to update its 1999 recommendations in light of new scientific evidence, and to review the risk assessment in support of the 2001 Drinking Water standard. NRC (2001) reviewed the methodology used in EPA's arsenic risk assessment (U.S. EPA, 2001) and provided a systematic analysis of and recommendations for applying the Taiwanese epidemiological data for assessing cancer risks from arsenic exposure in U.S. populations. Recommendations included the inclusion of a reference population in the dose-response assessment, the form of the dose-response model, exposure assumptions, and approaches for extrapolating risks to the U.S. population. As the committee noted, the cancer risk estimates that it developed were higher than those reported by U.S. EPA (2001), and reasons for those differences were reviewed. EPA examined and applied the NRC (2001) statistical methodology and submitted its revised analysis (U.S. EPA, 2005c) to the Agency's Science Advisory Board (SAB) for review and comment. The SAB (2007) provided additional discussion related to the treatment of arsenic exposure, and recommended expanded sensitivity analyses of other exposure-related assumptions. EPA adopted these recommendations, along with responses to comments from interagency reviewers, into the current assessment. The current quantitative risk assessment can thus be described as

EPA's reimplementation of the technical cancer risk modeling recommendations in NRC (2001), with additional examination of arsenic exposure assumptions and taking into account SAB's (2007) advice for the expansion of sensitivity analyses of modeling methods and choices.

Table 5-1. Historical Summary of Arsenic Risk Assessment Efforts

Assumption/Method	U.S. EPA (1988b)	NRC (1999)	Morales et al. (2000)	U.S. EPA (2001)	NRC (2001)	U.S. EPA (2005c)
Goals/Scope of Assessment	Revise EPA's 1984 risk assessment for skin cancer, evaluate evidence of arsenic essentiality	Review EPA's 1988b risk assessment, suggest alternative approaches, but was "not a risk assessment"	Test dose-response models, modeling assumptions	Estimate U.S. cancer risks in support of Drinking Water Standard	Review EPA's 2001 methods and results	Incorporate NRC (2001) recommendations for SAB Review
Critical Study	Taiwan skin cancer prevalence studies (Tseng et al. 1968; Tseng, 1977)	Taiwan epidemiological studies (Wu et al., 1989; Chen et al. 1988a, 1992)	Taiwan epidemiological studies (Wu et al., 1989; Chen et al. 1988a, 1992)			
Critical Study Endpoint(s)	Skin cancer incidence	Bladder cancer mortality	Bladder, lung, liver cancer mortality	Bladder, lung cancer mortality		
Dose-Response Model	Linear multistage	Weibull, Poisson Regression	Nine Poisson forms with varying age, dose representations, one multistage Weibull	Morales et al. "Model 1" (multiplicative linear dose, quadratic age)	Additive Poisson, linear dose, quadratic age	Additive Poisson, linear dose, quadratic age; UCLs on dose coefficients estimated by Bayesian Simulation
-Reference Population	Taiwanese outside of arseniasis endemic area	With and without All-Taiwan	None, southwest Taiwan, All-Taiwan	None	All-Taiwan, southwest Taiwan	southwest Taiwan
-As Concentration	Stratified; 0-300, 300-600, 600-900 µg/L in well water, unknown exposure	Median well As concentrations	Median well As concentrations	Median well As concentrations	Median; sensitivity analysis of other values	Median well As concentrations

Assumption/Method	U.S. EPA (1988b)	NRC (1999)	Morales et al. (2000)	U.S. EPA (2001)	NRC (2001)	U.S. EPA (2005c)
- Taiwanese Water Intake	3.5 L/day (M), 2.0 L/day (F)	3.5 L/day (M), 2.0 L/day (F)	Water intakes not specified	3.5 L/day (M), 2. 0 L/day (F) + 1.0 L/day cooking	Recommendations based on approx. 2 L/day. Sensitivity analysis of U.S./Taiwan intake ratios is presented.	2.0 L/day
- Taiwanese Body Weight	55 kg (M), 50 kg (F)	55 kg (M), 50 kg (F)	Body weights not specified	55 kg (M), 50 kg (F)	55 kg (M), 50 kg (F)	50 kg (M and F)
-Non-Water As Intake	None (0 µg/day)	Not explored	None (0 µg/day)	50 µg/day (exposed population)	None (0 µg/day) in baseline assessment; Sensitivity analysis showed little effect of adding 30 or 50 µg/day to study village exposure estimates.	30 µg/day exposed population only, sensitivity analyses of 0-50 µg/day
Risk Model for U.S. Population	Simple life table	Simple life table	Life table, 5-year age strata	Life table, 5-year age strata	BEIR IV survival model (relative risk)	
-U.S. Incidence, Mortality Data	Not specified	NCHS 1994 mortality data	NCHS 1996 mortality			
-U.S. Water Intake	2.0 L/day (approximate 90th percentile value)	2.0 L/day (approximate 90th percentile value)	Average US water Intake	1.0-1.2 L/day used as central tendency values; 2.1-2.3 L for 90th percentile risk in Monte Carlo model	1.0 L/day with sensitivity analyses	1.0 L/day
-U.S. Body Weight	70 kg (M and F)	70 kg (M and F)	Average US body weights	70 kg (M and F)		

Assumption/Method	U.S. EPA (1988b)	NRC (1999)	Morales et al. (2000)	U.S. EPA (2001)	NRC (2001)	U.S. EPA (2005c)
Endpoints Calculated	Unit risk = $3x10^{-5}$ per µg/L (females), $7x10^{-5}$ per µg/L (males); Slope factors = 1 to $2x10^{-3}$ per mg/kg-day (incidence)	Lifetime bladder cancer risk at 10 µg/L = $3x10^{-3}$ (males), $9x10^{-3}$ (females) ED_{01} = 404-431 µg/L, LED_{01} = 323-407 µg/L	"Model 1", no reference pop. Males (µg/L) ED_{01} LED_{01} Lung 364 294 Bladder 395 326 Females (µg/L) ED_{01} LED_{01} Lung 258 213 Bladder 252 211 Many other results presented	Slope Factors derived from Morales et al. (2000) ED_{01}, LED_{01} values. Unit risk, per µg/L: Male bladder= $2.5x10^{-5}$ (MLE), $3.1x10^{-5}$ (UCL) Male lung = $2.8x10^{-5}$ (MLE), $3.4x10^{-5}$ (UCL) Female bladder = $4.0x10^{-5}$ (MLE), $4.7x10^{-5}$ (UCL) Female lung= $3.9x10^{-5}$, (MLE), $4.7x10^{-5}$ (UCL)	Lifetime cancer risk incidence from 10 µg/L: Male lung = $1.8x10^{-3}$ bladder = $2.3x10^{-3}$ Female lung = $1.4x10^{-3}$ bladder = $1.2x10^{-3}$	Female lung + bladder incidence: Unit Risk = $1.6x10^{-4}$ per µg/L Incidence at 10 µg/L in drinking water = $1.6x10^{-3}$; Drinking water concentration for 10^{-4} incidence risk = 0.63 µg/L

The techniques and assumptions used in the arsenic risk assessment have evolved and changed over time, and it is not possible to do justice to all of the changes and innovations in each assessment in this chapter. Table 5-1 provides a general summary of the important data sources, techniques, and assumptions employed in each assessment. Where cells in the table are merged across the columns, this indicates that the same assumptions were used in more than one assessment, implying a solidification of a technical consensus. The major issues addressed in each study include:

- **The scope and goals of each assessment.** Some of the efforts in Table 5-1 (the NRC studies most importantly) were not intended to be comprehensive risk assessment, but to provide recommendations for EPA and other agencies. Some were pure modeling studies (Morales et al., 2000), and some were employed to derive quantitative risk estimates for regulatory support purposes (U.S. EPA, 2001) or for health criteria development. (U.S. EPA, 2005c, 2008)

- **Selection of critical studies for use in the risk assessment**. As noted above the EPA (1988b) assessment was based on skin cancer prevalence data (Tseng et al. 1968; Tseng, 1977). All of the subsequent assessments in the table use data from later epidemiological studies (Wu, 1989; Chen et al., 1988a, 1992), which provide information on person-years at risk and cancer mortality in narrowly defined age strata, and exposure concentrations from individual water supply wells.

- **Critical study endpoints.** Over time, assessments have moved from evaluating skin cancer (U.S. EPA, 1984, 1988b) to internal cancers (lung and bladder). As discussed below, the change in endpoint is the major reason that the cancer potency estimated in the current assessment is so different from that derived in 1988. Wu et al. (1989) and Chen et al. (1988a, 1992) also reported data on liver cancer, but in response to concerns related to a high incidence of viral hepatitis in Taiwan (U.S. EPA, 2001), liver cancer has not been included as an endpoint in recent assessments.

- **Dose-response models.** The form of the dose-response models used to assess risks in the Taiwanese population has evolved over time as different investigators explored the performance of various models under a wide range of exposure assumptions. In the early models, linear regression and multistage models were used for dose-response assessment in the Taiwanese population. In the more recent analyses, Poisson regression with linear dose terms and quadratic age terms have been employed, as recommended by NRC (2001), to derive primary risk estimates. In addition, sensitivity analyses of other Poisson models (different transformations of dose) have been conducted, as recommended by SAB (2007). Changes in the modeling approaches, like changes in the endpoints modeled, have resulted in substantial changes in estimated cancer potency.

- **Inclusion/exclusion of a reference population.** EPA's 2001 risk assessment was based on a dose-response model for the Taiwanese population that did not include a reference population (i.e., a group with similar characteristics not exposed to arsenic in drinking water). In keeping with NRC (2001) and SAB (2007) comments, the primary estimates in this chapter are derived based on the inclusion of a reference population from

southwest Taiwan; sensitivity analyses are provided for risk estimates with the reference population excluded and with a reference population from all regions of Taiwan (i.e., "all-Taiwan").

- **Arsenic concentration used in the dose-response model.** The available exposure data (Wu et al., 1989; Chen et al., 1992) consist of measurements from 155 village drinking water wells taken between 1964-1966 for the each of 42 exposed villages. Most of the assessments in Table 5-1 employed the median exposure concentrations for each group. That approach also is followed in this assessment; however, following SAB (2007) recommendations, a sensitivity analyses on the impacts of using minimum and maximum village arsenic concentrations in the risk assessment has been conducted.

- **Water intake and body weight of the exposed population.** As discussed in Section, 5.3.5, there is little precise data available concerning the distribution of daily drinking water intake volumes in the exposed populations. As shown in Table 5-1, past assessments have employed a range of assumptions; the basic consensus is that Taiwanese men appear to consume more water than men in the U.S. owing to the hotter climate, and because a large proportion of them engage in vigorous outdoor activity as part of their livelihood. Consistent with the limited information, the current analysis has followed this consensus. Following other analyses, this assessment assumes an average body weight of 50 kg for both Taiwanese men and women.

- **Non-water arsenic intake.** Because the risk modeling for the Taiwanese population is based on estimated daily arsenic dosage, it is important to include reasonable assumptions about the amount of arsenic intake coming from non-drinking water sources. This is an area where there is relatively little data, and considerable confusion about, for example, whether and how to include a contribution from cooking water, reasonable estimates of arsenic concentrations in food, and whether the arsenic-exposed and reference populations should be assumed to receive the same non-water arsenic intake. The various assumptions used in previous analyses are summarized in Table 5-1, and the basis for non-water arsenic intake estimates used in this assessment is discussed in Section 5.3.5. As is the case for many other assumptions, the approach to dealing with uncertainty in non-water arsenic intake is to conduct sensitivity analyses based on a reasonable range of values.

- **Risk model for the U.S. population.** The outputs of the dose-response modeling for the Taiwanese population were arsenic dose-response coefficients that described the relationship between estimated arsenic intake in the Taiwanese population and proportional increases in age-specific lung and bladder cancer mortality risk. Consistent with NRC (2001) recommendations, lifetime cancer incidence in U.S. populations was then estimated by using a modified version of the "BEIR IV" relative risk model, as described in Appendix E. A key assumption underlying this model is that the risk of arsenic-related cancer mortality or incidence for the U.S. population is a constant multiplicative function of the current "background" age profile of cancer risks in the same U.S. population.

- **U.S. mortality and cancer incidence data.** Models for extrapolating cancer risks for the U.S population require data on overall mortality, and the BEIR IV model requires non-arsenic related cancer incidence data for the U.S. population. One source of variation in the cancer risk estimates over time has been the use of more recent mortality and cancer incidence data in the most recent assessments.

- **U.S. water intake and body weight.** Another ingredient required to predict cancer risks in the U.S. population are estimates of the drinking water intake and typical body weight of the exposed population. All of the recent assessments assume body weight of 70 kg for males and females. For the primary risk estimates, the current assessment assumes a water intake of 2.0 L/day, as discussed in Section 5.3.5, with sensitivity analyses of other values. Adult water intake of 2.0 L/day is used as a standard factor in EPA IRIS assessments, and represents approximately the 90^{th} percentile of intake of community water in the U.S. population. Other intake assumptions (e.g., mean versus upper percentile) can be used in risk assessments, depending on target population characteristics and assessment needs.

- **Endpoints calculated.** As can be seen in Table 5-1, different assessments have calculated a range of risk endpoints, including ED_{01}s, LED_{01}s, lifetime cancer risks, cancer slope factors, and drinking water concentrations corresponding to various cancer risk levels. As discussed in Section 5.3.8.2, this can create some difficulty in comparing the results across assessment, since converting from one measure to another can require assumptions related to exposure that may not have been clearly specified. Where they have been calculated, the most commonly used and easily comparable endpoints are provided, including drinking water Unit Risks (lifetime cancer incidence associated with 1 µg/L exposure), estimate cancer risk at 10 µg/L in drinking water, and the drinking water concentration associated with a lifetime cancer risk of 10^{-4}.

Given the many features of the risk assessment for arsenic that have changed over time, it is not surprising that the magnitude of the risk estimates have also varied from assessment to assessment. As discussed above, the cancer slope factor from U.S. EPA's (1988b) assessment, which is derived based on skin cancer prevalence, is not directly comparable to slope factors derived from internal cancer data in the later assessments. Modeling methods and assumptions used in the current assessment, and precisely how they differ from previous analyses, are discussed in more detail in 5.3.8.2.

5.3.2 Choice of Study/Data, Estimation Approach, and Input Assumptions

As discussed in Section 4.2, the few animal carcinogenicity bioassays that have been conducted on inorganic arsenic compounds do not provide sufficient quality data to use in human dose-response modeling (NRC, 2001 and SAB, 2000, 2007). There are, however, several epidemiologic studies that relate human exposures to arsenic in drinking water to cancer risk. NRC (2001) and SAB (2007) agree that the epidemiological studies by Chen et al. (1988a, 1992) and Wu et al. (1989) that use the southwestern Taiwanese population provide the best available data for conducting a quantitative risk assessment for exposure to arsenic in drinking water. SAB (2007) cited the important strengths of the data, including the large population (40,421), extensive follow-up (almost 900,000 person years), large number of exposed villages (42), large number of lung and bladder cancer deaths (441), reliability of the population and mortality counts, and stability of residential patterns, stating that:

- "…in view of the size and statistical stability of the database relative to other studies, the reliability of the population and mortality counts, the stability of residential patterns, and the inclusion of long-term exposures, it is the Panel's view that this [the Taiwanese] database remains, at this time, the most appropriate choice for estimating cancer risk among humans. Supporting this view is the fact that the datasets from Taiwan have been subjected to many years of peer review as part of published studies."

In keeping with SAB's recommendations, epidemiological studies by Smith et al. (1998) and Ferreccio et al. (2000) on arsenic-related lung cancer in Chile, as well as studies by Chiou et al. (2001) and Chen et al. (2004a), were evaluated (see Section 4.1 and Appendix B); however, these studies were not considered to be of comparable quality to the Taiwanese data set for use in the quantitative assessment. The dose-response estimation discussed below, like previous analyses, is based on the southwest Taiwanese data and incorporates the NRC and SAB recommendations for modeling approaches and sensitivity analyses, to the extent practicable within resource and time constraints.

5.3.3 Dose-Response Model Selection for Cancer Mortality in Taiwan

Despite the high quality of the data set, estimation of dose-response relationships based on the Taiwanese data is challenging for a number of reasons. First, owing to the "ecological" nature of the study, drinking water exposure information is not available for individual study subjects. Instead, drinking water arsenic exposure must be estimated based on measured arsenic concentrations in wells serving the 42 population groups ("villages") that constitute the study population. For 20 of the 42 villages, water was supplied by a single well at the time of sampling; for other 10 villages water was supplied by 2 wells; however, the remaining villages used more than two wells. Data provided are related to all the arsenic measurements for each well in each village, but no information is available concerning the time variability of arsenic levels in individual wells.

In addition to villages where drinking water arsenic concentrations were measured, the epidemiological data used in this assessment includes information on the cancer mortality in two

reference populations (southwest Taiwan and all of Taiwan) for the same period covered by the Chen et al. (1988a, 1992) studies. Drinking water concentrations for the reference populations were not measured, but are assumed to be lower than those seen in the 42 arsenic-exposed villages (zero drinking water arsenic intake was assumed for the reference populations). As discussed below, the data on the non-water arsenic intakes available for both the exposed and reference populations are very limited (Schoof et al. 1998), so the impacts of different assumptions are explored through a sensitivity analysis.

It is clear that cancer mortality in the reference population and in the arsenic-exposed villages is strongly age-dependent, with the older study subjects generally exhibiting higher mortality. The age-dependence does not appear to be monotonic, however, but rather peaks around age 60 and declines thereafter. This non-linear age-dependence complicates the estimation of dose-response relationships because it requires the estimation of models using non-standard methods.

Chen et al. (1992) used an Armitage-Doll time-to-tumor model to estimate cancer risks as a function of dose in this population for 20-year age strata, but the model they used assumed monotonically increasing cancer risk with age. As discussed below, using narrower age strata (5 years), the non-monotonic dependence of cancer risk on age becomes more apparent. Morales et al. (2000) used a variety of non-linear models to fit dose-response functions to data derived from the Chen et al. (1988a, 1992) and Wu et al. (1989) studies. They derived cancer slope estimates for arsenic-associated cancers of the bladder, liver, and lung by using Poisson regression with a number of different methods for expressing the dependence of risks on age and arsenic intake. When no reference population was included in the data, the best-fitting model included a quadratic function of age and a linear exponential term for dose. When the southwest Taiwan reference population was included in the risk modeling, the best fitting model again included a quadratic age model, but an exponential function of log-transformed dose. A number of other models with different age and dose terms were found to fit nearly as well as judged by the Akaike Information criterion (AIC). Many of the models also were very sensitive to changes in input assumptions.

NRC (2001) reviewed the U.S. EPA (2001) cancer assessment including application of the model from the Morales et al. study and conducted independent analyses of the data in order to systematically evaluate the effects of different modeling approaches, assumptions related to background cancer rates, and individual variability in exposures. As noted above, they recommended two specific changes to EPA's modeling approach; the inclusion of a reference population, and the use of an additive (rather than multiplicative) linear dose term in the Poisson regression. SAB (2007) also reviewed EPA's modeling procedures. Given the NRC recommendations and results of the SAB review, the current model (see Section 5.3.7) employs the following approaches:

- Poisson regression (of cancer mortality against age and dose) fit by maximum likelihood estimation (MLE),
- A quadratic age model,
- Additive linear dose term,
- Confidence limits on the dose terms estimated by profile likelihood, and

- Primary risk estimates derived for the data set that includes the southwest Taiwan reference population.

As recommended by SAB, sensitivity analyses were conducted to evaluate the impacts of different modeling assumptions (non-water arsenic intake, daily water intake, and reference population) on risk estimates. Several different model forms (quadratic, exponential linear, and exponential quadratic dose transformations) also were evaluated (see Section 5.3.8.4 for further detail).

5.3.4 Selection of Cancer Endpoints and Estimation of Risks for U.S. Populations

Lung and bladder cancer mortality in the Taiwanese population have been chosen as endpoints in the dose-response modeling because they are the internal cancers most consistently observed and best characterized in epidemiological studies of arsenic exposure (U.S. EPA, 2001 and NRC, 2001). Oral slope factors and other risk metrics were calculated separately for each endpoint and gender.

Although liver cancer risks also were examined by Morales et al. (2000), they were not included in the quantitative risk assessment because the observed liver cancer mortality in the southwest Taiwanese population were thought to be affected by a high incidence of viral hepatitis, which made attribution of risks to arsenic problematic. As noted in Section 4.1, arsenic-related skin cancer also has been noted in the Taiwanese population (and in other arsenic-exposed groups), but this endpoint was not included in the cancer risk assessment for several reasons. The high mortality rates for internal cancers, compared to skin cancers which are rarely fatal, makes the internal cancers an appropriate critical health endpoints for the cancer risk assessment. In addition, the internal cancers were identified as the critical endpoints because the estimated cancer potency of arsenic for lung and bladder cancers were much greater than the potency estimated for skin cancers (see Section 5.3.8.1). The development of pre-cancerous skin lesions (as reported by Ahsan et al., 2006) is being addressed separately in EPA's noncancer risk assessment.

The current risk model includes multiplicative terms for age and dose. Therefore, the risk calculated for a target population (e.g., a U.S. population exposed to arsenic in drinking water) depends on the "background" cancer risk, i.e., the expected age-specific cancer risk in the U.S. population in the absence of arsenic exposure. Morales et al. (2000) calculated lifetime arsenic-related mortality risks for the U.S. population exposed to different drinking water concentrations by applying age-specific hazard functions (derived from the dose-response models estimated for the Taiwanese population) to a "life table" of age-specific probabilities of death for the U.S. population. These calculations were based on data from 1996.

In response to comments from NRC and SAB, a slightly different approach to estimate cancer risks for U.S. populations is being used. In the following analysis, arsenic concentrations corresponding to an additional one percent lifetime cancer incidence (effective dose; ED_{01} values) above "background" are derived for each endpoint. Also derived are lowest effective dose (LED_{01}) values, which represent the lower confidence limits on the dose corresponding to a one percent lifetime incidence risk in the U.S. population. Consistent with EPA's *Guidelines for*

Carcinogen Risk Assessment (U.S. EPA, 2005a) and the NRC (2001) cancer assessment, risk estimates are derived based a linear extrapolation from the points of departure (LED_{01}s for lung, bladder, and combined cancers).

The ED_{01} and LED_{01} values are estimated using a variation on the "BEIR IV" model derived for use in estimating population cancer risks for radionuclide exposures (NRC, 2001). This method, which is described further in Section 5.3.7.3 and Appendix E.2, includes the application of relative cancer risk estimate derived from the Taiwanese dose-response assessment multiplicatively to age-specific cancer risks for the U.S. In this model, the background hazard consists of age-specific cancer incidence data for bladder and lung cancer from the United States for the years 2000-2003 (NCI, 2006). The ratios of cancer mortality to incidence for arsenic-related cancers are assumed to be the same in the U.S. and Taiwanese populations.

5.3.5 Non-Water Arsenic Intake and Drinking Water Consumption

It is important to clarify that the non-water arsenic intake value corresponds to the arsenic amount from dietary sources (rice and yam, the staple diet in Taiwanese population in endemic area) only. It does not include the arsenic intake value from water used for cooking rice or produce, which was addressed separately via sensitivity analysis modeling with higher water intake values (see below).

For the baseline risk calculations, the non-water arsenic intake was assumed to be 10 µg/day for the reference and exposed populations. Although the data supporting this value are scarce, it appears to be a reasonable intake estimate for the reference populations based on the available information. U.S. EPA (1989) estimated the arsenic intake based on soil arsenic level and rice consumption in Taiwan to be between 2-16 µg/day. The higher value was presumed to result from possible soil contamination by organic arsenical herbicides applications. U.S. EPA (1989) found no reliable data to estimate arsenic intake from sweet potatoes (yam) consumption by the southwest Taiwanese population. In a separate study, Schoof et al. (1998) estimated that the total inorganic arsenic intake from food sources in the endemic area in Taiwan ranging between 15 and 211 µg/day, with the average intake value as 50 µg/day. This arsenic intake value is based on analysis of limited rice and yam samples collected in the endemic area of Taiwan during 1993 and 1995 (Schoof et al., 1998). It is likely that the arsenic intake in the non-endemic area (background arsenic intake value for reference population) is lower than that reported in the endemic area.

EPA also examined the arsenic intake value from food sources in countries where the arsenic exposures are much lower than in Taiwan. The average non-water inorganic arsenic intake from food consumption is reported to range from 8.3 to 14 µg/day in the United States and from 4.8 to 12.7 µg/day in Canada, with variation across age groups (Yost et al. 1998). Based on the available information, EPA selected 10 µg/day as the best estimate for non-water arsenic intake (food sources) in baseline calculations. Alternate values of non-water arsenic intake were also explored in the sensitivity analysis (Section 5.3.8.3).

NRC (1999) reported the background arsenic intake of 50 µg/day in endemic areas based

on the Schoof et al. (1998) findings. It is not clear if this value was ever used for dose-response modeling in estimating bladder cancer risk. However, NRC (2001) included the background intake of 30 µg/day in the dose-response modeling; the basis for the latter value is not clear. NRC (2001) also reported that there was no difference in the lung and bladder cancer risk estimates when 30 or 50 µg/day were used as the non-water intake values in the exposed populations. It is not clear if NRC (2001) assumed any non-water arsenic intake value for the reference populations. In the draft Toxicological Review submitted to SAB in 2005 (U.S. EPA, 2005c), non-water arsenic intake values of 0, 30, and 50 µg/day were assumed for the exposed populations only, and the background inorganic arsenic intake was assumed to be zero for the reference populations. SAB (2007) recommended that the background arsenic intake for reference (control) populations should not be assumed to be zero. However, SAB did not specify a non-water inorganic arsenic intake value for the reference population.

Given the state of the available data and the recommendations from SAB, EPA has assumed 10 µg/day non-water arsenic intake for the current assessment for both reference and exposed populations in the baseline risk calculations. EPA also evaluated 0, 30, and 50 µg/day for dietary arsenic intake assumption for reference populations, and up to 200 µg/day for exposed populations. The high-end background arsenic intake value was recommended by SAB in 2007 (i.e., the background arsenic intake value in the exposed populations as high as 200 µg/day should be included to assess the impact in lung and bladder cancer risk estimates) (Section 5.3.8.3).

In the current assessment, the drinking water consumptions for Taiwanese males and females are assumed to be 3.5 L/day and 2.0 L/day, respectively, in the baseline risk calculations. These values are consistent with the assumptions applied by U.S. EPA (1988b), Chen et al. (1992), and in NRC reports (1999 and 2001) for cancer risk estimations. There is conflicting information concerning the extent to which these values include both direct drinking water consumption and water used for cooking. To examine the impact of additional water consumption in cancer risk estimations, NRC (2001) also examined different ratios of water intake-rates between Taiwanese and U.S. populations (up to ratio of 3.0).

In the U.S. EPA (1989) report, the arsenic workgroup estimated that the total water consumption for the Taiwanese men, including the water used for cooking rice and yam (staple diets in southwest Taiwanese population), was 4.5 L/day since Taiwanese workers could drink 3.0 to 4.0 L/day of water and the 3.5 L/day seemed to be a reasonable estimate for direct water consumption. Indirect water consumption from cooking rice and yams was estimated to be 1.0 L/day. The basis for the derivation of the drinking water values in the U.S. EPA (1989) report is approximate and gathered from very limited populations (three or four residents were surveyed). In the Arsenic Rule (U.S. EPA, 2001), the total water Taiwanese consumption rates (including water used for cooking) were assumed to be 4.5 L/day for males and 3.5 L/day for females.

SAB (2007) did not recommend specific water intake values to be used for cancer risk modeling in the Taiwanese populations. Therefore, in the current assessment, the baseline water intake values modeled are 3.5 L/day for males and 2.0 L/day for females, to be consistent with NRC (1999) recommendations. In addition, a range of water consumption values (up to 5.1 L/day in males and 4.1 L/day in females) were evaluated in the sensitivity analysis to study the

impact of alternate water consumption in the cancer risk estimates. The water consumption values modeled in the baseline calculations for Taiwanese populations are also close to the average estimates provided for populations in West Bengal, India (Chowdhury et al., 2001) where the climate is close to Taiwan. The average drinking water intake values for children, adult females and adult males were reported as 2.0, 3.0, and 4.0 L/day, respectively.

The drinking water consumption for the U.S. reference population is estimated to be 2.0 L/day for both men and women. This is approximately equal to the 90th percentile estimate (2.014 L/day) from the 1994-1996 and 1998 data gathered as part of the Continuing Survey of Food Intake by Individuals (U.S. EPA, 2004), and is consistent with upper percentile estimates from previous surveys. Alternative assumptions about U.S. drinking water consumption result in simple reciprocal adjustments to slope factor estimates (discussed further in Section 5.3.8.3). Within the range analyzed, changes in the assumptions about Taiwanese drinking water consumption also result in nearly linear effects on estimated dose-response slope estimates.

5.3.6 Dose-Response Data

Table 5-2 summarizes the cancer mortality data from the Morales et al. (2000) study. For this assessment, the original data set containing age-specific person-years at risk (PYR), mortality statistics, and village water concentration data was obtained from Dr. Morales (Morales et al., 2000).

Water arsenic concentration data were provided for each village. Single concentration measurements were provided for each well. Data from only one well were available for twenty-one of the 42 villages. Between 2 and 47 well concentrations were provided for the other 22 villages (NRC, 1999). For dose-response estimation, models were fit to the median well concentration for each village. As part of the sensitivity analysis, the reported maximum or minimum well arsenic concentrations were also applied to the models.

Table 5-2. Cancer Mortality Data used in the Arsenic Risk Assessment

Gender	Village Water Concentration, µg/L	Age	20-30	30-49	50-69	>70	Total
Male	<100	PYR[a]	35,818	34,196	21,040	4,401	95,455
		Deaths[b]	(0, 0, 0)	(1, 10, 2)	(6, 17, 12)	(10, 4, 14)	(17, 31, 28)
	100-299	PYR	18,578	16,301	10,223	2,166	47,268
		Deaths	(0, 0, 0)	(0, 4, 3)	(7, 15, 14)	(2, 4, 13)	(9, 23, 30)
	300-599	PYR	27,556	25,544	15,747	3,221	72,068
		Deaths	(0, 3, 0)	(5, 7, 9)	(15, 23, 30)	(12, 6, 14)	(32, 39, 53)
	>600	PYR	16,609	15,773	8,573	1,224	42,179
		Deaths	(0, 0, 1)	(4, 12, 3)	(15, 15, 23)	(8, 2, 6)	(27, 29, 33)
	Total	PYR	98,561	91,814	55,583	11,012	256,970
		Deaths	(0, 3, 1)	(10, 33, 17)	(43, 70, 79)	(32, 16, 47)	(85, 122, 144)
Female	<100	PYR	27,901	32,471	21,556	5,047	86,975
		Deaths	(0, 0, 0)	(3, 1, 5)	(9, 6, 18)	(9, 5, 5)	(21, 12, 29)
	100-299	PYR	13,381	15,514	11,357	2,960	43,212
		Deaths	(0, 0, 0)	(0, 3, 4)	(9, 6, 10)	(2, 5, 5)	(11, 14, 19)
	300-599	PYR	19,831	24,343	16,881	3,848	64,903
		Deaths	(0, 0, 0)	(0, 5, 6)	(19, 6, 20)	(11, 2, 10)	(30, 13, 36)
	>600	PYR	12,988	15,540	9,084	1,257	38,869
		Deaths	(0, 0, 0)	(0, 4, 6)	(21, 7, 28)	(7, 1, 4)	(28, 12, 38)
	Total	PYR	74,101	87,868	58,878	13,112	233,959
		Deaths	(0, 0, 1)	(3, 13, 21)	(58, 25, 76)	(29, 13, 24)	(90, 51, 122)

[a] PYR = person-years at risk

[b] Numbers in parentheses = number of cancer deaths due to bladder, liver, and lung cancer, respectively.

5.3.7 Risk Assessment Methodology

The cancer risk assessment for U.S. population exposure to arsenic in drinking water was conducted in four steps:

- Models were fit to the data using mg/kg-day intake metrics calculated from the estimated water consumption values for the Taiwanese population and village water arsenic concentrations, assuming a 10 µg/day non-water dietary intake in the baseline analysis. Dose-response models were fit to the Morales et al. (2000) data for bladder and lung cancer in both genders using maximum likelihood methods (see Section 5.3.7.1).

- Upper confidence limits (UCLs) on the dose coefficients from the fitted models were estimated using the profile likelihood method (see Section 5.3.7.2).

- LED_{01} values for U.S. populations were calculated for each endpoint and gender based on the dose coefficient UCLs calculated for the Taiwanese populations in the previous step. Using the "BEIR IV" methodology, U.S. bladder and lung cancer incidence data for the years 2000-2003 (NCI, 2006) were used as the reference values for calculating U.S.

lifetime cancer risks. Thus, the LED_{01} values are expressed in terms of lifetime cancer incidence for the U.S. population (see Section 5.3.7.3).

The LED_{01} values were used to calculate ingestion drinking water unit risks for lung and bladder cancer for arsenic-exposed men and women in the United States. This step involved linear extrapolation from the LED_{01} values to zero dose and risk, yielding estimates of low-dose cancer slope factors. Unit Risks and slope factors calculations adjusted for differences between body weights and drinking water ingestion rates in Taiwan and the U.S. Other risk metrics (estimated lifetime incidence risk per mg/kg-day arsenic intake and corresponding to specific drinking water concentrations) were calculated for each endpoint from the LED_{01} values (see Section 5.3.7.4).

5.3.7.1 Dose-Response Estimation Based on Taiwan Cancer Mortality Data

A "Poisson model" was used to fit the cancer mortality data for the Taiwanese population. The general form of the Poisson model is:

$$h(x,t) = h_0(t) \times g(x) \qquad (5\text{-}1)$$

Where: $h(x,t)$ = cancer mortality risk at dose "x" and age "t"
$h_0(t)$ = cancer mortality risk in the reference population at age "t"
$g(x)$ = risk attributable to arsenic exposure at dose "x" (mg/kg-day)

Taiwanese cancer mortality and person years at risk (PYR) data were available for five-year ranges for ages 20-84. Cancer mortality data for the southwest Taiwan reference groups also were included in the preferred version of the model; estimates were derived without the reference population and with cancer mortality statistics from all regions of Taiwan. In the Poisson model, which is widely applied in the analysis of epidemiology data, cancer deaths are assumed to be "rare" events and Poisson-distributed within each age-dose group. When $h_0(t)$ and/or $g(x)$ are non-linear functions, as is the case for arsenic, the model cannot be fit using conventional least-squares regression methods or general linear models (GLM). Based on recommendations from NRC (2001) and after testing a number of different models, the following model form was selected for primary risk estimates based on goodness-of-fit and parsimony criteria[4]:

$$h(x,t) = \exp(a_1 + a_2*\text{age} + a_3*\text{age}^2) * (1 + b* \text{dose}) \qquad (5\text{-}2)$$

Where: a_1, a_2, a_3 = age coefficients
b = dose coefficient

[4] Results obtained using alternative model forms are discussed in Section 5.3.8.4.

Specifically, the model parameters in h(x,t) in Equation 5-2 were obtained by assuming that the number of cases in each exposure-age category has a Poisson distribution with parameter $\lambda(x,t)$, Cases ~ Poisson ($Py * \lambda(x,t)$), where *Py* is person years, and λ is the intensity of Poisson parameter at the exposure-age,(x,t), category. Because data are given in five-year age intervals, the parameter λ is related to hazard rate *h* which is equal to $\lambda/5$.

In this model, the exponential term represents "$h_0(t)$"in Equation 5-1, the age-dependent risk of cancer at the "background" doses of arsenic (zero from drinking water and 10 µg/day from diet in the preferred model). The last term in the equation captures the dependency of risk on the daily ingestion dose of arsenic.

Cancer mortality data were stratified across 13 five-year age groups and 43 villages (42 exposed villages plus the reference population). This stratification yielded 559 data points per cancer endpoint for model fitting. Mid-range values for the age ranges were standardized to their mean values and treated as nuisance parameters.

The unit of dose used in the modeling was mg/kg-day. In the primary (baseline) risk model, the estimated non-water arsenic intake was 10 µg/day for both the exposed and reference populations. The total arsenic dose received by the population of any village was estimated as the sum of the non-water dietary intake plus the median arsenic well water concentration for the village (baseline model), multiplied by the estimated water Taiwanese consumption rates (3.5 L/day for men, 2.0 L/day for women) and divided by estimated average body weights for Taiwanese men and women (50 kg for both genders; Chen et al., 1992). The southwest Taiwanese population outside of the arseniasis-endemic area (Morales et al., 2000) served as the reference population in the baseline model.

Values for the coefficients a_1, a_2, a_3, and b were fit using MLE methods. Likelihood maximization was performed using the Solver add-in of Excel®. The MLE fits for the baseline model were replicated using the Non-linear Estimation module of Statistica®. Replicated results (estimated age and dose coefficients) were identical to Solver estimates to the third decimal place for all endpoints.

5.3.7.2 Estimation of Confidence Limits on Cancer Slope Parameters

The LED_{01} values were derived based on estimated upper confidence limits on the estimated dose coefficients ("b") for each endpoint and gender. The confidence limits were calculated using the likelihood profile method (Venson and Moolgavkar, 1988). In this approach, the value of the dose parameter, b, was varied from its estimated mean value. The ratio of the log likelihood for the best-fit model to the log likelihood for other values of "b" is known to follow an approximate chi-squared distribution with one degree of freedom. Thus, the 5^{th} and 95^{th} confidence limits on the dose coefficient "b" correspond to the values where the likelihood ratio is equal to 1.92. Upper and lower confidence limits were calculated using Solver®. The fact that the profile likelihood method ignores the likelihood impact of the age "nuisance parameters" implies that the calculated confidence limits are only approximate.

Confidence limit calculations using other methods (empirical Bayesian simulation[5] and "bootstrap-t") gave comparable results (within a few percent of the values estimated by profile likelihood).

5.3.7.3 Estimation of LED_{01} Values Using Relative Risk Models

Once the dose coefficients were calculated, they were used to estimate arsenic-associated lifetime risks in the U.S. population. In this analysis, LED_{01} values for the U.S. population were calculated using a variant of the "BEIR IV" relative risk model recommended by NRC (2001). The method applied the relative risk estimated as $(1 + b_{UCL} * \text{dose})$ to the age profile of cancer incidence for the reference (U.S. male or female) population, where b_{UCL} is the 95% upper confidence limit on "b" (the arsenic coefficient from the dose-response model for the Taiwanese population, estimated as explained in the previous section). The BEIR model also takes into account the effect of noncancer mortality, cancer mortality, and previous cancer incidence on the number of individuals in the exposed population who survive to the start of each 5-year age stratum. To estimate cancer risks in the U.S. population, incidence risks are calculated for each 5-year age stratum and summed to give an estimate of lifetime incidence. The dose is then adjusted until the estimated extra incidence risk from arsenic-associated cancer risk equaled 0.01 (1%) for the U.S. reference population. The dose (in mg/kg-day) that fulfills this condition is the LED_{01}, which becomes the point of departure (POD) for estimating the cancer slope factor.

The BEIR IV model takes as its input, age-specific mortality data and lung and bladder cancer incidence for the U.S. reference population.[6] U.S. cancer incidence was estimated in this analysis based on mortality data for the year 2000 (NCHS, 2000). Lung and bladder incidence data for the years 2000-2003 were obtained from the National Cancer Institute's SEER (surveillance epidemiology and end result) program (NCI, 2006). Arsenic intakes resulting in 10^{-2} lifetime risks were estimated using Solver®. Details of the relative risk methodology are provided in Appendix E.2.

5.3.7.4 Estimation of Unit Risks

For each endpoint and gender, the slope of a line from the LED_{01} dose through the intercept (water-related arsenic dose = 0, water related arsenic risk = 0) was calculated. The slopes of these lines represent the oral Cancer Slope Factor for the endpoint:

$$\text{Oral Cancer Slope Factor (per mg/kg-day)} = 0.01/LED_{01} \qquad (5\text{-}3)$$

[5] The Empirical Bayes modeling involved taking random samples within the neighborhoods of the MLE coefficient values, calculating the log likelihood, and after many iterations, building up an estimate of the posterior distribution of the "b" coefficient (mean and standard error). Confidence limits were then estimated assuming the posterior probability of b was normally distributed.

[6] Note that the age dependence estimated for the Taiwanese population – represented by the parameters a_1, a_2, and a_3 – is specific to that population, and is not carried over to the U.S.

Linear low-dose extrapolation was employed consistent with EPA's finding that insufficient mode of action data are available to justify the use of non-linear, low-dose models (Section 4.6.3.2). Unit risks (cancer risk per µg/L arsenic in drinking water) also were estimated:

Unit risk (per µg/L) = Cancer Slope Factor (per mg/kg-day) * 0.001 * DW/BW (5-4)

Where:
- 0.001 = conversion from milligrams to micrograms
- BW = body weight for exposed population in kilograms (U.S. male and female)
- DW = daily drinking water consumption for exposed population in liters (U.S. male and female)

As discussed previously, the estimated drinking water consumption for the U.S. adult population is 2.0 L/day for both males and females. U.S. male and female body weights are estimated to be 70 kg. The 2.0 L/day is a standard factor used in EPA IRIS assessments, and represents approximately the 90th percentile of intake of community water in the US population. Other intake assumptions (e.g., mean versus upper percentile) can be used in risk assessments, depending on target population characteristics and assessment needs.

5.3.8 Results

5.3.8.1 Ingestion Pathway Cancer Slope Factors and Unit Risks

Table 5-3 presents the estimated risk metrics for lung, bladder, and combined cancers in males and females under baseline assumptions (see Footnote "a" to the table for baseline modeling assumptions).

The estimated oral slope factor for female lung cancer (16.6 per mg/kg-day) is higher than that for males (6.7 per mg/kg-day), but the bladder cancer oral slope factors for males and females are comparable (11.2 and 10.5 per mg/kg-day, respectively). Drinking water unit risks for lung cancer are 1.9×10^{-4} and 4.8×10^{-4} per µg/L, respectively, for males and females while the drinking water unit risks for bladder cancer are 3.2×10^{-4} and 3.0×10^{-4} per µg/L, respectively. Estimated lifetime incidence risks corresponding to 10 µg/L arsenic in drinking water follow similar patterns for the various endpoints. Estimated drinking water concentrations associated with 10^{-4} lifetime incidence range from 0.21 µg/L (female lung cancer) to 0.52 µg/L (male lung cancer).

Table 5-3. Cancer Incidence Risk Estimates for Lung and Bladder Cancers in Males and Females[a]

Metric	Lung Cancer	Bladder Cancer
Males		
ED_{01}, mg/kg-day	1.9E-03	1.1E-03
LED_{01}, mg/kg-day	1.5E-03	8.9E-04
Oral slope factor, per mg/kg-day	6.7	11.2
Unit risk, per µg/L drinking water	1.9E-04	3.2E-04
Lifetime incidence risk at 10 µg/L in drinking water	1.9E-03	3.2E-03
Water Concentration for 10^{-4} Risk, µg/L	0.52	0.31
Females		
ED_{01}, mg/kg-day	7.5E-04	1.2E-03
LED_{01}, mg/kg-day	6.0E-04	9.5E-04
Oral slope factor, per mg/kg-day	16.6	10.5
Unit risk, per µg/L drinking water	4.8E-04	3.0E-04
Lifetime incidence risk at 10 µg/L in drinking water	4.8E-03	3.0E-03
Water Concentration for 10^{-4} Risk, µg/L	0.21	0.33

[a] Baseline assumptions: Reference population = southwest Taiwan; Taiwanese male and female body weight = 50 kg, Taiwanese male water intake = 3.5 L/day, Taiwanese female water intake = 2.0 L/day; reference and exposed population non-water arsenic intake = 10 µg/day. Male and female U.S. body weights are assumed to be 70 kg, and U.S. water intake for both males and females is assumed to be 2.0 L/day.

Arsenic-related cancer risks also are calculated for the population as a whole, that is, for combined bladder and lung cancer incidence in a population composed of both men and women. In this analysis, total cancer risk (lung plus bladder) for males and females are calculated by combining the risk for the individual tumor types. Upper confidence limits on the combined cancer risks can be calculated based in the assumption that the uncertainty in the two cancer slope factors are both normally distributed. If this is the case, the 95% upper bound, U, for the combined cancer potency can be calculated as:

$$U = (m_1 + m_2) + \sqrt{(u_1 - m_1)^2 + (u_2 - m_2)^2} \qquad (5\text{-}5)$$

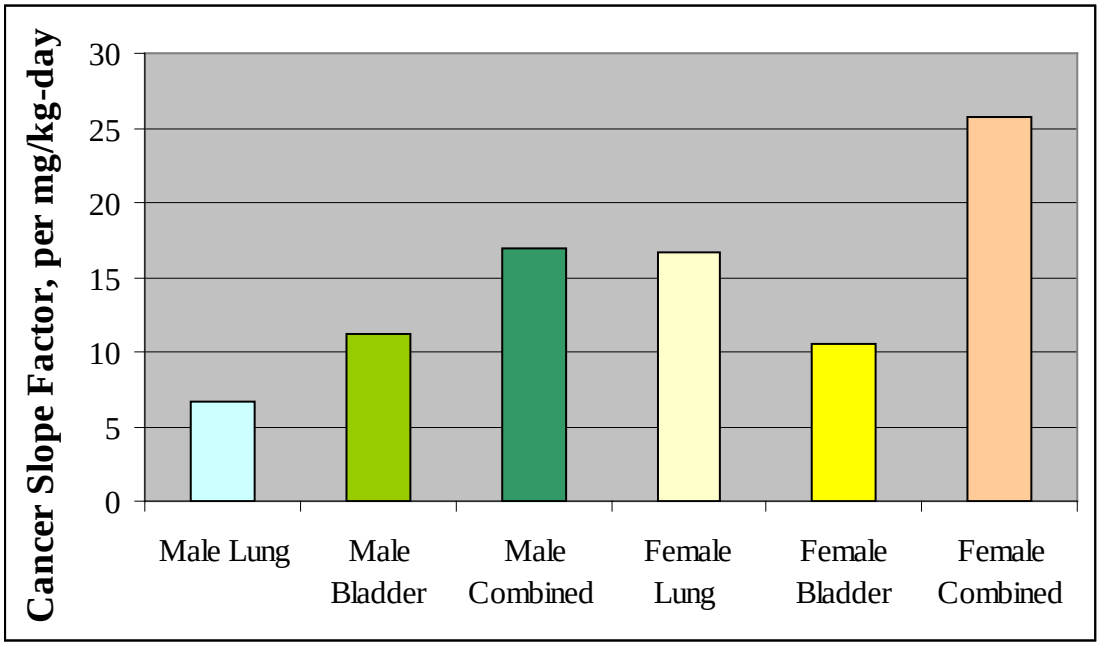
where m_i and u_i, i=1,2, are respectively mean and 95% upper bound cancer potency for the two tumor types. The results of these calculations are summarized in Table 5-4. Using this approach, the combined cancer potency factor estimate for males is 16.9 per mg/kg-day for males and 25.7 per mg/kg-day for females. The estimated drinking water unit risk for combined male lung and bladder cancer is 4.8×10^{-4} per µg/L; for females, the estimated value is 7.3×10^{-4} per µg/L. The drinking water concentrations corresponding to 10^{-4} combined cancer risks for males and females are 0.21 and 0.14 µg/L, respectively.

Table 5-4. Combined Lung and Bladder Cancer Incidence Risk Estimate for the U.S. Population (Males and Females)

Metric	Male Combined Lung+Bladder	Female Combined Lung+Bladder
Oral slope factor, per mg/kg-day	16.9	25.7
Unit risk, per µg/L drinking water	4.8E-04	7.3E-04
Lifetime incidence risk at 10 µg/L in drinking water	4.8E-03	7.3E-03
Water Concentration for 10^{-4} Risk, µg/L	0.21	0.14

Figure 5-1 shows the estimated oral slope factors for each of the endpoints separately, along with oral slope factor estimates for the combined cancers in males and females. **In keeping with EPA policy, the combined cancer slope factor for women (25.7 per mg/kg-day) is appropriate for use in establishing health criteria, since, based on the available data, women appear to be the more sensitive group.**

Figure 5-1. Estimated Oral Slope Factors for Individual and Combined Cancer Endpoints



5.3.8.2 Comparison to Previous Cancer Risk Estimates

As discussed in Section 5.3.1, a number of risk assessments have been conducted by EPA and others. Results of the present dose-response assessment were compared to cancer risk estimates derived from the same and other data sets in previous studies (NRC, 2001 and U.S.

EPA, 2005c). Note that the results of the U.S. EPA (1988b) analysis, which estimated a cancer slope factor of 1.0 – 2.0 per mg/kg-day, are not comparable to the results of the current assessment (slope factor 25.7 per mg/kg-day), because the former was based on skin cancer, while all of the more recent analyses estimate risks of internal (lung and bladder) cancers. Thus, the detailed comparisons in this section are limited to assessments that also address lung and bladder cancer. The Drinking Water Standard (U.S. EPA, 2001) also provides numerical risk estimates for exposures to arsenic in drinking water. However, Tables III.D-2(a) and (b) of the Rule display ranges of cancer risks for populations exposed to distributions of arsenic concentrations in drinking water at and above the proposed MCL options. Thus, the numerical risk results of that analysis are also not directly comparable to the NRC (2001), U.S. EPA (2005c) and current assessments, which apply to populations exposed to single concentrations. In the analyses that follow, some of the risk comparisons are based on mortality estimates that have been converted to incidence using recent U.S. incidence-mortality ratios. This conversion introduces additional uncertainty into the comparisons; different results would have been obtained had the incidence been modeled directly rather than estimated after-the-fact.

ED_{01} and LED_{01} Estimates from Chen et al. (1988a, 1992), Ferreccio (2000), and Chiou et al. (2001)

Consistent with SAB (2007) recommendations, Table 5-5 presents risk estimates from previous studies, and compares them to estimates derived in this analysis. The estimates in Table 5-5 come from Table 5-3 of NRC (2001), and include ED_{01} and LED_{01} estimates (expressed as μg/L arsenic in drinking water) from a number of studies of arsenic-related cancer risks in Chile (Ferreccio et al., 2000) and Taiwan (Chiou et al., 2001 and Chen et al., 1988a, 1992).

NRC calculated ED_{01} and LED_{01} values for lung and bladder cancer mortality from the same Taiwanese cohort as used in the current assessment, based on the results presented in Chen et al. (1988a, 1992), but without a reference population. In addition, these values do not account for differences in drinking water consumption between the U.S. and Taiwanese populations, and did not apply life-table adjustments.

Table 5-5. Comparison of ED_{01} and LED_{01}[a] Estimates From Past Studies[b] with Those from the Current Analysis

Study	Male Lung		Female Lung		Male Bladder		Female Bladder	
	ED_{01}	LED_{01}	ED_{01}	LED_{01}	ED_{01}	LED_{01}	ED_{01}	LED_{01}
Chen et al. (1988a, 1992), Taiwan	38-84	37-72	33-94	31-84	102-317	94-286	138-443	125-406
Ferreccio et al. (2000), Chile	5-17	3-14	7-27	5-21	--	--	--	--
Chiou et al. (2001), Taiwan	--	--	--	--	129-500+	42-500+	231-500+	88-500+
Current Analysis	66	52	26	21	40	31	41	33

[a] Units = μg/L arsenic in drinking water
[b] Source of estimates: NRC (2001)

NRC also estimated ED_{01} and LED_{01} values based on data from the Ferreccio et al. (2000) case-control study of male and female lung cancer data from a Chilean population that included 151 lung cancer cases and 419 controls. The ED_{01} and LED_{01} derived by NRC were obtained by linear regression of mortality odds ratio estimates on exposures, with the intercept forced to a value of 1.0 at zero exposure. These estimates, are shown in the second row of Table 5-5. Multiplicative linear dose and log dose models were used to derive ED_{01} and LED_{01} estimates from the study by Chiou et al. (2001) of urinary tract cancer incidence over a four-year period in 8000 Taiwanese exposed to arsenic in drinking water. These results are presented in the third row of Table 5-5. Where ranges are given in the table, the minimum and maximum values represent the lowest and highest ED_{01} or LED_{01} estimates that were derived when different models were used.

The bottom row of the table shows the ED_{01} and LED_{01} values for cancer incidence derived in this analysis using the Poisson regression and BEIR IV models. The ED_{01} and LED_{01} values for lung cancer derived in the current assessment fall within, or are close to, the ranges estimated from the Chen et al. (1988a, 1992) data. This finding is not surprising because the results are estimated for the same cohort in both cases, and because the case mortality for lung cancer is so high (nearly 100%). The ED_{01} and LED_{01} values derived in the current assessment are, however, higher than those estimated by Ferreccio et al. (2000). One possible explanation involves differences in modeling methods; to estimate ED_{01} and LED_{01} values from the Ferreccio study, NRC applied linear regression to the odds ratio estimates, forcing the intercept through 1.0 at zero dose. Thus, these values must be considered highly uncertain. The differences also may be due to differences in exposure conditions (e.g., NRC did not account for differences in drinking water intake between the Chilean and U.S. populations) or other covariates (e.g., smoking) between the two studies.

For bladder cancer, the ED_{01} and LED_{01} values estimated in this analysis are lower (2.5- to 10-fold) than those derived from the Chen et al. studies (1988a, 1992). In addition to the differences in modeling approaches outlined above, another possible reason for this difference is that the Chen et al. (1988a, 1992) studies are based on bladder cancer mortality, while the ED_{01} and LED_{01} values in this analysis are for bladder cancer incidence. Adjustment for bladder cancer case mortality (in the order of 16-20%) would make the EPA's current results much more similar to those of Chen et al. (1988a, 1992).

Finally, the ED_{01} and LED_{01} values from the current analysis are below the lower end of the ranges estimated by Chiou et al. (2001). Reasons for this finding are not entirely clear. The sensitivity of the Chiou et al. study may have been limited by the short follow-up period (NRC, 2001), and only 18 total urinary tract cancers were identified in the study. Only four exposure categories were analyzed (less than 10 µg/L, 10-50, 50-100, and greater than 100 µg/L in water; non-water exposures were not evaluated). The low sensitivity could have caused the ED_{01} and LED_{01} estimates derived by Chiou et al. (2001) to be biased upward from what would have been seen with a more extended follow-up period.

Estimated Risk Associated with 10 µg/L Drinking Water Arsenic from NRC (2001) and U.S. EPA (2005c)

Table 5-6 provides an additional set of comparisons between the current risk estimates and the results from two previous analyses by NRC and EPA. Lifetime incidence risks are presented for a hypothetical U.S. population exposed to 10 µg/L arsenic in drinking water. NRC (2001) estimated arsenic-associated risks using an "additive Poisson model with dose entered as a linear term and using the BEIR IV formula" (p. 201). As expected, the minor differences in assessment methods between the NRC (2001) and U.S. EPA (2005c) analyses had very little impact on the risk estimates. For all four endpoints, the difference in risks estimated in the two studies is less than 10%.

Table 5-6. Comparison of Cancer Risk Assessment Results with Estimates from NRC (2001), and U.S. EPA (2005c)

Source of Estimate	Estimated Cancer Incidence at 10 µg/L Arsenic in Drinking Water (per 10,000 exposed population)			
	Bladder		Lung	
	Male	Female	Male	Female
NRC (2001), Taiwan	23	12	14	18
EPA (2005c)[a]	21	11	13	18
Current Analysis	32	30	19	48

[a] The original mortality risk estimates from U.S. EPA (2005c) were multiplied by incidence-mortality ratios for the various endpoints to obtain incidence estimates. For the Taiwanese populations, case mortality for lung cancer was assumed to be 100% and mortality for bladder cancer was assumed to be 80% (NRC, 2001).

The incidence risks derived in the current analysis, however, are reasonably close, but not identical, to the NRC (2001) estimates. Differences in the calculated cancer potency relate to several factors. Changes in the assumed drinking water intake in females in the current assessment compared to the NRC (2001) and U.S. EPA (2005c) analyses are summarized in Table 5-7. In particular, the change in the assumed ratios of Taiwanese/U.S. female water intake from 2.8 in the earlier assessments to 1.4 in the current analysis are relevant to the differences in risk seen in Table 5-6. The lower ratio in the current analysis translates into a two-fold greater estimated risk for females in the current assessment than in the NRC (2001) and U.S. EPA (2005c) analyses.

Table 5-7. Drinking Water Intake and Body Weight Assumptions in Females in Recent Arsenic Risk Assessments

Assessment	Body Weight, kg		Water Intake, L/day		Ratio of Taiwan/U.S. Drinking Water Intake
	Taiwan	U.S.	Taiwan	U.S.	
NRC (2001)	50	70	2	1	2.8
U.S. EPA (2005c)	50	70	2	1	2.8
Current Analysis	50	70	2	2	1.4

In addition, the NRC (2001) risk estimates are based on maximum likelihood estimates (MLE) of the arsenic slope parameters in the Poisson regression, while U.S. EPA (2005c) and the current assessment derive risks based on the statistical upper confidence bounds on these parameters. As shown in Table 5-3, the difference between the MLE estimates (ED_{01} values) compared to the upper confidence limit (LED_{01}) is on the order of 20%. This would translate into approximately 20% greater risks calculated based on the upper confidence limit values compared to the MLE estimates.

The use of more recent cancer incidence and mortality data in the BEIR IV model than in the previous risk assessments also probably contributes to the differences in risks in Table 5-6. Also, the current assessment includes a modification to the BEIR IV model suggested by Gail et al. (1999) for obtaining more accurate estimates of incidence within multi-year age strata. The modifications to the model are described in detail in Appendix E.2.

Changes in the assumptions related to non-water arsenic intake also would be expected to have small to moderate affects on the results within the range in question. In this assessment, both the reference and exposed populations are assumed to receive 10 µg/day non-water arsenic intake (see Section 5.3.5). Section 5.3.8.3 presents the results of uncertainty analyses which explore the effects of changes in selected modeling assumptions, including non-water arsenic intake, on the risk estimates.

5.3.8.3 Sensitivity Analyses of Cancer Risk Estimates to Changes in Parameter Values

NRC (2001) and SAB (2007) recommended that the impacts of different modeling assumptions and input parameter values be investigated in the risk assessment for arsenic in drinking water. EPA, therefore, examined several aspects of the cancer risk modeling through single-value sensitivity analysis. The Agency felt that the currently available data were insufficient to support detailed probabilistic uncertainty and variability estimation. In response to SAB comments, EPA evaluated the impacts of:

- Varying the assumed daily non-water arsenic intake of the exposed and reference populations. Sensitivity cases were run in which the non-water arsenic intake in the exposed populations was varied from its default value of 10 µg/day to 0, 100, and 200 µg/day. An additional case was run in which both the exposed and reference populations were assumed to receive 0, 30, and 50 µg/day non-water arsenic exposure. Because the Poisson risk model for female bladder cancer is particularly sensitive to changes in assumptions related to non-water arsenic intakes (see below), non-water arsenic intake was limited to below 50 µg/day in reference populations.

- Varying assumptions related to drinking water intake by the exposed Taiwanese population. Cases were run in which the male drinking water consumption was varied from its baseline value of 3.5 L/day to 5.1 L/day, 3.0 L/day, and 2.75 L/day. Female drinking water intake in the Taiwanese population was varied from its baseline value of 2.0 L/day to 2.75 and 4.1 L/day.

- Varying the arsenic well concentrations used to fit the dose-response model for the Taiwanese population. The baseline risk model used the median village arsenic concentrations as the exposure metric. In the sensitivity analysis, cases also were run using the minimum and maximum well concentrations in each village.

- Including different Taiwanese reference populations in the dose-response assessment. The baseline (southwest Taiwan) reference population was replaced by data from all Taiwan. The model also was run without any distinct reference population.

Tables 5-8 and 5-9 summarize the results of the sensitivity analysis runs. Table 5-8 shows the estimated (incidence) risks associated with a drinking water concentration of 10 µg/L for the U.S. population estimated when calculated using the assumptions specified in the left-hand column of the table. Table 5-9 shows the proportional changes in estimated risks in relations to the baseline estimate. Figure 5-2 summarizes the impact of alternative modeling assumptions, showing the ratios of estimated cancer risks to the base case estimates for changes in input variables having a substantial (>20%) effect on the risk estimates.

Table 5-8. Sensitivity Analysis of Estimated Cancer Incidence Risks Associated with 10 µg/L to Changes in Modeling Assumptions and Inputs

Estimated Cancer Risk at 10 µg/L	Male Lung	Female Lung	Male Bladder	Female Bladder
Baseline (all default values)[a]	1.9E-03	4.8E-03	3.2E-03	3.0E-03
Non-water As intake = 0µg/day (reference and exposed populations)	1.9E-03	4.6E-03	3.0E-03	2.6E-03
Non-water As intake = 30 µg/day (reference and exposed populations)	2.0E-03	5.1E-03	3.5E-03	4.5E-03
Non-water As intake = 50 µg/day (reference and exposed populations)	2.0E-03	5.5E-03	3.9E-03	1.1E-02
Non-water As intake (exposed population) = 0 µg/day	1.9E-03	4.8E-03	3.2E-03	3.0E-03
Non-water As intake (exposed population) = 100 µg/day	1.8E-03	4.4E-03	3.0E-03	2.8E-03
Non-water As intake (exposed population) = 200 µg/day	1.7E-03	3.9E-03	2.8E-03	2.4E-03
Taiwan H_20 consumption = 3.0 L/day (M), 2.0 L/day (F)	2.3E-03	4.8E-03	3.8E-03	3.0E-03
Taiwan H_20 consumption = 5.1 L/day (M), 4.1 L/day (F)	1.3E-03	2.3E-03	2.2E-03	1.4E-03
Taiwan H_20 consumption = 2.75 L/day (M, F)	2.5E-03	3.4E-03	4.1E-03	2.1E-03
Village H_20 As concentrations = minimum values	2.5E-03	5.7E-03	4.0E-03	4.0E-03
Village H_20 As concentrations = maximum values	1.4E-03	3.5E-03	2.3E-03	2.1E-03
Reference population = none	1.2E-03	1.5E-03	8.3E-04	3.5E-04
Reference population = all Taiwan	2.4E-03	3.9E-03	4.8E-03	6.2E-03

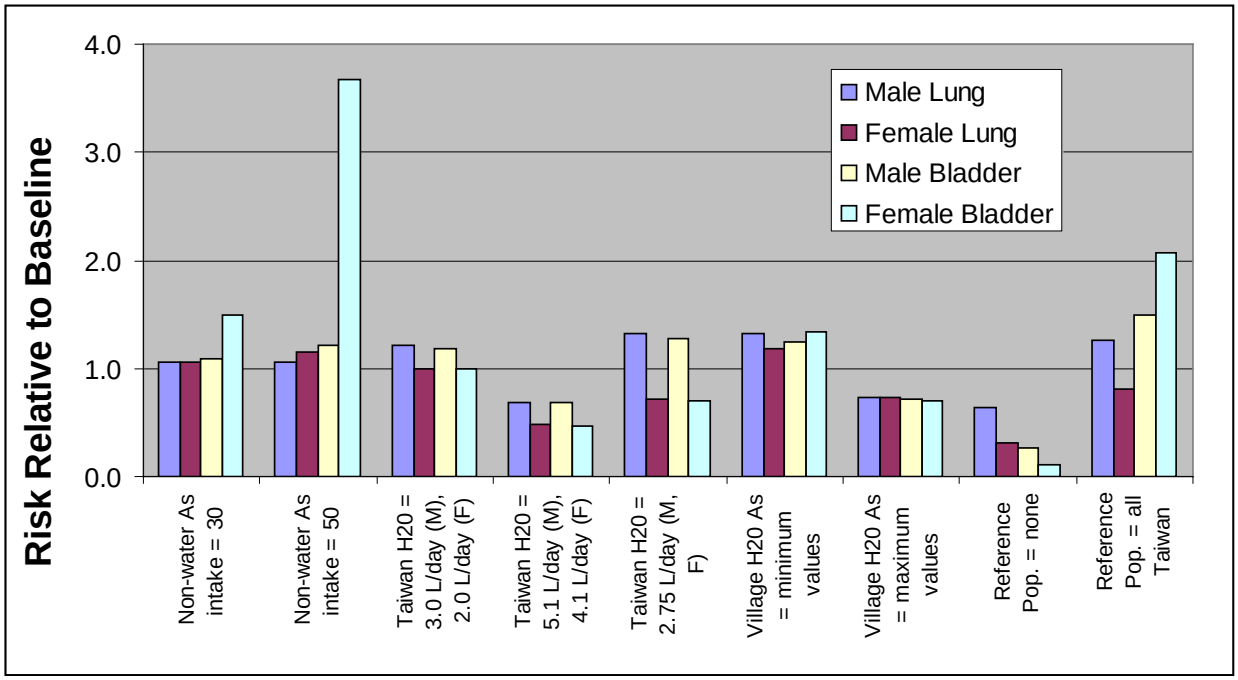
[a]Baseline inputs: Reference population = southwest Taiwan; male and female body weight = 50 kg, male water intake = 3.5 L/day, female water intake = 2.0 L/day, reference and exposed population non-water arsenic intake = 10 µg/day. U.S. Population male and female body weights = 70 kg, male and female water consumption = 2.0 L/day.

Table 5-9. Proportional Changes in Cancer Risks at 10 µg/L Associated with Changes in Modeling Inputs and Assumptions

Modeling Assumptions/Input Values	Male Lung	Female Lung	Male Bladder	Female Bladder
Baseline (all default values)[a]	0%	0%	0%	0%
Non-water As intake = 0 µg/day (reference and exposed populations)	0%	-4%	-6%	-13%
Non-water As intake = 30 µg/day (reference and exposed populations)	5%	6%	9%	50%
Non-water As intake = 50 µg/day (reference and exposed populations)	5%	15%	22%	267%
Non-water As intake (exposed population) = 0 µg/day	0%	0%	0%	0%
Non-water As intake (exposed population) = 100 µg/day	-5%	-8%	-6%	-7%
Non-water As intake (exposed population) = 200 µg/day	-11%	-19%	-13%	-20%
Taiwan H_20 consumption = 3.0 L/day (M), 2.0 L/day (F)	21%	0%	19%	0%
Taiwan H_20 consumption = 5.1 L/day (M), 4.1 L/day (F)	-32%	-52%	-31%	-53%
Taiwan H_20 consumption = 2.75 L/day (M, F)	32%	-29%	28%	-30%
Village H_20 As concentrations = minimum values	32%	19%	25%	33%
Village H_20 As concentrations = maximum values	-26%	-27%	-28%	-30%
Reference population = none	-37%	-69%	-74%	-88%
Reference population = all Taiwan	26%	-19%	50%	107%

[a] Baseline inputs as described in footnote to Table 5-8.

Figure 5-2. Change in Arsenic-Related Unit Risk Estimates Associated with Variations in Input Assumptions



These results indicate that varying most of the risk modeling inputs within the tested ranges have a small or moderate effect on risk estimates for most endpoints. For all of the endpoints except female bladder cancer, changing assumptions related to non-water arsenic intake for the reference and/or exposed populations results in small changes (<25%) in the estimated oral slope factor and cancer risks at 10 µg/L in drinking water. Risk estimates for female bladder cancer, in contrast, are quite sensitive to changes in non-water arsenic intake in the range from zero to 50 µg/day. When non-water arsenic intake is assumed to be 30 µg/day (rather than 10 µg/day in the baseline estimate), estimated female bladder cancer risks are approximately 50% higher than under baseline assumptions. When non-water arsenic intake increases to 50 µg/day, female bladder cancer risks increases by 267% compared to baseline. The sensitivity of the risks estimates are greater for changes in reference population arsenic intake; when non-water intake increases to 100 and 200 µg/day for the exposed populations alone, the impact on female bladder cancer risks is much less (7 and 20%, respectively).

As expected, the risk estimates obtained when making different assumptions concerning Taiwanese drinking water consumption are very nearly inversely proportional to the assumed water intake. For example, when male drinking water consumption is assumed to be 5.1 L/day, rather than 3.5 L/day in the baseline case, estimated cancer risks for male lung and bladder cancer are both approximately 0.69 (= 3.5/5.1) times the values derived using baseline assumptions. Similar results are seen for the other endpoints.

Using different exposure concentration metrics also show relatively limited impacts on the estimated cancer risks. When the village minimum water concentrations are used as inputs to the Poisson risk model, the estimated cancer risks increase slightly (32, 19, 25, and 33% over baseline) for male and female lung and male and female bladder cancer, respectively. When village maximum water concentrations are used as model inputs, the estimated cancer incidence risks decrease between 26 and 30% relative to baseline. These changes are roughly reciprocal to the changes in average exposure concentrations, as expected.

The final two rows of Tables 5-8 and 5-9 illustrate the impact of alternative assumptions about which reference populations are included in the Taiwanese risk assessment model. When no reference population is included (the Poisson model is fit only to the data from the 42 exposed villages), the estimated risks for all four endpoints are considerably lower than under the baseline case, which included the southwest Taiwan population. This finding is not unexpected, because the addition of the relatively large reference population serves to “anchor” the low-exposure end of the model and decrease the impact of the high variability (“noise”) in the exposed population data. When the reference population is excluded from the assessment, estimated cancer risks are reduced between 37% (male lung) and 88% (female bladder cancer) compared to the baseline model that included the southwest Taiwan reference populations. All of the exposure-response “b” parameters retain statistical significance, however, even when the reference population is excluded. Finally, including the “all Taiwan” reference population, rather than southwest Taiwan, has smaller and variable effects on the risk estimates. Predicted risks for male lung and bladder cancer are increased (decreased) by approximately 26 and 19%, respectively, while risks for female lung and bladder cancer are increased by 50% and 107%, respectively, compared to baseline.

Based on these outcomes, it appears that the risk model results are relatively stable and react predictably to reasonable changes in exposure assumptions. The exception is female bladder cancer, for which the dose-response parameter estimated in the Poisson model is very sensitive to the assumed non-water arsenic intake by the reference population in the range between zero and 50 µg/day. In addition, risk estimates for all endpoints are strongly affected by the inclusion or exclusion of a low-dose reference population in the Poisson risk model.

5.3.8.4 Sensitivity Analyses of Cancer Risk Estimates to Dose-Response Model Form

In the course of this analysis, EPA has investigated the impact of alternative model forms on the cancer risks estimated for the Taiwanese and U.S. populations for individual endpoints (lung and bladder cancer). Based on the past experience of Morales et al. (2000) and modeling results presented by NRC (2001), this effort was limited to exploring alternative forms for the dose dependence of risks. Equation 5-2 shows EPA's baseline model, which is "linear Poisson" with the form:

$$h(x,t) = \exp(a_1 + a_2*age + a_3*age^2) * (1 + b* dose) \quad (5\text{-}2)$$

In addition to the linear model, three other models were evaluated. First, the quadratic form of dose dependence:

$$h(x,t) = \exp(a_1 + a_2*age + a_3*age^2) * (1 + b1* dose + b_2*dose^2) \quad (5\text{-}6)$$

In addition, two models in which the dose dependence was exponential, one linear and one quadratic, also were tested:

$$h(x,t) = \exp(a_1 + a_2*age + a_3*age^2) * \ Exp(b0 + b_1*dose) \quad (5\text{-}7)$$

$$h(x,t) = \exp(a_1 + a_2*age + a_3*age^2) * \ Exp(b0 + b_1*dose + b_2*dose^2) \quad (5\text{-}8)$$

The last model (Equation 5-8) was specifically recommended by SAB (2007) for evaluation. In the discussion that follows, these four models will be referred to, respectively, as the "linear" (baseline) model (Equation 5-2), quadratic model (Equation 5-6), linear exponential model (Equation 5-7), and quadratic exponential model (Equation 5-8).[7]

All four models were fit to lung cancer data from the Taiwanese population, using the baseline exposure parameter values and including the southwest Taiwanese reference population. Models were fit using the Non-linear Estimation module of Statistica®. For males, the quadratic and quadratic exponential models curve sharply downward at high doses, whereas the linear exponential model curves sharply upward. Over the dose range from zero to 0.05 mg/kg-day in males, which corresponds to an arsenic drinking water concentration range of 0 to 710 µg/L

[7] "Absolute risk" models, (models where arsenic exposure was assumed to result in additive, rather than multiplicative, increments in risks), were found to fit the data much less well than the multiplicative forms shown in Equations 5-6 to 5-8 and are not discussed further.

(which covers approximately 95% of the exposed population years at risk), predictions from the non-linear models are never more than 22% higher or 24% lower than the predictions from the linear (baseline) model. As noted previously, these differences are relatively small compared to the degree of statistical uncertainty in the estimates of the dose-response coefficients.

For females, two of the models (quadratic and quadratic exponential) predict lung cancer risks for 60-65 year-olds that are very close to those predicted by the linear model. The linear exponential model, however, curves strongly upward at high doses. Over the dose range from 0 to 0.03 mg/kg-day in females (corresponding to 0 to 750 µg/L arsenic in drinking water, about 95% of the exposed population years at risk), the cancer risks predicted by the non-linear models are never more than 9% above or 37% below the risks predicted by the linear (baseline) model.

These analyses indicate that, within the range of exposures covered by the epidemiological data, the alternative model forms predict very similar risks (i.e., variations in risk estimates across models are well within the estimated statistical uncertainty of the models). The behavior to the various models at the extremes of the data (high and low exposures) depends to a large extent on the model specification; models with non-linear dose specifications will predict risks that increase more or less rapidly in the extremes than the linear additive Poisson regression, depending on the form of the dose term. As discussed in Section 4.6.3, given the limitations in data related to mode of action, there is no compelling reason to prefer non-linear models, and the additive Poisson model is the simplest, best fitting, and most parsimonious model currently available for establishing a point of departure for establishing health criteria.

5.3.8.5 Significance of Cancer Risks at Low Arsenic Exposures

Several recently published studies have called into question the strength and significance of the exposure-response relationship for arsenic in the Taiwanese population studied by Chen et al. (1988a, 1992) and Wu et al. (1989) that have been used by EPA for estimating cancer risk. Based on "graphical and regression analysis," Lamm et al. (2003) found no significant dose-response relationship for arsenic-related bladder cancer in the subset of the Taiwanese population with median drinking water well concentrations less than 400 µg/L. Kayajanian et al. (2003) found that combined male and female lung, bladder, and liver cancer were relatively elevated at low arsenic exposures, then decreased to minimums for villages with water arsenic concentrations in the range between 42 and 60 µg/L, and then again increased with increasing arsenic exposure. In a more recent analysis, Lamm et al. (2006) found that (1) dummy variables related to "township" location were significant (along with arsenic well concentration) when all the townships were included in the analysis and (2) the dose-response parameter for arsenic exposure became insignificant for arsenic well concentrations less than 151 µg/L when only a subset of the data was included in the regression.

The studies by Lamm et al. (2003, 2006) and Kayajanian (2003) have severe limitations. In evaluating the findings of these studies, it is important to recognize the complexity and limitations of the Taiwanese data set. Cancer mortality and person-years at risk observations are provided for a large number (n = 559) of relatively small age- and village-stratified populations (median person-years at risk ~ 340 for both males and females). Most population groups have

zero cancer deaths, and the data are very "noisy." Cancer mortality is strongly age-dependent, and simultaneously evaluating the age-and dose-dependence of cancer mortality based on a data set in which cancer deaths are "rare events" requires appropriately structured models. All of these features of the data drove the selection of the Poisson regression methods described in Section 5, and the use of simpler models (linear regression, for example) can (and did) produce misleading results.

With regard to the Lamm et al. (2003) paper, it is likely that the use of linear regression and the failure to correctly account for the age-dependency of bladder cancer risks combined to make it impossible to detect a significant exposure-response relationship in villages with water arsenic levels less than 400 µg/L. U.S. EPA (2005d) evaluated this study and noted the following weaknesses:

- Classification of wells as artesian or shallow was based solely on arsenic concentration.
- Age was not included as a variable in the regression analysis, despite the clear strong dependence of cancer risks on age.
- Previous studies have found little evidence for the presence of other potential carcinogens in the sampled wells.

The major limitation of Kayanjanian's (2003) analysis of the Taiwanese data is that it breaks the data into strata that are too small to be used to calculate reliable mortality risks, and that it is very sensitive to the specific way that the data are stratified. The observed trend in cancer mortality versus arsenic dose would be very different if only few cancer deaths were misclassified, or if the pattern of cancer deaths had been slightly different by chance. Lamm et al.'s (2006) failure to find a significant exposure-response relationship in villages with arsenic water concentrations below 151 µg/L can also be explained by (1) the use of linear regression without age-adjustment and (2) the omission of data from three of the six townships from the regression.

Appendix F provides additional analyses supporting the significance and robustness of the dose-response relationship for arsenic at low doses and in the defined subsets of the population studied by Lamm et al. (2006).

5.4 CANCER ASSESSMENT (Inhalation Exposure)

An inhalation cancer slope factor was developed for inorganic arsenic and posted on the IRIS database in 1988. A re-assessment of the cancer dose-response estimation for inhalation exposure to inorganic arsenic is not addressed in this document.

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

6.1 HUMAN HAZARD POTENTIAL

Arsenic is readily absorbed from the GI tract either from drinking water or food sources. Although dermal absorption is not significant compared to absorption from oral exposure, it may

have contributed to the total arsenic exposures and health effects reported in many epidemiological studies in the literature. There appears, however, to be little if any dermal absorption (NRC, 1999) except at high occupational exposures (Hostynek et al., 1993). Inhalation is not being addressed in this document.

After absorption, inorganic As can undergo a complicated series of enzymatic and non-enzymatic reduction, enzymatic oxidative methylation and conjugation reactions. Although these reactions occur throughout the body, the rate at which they occur varies greatly from organ to organ with major metabolism occurring in liver. While there are two proposed pathways (Figures 3-1 and 3-2) for arsenic metabolism with each pathway likely to occur depending on exposure level and/or individual, the main urinary excretion products in humans are MMA and DMA and the parent compound. Arsenic metabolism (mainly methylation) varies greatly across different species (Vahter, 1994, 1999a), which may explain why there has been no adult animal model for the carcinogenic potential of arsenic. Although a few animal bioassays have been conducted, they have all been negative. Arsenic-induced cancers have been observed with transplacental exposure in mice. Transplacental exposure to arsenic in mice has found increases in the development of lung, liver, reproductive, and adrenal tumors. Skin tumors in animals have only been induced in transgenic models or in co-carcinogenesis studies.

Despite the lack of a good animal model for arsenic carcinogenesis, there have been numerous epidemiological studies examining the carcinogenic potential of inorganic As via oral exposure. Although each of the investigations has their own inherent strengths and weaknesses, the combination of all the study results supports an association between oral exposure to inorganic As and cancer including bladder, kidney, skin, lung, liver, and prostate. Because the association between arsenic and these cancers have been found in different populations, it is unlikely that any single attribute (e.g., nutritional habits) associated with a single population is responsible for the increased cancer rates. However, genetic polymorphisms have been found to be an important factor in the methylation of arsenic. Evidence suggests that individuals that have a greater capacity to methylate arsenic completely to DMA are at a lower risk for developing arsenic related cancers. Nutritional and personal habits including smoking also affect the methylation rate. Therefore, genetic, nutritional, and life-style factors contribute to the interindividual variations.

Although there has been dose-response relationships observed for the majority of cancers noted in areas with high-levels of arsenic in their drinking water, results for low-level arsenic epidemiologic investigations (primarily from the United States and Europe) have been equivocal in the relationship between these cancers and arsenic exposure. This could be due to the fact that none of the studies accounted for arsenic exposure through food sources, which would be a significant source as the levels in the drinking water decreased (Uchino et al., 2006 and Kile et al., 2007). Because cancer has a long latency period, misclassification also occurs due to lack of data on disease-relevant exposures (Cantor and Lubin, 2007), which would be more significant in studies examining lower exposures. Therefore, studies with low levels of exposure that are ecological in nature (no individual exposure) are more prone to exposure misclassification, which means they are biased towards the null hypothesis. Despite all these numerous limitations in low-level exposure studies, positive associations have been observed for cancers of the prostate (Hinwood et al., 1999 and Lewis et al., 1999), skin (Hinwood et al., 1999; Karagas et al,

2001; Beane-Freeman et al., 2004; and Knobeloch et al., 2006), and bladder (Kuritto et al., 1999; Steinmaus et al., 2003; and Karagas et al, 2004). In most cases, however, there is no dose-response with increases observed at the highest concentrations only and in many cases significant results occurred in smokers only.

Based upon current EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 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.

The available evidence is inadequate to establish a MOA by which arsenic induces tumors. The genotoxicity data for arsenic are equivocal. Chromosomal aberrations have been observed in humans and animals exposed to arsenic, but arsenic has been generally negative in bacterial mutagenicity tests and has only been observed to be a weak mutagen at the *hprt* locus in Chinese hamster V79 cells at toxic concentrations (Li and Rossman, 1989a). In addition, even though it appears genotoxic in animal models, it does not generally induce tumors in animal models. 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 doses causing overt cytotoxicity (Rossman, 2003). It has been suggested that arsenic acts as an aneugen (affects the number of chromosomes) at low doses, but as a clastogen (causes chromosomal breaks) at high doses (Rossman, 2003). However, arsenic has also been demonstrated to affect other processes possibly involved with carcinogenesis including aberrant gene/protein expression, ROS, DNA repair inhibition, signal transduction, and cancer promotion. Therefore, it is likely that arsenic acts via multiple MOAs, which would explain the number of different internal cancers associated with arsenic.

6.2 DOSE RESPONSE

Only the oral cancer assessment is addressed in this document. Lung and bladder cancer mortality in the Taiwanese population were selected as endpoints in the dose-response modeling because they are the internal cancers with the most consistent results and are best characterized in epidemiology studies of arsenic exposure (NRC, 1999, 2001; SAB 2000, 2007). Dose-response models were estimated for the Taiwanese population using additive Poisson regression with linear dose terms and quadratic age terms.

ED_{01} values were derived from the MLE dose-response parameter estimates. LED_{01} estimates were derived from the 95% upper confidence limits on the dose-response parameters, as described in Appendix E. The analysis was done in two phases. The first phase consisted of the derivation and fitting of dose-response models using the Taiwanese epidemiology data from Chen et al. (1988a, 1992) and Wu et al. (1989). The outputs of this phase of the analysis were arsenic dose-response coefficients that described the relationship between estimated arsenic intake in the Taiwanese population and proportional increases in age-specific lung and bladder cancer mortality risk. Lifetime cancer incidence in U.S. populations were then estimated by

using a modified version of the "BEIR IV" relative risk model. A key assumption underlying this model is that the risk of arsenic-related cancer is a constant multiplicative function of the "background" age profile of cancer risks in the target U.S. population. Estimates of arsenic-related cancer risks in a (hypothetical) U.S. population exposed to arsenic at varying levels in drinking water were then derived.

The oral slope factors for lung and bladder cancers in U.S. males and females were derived using the following assumptions: non-water arsenic intake for the reference and exposed populations was 10 µg/day; drinking water consumption was 3.5 and 2.0 L/day in Taiwanese men and women, respectively; 50 kg was the average Taiwanese body weight; and a 70 kg individual in the U.S. consumes 2.0 L/day of water (Section 5.3.5). The oral slope factor is dependent on assumptions related to the volume of contaminated water consumed over the course of a day and the amount of arsenic consumed through the diet. Changes in these assumptions would result in different cancer potency estimates (as discussed in Section 5.3.8.3), and corresponding changes in the other risk criteria (drinking water unit risk, drinking water concentration associated with 10^{-4} lifetime cancer risk, etc.). Sensitivity analyses were performed to test the effects of differences in drinking water intake assumptions, non-water arsenic intake assumptions, using median well water values compared to minimum and maximum values, and including different Taiwanese reference populations on the estimates (Section 5.3.8.3). Based on the results of the sensitivity analyses, the risk model results, with the exception of female bladder cancer, appear to be relatively stable and react predictably to reasonable changes in exposure assumptions. Female bladder cancer estimates were particularly sensitive to variations in non-water arsenic intake.

Estimated cancer potency factors for lifetime U.S. male lung and bladder cancer incidence were 6.7 and 11.2 per mg-kg-day, respectively. The corresponding values for females were 16.6 and 10.5 per mg/kg-day (Table 5-3). Cancer potency for combined lung and bladder cancer risks were estimated for males and females, as described in Section 5.3.8.1. The estimated cancer potency factors for combined (lung plus bladder) cancer incidence were 16.9 and 25.7 per mg/kg-day, respectively. **The potency factor estimate for women (25.7 per mg/kg-day) was identified as the recommended point of departure for derivation of health criteria, with women being the more sensitive population.**

The cancer potency estimates derived in this analysis are not directly comparable to those estimated in EPA's 1988 assessment (U.S. EPA, 1988b). That analysis derived a much lower potency factor estimate (1.0-2.0 per mg/kg-day) based on an analysis of skin cancer incidence in the Taiwanese population studied by Tseng et al. (1968; Tseng, 1977). Since the exposure-response data on internal cancers has become available, all the subsequent assessments (including this one), have been based on internal (bladder and/or lung) cancer (see Section 5.3.1). The difference in endpoints (skin versus internal cancers) is the main reason for the relatively large difference in estimated cancer potency in the more recent assessment compared to the 1988 assessment.

As discussed in Section 5.3.8.2, the lifetime risk estimates for male and female lung and bladder cancer calculated in this assessment are generally consistent with the risk estimates from previous analyses that used the internal cancers (NRC, 2001 and U.S. EPA, 2005c). The bulk of

the difference between the cancer potency estimates in this assessment and those from previous analyses can be explained by differences in dose-response models, changes in the assumptions relating to the relative drinking water consumption by women in Taiwan and the U.S., and by the use of more recent data on U.S. population mortality and cancer incidence in the BEIR IV relative risk model.

The *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) indicates that age-dependent adjustment factors should be applied to the slope factor and combined with early-life exposure estimates when estimating cancer risks from exposures to carcinogens with a mutagenic MOA. As discussed in Section 4.6.3, insufficient data are available to adequately demonstrate a mutagenic mode of action for inorganic arsenic. Therefore, the application of age-dependent adjustment factors is not recommended.

The overall level of confidence in the data is high, first, because the data used in the dose-response assessment come from human epidemiology rather than animal bioassays. The Taiwanese studies characterize the cancer risks of an extremely large, well-characterized population with a wide range of exposure concentrations. Reliability and accuracy of mortality records, verification of endpoints with histological examinations, several decades of exposure to arsenic in drinking water to detect internal cancer outcomes, apparent similarities in lifestyle habits (similar urbanization in endemic area versus rest of southwestern Taiwan) between exposed and reference populations and the residential stability of the population (i.e. little migration or emigration) are high. The data demonstrate a statistically significant dose-related effect in humans, across the entire range of exposures (i.e., 10-934 ppb median levels) evaluated. The currently used BEIR IV model is an improvement over previous models because it contains a quadratic age model, an additive linear dose term, a reference population, and adjusts for differences between the exposed and target (i.e., U.S.) populations.

Despite all their strengths, the Chen et al. (1988a, 1992) and Wu et al. (1989) studies are "ecological"; data on individual exposure (which are a function of both water consumption rates and concentrations) are not available. In addition, smoking information was not provided in the critical studies (however, it appears comparable- 40% vs. 32% in endemic area versus the rest of Taiwan according to Chen et al., 1985). Lacking this information introduces an unquantifiable degree of uncertainty into the risk estimates. In EPA's judgment, these factors are equally likely to have resulted in overestimates or underestimates of risks.

6.2.1 Choice of Models

As discussed in Section 5.3.1, the Taiwanese data have been used as the basis for quantitative risk assessment by a number of investigators. In this current analysis, EPA is building on the experience of previous efforts by the Agency and others, and has incorporated comments and recommendations by NRC (2001) and the Science Advisory Board (SAB, 2007) in the selection of statistical methods for use in the risk assessment. As discussed in Section 5.3.7.1, the current assessment employs a Poisson regression model with additive linear dose terms and quadratic age terms for dose-response model fitting in the Taiwanese population. This model was found to be the simplest, best fitting model among a number of alternatives tested.

Sensitivity analyses of other models (quadratic, exponential linear, and exponential quadratic dose transformation) were also evaluated (see Section 5.3.8.4 for further details).

To extrapolate arsenic-related cancer risks to the U.S. population, the current assessment employs a variant of the "BEIR IV" relative risk model (Section 5.3.7.3). This model takes as its inputs the dose-response coefficients from the Poisson regressions and "background" cancer incidence and population mortality data from the target (U.S.) population. Population mortality data for the year 2000 (NCHS, 2000) and background lung and bladder cancer incidence for the 2000-2003 (NCI, 2006) were used as inputs to the BEIR IV model.

6.2.2 Dose Metric

Inorganic As is metabolized *in vivo*, with some of the known metabolites being more toxic than the parent compound. However, it is not known whether it is a metabolite, the parent compound, or a combination of the two that is responsible for the observed carcinogenic potential. An increase in MMA or decreased DMA in the urine has been associated with an increase in disease risk (Yu et al., 2000; Chen et al., 2005a; Steinmaus et al, 2005; Valenzuela et al., 2005; Ahsan et al., 2007; Huang et al., 2007b; and McCarthy et al., 2007a); therefore, the actual carcinogenic moiety may not be proportional to administered exposure and use of administered exposure may provide a bias in the model. However, the exposure assessment for the model is ecological in nature and provides its own inherent bias. Detailed arsenic speciation data are not available for the Taiwanese population used in the risk assessment. Therefore, estimated total daily arsenic dose (water + other dietary) has been used as the dose metric in the risk assessment. Arsenic dose is estimated based on well water concentration data, and it is assumed that the arsenic concentrations have been constant over the period of exposure. Since there are no data related to the temporal variability in the well water concentrations, this introduces uncertainty into the dose estimates for the 43 villages. Sensitivity analyses were conducted to investigate the impact of using alternative exposure indices, as discussed in Section 5.3.8.3.

6.2.3 Human Population Variability

Although the extent of inter-individual variability in arsenic metabolism has not been adequately characterized, genetic polymorphism, nutritional status, and personal habits (e.g., smoking) have all been associated with differences in arsenic methylation. Data exploring whether there is a differential sensitivity to arsenic carcinogenicity across life stages is limited. Data by Waalkes et al. (2003, 2004a) indicate that transplacental exposure in mice is a sensitive stage for carcinogenic potential. These are the only studies in which inorganic As exposure has been associated with cancer in rodents. Lung, liver, reproductive, and adrenal tumors were associated with arsenic administration during gestation (10 days only). A single epidemiological study by Smith et al. (2006) examined lung cancer rates (and other respiratory diseases) in cohorts exposed during childhood and cohorts likely exposed *in utero* to arsenic concentrations of 860 ppb that subsequently dropped to 100 ppb. Results demonstrated that exposure during either period of development caused increased risk of lung cancer in females aged 40-49 born between 1950-1957 and in males aged 30-49 born between 1950 and 1970. However, the risks associated with early childhood exposures and/or *in utero* exposures were not compared to risks

from exposures during adulthood. Thus, the available data do not allow for a quantitative assessment of the relative sensitivity to arsenic exposures between the Taiwanese population used in the dose-response assessment and U.S. populations exposed to arsenic in drinking water.

SAB (2007) acknowledged "the possible issue of compromised nutrition among segments of the exposed population" in the Taiwanese study population, along with the lack of data related to smoking history. However, data are not available that would allow quantitative evaluation of these factors. Therefore, this risk assessment assumes that the observed carcinogenic potency in the Taiwanese population, with suitable corrections for differences in drinking water intake and background cancer incidence, is an appropriate predictor of the potential for human cancer risk in the U.S. population.

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

The *Toxicological Review of Inorganic Arsenic* has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2000a). For the external peer review, the reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific charge questions, addressing key scientific issues of the assessment. While the external peer review panel (SAB) was supplied with questions regarding both DMA^V and inorganic arsenic, only questions and responses pertaining to inorganic arsenic will be addressed in this Appendix. Charge question B3 asked the SAB to comment on EPA's hypothesis that inorganic arsenic acts via different modes of action for carcinogenicity. SAB agreed with EPA's conclusion, but during a discussion on the mode of action of DMA^V, a member of the panel stated that the description for inorganic arsenic's mode of action could be strengthened. In addition to strengthening the mode of action discussion, studies on the mode of action for inorganic arsenic have been placed in a table in Appendix C. Section 4.4.1 provides a summary of the specifics in the tables instead of detailed write-ups for all the studies. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. Public comments were submitted to the SAB and were taken into consideration by the panel during their review. The summary of significant comments and responses below is inclusive of the major issues raised by public commenters which specifically focused on the choice of study for cancer quantitation and the nature of the dose response. Editorial comments were considered and incorporated into the document as appropriate and are not discussed further.

Charge Question B3

EPA concluded that iAs causes human cancer most likely by many different modes of action. This is based on the observed findings that iAs undergoes successive methylation steps in humans and results in the production of a number of intermediate metabolic products and that each has its own toxicity. EPA asked the SAB to comment on the soundness of its conclusion.

SAB Comments

The Panel concluded that:

i) Multiple modes of action may operate in carcinogenesis induced by iAs because there is simultaneous exposure to multiple metabolic products as well as multiple target organs and the composition of metabolites can differ in different organs.

ii) Each arsenic metabolite has its own cytotoxic and genotoxic capability.

iii) Inorganic arsenic (iAs^{III}) and its metabolites are not direct genotoxicants because these compounds do not directly react with DNA. However, iAs^{III} and some of its metabolites can exhibit indirect genotoxicity, induce aneuploidy, cause changes in DNA methylation, and alter signaling and hormone action. In addition, iAs can act as a transplacental carcinogen and a cocarcinogen.

iv) Studies of indirect genotoxicity strongly suggest the possibility of a threshold for arsenic carcinogenicity. However, the studies discussed herein do not show where such a threshold might be, nor do they show the shape of the dose-response curve at these low levels. In addition, a threshold has not been confirmed by epidemiological studies. This issue is an extremely important area for research attention, and it is an issue that should be evaluated in EPA's continuing risk assessment for iAs.

v) Arsenic essentiality and the possibility of hormetic effects are in need of additional research to determine how they would influence the determination of a threshold for specific arsenic-associated health endpoints.

EPA Response

EPA agrees that the available data potentially support multiple modes of action for inorganic arsenic. The Agency believes that, at this point, the data concerning mode of action are not well-enough understood to support their use in quantitative risk assessment.

Charge Question C2

EPA reviewed the available epidemiologic studies including those published since the NRC 2001 review for U.S. populations exposed to inorganic arsenic via drinking water. EPA concluded that the Taiwanese dataset remains the most appropriate choice for estimating cancer risk in humans. The SAB was asked to comment on the soundness of this conclusion and also on whether these data provide adequate characterization of the impact of childhood exposure to iAs.

SAB Comments

The Panel concluded that:

i) Because of various factors (e.g., size and statistical stability of the Taiwanese database relative to other studies, the reliability of the population and mortality counts, the stability of residential patterns, and the inclusion of long-term exposures), this database remains, at this time, the most appropriate choice for estimating bladder cancer risk among humans, though the data have considerable limitations that should be described qualitatively or quantitatively to help inform risk managers about the strength of the conclusions.

ii) There are other epidemiologic databases from studies of populations also exposed at high levels of arsenic, and the Panel recommends that these be used to compare the unit risks at the higher exposure levels that have emerged from the Taiwan data.

iii) The Panel also suggests that published epidemiology studies of US and other populations chronically exposed from 0.5 to 160 μg/L inorganic arsenic in drinking water be critically evaluated, using a uniform set of criteria and that the results from these evaluations be transparently documented in EPA's assessment documents. If, after this evaluation, one or more of these studies are shown to be

of potential utility, the low-level studies and Taiwan data may be compared for concordance. Comparative analyses could lead to further insights into the possible influence of these differences on population responses to arsenic in drinking water.

iv) Regarding childhood exposure to iAs, it was the Panel's view that, based on available data, it is not clear whether children differ from adults with regard to their sensitivity to the carcinogenic effects of arsenic in drinking water. However, the possibility of a different response in degree or kind should not be ignored and needs to be investigated.

EPA Response

After consideration of additional studies, EPA agreed with the SAB that the Taiwanese data were the best available for quantitative analysis. Studies assessed, but not used in the analysis, are summarized in Section 4.1 of the document. The studies were systematically evaluated for their suitability in risk assessment based on a uniform set of criteria including the study type, the size of the study population and control population, and the relative strengths and weaknesses of the study based on SAB recommended criteria (i.e., estimates of the level of exposure misclassification; temporal variability in assigning past arsenic levels from recent measurements; the extent of reliance on imputed exposure levels; the number of persons exposed at various estimated levels of waterborne arsenic; study response/participation rates; estimates of exposure variability; control selection methods in case-control studies; and the resulting influence of these factors on the magnitude and statistical stability of cancer risk estimates). Study summaries are also provided in Tabular form in Appendix B for ease of comparison. Studies are arranged geographically and include other areas of high arsenic exposure (e.g., South America) as well as areas of low exposure (e.g., U.S. and Europe). With regard to children, studies examining children were evaluated and are discussed in Section 4.7.1 of the document, but EPA believes that the available data do not yet allow a definitive conclusion as to the differential susceptibility of children to arsenic exposure. EPA notes that recent animal studies demonstrating the potential for cancer after *in utero* arsenic exposures gives rise to additional concerns regarding exposures early in development.

Charge Question D2

EPA determined that the most prudent approach for modeling cancer risk from iAs is to use a linear model because of the remaining uncertainties regarding the ultimate carcinogenic metabolites and whether mixtures of toxic metabolites interact at the site(s) of action. EPA asked the SAB if it concurred with the selection of a linear model following the recommendations of the NRC (2001) to estimate cancer risk in light of the multiple modes of carcinogenic action for iAs.

SAB Comments

The Panel concluded that:

i) Inorganic arsenic has the potential for a highly complex mode of action.

ii) Until more is learned about the complex PK and PD properties of iAs and its metabolites there is not sufficient justification for the choice of a specific nonlinear form of the dose-response relationship.

iii) The NRC (2001) recommendation to base risk assessments on a linear dose response model that includes the Southwestern Taiwan population as a comparison group seems the most appropriate approach.

iv) The Panel also recommends that EPA perform a sensitivity analysis of the Taiwanese data with different exposure metrics, with the subgroup of villages with more than one well measurement, and using a multiplicative model that includes a quadratic term for dose.

EPA Response

As discussed in Section 5.3, EPA investigated a range of model forms for use in the risk assessment, building on previous efforts, including EPA (2001) and Morales et al. (2000). The model used in the derivation of the preferred risk assessments (see Section 5.3.3) employs:

- Poisson regression (of cancer mortality against age and dose) fit by maximum likelihood estimation (MLE)
- A quadratic age model
- A linear multiplicative dose term
- Confidence limits on the dose term estimated by profile likelihood
- Estimates derived for the data set that includes the southwest Taiwan reference population

A range of alternative model forms were investigated, as discussed in Section 5.3.8.4, and the impacts of alternative assumptions about non-water arsenic intake, drinking water consumption, and other exposure factors were investigated through sensitivity analyses, as described in Section 5.3.8.3. EPA also investigated the properties of the dose-response relationship in the low-dose range of the Taiwanese data, and found that arsenic slope coefficients were positive and statistically significant even when high-exposure groups were excluded from the analysis. EPA's dose-response modeling found no indication of the existence of a threshold arsenic exposure below which cancer risks are not elevated. As discussed in Section 4.6.3, EPA believes that the available mode of action data do not justify the use of non-linear low-dose extrapolation from the point of departure (POD).

Charge Question D3

EPA employed the Microsoft Excel software that was previously used by the NRC (2001) to project estimated cancer risks from iAs exposure. The SAB was asked to comment on the precision and accuracy of this program.

SAB Comments

The Panel concluded:

i) That the EPA program conformed to the NRC (2001) recommendation for modeling cancer hazard as a function of age and the average daily dose of exposure to arsenic through drinking water sources.

ii) The panel did, however, identify and report to the EPA on two potential discrepancies in the data inputs and one computational error in the portion of the program that employs the BEIR-IV formula to evaluate excess lifetime cancer risk from arsenic exposure.

iii) The panel made several suggestions for improvements in the model's programming and documentation conventions as well as recommendations for specific sensitivity analyses designed to test the robustness of the model to alternative formulations of the hazard function and aggregate population data inputs.

EPA Response

EPA made a number of changes to the model implementation in response to the SAB comments. As in the previous analyses, the linear Poisson dose-response models were estimated using maximum likelihood methods; models were implemented in Excel® and replicated using Statistica®. In the latest analyses, confidence limits on the arsenic dose-response coefficients were estimated using profile likelihood, rather than Bayesian simulation. The confidence limit estimates derived using profile likelihood were very similar to those obtained using Bayesian simulation and estimates derived by "bootstrap" methods.

In this latest analysis, the BEIR IV formula for estimating lifetime cancer incidence risks was modified in response to SAB and internal EPA comments. The revised model estimates lifetime cancer incidence data based on "background" cancer incidence and mortality data from the NCI SEER program (see Section 5.3.7.3). The revised approach is discussed in detail in Appendix E.2.

As discussed in the previous response, EPA conducted sensitivity analyses on a number of model parameters. These analyses are described in Section 5.3.8.3.

Charge Question D4

In calculating estimated cancer risk to the US general population from drinking water exposure to iAs, the EPA utilized epidemiologic data from Taiwan. EPA followed the NRC (2001) recommendations to account for the differences in the drinking water consumption rates for the Taiwanese population and U.S. populations. On the basis of more recent data (noted in USEPA, 2005b), EPA utilized water intake adjustments for 2 to 3.5 liters/day. EPA asked the SAB to recommend a drinking water value.

SAB Comments

The Panel agreed that water consumption (via drinking as water, in beverages, or in cooking water) assumptions have a substantial impact on the assessment of arsenic's risk. However, the Panel did not recommend specific values for EPA to use in evaluating dose-

response in the Taiwanese study nor for levels of exposure in the U.S. population risk estimates. It did recommend that uncertainty in this parameter be evaluated for both the Taiwanese study population and the U.S. populations at risk. The Panel recommended that EPA should:

i) Evaluate the impact of drinking water consumption rates associated with more highly exposed population groups with differing exposures and susceptibilities (e.g., children, pregnant women).
ii) Incorporate variability parameters for individual water consumption into their analysis for dose-response in the Taiwanese population as they have done for the U.S. population.
iii) Conduct sensitivity analyses of the impact of using a range of consumption values for the Taiwanese population.
iv) Provide a better justification for assuming different consumption levels by gender or in the absence of such a justification, conduct additional sensitivity analyses to examine the impact of equalizing the gender-specific consumption level.
v) More fully articulate and document how different sources of water intake, as well as variability, are incorporated into the risk model (e.g. data for intake from beverages and cooking water).

EPA Response

Data are not available regarding individual water consumption rates and background (non-water) arsenic intake in the Taiwanese study populations. EPA, therefore, conducted a series of sensitivity analyses involving ranges of drinking water consumption and "background" (non-water) arsenic consumption that the Agency believes spans a reasonable range of values for these parameters. Arsenic dose-response models were fit assuming non-water arsenic intakes of 0, 10, 30, 50, 100, and 200 µg/day in the exposed populations, non-water arsenic intake of 0, 30, and 50 µg/day in the reference population, and for male (Taiwanese) daily water consumption ranging from 2.75-5.1 L/day and female water consumption ranging from 2.0-4.1 L/day. Risk models also were fit using three different sets of village arsenic drinking water concentrations (median, minimum, and maximum), and three sets of assumptions related to reference (unexposed) populations (southwest Taiwan, all Taiwan, and none). The results of these analyses are summarized in Tables 5-8 and 5-9. Overall, EPA found that cancer slope estimates for male and female lung cancer and male bladder cancer were relatively insensitive to assumptions related to non-water arsenic intake and varied more or less inversely with the assumed daily water consumption, and with drinking water arsenic concentration estimates. When alternative reference populations were assumed (all Taiwan or none), cancer slope coefficients were lower than when the southwest Taiwan comparison group was included in the analysis. The cancer slope estimates for female bladder cancer were generally more sensitive to changes in exposure assumptions than the other endpoints.

Charge Question D5

As recommended by the NRC (2001) EPA considered the background dietary intake of iAs and incorporated adjustment values of 0, 10, 30, and 50 µg per day into the cancer modeling based on available new data. The SAB was asked to recommend a value for the background

dietary intake of iAs for both the control population and study population of Southwestern Taiwan.

SAB Comments

The Panel agreed that arsenic levels in food are important considerations for EPA's assessment of lung and bladder cancer risk associated with exposures to arsenic in drinking water. However, the Panel did not recommend a specific value for EPA to use in its base risk assessment. It did recommend a range of values for consideration by EPA in its sensitivity analysis and the Panel offered suggestions to EPA for additional analytical steps to clarify the impact of food levels of arsenic on dose-response and exposure as it revises its risk estimates. These Panel recommendations include that EPA should:

i) Conduct sensitivity analyses using a range of total arsenic food intake values from at least 50 to 100 µg /day to perhaps as high as 200 µg/day to assess the impact of this range of dietary intakes on risk of lung and bladder cancer from exposure *via* drinking water in the Taiwan cohort.

ii) Not assume that the control population has an intake value of zero arsenic from food.

iii) Apply greater rigor in their discussions of data used in these assessments (e.g., sources, methodological and analytical issues, bioavailability).

iv) Give immediate research attention to the issue of arsenic bioavailability.

EPA Response

As discussed in the previous response, EPA conducted sensitivity analyses that assumed non-water arsenic intakes (doses) for the exposed populations ranging from 0-200 µg/day and ranging from 0-50 µg/day in the reference population. EPA did not specifically conduct sensitivity analyses related to arsenic bioavailability. The Agency notes, however, that the range of absorbed dose that was evaluated implicitly addresses potential bioavailability differences. For example, assuming 50 µg arsenic intake absorbed dose is equivalent to assuming 50% of absorption of a 100 µg/day dose, etc. The Agency believes that the range of arsenic intake that was considered covers the plausible ranges of non-water dietary arsenic and bioavailability thereof.

Appendix B: Tabular data on cancer epidemiology studies

The SAB Arsenic Review Panel provided comments on key scientific issues associated with arsenicals on cancer risk estimation in July of 2007 (SAB, 2007). It was concluded that the Taiwanese database is still the most appropriate source for estimating bladder and lung cancer risk among humans (specifics provided in Section 5) because of 1) the size and statistical stability of the database relative to other studies; 2) the reliability of the population and mortality counts; 3) the stability of residential patterns; and 4) the inclusion of long-term exposures. However, SAB also noted considerable limitations within this data set (EPA-SAB-07-008, http://www.epa.gov/sab). The panel suggested that one way to mitigate the limitations of the Taiwanese database would be to include other relevant epidemiological studies from various countries. For example, SAB referenced other databases that contained studies of populations also exposed to high levels of arsenic (e.g., Argentina and Chile), and recommended that these alternate sources of data be used to compare the unit risks at the higher exposure levels that have emerged from the Taiwan data. The SAB also suggested that, along with the Taiwan data, published epidemiology studies from the United States and other countries where the population is chronically exposed to low levels of arsenic in drinking water (0.5 to 160 ppb) be critically evaluated, using a uniform set of criteria presented in a narrative and tabular format. The relative strengths and weaknesses of each study should be described in relation to each criterion.

Additionally, SAB (2007) recommended that the following issues be considered when reviewing "low-level" and "high level" studies: a) estimates of the level of exposure misclassification; b) temporal variability in assigning past arsenic levels from recent measurements; c) the extent of reliance on imputed exposure levels; d) the number of persons exposed at various estimated levels of waterborne arsenic; e) study response/participation rates; f) estimates of exposure variability; g) control selection methods in case-control studies; and h) the resulting influence of these factors on the magnitude and statistical stability of cancer risk estimates.

In light of the SAB recommendations, epidemiological studies in the literature from 1968 to 2007 have been reviewed. The report includes data from all populations that have been examined in regards to cancer from arsenic exposure via drinking water. Earlier publications were reviewed and are included as needed to facilitate the understanding of results from certain study populations. As recommended by the SAB, studies were presented in both a narrative (Section 4.1) and tabular (below) format. Each publication was evaluated using a uniform set of criteria, including the study type, the size of the study population and control population, and the relative strengths and weaknesses of the study, focusing on the major strengths and weaknesses. While the information in the tables mirrors the information in the narrative, the narrative may provide additional important information concerning the investigation. The studies are presented by country of origin, then in chronological order by publication year. Below also are definitions of terms that are used in the tables (and the narratives in Section 4.1).

Cross-sectional studies have inherent limitations including: 1) difficulty in making causal inference; 2) data collected for only one point in time, and different results may be found if another time-frame had been chosen; and 3) prevalence-incidence bias (also called Neyman bias), which is especially prevalent for longer-lasting diseases, where any risk factor that results in death will be under-represented among those with the disease.

Ecological studies provide low cost, convenience, simplicity of analysis, and ease of exposure measurement at population or group level rather than at the individual level; therefore, a wider range of exposures can often be obtained. Concerns about the methodological weakness of ecological studies arise from the fact that estimates of effect do not equate to estimates of biological effect obtained from individual level analysis, that exposure data from this design cannot be used to obtain direct estimates of the rate of injury in exposed and unexposed populations, existing data sources are often flawed, and it is difficult to control confounding.

Cohort studies are research studies in which the medical records of groups of individuals, who are alike in many ways, but differ by a certain characteristic (for example, individuals who smoke and those who do not smoke) are compared for a particular outcome (such as lung cancer). Cohort studies are generally used to follow large groups over a long period to study rare or long-latency diseases.

A case-control study is a retrospective study that compares two groups of people: those with the disease or condition under study (cases) and a very similar group of people (matched controls) who do not have the disease or condition. Researchers study the medical and lifestyle histories of the people in each group to determine which factors may be associated with the disease or condition under investigation. An example is where one group may have been exposed to a particular substance that the other was not.

In a nested case-control study, cases of a disease that occur in a defined cohort are identified and, for each, a specified number of matched controls is selected from among those in the cohort who have not developed the disease by the time of disease occurrence in the case. The nested case-control design can potentially offer a lower cost and effort for data collection and analysis compared with the full cohort approach, with relatively minor loss in statistical efficiency. The nested case-control design is particularly advantageous for studies of biologic precursors of disease.

Recall bias is a type of systematic bias that occurs when the way a survey respondent answers a question is affected not just by the correct answer, but also by the respondent's memory.

Selection bias is the error of distorting a statistical analysis due to the methodology of how the samples were collected. As an example, sample selection may involve pre- or post-selecting the samples that may preferentially include or exclude certain kinds of results. Selection bias is possible whenever the group of people being studied has any form of control over whether to participate making the participants a non-representative sample. Selection bias may also occur when investigators preferentially select individuals to be included as cases or controls based on prior knowledge of study hypotheses or outcomes. Selection bias in epidemiology is a distortion of data that arises from the way that the data have been collected. If the selection bias is not taken into account, conclusions drawn from the results obtained may be wrong. Self-selection bias is when individuals that comprise the study population have any control over whether or not they are allowed to participate. An individuals' decision to participate in a study may be associated with other factors that affect the study, which results in

the participants being a non-representative sample.

The standardized mortality ratio (SMR) in epidemiology is the ratio of observed deaths to expected deaths in a population for a specific health outcome. The SMR also serves as an indirect means for adjusting a rate. The number of observed deaths is obtained for a particular sample of a population that is under investigation, and the number of expected deaths reflects the number of deaths for a larger population from which the study sample has been taken. The calculation used to determine the SMR is simply the number of observed deaths divided by the number of expected deaths. The SMR may be displayed as either a ratio or sometimes as a percentage. If the SMR is shown as a ratio and is equal to 1.0, this means the number of observed deaths equals that of expected cases. If the SMR is greater than 1.0 there is a higher number of deaths than expected, and if the SMR is less than 1.0 there is a lower number of observed than expected deaths.

The standardized incidence ratio (SIR) is a common tool for monitoring disease rates. Incidence is the number of newly diagnosed cases in a given location during a given time period. An SIR compares the actual number of cases for a given place and time to the number that would be expected based on disease rates in some comparison area.

In statistics and epidemiology, relative risk (RR) is the risk of an event (or of developing a disease) relative to exposure. Relative risk is a ratio of the probability of the event occurring in the exposed group versus the control (non-exposed) group.

Time-weighted average (TWA) is the average exposure to a contaminant or condition (such as noise) to which workers are exposed over a period, such as in an 8-hour work day.

Table B-1. Taiwan Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
Not indicated	19,269 males 21,152 females (40,421 total)	Arsenic concentration in well water (ppb): Low (L) - 0-290 Mid (M) - 300-590 High (H) - ≥ 600 Undetermined (U)	Age/ gender specific skin cancer prevalence rate (1/1000) by arsenic concentration (L, M, H, U): Males, 20-39 yrs.- L= 1.5, M= 4.3, H= 22.4, U= 1.7 Males, 40-59 yrs.- L= 6.5, M= 47.7, H= 98.3, U= 51.7 Males, 60 yrs. and over- L=48.1, M=163.4, H=255.3, U=148.2 Total all males combined- L= 4.0, M=14.4, H= 31.0, U= 16.5 Females, 20-39 yrs.- L= 0.1, M= 0.7, H= 3.5, U= 0.9 Females, 40-59 yrs.- L= 3.6, M= 19.7, H= 48.0, U= 9.2 Females, 60 yrs. and over- L= 9.1, M= 62.0, H= 110.1, U= 62.9 Total all females combined- L= 1.3, M= 6.3, H= 12.1, U= 4.7 Both genders, 20-39 yrs.- L= 1.3, M= 2.2, H= 11.5, U= 1.2 Both genders, 40-59 yrs.- L= 4.9, M= 32.6, H= 72.0, U= 28.3 Both genders, 60 yrs. and over- L=27.1, M= 106.2, H= 192.0, U= 107.9 Total both genders combined- L= 2.6, M= 10.1, H= 21.4, U= 10.4	Strengths: -Large number of participants. -Dose response information provided. Weaknesses: -No individual exposure data. -Possible recall bias among study participants in determining the age of cancer onset and length of residence in the study area. -Water supply changes over time were not collected nor were information on smoking histories, and the arsenic concentration from individual wells varied over time.	Tseng et al., 1968 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			Observed rate/1000: Hyperpigmentation = 183.52 Keratosis = 70.95 Skin cancer = 10.59 Blackfoot disease = 8.91		
1958-1975	40,421 individuals	Arsenic concentration in well water (ppb): <300 = Low (L) 300-600 = Mid (M) >600 = High (H)	Age-specific prevalence (per 1000): Skin cancer 20-39 years- L =1.3, M = 2.2, H = 11.5 40-59 years- L = 4.9, M = 32.6, H = 72.0 60+ years- L = 27.1, M = 106.2, H = 192.0 Blackfoot disease 20-39 years- L = 4.5, M = 13.2, H = 14.2 40-59 years- L = 10.5, M = 32.0, H = 46.9 60+ years- L = 20.3, M = 32.2, H = 61.4 Skin cancer and BFD combined- Observed – 61 cases, 1.51/1000 Expected – 4 cases, 0.09/1000 Observed to expected ratio = 16.77	Strengths: -Large study population. -Adjusted for age and gender. Weaknesses: -No individual monitoring data. -Possible recall bias among study participants (interviews and mailed surveys) in determining the age of cancer onset and the length of residence in the area.	Tseng, 1977 Ecological
1968-1982	Subjects from BFD endemic area	Median arsenic concentration (ppb): artesian well water- 780 (range, 350-1140) shallow well water- 40 (range, 0-300)	Cancer SMRs (95% CI, p value <0.05): Males – bladder = 11.00 (9.33-12.67) kidney = 7.72 (5.37-10.07) skin = 5.34 (3.79-8.89) lung = 3.20 (2.86-3.54) liver = 1.70 (1.51-1.89) colon = 1.60 (1.17-2.03) Females –	Strengths: -The SMRs for the study cohort taken from BFD endemic area in Taiwan were determined using the general population of Taiwan and world population. -Controlled for the potential confounders age and gender. Weakness:	Chen et al., 1985 ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			bladder = 20.09 (17.02-23.16) kidney = 11.19 (8.38-14.00) skin = 6.52 (4.69-8.35) lung = 4.13 (3.60-4.66) liver =2.29 (1.92-2.66) colon = 1.68 (1.26-2.10)	-Arsenic measurements not linked to cancer mortality. - Death certificates list the main cause of death rather than all causes - SMRs were only presented by township and villages	
January 1980-December 1982	Deceased cancer cases- 69 bladder 76 lung 59 liver Controls-368 (community matched)	Median arsenic concentration: artesian well water- 780 ppb (range, 350-1140) shallow well water- 40 ppb (range: 0 - 300)	Age-sex adjusted odds ratios, well water use ≥ 40 years: bladder cancer = 3.90 lung cancer = 3.39 liver cancer= 2.67 Mantel-Haenszel x2: Bladder cancer = 13.74* Lung cancer = 8.49* Liver cancer = 9.01* *P< 0.01 Multivariate logistic regression: Improvement x2 value- Bladder cancer = 11.45* Lung cancer = 9.04* Liver cancer = 6.34* *P< 0.01	Strengths: -Cases confirmed using histology or cytology findings. -Cancer cases and controls were from the same BFD community. -Potential confounders adjusted for in the analysis included age, gender, smoking, tea drinking, vegetable consumption, and fermented bean consumption. Weaknesses: -Confounders not controlled for included recall bias from case and control interviews regarding lifestyle, diet, and daily water consumption and source of water. -Selection bias (control selection).	Chen et al., 1986 Case-control
1973-1986	Blackfoot endemic area residents Population of Taiwan as reference population World population as	Three exposure categories (ppb): <300 300-590 ≥ 600	Age standardized mortality per 100,000 for various cancers: World Population: <300 ppb Males – all sites= 154.0; liver= 32.6, lung= 35.1, skin= 1.6, prostate= 0.5, bladder = 15.7, kidney = 5.4 Females – all sites= 118.8, liver= 14.2, lung= 26.5, skin= 1.6, bladder= 16.7, kidney = 3.6	Strengths: -Data from arsenic monitoring conducted in 1962-64 and 1974-76 found similar results. Weaknesses: -Individual Arsenic exposure levels were not presented.	Chen et al., 1988a Cohort

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
	reference population		300-590 ppb Males – all sites= 258.9, liver=42.7, lung= 64.7, skin= 10.7, prostate = 5.8, bladder = 37.8, kidney = 13.1 Females – all sites= 182.6, liver= 18.8, lung= 40.9, skin= 10.0, bladder = 35.1, kidney = 12.5 ≥ 600 ppb Males – all sites= 434.7, liver= 68.8, lung= 87.9, skin= 28.0, prostate= 8.4, bladder = 89.1, kidney = 21.6 Females – all sites= 369.4, liver= 31.8, lung= 83.8, skin= 15.1, bladder= 91.5, kidney = 35.3 Taiwan: Males – all sites= 128.1, liver= 28.0, lung= 19.4, skin= 0.8, prostate= 1.5, bladder= 3.1, kidney= 1.1 Females – all sites = 85.5, liver = 8.9, lung = 9.5, skin = 0.8, bladder = 1.4, kidney = 0.9		
January 1968-December 1983	241 cases 759 controls General population of Taiwan Local endemic	Arsenic concentration (ppb): artesian well water – median = 780 range = 350-1140 shallow well water median = 40 range = 0-300	Significant SMRs (p values) (compared to population of Taiwan): Cancers– bladder = 38.80 (<0.001) skin = 28.46 (<0.01) lung = 10.49 (<0.001) liver = 4.66 (<0.001) colon = 3.81 (<0.05)	Strengths: -Cases consisted of black foot disease cases, matched to healthy community controls for age, sex and residence. -Recall bias was minimized through interview techniques. -SMRs were determined using both the national Taiwanese population and the local endemic area population.	Chen et al., 1988b Cohort/ Nested case-control

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
	area population		Significant SMRs (p values) (compared to population of BFD endemic area): Cancers – bladder = 2.55 (<0.01) skin = 4.51 (<0.05) lung = 2.84 (<0.01) liver = 2.48 (<0.01)	Weakness: -Arsenic dose levels were not provided.	
August 1983-February 1987	246 BFD bladder cancer cases 444 BFD-endemic area residents 286 residents neighboring the endemic area 731 non-endemic area residents	Percent of area well water with arsenic content of ≥ 50 ppb: Pei-men = 81 Hsueh-Chia=27 Pu-Tai = 58 Jinag-Jium=24 Tai-Pao =45 Pao-Chung=54; ≥ 350 ppb: Pei-men = 62 Hsueh-Chia =7 Pu-Tai = 8 Jinag-Jium =0 Tai-Pao = 6 Pao-Chung = 0	Positive cytology (bladder cancer/atypia) prevalence rate (%): BFD cases= 4.5 endemic area= 2.5 neighboring area= 0.7 non-endemic area= 0.13	Strengths: -Histological confirmation of bladder cancer diagnoses. Weaknesses: -Lack of individual exposure data.	Chiang et al., 1988 Case-control
1973-1986	Residents of 42 villages 1976 world population used as comparison	Three exposure categories (ppb): <300 300-590 >600	Trend test of the extension of the Mantel-Haenszel Chi square test-Cancers- both genders: bladder, skin, lung- P<0.001 males only: kidney, liver, prostate - P <0.05 females only: kidney- P<0.001	Strengths: -Adjustments made for age and gender. -Life-style, access to medical care, and socioeconomic status were similar among the study groups. Weaknesses: -Limitations of mortality data. -Associations observed at the local level may not be accurate at the individual level (ecological fallacy).	Wu et al., 1989 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1972-1983	Arsenic exposed subjects from 314 townships and precincts	Total wells tested = 83,656, ≥ 50 ppb in 15,649 wells (18.7%), ≥ 350 ppb in 2,224 wells (2.7%) Concentrations of the remainder of the wells were not given.	Multivariate adjusted regression coefficient for cancers (SE): Males- liver = 6.8 (1.3), nasal cavity = 0.7(0.2), lung = 5.3 (0.9), skin = 0.9 (0.2), bladder = 3.9 (0.5), kidney = 1.1 (0.2), prostate = 0.5 (0.2); Females- liver = 2.0 (0.5), nasal cavity = 0.4 (0.1), lung = 5.3 (0.7), skin = 1.0 (0.2), bladder = 4.2 (0.5), kidney = 1.7 (0.2) No p values indicated.	Strengths: -Potential confounders controlled for included socioeconomic differences, i.e., urbanization and industrialization. -Cancer rates in endemic BFD townships were compared with cancer rates in non-endemic townships of Taiwan. -Ecological correlations reported between arsenic content in well water and mortality from various cancers. Weaknesses: -Potential confounders not controlled for were gender and other potential well water contaminants. -No individual arsenic exposures.	Chen and Wang, 1990 Ecological
1973-1986	Arsenic exposed subjects from 42 villages	Well water arsenic exposure categories (ppb): <100 100-290 300-590 ≥ 600 Overall range: 10-1,752	Cancer development potency index (daily arsenic intake of 10 µg/kg): males- liver = 4.3 x 10-3 lung = 1.2 x 10-2 bladder = 1.2 x 10-2 kidney = 4.2 x 10-3 women – liver = 3.6 x 10-3 lung = 1.3 x 10-2 bladder = 1.7 x 10-2 kidney = 4.8 x 10-3	Strengths: -Potential confounders included age, gender, access to medical care, socioeconomic status, and life-style and were all controlled for in the analysis. -Villages share similar socioeconomic status, living environments, lifestyles, dietary patterns and even medical facilities. Weaknesses: -Armitage-Doll model constrains risk estimates to be monotonically increasing function of age -Age stratification only available for 20-year strata -Possible underestimation of risk because the assumption was made that an individuals arsenic intake remained	Chen et al., 1992 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
				constant from birth to the end of the follow-up period. -Assumption that an individual's arsenic intake remained constant from birth to the end of the follow-up period and the possible underestimation of risk because other sources of arsenic exposure were not considered.	
Followed-up for 0.05-7.69 years [4.97 ±1.72 (SD) years] until January 1993	263 BFD cases 2293 healthy residents	Artesian well water median arsenic level= 780 ppb Shallow well water median arsenic level= 40 ppb	Multivariate adjusted RR (95% CI), cancer: All sites- Age: Every-l-yr increment =1.05 (1.03-1.06)* Sex : Men =1.00, Women = 0.72 (0.43-1.18)* Cigarette smoking: No = 1.00, Yes =1.52 (1. 00-2.48)* Status of blackfoot disease: No = 1.00, Yes = 2.69 (l.80-4.01)* Cumulative arsenic exposure (mg/liter x yr) : 0 = 1.00 0.1-19.9 = 1.39 (0.82-2.37) 20+ = 1.76 (1.01-3.06)* Unknown = 0.72 (0.42-1.22) Lung- Age: Every-l-yr increment = 1.06 (1.02-1.10)* Sex: Men = 1.00, Women = 1.79 (0.44-7.32)* Cigarette smoking: No = 1.00, Yes = 4.31 (1.08-17.20)* Status of blackfoot disease: No = 1.00, Yes = 2.45 (1.07-.57)* Cumulative arsenic exposure	Strengths: -Showed a significant dose response relationship with increasing concentrations of arsenic. -Analysis adjusted for BFD status, age, sex, and smoking. -Reported incidence data. Weaknesses: -Artesian well water arsenic concentration was unknown for some study subjects.	Chiou et al., 1995 Cohort

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			(mg/liter x yr) : 0 = 1.00 0.1-9.9 = 2.74 (0.69-11.0) 20+ = 4.01 (1.00-16.12)* Unknown 2.01 (0.55-7.36) Bladder- Age: Every l-yr increment = 1.04 (1.05-1.08)* Sex: Men = 1.00, Women = 0.45 (0.18-1.16) Cigarette smoking: No = 1.00, Yes = 1.00 (0.37-2.31) Status of blackfoot disease: No = 1.00, Yes = 4.41 (2.06-9.45)* Cumulative arsenic exposure (mg/liter x yr) : 0 = 1.00 0.1-19.9 = 1.57 (0.44-5.55) 20+ = 3.58 (1.05-12.l9)* Unknown 1.25 (0.38-4.12) *P<0.05		
January 1980-December 1987	2,915 urinary cancer cases	6 categories of arsenic exposure (ppb): <50 50-80 90-160 170-320 330-640 >640	Rate differences (SE)* with positive associations: Males- Bladder cancer: transitional cell >640 ppb = 0.57(0.07), Adenocarinoma >640 ppb = 0.027(0.008) Kidney cancer: transitional cell 330-640 ppb = 0.05(0.02) Females- Urethral cancer, all cell types combined >640 ppb = 0.027(0.007) *estimates for 1 unit increase (1%) in predictor (exposure category)	Strengths: -Adjusted for age, gender, urbanization, and smoking. Weaknesses: - Limitations of ecological study design.	Guo et al., 1997 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1971-1994	11,193 mortalities from all causes of disease Local reference population National reference population	Median artesian wells water arsenic content: 780 ppb (range=250-1140 ppb) Individual exposure data not available.	Males- BFD area compared to local reference – SMR (95% CI): all cancers = 2.19 (2.11-2.28) BFD area compared to national reference – SMR (95% CI): all cancers = 1.94 (1.87-2.01) Females- BFD area compared to local reference – SMR (95% CI): all cancers = 2.40 (2.30-2.51) BFD area compared to national reference – SMR (95% CI): all cancers = 2.05 (1.96-2.14) P<.05	Strengths: -Exposed group and local reference group had similar lifestyle factors. -All cancers were pathologically confirmed. -Controlled for gender, a potential confounder. Weaknesses: –Only one underlying cause of death (not multiple causes) was indicated on death certificate, resulting in possible distortion of association between exposure and disease. -Lack of individual exposure data. -Potential confounders not controlled for were age, smoking, alcohol consumption, and occupational exposures.	Tsai et al., 1999 Cross-sectional
1973-1986	42 arseniasis endemic villages Population of Taiwan	Arsenic exposure categories (ppb) = 0-50 50-100 100-200 200-300 300-400 400-500 500-600 600+	SMRs (male and female combined.) Bladder cancer SMRs*: 0-50 ppb = 10.02 50-100 ppb = 4.15 100-200 ppb = 10.47 200-300 ppb = 7.66 300-400 ppb = 7.44 400-500 ppb = 29.68 500-600 ppb = 14.90 600+ ppb = 32.71 Lung cancer SMRs*: 0-50 ppb = 1.56 50-100 ppb = 1.43 100-200 ppb = 2.43 200-300 ppb = 3.08 300-400 ppb = 1.97 400-500 ppb = 3.65	Strengths: -Person years at risk stratified by age, gender, and arsenic level. -Individual well concentrations were available for each village. Weaknesses: -Ecological study design (no individual monitoring data, individual exposures not available). -Potential confounding by smoking, use of bottled water, and dietary intake since this information was not available.	Morales et al., 2000 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			500-600 ppb = 3.32 600+ ppb = 5.14 Liver cancer SMRs*: 0-50 ppb = 1.18 50-100 ppb = .65 100-200 ppb = 1.74 200-300 ppb = 1.44 300-400 ppb = .77 400-500 ppb = 1.60 500-600 ppb = 1.59 600+ ppb = 2.17 Bladder, lung, and liver combined cancer SMRs*: 0-50 ppb = 1.83 50-100 ppb = 1.16 100-200 ppb = 2.51 200-300 ppb = 2.47 300-400 ppb = 1.63 400-500 ppb = 3.93 500-600 ppb = 3.06 600+ ppb = 4.86 *No significance levels presented.		
October 1991-September 1994 with follow-up through the end of 1996	8,102 residents (4,056 men and 4,046 women) General population of Taiwan used as comparison	Exposure categories (ppb) : ≤10.00 10.1-50.0 50.1-100.0 ≥ 100.0	Standardized incidence ratio (95% CI)=: urinary cancer = 2.05 (1.22, 3.24) bladder = 1.96 (0.94-3.61) kidney = 2.82 (1.29-5.36) P<0.05 Multivariate adjusted RR (95% CI)- Well water arsenic concentration (ppb): Urinary organs- 10.1-50.0= 1.5 (0.3-8.0)	Strengths: - Showed a significant dose response relationship with increasing concentrations of arsenic. -Potential confounders adjusted for included age, gender, and smoking. -Individual exposure estimates were available. Weaknesses: -Possible diagnosis bias since data was collected from various community	Chiou et al., 2001 Cohort

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			50.1-100.0= 2.2 (0.4-13.7) >100.0= 4.8 (1.2-19.4) TCC 10.1-50.0= 1.9 (0.1-32.5) 50.1-100.0= 8.2 (0.7-99.1) >100.0=15.3 (1.7-139.9)	hospitals. -Possible recall bias resulting from self-reported information. - Short duration of follow-up, which limited the number of person-years of observation. -Possible misclassification especially in the low-dose region due to lack of arsenic exposure information in the food.	
January 1980-December 1989	2,369 skin cancer cases (1,415 men and 954 women)	6 categories of arsenic exposure (ppb): <50, 50-80, 90-160, 170-320, 330-640, >640	Statistically significant rate differences per 100,000 person years (SE)* : Males- Basal cell carcinoma >640 ppb = 0.128(0.025)** Squamous cell carcinoma 170-320 ppb = 0.073(0.024)** 330-640 ppb= -0.10(0.031)** >640 ppb = 0.155(0.028)** Females- Squamous cell carcinoma 330-640 ppb = -0.064(0.027)* >640 ppb = 0.212(0.024)** *p <0.05 **p <0.01	Strengths: -Cases were identified from government operated National Cancer Registration Program. -Pathological classifications determined by board certified pathologists. -Potential confounders adjusted for in the analysis included gender and age. Weakness: -Limitations of ecological study design. (No monitoring data were presented).	Guo et al., 2001 Ecological
January 1980-December 1999	40,832 liver cancer patients (32,034 men and 8,798 women)	BFD area average arsenic concentration = 220 ppb Non BFD area average arsenic concentration = 20 ppb.	No statistically significant (P > 0.05) differences were noted for cell types of liver cancer between the BFD area and the other areas.	Strengths: -Cases identified from government operated National Cancer Registration Program. -Pathological classifications were determined by board certified pathologists. -Potential confounders adjusted for included gender and age. Weaknesses:	Guo, 2003 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
				-Limitations of ecological study design. (No monitoring data were presented).	
January 1985 – December 2000; average follow-up of 8 years	2,503 residents in southwestern area 8,088 residents in northeastern area	Southwestern area average arsenic exposure categories (ppb): <10 10-99.9 100-299.9 300-699.9 ≥ 700 Unknown	Multivariate-adjusted RR of lung cancer for average arsenic level in well water (ppb): <10 = 1.00 (Referent) 10-99.9 = 1.09(0.63-1.91) 100-299.9 =2.28 (1.22-4.27) 300-699.9 = 3.03 (1.62-5.69) ≥700 = 3.29 (1.60-6.78) Unknown = 1.10 (0.60-2.03)	Strengths: -Confounders controlled for were age, gender, education, and alcohol consumption. -Long follow-up period and the use of a national computerized cancer case registry. -All lung cancer cases were pathologically confirmed. Weaknesses: -Historical monitoring data not available. -Possible misclassification bias because exposure measurements were based on one survey.	Chen et al., 2004a Cohort
1971-2000	Residents of 4 BFD endemic townships	Median well water arsenic level, early 1960s = 780 ppb	SMR liver cancer: Males, 1989-91 = 1.868 1998-2000 = 1.242 Females, 1983-85 = 2.041 1998-2000 = 1.137	Strengths: -Residents in the study area were similar in terms of socioeconomic status, living environments, lifestyles, dietary patterns, and health service facilities. -Accurate death registration system. Weaknesses: -Limitations of mortality data.	Chiu et al., 2004 Cohort
January 1971-December 1990	1078 lung cancer mortality cases	Arsenic exposure levels (ppb): <050 50-80 90-160 170-320 330-640 >640	Lung cancer mortality increase with 1,000 ppb increase in mean arsenic level (P=0.01)- Men: 27.45/100,000 person years Women: 18.93/100,00 person years	Strengths: -Adjusted for gender and age. -Cases were ascertained using information from household registry offices in each township. Taiwanese law requires timely reporting of deaths to these offices.	Guo, 2004 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
				Weaknesses: -Limitations of ecological studies. -Smoking was not controlled for in the analysis.	
1971-2000	Residents of 4 BFD endemic townships	Median arsenic level (ppb), early 1960s= 780 (range = 350 -1140).	Kidney cancer SMR (observed vs. expected)- 1971 Men = 19.04 (4 vs. 0.21) Women = 23.52 (8 vs. 0.34) 2000 Men = 4.46 (8 vs. 1.79) Women = 6.52 (9 vs. 1.38)	Strengths: -Adjusted for gender and age. -Mandatory registering of all births, deaths, marriages, divorces, and migration to the Household Registration Office in Taiwan making it an accurate data source. -Most residents had similar socioeconomic status, living environments, lifestyles, dietary patterns, and health service facilities and worked in farming, fisheries, or salt production. -All kidney cancer cases in the area probably had similar access to medical care. Weaknesses: -Mortality data limitations. -Cross-sectional study limitations. -Smoking may possibly have been a confounder not adequately controlled for.	Yang et al., 2004 Cross-sectional
1988-2001	7 females 14 males	No exposure data.	Chi square: (Taiwan case series compared to 3 U.S. case series studies)- Males - urethral adenocarcinoma: P<0.0001	Strengths: -Cases were pathologically confirmed. Weaknesses: -Limited number of cases. -No exposure information.	Tsai et al., 2005 Cross-sectional
1971-2000	Residents in 4 BFD endemic area townships	Median arsenic level early 1960s = 780 ppb	Bladder cancer SMRs (observed vs. expected)- 1971: Males = 10.25 (8 vs. 0.78)	Strengths: -All bladder cancer cases in the area probably had similar access to medical care.	Yang et al., 2005 Cross-sectional

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			Females = 14.89 (7 vs. 0.47) 2000: Males = 2.15 (5 vs. 2.32) Females = 7.63 (10 vs. 1.31)	-Adjusted for age and gender. -Mandatory registering of all births, deaths, marriages, divorces, and migration to the Household Registration Office in Taiwan making it an accurate data source. Weaknesses: -Limitations of a cross-sectional mortality study. -Smoking may possibly have been a confounder.	

Table B-2. Japan Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1959-1992	454 residents	Well arsenic concentration (ppb): <50 50-990 ≥ 1000	≥ 1000 ppb SMRs (95% CI): males- all deaths= 1.88 (1.17-2.96) all cancers= 4.19 (2.20-7.56) lung cancer = 19.08 (8.88-38.76) urinary cancer= 33.16 (5.92-121.58) all cancers except lung= 2.22 (0.87-5.22) females- all deaths=1.31 (0.76-2.18 all cancers= 3.00 (1.40-6.13) lung cancer= 7.15 (0.36-41.11) urinary cancer= 27.85 (1.42-159.89) all cancers except lung= 2.73 (1.19-6.04) Cox's proportional hazard analysis (95% CI), highest group vs. background: concentration categories (ppb) ≥ 1 000 vs. 1 all deaths= 1.74 (1.10-2.74) all cancers= 4.82 (2.09-11.14) lung cancer= 1,972.16 (4.34-895,385.11)	**Strengths:** -Cohort examined by 3 exposure categories. -Included information on smoking, age and gender. **Weaknesses:** -Lacking detailed arsenic intake information. -Small study population. -Possible misclassification bias. -Recall bias (smoking history). .	Tsuda et al., 1995 Cohort

Table B-3. South America Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1986-1991	Bladder cancer deaths in 26 Cordoba counties Population of Argentina	Exposure categories: low medium high (crude average estimate of 178 ppb) Two counties in high exposure group.	Bladder cancer SMR (95% CI) by exposure category- Men low: 0.80 (0.66-0.96) medium: 1.42 (1.14-1.74) high: 2.14 (1.78-2.53) test for trend- P=0.001 Women low: 1.21 (0.85-1.64) medium: 1.58 (1.01-2.35) high: 1.82 (1.19-2.64) test for trend- P=0.04	**Strengths:** -Adjusted for age and gender.-Analysis restricted to rural counties to limit confounders. -To account for cancer diagnosis and detection bias, stomach cancer, which is known not to be related to arsenic exposure, was used as a comparison cancer. **Weaknesses:** -Limitations of ecological studies**.** -Lack of comprehensive, systematic monitoring data. -No arsenic exposure levels in low and medium groups reported. -Lack of individual smoking history.	Hopenhayn-Rich, et al. 1996a Ecological
1986-1991	Population from 26 counties in Cordoba Population of Argentina	Exposure categories: low medium high (crude average estimate of 178 ppb)	SMRs (95% CI) by exposure categories: Kidney cancer- Men low= 0.87 (0.66-1.10) medium= 1.33 (1.02-1.68) high= 1.57 (1.17-2.05) Women low= 1.00 (0.71-1.37) medium= 1.36 (0.94-1.89) high= 1.81 (1.19-2.64) Lung cancer Men low= 0.92 (0.85-0.98) medium= 1.54 (1.44-1.64) high= 1.77 (1.63-1.90) Women low= 1.24 (1.06-1.42)	**Strengths:** -Adjusted for age and gender. -Analysis restricted to rural counties to limit confounders. -To account for cancer diagnosis and detection bias, stomach cancer, that is known not to be related to arsenic exposure, as a comparison cancer. **Weaknesses:** -Limitations of ecological studies**.** -Lack of comprehensive, systematic monitoring data. -No arsenic exposure levels in low and medium groups reported. -Lack of individual smoking history.	Hopenhayn-Rich et al. 1998 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			medium= 1.34 (1.12-1.58) high= 2.16 (1.83-2.52) P < 0.001 in trend test		
1989-1993	390,340 residents national mortality data from 1991 Population of Chile used as reference group	Region II average water arsenic level (ppb): 1950-54 = 123 1955-59 = 569 1960-64 = 568 1965-69 = 568 1970-74 = 272 1975-79 = 176 1980-84 = 94 1985-89 = 71 1990-94 = 43	SMRs (95% CI, p value) ≥ 30 years old: men- bladder - 6.0 (4.8-7.4, <0.001) kidney - 1.6 (1.1-2.1, 0.012) liver - 1.1 (0.8-1.5, 0.392) lung - 3.8 (3.5-4.1, <0.001) skin - 7.7 (4.7-11.9, <0.001) women- bladder - 8.2 (6.3-10.5, <0.001) kidney - 2.7 (1.9-3.8, <0.001) liver - 1.1 (0.8-1.5, 0.377) lung - 3.1 (2.7-3.7, <0.001) skin - 3.2 (1.3-6.6, 0.016)	**Strengths:** -Large study size. -Used national data for comparison. No other major populations in Chile were exposed to arsenic in drinking water. -SMRs adjusted for age and gender. **Weaknesses**: -Arsenic levels in drinking water available only by city or town. -Deaths were not linked to town so individual exposure is not known. -Limited smoking data. -No dose response information provided.	Smith et al., 1998 Ecological
1994-1996	152 lung cancer cases 419 controls	Average water arsenic concentration (ppb) during peak exposure years: 0-10 10-29 30-59 60-89 90-199 200-399 400-699 700-999	Lung cancer odds ratio (95% CI): Age/gender adjusted: 0-10 ppb = 1 (referent) 10-29 ppb = 0.4 (0.1-0.5) 30-59 ppb = 0.0 (0.6-7.2) 60-89 ppb = 0.1 (1.8-9.2) 90-199 ppb = 0.8 (1.1-7.0) 200-399 ppb = 0.4 (2.0-10.0) 400-699 ppb = 0.9 (2.4-19.8) 700-999 ppb = 0.3 (3.1-12.8) Male vs. female = 0.7 (1.1-2.7) Full model (95% CI) (included smoking and copper smelting): 0-10 ppb = 1 (referent) 10-29 ppb = 0.3 (0.1-1.2) 30-59 ppb = 1.8 (0.5-6.9)	**Strengths:** -Odds ratios adjusted for age, gender, cumulative lifetime cigarette smoking, working in copper smelting, and socioeconomic status. -Because the control group selection was complex, several validity checks were completed. **Weaknesses:** -Relatively more controls were chosen from the highly exposed city of Antofagasta than from the lower exposure cities of Arica and Iquique resulting in possible underestimation of risk.	Ferreccio et al., 2000 Case-control

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			60-89 ppb = 4.1 (1.8-9.6) 90-199 ppb = 2.7 (1.0-7.1) 200-399 ppb = 4.7 (2.0-11.0) 400-699 ppb = 5.7 (1.9-16.9) 700-999 ppb = 7.1 (3.4-14.8) Male vs. female=1.1 (0.6-1.8) Ever vs. never smoked = 4.3 (2.6-7.3) SES medium vs. low = 1.3 (0.7-2.5) SES high vs. low = 2.3 (0.5-12.1) Copper smelting (ever/never) = 1.7 (0.7-4.4)		
1996-2000	114 bladder cancer cases 114 individuals without bladder cancer	Average arsenic concentration (ppb) of 5 years of highest exposure during the period of 6-40 years prior to interview : 0-50 51-100 101-200 >200 (Mean – 164 ppb)	Bladder cancer Odds ratio (95% CI)–ever smokers by time before interview: 51-60 years earlier = 2.65 (1.2-5.8) 61-70 years earlier = 2.54 (1.0-6.4) periods combined = 2.5 (1.1 - 5.5)	**Strength:** -Potential confounders controlled included age, gender, smoking, and county of residence. **Weaknesses:** -Lack of a cancer registry, arsenic exposure misclassification (use of current water source arsenic measurements possibly causing underestimation of exposure), and recall bias. -Possible selection bias since controls had a significantly reduced rate of participation than cases and cases were selected from the tumor registry. -Other harmful exposures not measured.	Bates et al., 2004 Case-control
1989-2000	~200,000 residents	Water arsenic levels: Prior to 1958, ~90 ppb. In the late 1950s, water supplementation from a nearby river where arsenic levels approached 1000 ppb was added to the existing city	SMRs (95% CI): 1950-1957 birth cohort (early childhood exposure): lung cancer = 7.0 (5.4-8.9, p<0.001) High exposure period (1958-	**Strengths**: -Extensive documentation of arsenic in drinking water in the Antofagasta water system. **Weaknesses**: -Residence was determined from death	Smith et al., 2006 Cohort

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
		water supply.	1971) with probable exposure in utero and early childhood: lung cancer = 6.1 (3.5-9.9, p<0.001)	certificates and relates to residence at the time at death. -Reliance on death certificates resulting in potential diagnostic bias. -Information bias (smoking history).	
1950-2000	Region II residents Region V residents as comparison group Population of Chile	Average arsenic concentration (ppb): Region II 1950-54 = 123 1955-59 = 569 1960-64 = 568 1965-69 = 568 1970-74 = 272 1975-79 = 176 1980-84 = 94 1985-89 = 71 1990-94 = 43 Region V Unexposed	Peak Rate Ratios (95% CI) compared to Region V and Chile: <u>Lung Cancer</u> 1992-94 Men 3.61 (3.13-4.16) (Region V) 4.20 (3.76-4.70) (Chile) 1989-91 Women 3.26 (2.50-4.23) (Region V) 3.41 (2.76-4.22) (Chile) <u>Bladder Cancer</u> 1986-88 Men 6.10 (3.97-9.39)(Region V) 5.99 (4.41-8.14)(Chile) 1992-1994 Women 13.8 (7.74-24.5)(Region V) 9.32 (6.67-13.0)(Chile)	**Strengths:** -Large population size. -Accurate past exposure data. -Known exposure pattern. -Controlled for potential confounding by age, gender, and smoking. **Weaknesses:** -Could not account for migration. -No individual exposure data or data on other risk factors (smoking and occupation).	Marshall et al., 2007 Ecological
1950-2000	314,807 exposed 1,230,498 unexposed	Average water concentration (ppb) in Region II: Before arsenic removal plant- 1950-1957 = 90 1958-1970 = 870 After arsenic removal plant- 1971-1985 = 110 1986-2000 = 40 Present = 10	Excess deaths as percentage of total deaths (%) due to acute myocardial infarction, lung cancer, and bladder cancer combined Males 1950-1957 = 1.00 1958-1964 = 4.19 1965-1970 = 6.03 1971-1979* = 6.48	**Strengths:** -Almost all drinking water came from a few municipal water sources which had known arsenic concentrations. -The study involved a large population that experienced a rapid increase in arsenic exposure followed by a rapid decrease in arsenic exposure. -To ensure that an appropriate comparison population was chosen,	Yuan et al., 2007 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			1980-1985 = 8.94 1986-1990 = 10.07 1991-1995 = 10.87 1996-2000 = 7.92 Total = 6.93 Females 1950-1957 = 0.48 1958-1964 = 1.59 1965-1970 = 3.11 1971-1979* = 3.78 1980-1985 = 2.75 1986-1990 = 3.85 1991-1995 = 4.00 1996-2000 = 3.36 Total = 2.94 *No data available for 1976	preliminary investigations were conducted to compare income, smoking, and quality of death certificate information. **Weaknesses:** -Possible biases resulting from a lack of individual exposure data and confounders.	

Table B-4. North America Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
39 years (endpoint-1978 diagnosis)	71 National Bladder Cancer Study participants 160 National Bladder Cancer Study participants without bladder cancer	Mean arsenic level (ppb) = 5.0 (range = 0.5-160) Exposure indices: Index 1-cumulative dose (<19, 19 to <33, 33 to <53, ≥ 53 mg) Index 2-Intake concentration adjusted to fluid intake (<33, 33 to <53, 53 to<74, ≥74 mg-years).	Odds ratio for bladder cancer and arsenic exposure: no association of bladder cancer with Index 1 or Index 2. Among smokers, positive trend in 10 year intervals.	**Strengths:** -Age, gender, smoking status, years of chlorinated surface water exposure, history of bladder infection, education, occupation, population size of geographic area, and urbanization were addressed. -Cases were histologically confirmed. **Weaknesses:** -Small size of study population. -Absence of historical monitoring data and arsenic levels in public water supplies were collected in 1978-1979. -The subjects were mostly males and the data on females were inadequate. -Arsenic exposure levels were based on measurements close to the time that cases were diagnosed. -Arsenic from food was not considered.	Bates et al., 1995 Case-control
1996	2,203 deceased individuals from Millard County General Utah population used as comparison	Arsenic exposure index (ppb-years)- low: <1000 medium: 1000-4999 high: ≥5000	Cancer SMRs (95% CI): kidney- males = 1.75 (0.80-3.32) females = 1.60 (0.44-4.11) bladder and other urinary organs- males = 0.42 (0.08-1.22) females = 0.81 (0.10-2.93) melanoma of the skin- females = 1.82 (0.50-4.66) prostate = 1.45* (1.07-1.91) *p ≤ 0.05	**Strengths:** -Major strength of the study is that it measured the effects of chronic arsenic exposure in U. S. population. -Advantages of cohort design including the fact that the exposure precedes the effect being measured and that the cohort design has the ability to measure a variety of effects from a single type of exposure. **Weaknesses:** -Exposure assessment - study power -Exposure to atmospheric arsenic and	Lewis et al., 1999 Cohort

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
				arsenic from food were potential confounder.	
1993 - 1996	587 BCC cases 284 SCC cases 524 controls	Toenail arsenic level (μg/g), BCC cases= 0.01-2.03; SCC cases= 0.01-2.57; controls= 0.01-0.81	OR (95% CI), toenail arsenic concentrations above the 97th percentile: SCC = 2.07 (0.92- 4.66) BCC = 1.44 (0.74- 2.81)	**Strengths:** -Evaluated the effects of age, gender, race, educational attainment, smoking status, skin reaction to first exposure to the sun, history of radiotherapy (potential confounders). -Toenail concentrations individualize exposure and account for arsenic from other sources. **Weaknesses:** -Latency of arsenic induced skin cancer unknown, follow-up period may have been inadequate. -Toenail arsenic measurements only account for recent past exposure.	Karagas et al., 2001 Case-Control
1979-1999	not applicable	Arsenic exposure categories-(ppb): low <10 medium= 10-25 high= 35-90	SIR (95% CI), childhood leukemia and all childhood cancers excluding leukemia: Low exposure group: leukemia= 1.02 (0.90-1.15) all cancers= 0.99 (0.92-1.07) Medium exposure group: leukemia = 0.61 (0.12-1.79) all cancers = 0.82 (0.47-1.33) High exposure group: leukemia =0.86 (0.37-1.70) all cancers = 1.37 (0.96-1.91)	**Strengths:** -The analysis was stratified by age. -Low arsenic exposure study. -Findings were reported for different concentration ranges. **Weaknesses:** -Small study size. -Limitations of ecological study design. -Arsenic from food was not measured leading to possible exposure misclassification.	Moore et al., 2002 Ecological
1994-2000	181 cases 328 controls	Exposure categories (ppb): 0-19 20-79 80-120 >120 Arsenic exposure indices: 1) highest average daily	Bladder cancer OR (95% CI): >80 μg/day= 0.94 (0.56-1.57) linear trend, p = 0.48 >80 μg/day, ≥ 40 years ago - smokers= 3.67 (1.43-9.42) linear trend, p < 0.01	**Strengths:** -Potential confounders adjusted included gender, age, smoking history, education, occupation associated with elevated rates of bladder cancer, and income. -Use of cancer registry. -Individual exposure levels.	Steinmaus et al., 2003 Case-control

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
		arsenic intake for any one year, 2) highest average daily arsenic intake averaged over any contiguous 5 years, 3) highest average daily arsenic intake averaged over any contiguous 20 years, and 4) total lifetime cumulative exposure.		**Weaknesses:** -Information bias (next- of -kin interviews). -Arsenic exposures outside the study area were not incorporated. -In the arsenic exposed areas, the percentage of nonparticipants was five percent higher among cases than controls. This difference would probably mean that more exposed cases were missed in analyses of recent exposure, biasing the odds ratio toward the null. -Arsenic exposure from food was not considered.	
1999-2000	368 cutaneous melanoma cases 373 colorectal cancer controls	Median toenail arsenic concentration: cases = 0.06 µg/g, controls = 0.04 µg/g.	OR=2.1 (95% C.I. = 1.4-3.3, p-trend = 0.001) for increased risk of melanoma with elevated toenail arsenic concentrations OR=6.6 (C.I. = 2.0-21.9) for increased risk of melanoma with previous diagnosis of skin cancer and elevated toenail arsenic concentrations	**Strengths:** -Potential confounders controlled for were age, gender, skin color/skin type, prior history of sunburn, education, and occupational exposure(s). -Ascertainment of cases and controls was accomplished by using the Iowa Cancer Registry, a Surveillance, Epidemiology, and End Results Program registry. This allowed newly diagnosed melanoma cases to be identified for a specific period and assured a greater degree of certainty regarding the accuracy of diagnosis. -Toenail arsenic measurements individualize exposure and account for arsenic exposure from other sources. **Weaknesses:** -A limitation was that toenail samples were collected 2-3 years after diagnosis, resulting in possible exposure	Beane-Freeman et al., 2004 Case-control

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
				misclassification.	
July 1, 1994 and June 30, 1998	383 transitional cell bladder cancer cases 641 controls	Toenail arsenic level (μg/g): cases- 0.014-2.484 controls- 0.009 -1.077	Odds ratio (95% CI)- bladder cancer among smokers: > 0.330 μg/g = 2.17 (0.92–5.11)	**Strengths:** -Evaluated the following potential confounders: age, gender, race, educational attainment, smoking status, family history of bladder cancer, study period and average number of glasses of tap water consumed per day. -Conducted stratified analyses according to how long subjects used their current water system (<15 years, ≥15 years) to evaluate the possibility that an extended latency period is required for bladder cancer development. -Attempted to minimize misclassification by using biomarker (toenails). **Weaknesses:** -Possible misclassification at lower end of exposure range. -Limited data at extreme ends of exposure. -Lifetime exposure could not be calculated since data from previous residences could not be determined.	Karagas et al., 2004 Case-Control
1950-1979	2,498,185 white males 1970 U.S. standard population	Median water arsenic concentration (ppb): 3.0-3.9 4.0-4.9 5.0-7.4 7.5-9.9 10.0-19.9 20.0-49.9 50.0-59.9	Bladder cancer SMRs (95% CI), white males by median arsenic concentration in groundwater (ppb): 3.0-3.9 = 0.95 (0.89-1.01) 4.0-4.9 = 0.95 (0.88-1.02) 5.0-7.4 = 0.97 (0.85-1.12) 7.5-9.9 = 0.89 (0.75-1.06) 10.0-19.9 = 0.90 (0.78-1.04) 20.0-49.9 = 0.80 (0.54-1.17) 50.0-59.9 = 0.73 (0.41-1.27)	**Strengths:** -Large study population. -Study was nationwide. -Included over 75 million person-years of observation. **Weaknesses:** -No individual exposure data. -Assumed that study participants consumed local drinking water. -Available data assumed to represent	Lamm et al., 2004 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			All levels combined = 0.94 (0.90-0.98)	actual arsenic content of water. -Analysis did not directly adjust for smoking, urbanization, and industrialization. -Arsenic contribution from food was not measured.	
July 2000-January 2002	6,669 residents	Three arsenic exposure categories (ppb): <1.0 1.0-9.0 ≥ 10	Skin cancer adjusted odds ratio (95% CI): Arsenic level (ppb)- <1.0 = referent 1-9.9 = 1.81 (1.10-3.41) ≥ 10 = 1.92 (1.10-3.68) Age (years)- 35-64 = referent ≥ 65 = 4.53 (2.79-7.38) Gender- female = referent males = 2.25 (1.33-3.79) Cigarette use- no = referent yes = 1.37 (0.84-2.24)	**Strengths:** -Large sample size. -History of individual tobacco use. -Arsenic well water analysis for each household. -Participants consumed water from the tested wells for at least 10 years. -Analysis controlled for age, gender, and tobacco use. **Weaknesses:** -Skin cancers were self-reported and not confirmed by a medical records review. -Few people could provide information about specific types of cancer. -Families that participated may have been especially concerned about arsenic exposure or family members may have had existing health conditions. -Not controlled for sun exposure or occupation. -Arsenic contribution from food was not measured.	Knobeloch et al., 2006 Cross-sectional
1979-1997	Residents of six Michigan counties Remainder of Michigan population as comparison	Population-weighted mean arsenic concentration (ppb) exposed counties = 11.00 remainder of Michigan= 2.98	Elevated cancer SMRs (95% CI) males- liver/biliary = 0.85 (0.72-1.00) trachea, bronchus, lung = 1.02 (0.98-1.06) melanoma = 0.99 (0.79-1.22) other skin cancer = 1.24 (0.86-1.72)	**Strengths:** -Mortality data gathered from Michigan Resident Death Files for 20 year period. -Mortality rates stratified by gender, age and race. **Weaknesses:** -Possible differences in reporting and	Meliker et al., 2007 Ecological

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
			bladder = 0.94 (0.82-1.08) kidney/urinary =1.06 (0.91-1.22) females- liver/biliary = 1.04 (0.89-1.20) trachea, bronchus, lung = 1.02 (0.96-1.07) melanoma = 0.97 (0.73-1.27) other skin cancer = 1.06 (0.60-1.72) female reproductive organs = 1.11* (1.03-1.19) bladder = 0.98 (0.80-1.19) kidney/urinary organs = 1.00 (0.82-1.20) *p<0.01	classification of underlying causes of death. -No assessment of individual exposures and case migration. -Smoking and obesity, possible confounders, were not included in the analysis. -Preferential sampling based on home owners' request. -Arsenic contribution from food was not measured.	

Table B-5. China Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1990	3179 residents	HAC (ppb): <10 10- 30- 50- 60- 100- 150- 500+ CAE (ppb-year): <10 10- 32- 100- 316- 1000- 3162- 10000+	Crude and (age adjusted) skin cancer prevalence rates by HAC: <10 = 0.0 (0.0) 10- = 0.0 (0.0) 50- = 0.0 (0.0) 150- = 1.2 (1.0) 500+ = 7.1 (5.9) Crude and (age-adjusted) skin cancer rates by CAE: <10 = 0.0 (0.0) 10- = 0.0 (0.0) 32- = 0.0 (0.0) 100- = 0.0 (0.0) 316- = 0.0 (0.0) 1000- = 0.4 (0.3) 3162- = 0.8 (0.2) 10000+ = 2.7 (2.0)	**Strengths:** -Large study population. -Used both HAC and CAE in the analyses. -Arsenic concentrations measured in 184 wells. -Controlled for age and differences in cumulative arsenic exposure dose and duration of exposure. **Weaknesses:** -Possible recall and misclassification bias resulting from the collection of exposure histories through interviews. -Inherent limitations of ecologic study design. -Did not control for sun exposure.	Lamm et al., 2007 Ecological

Table B-6. Finland Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1981-1995	61 bladder cancer cases and 49 kidney cancer cases 275 referents	Water arsenic concentration (ppb)- <0.1 0.1-0.5 ≥ 0.5 Arsenic daily dose (µg/day) <0.2 0.2-1.0 ≥1.0 Cumulative dose (µg) <500 500-2000 ≥ 2000	Bladder cancer risk ratios (95% CI): Shorter latency- Water arsenic concentration (ppb): 0.1-0.5 = 1.53 (0.75-3.09) ≥ 0.5 = 2.44 (1.11-5.37) Daily arsenic dose (µg/day): 0.2-1.0 = 1.34 (0.66-2.69) ≥ 1.0 = 1.84 (0.84-4.03) Cumulative dose (µg): 500-2000 = 1.61 (0.74-3.54) ≥ 2000 = 1.50 (0.71-3.15) Longer latency- Water arsenic concentration (ppb): 0.1-0.5 = 0.81 (0.41-1.63) ≥ 0.5 = 1.51 (0.67-3.38) Daily arsenic dose (µg/day): 0.2-1.0 = 0.76 (0.38-1.52) ≥ 1.0 = 1.07 (0.48-2.38) Cumulative dose (µg): 500-2000 = 0.81 (0.39-1.69) ≥ 2000 = 0.53 (0.25-1.10)	**Strengths:** -Cases were identified through the Finnish Cancer Registry. -The 1985 Population Census file of the Statistics Finland was used to identify areas in which less than 10% of the population used the municipal water supply. -Risk ratios adjusted for age, gender and smoking. **Weaknesses:** -Possible misclassification and possible recall bias resulting from the study choosing to use water consumption from the 1970. -Lacks other sources of arsenic exposure.	Kurttio et al., 1999 Case cohort
1985-1988 and April 1999	280 incident bladder cancer cases 293 controls	Arsenic exposure quartiles (µg/g): I - <0.050 2 - 0.050 - 0.105 3 - 0.106 - 0.161 4 - >0.161	Bladder cancer odds ratio (95% CI)- highest vs. lowest quartile of toenail arsenic =1.13, (0.70, 1.81) *p* trend = 0.65 for the highest vs. lowest quartile).	**Strengths:** -Study used toe nail arsenic as biomarkers of exposure. -Cases and controls matched according to age, toenail collection date, intervention group (Alpha tocopherol and beta carotene), and smoking duration. -Study adjusted for matching factors, smoking, educational level, beverage	Michaud et al., 2004 Cohort/ Nested case- control

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
				intake, and place of residence. -Cut point of > 0.09 µg/g used to avoid sample misclassification. -Potential confounders including smoking cessation, smoking inhalation, educational level, beverage intake, and place of residence, were controlled for in the study analysis. **Weaknesses:** -Water intake was not included in the total beverage variable. -Toenail arsenic measures recent past exposures.	

Table B-7. Denmark Cancer Studies

Study Period	Subjects/ Controls	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1970-2003	39,378 Copenhagen residents 17,000 Aarhus residents	TWA arsenic exposure (ppb) from 41 years old to date of enrollment:: Copenhagen: min = 0.05 max = 15.8 Aarhus: min = 0.09 max = 25.3 Entire cohort: min = 0.05 max = 25.3	Cancer Incidence rate ratios (95% CI)- Time weighted average exposure: Copenhagen- melanoma = 0.73 (0.46-1.14) non-melanoma = 1.09 (0.95-1.24) breast = 1.04 (0.88-1.22) Aarhus- melanoma =0.85 (0.61-1.20) non-melanoma = 0.97 (0.90-1.05) breast = 1.06 (1.01-1.11) Cumulative exposure: Copenhagen- melanoma = 0.94 (0.81-1.08) non-melanoma = 1.01 (0.97-1.06) breast =1.01 (0.95-1.06) Aarhus- melanoma = 0.97 (0.90-1.05) non-melanoma = 0.98 (0.95-1.01) breast = 1.01 (0.99-1.03)	**Strengths:** -Large study population. -Socioeconomic/demographic similarities of the cohorts. -Potential confounders adjusted were smoking, alcohol consumption, education, body mass index, daily intake of fruits/vegetables, red meat, fat and dietary fiber, skin reaction to the sun, hormone replacement therapy use, reproduction, occupation, and enrollment area. **Weaknesses:** -Possible misclassification bias. -Overall low arsenic concentration in drinking water in Denmark. -Lack of data regarding other sources of arsenic.	Baastrup et al., 2008 Cohort

Table B-8. Australia Cancer Studies

Study Period	Subjects/Control	Exposure Assessment	Study Outcome	Strengths/ Weaknesses	Reference/ Type of Study
1982-1991	Victoria Cancer Registry cancer data Australian Bureau of Statistics denominator data	Water/ soil exposure groups: High water/High Soil - >10 ppb/ >100 mg/kg High Water /Low soil >10 ppb/ <100 mg/kg High Soil /Low water <10 ppb/ >100 mg/kg	Cancer SIRs (95% CI), males and females: all cancers- 1.06 (1.03-1.09) prostate- 1.14 (1.05-1.23) kidney- 1.16 (0.98-1.37) melanoma- 1.36 (1.24-1.48) chronic myeloid leukemia- 1.54 (1.13-2.10) females, breast- 1.10 (1.03-1.18)	**Strengths:** -Study included both water and soil in exposure categories. -Twenty-two areas included in the study. **Weaknesses:** -Socioeconomic status, race, occupation and living in a rural area were possible confounders. -Possible exposure misclassification. -Ecological study limitations.	Hinwood et al., 1999 Ecological

Appendix C. Tables for Studies on Possible Mode of Action for Inorganic Arsenic

This appendix contains three tables that deal with possible modes of action (MOA) of arsenic in the development of cancer based on (1) *in vivo* human studies (Table C-1), (2) *in vivo* experiments on laboratory animals (Table C-2), and (3) *in vitro* studies (Table C-3). They describe numerous experiments published from 2005 through August of 2007, as well as earlier experiments that were mentioned in the (a) Science Advisory Board Arsenic Review Panel comments of July of 2007 (SAB, 2007), (b) 2001 NRC document on arsenic (NRC, 2001), or (c) a detailed early draft of this document that lacked MOA tables. The data from these studies are distributed among 22 key-event categories, with the data from different experiments from a single publication often being summarized under different key-event categories. For example, the results in Wang et al. (1996) are summarized by rows under Apoptosis, Cytotoxicity, and Effects Related to Oxidative Stress (ROS). The advantage of distributing the data in this way is that it helped to focus on a particular key event for each set of data. The disadvantage of using this approach is that it spatially separated the different parts of each experiment. An exception to this procedure is the category Immune System Response, in which results from different parts of each experiment are presented in successive rows.

A brief discussion of the approaches and conventions used in preparing the tables is included here. Abbreviations are used liberally in an attempt to reduce the size of the table. An attempt was made to provide a summary of the main findings of each experiment, with the expectation that any reader wanting more detail would read the publication. A search for any specific citation should make it easy to pull together the information from the numerous parts of some studies that related to different categories. Although, for example, cytotoxicity data are generally summarized in the Cytotoxicity category, exceptions sometimes were made in an attempt to decrease the size of the table. For example, if data presented on apoptosis contained only slight, but interesting, data on cytotoxicity, a brief summary of those cytotoxicity findings was sometimes added at the end of the results column in the row that described the results on apoptosis. When an experiment that tested only one concentration yielded interesting results, the results column is sometimes merged with one or more columns to its left in that same row so that the expansion of the vertical height of the table was not be impacted so much by the description of the results. In such a case, the only dose tested was obviously the LOEC or LOEL.

In vivo experiments on laboratory animals were almost always restricted to experiments in which the route of exposure was oral. In most cases this meant that the arsenical was administered in drinking water or was given by gavage. A few experiments had the arsenical in the feed. Two experiments on chicken embryos had a solution (with concentration in μM) put onto the embryo, and one genetic assay done on *Drosophila melanogaster* had the concentration (given in mM) reported for the media. All other *in vivo* experiments were done on mice or rats. Numerous studies were excluded on other non-mammalian species, including, for example, fish, nematodes, and algae.

Tables C-2 and C-3 list all doses or concentrations tested as well as the duration of testing. It was often necessary to estimate the concentrations or doses tested from figures. For brevity, the control dose of 0 is not listed as a concentration tested. In the rare instances in which there was no zero-dose control group, this omission is mentioned in the results section. In

many cases the papers themselves did not specify the LOECs or LOELs, and those values were estimated from tables or figures. Because of the large variation in the way that papers presented data and variability in their findings, and because of the rather common failure to clearly define the error bars around data points in figures, there was often subjectivity involved in selecting the LOEC or LOEL. There was no strict requirement that the LOEC or LOEL declared for each experiment had to be shown to be statistically significantly higher than the control, although it was not uncommon for that to be the case. The wording in the results column often helps to clarify this situation. If, for example, there were 6 concentrations tested, and if the second from the lowest concentration had error bars that did not overlap those of the control, and if the third from the lowest concentration was identified as being statistically significantly higher then the control, then the second from the lowest concentration tested would have been declared the LOEC. The LOEC, for example, should be viewed as the lowest concentration that was "quite likely" to have caused an effect—without any specific statistical interpretation being attached to it. As long as this is made clear, it was felt that this approach would be most useful to readers who want to know the lowest concentration level at which a particular effect would probably occur.

Arrows are used to indicate changes that were increases or decreases from the control. If the change was relative to some other group, it was clearly indicated as such. In most cases, the changes in magnitude of effects relative to the control were described as, for example, 2.34x or 0.46x, which mean that they are 2.34 times higher than the control or only 46% as high as the control. When those ratios were based on estimates made from a graph, they are generally preceded by a "~" mark; however, if they were calculated from tabulated values, they are generally presented without that mark.

In Table C-2 the doses are presented in terms of the amount of arsenic. When doses were reported in mg arsenic/L or in ppm As, it was assumed that the doses included adjustment to determine the amount of As administered. In a few publications it was unclear if the reported doses were for the compound or for the amount of arsenic administered. Partly because of this uncertainty, all doses shown in the table that were corrected to the amount of arsenic from values that were clearly reported as concentrations of some arsenical compound (or for which that was assumed to be the case) are preceded by an asterisk. Species of arsenic are shown in Tables C-2 and C-3, and As^V is almost always sodium arsenate.

Abbreviations for Tables in Appendix C

⇑	increase
⇓	decrease
~	approximately (if before a listing of concentrations, it applies to all)
≈	approximately equal
AA	ascorbic acid (Vitamin C)
AB assay	AlamarBlue assay
ABTS	2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid
AC	Arsenic chloride
ADM	Adriamycin
ADSB	apparent DNA strand break
AFP	α-fetoprotein
AG06 cells	SV40-transformed human keratinocytes
AGT	Average generation time
Ahr$^{+/+}$ MEFs	mouse embryo fibroblasts of genotype Ahr$^{+/+}$ from C57BL/6J mice, which are cells known to respond to a B(α)P or TCCD challenge by activation of the AhR
Akt1	V-akt murine thymoma viral oncogene homolog 1 (a human gene)
ALAD	δ-aminolevulinic acid dehydratase
ALAS	δ-aminolevulinic acid synthetase
A_L hybrid cells	cell line that contains structural set of CHO-K1 chromosomes and one copy of human chromosome 11
AMs	Alveolar macrophages
AML	Acute myelogenous leukemia
AMPK	adenosine monophosphate-activated protein kinase
AO	Acridine orange
APE/Ref-1	apurinic/apyrimidinic endonuclease (*hAPE1*)
AP sites	sites of base loss [apurinic/apyrimidinic (AP) sites]
AP-PCR	arbitrarily-primed polymerase chain reaction
Aprt	adenosine phosphoribosyl transferase
ARE	Antioxidant response element
AR230 cells	a CML cell line that expresses large amounts of Bcr-Abl
AR230-r cells	AR230 cells that are resistant to the Bcr-Abl inhibitor imatinib mesylate
AR230-s cells	AR230 cells that are sensitive to the Bcr-Abl inhibitor imatinib mesylate
AS52 cells	pSV2 gpt-transformed Chinese hamster ovary cell line. They carry a single copy of a transfected *E. coli gpt* gene.
As	Arsenic
As^{III}	Arsenite
As^{V}	Arsenate
ASK1	Apoptosis signal-regulating kinase 1
ATO	Arsenic trioxide

A2780 cells	human ovarian carcinoma cell line
A431 cells	human epidermoid carcinoma cell line
A5/SG assays	A5 (Annexin V-Alexa568) and SG (a green fluorescent DNA dye) staining assays; A5+/SG- cells are apoptotic
A549 cells	human non-small cell lung cancer (NSCLC) cell line (alveolar basal epithelial cell line)
B[*a*]P	benzo[*a*]pyrene
BAEC	Bovine aortic endothelial cells
BALF	Bronchoalveolar lavage fluid
BEAS-2B cells	human bronchial (pulmonary) epithelial cell line
BCS	Bathocuproinedisulphonic acid
BER	base excision repair
BFTC905 cells	a human urothelial carcinoma cell line
BFU	burst-forming units
BHMT	betaine-homocysteine methyltransferase
BHT	Butylated hydroxytoluene
Bid	a BH3 domain-containing proapoptotic Bcl2 family member that is a specific proximal substrate of Casp8 in the Fas apoptotic signaling pathway
BPDE	Benzo[a]pyrene diol epoxide
BrdU	bromodeoxyuridine
BSO	L-buthionine-S,R- sulphoximine (depletes GSH, γ-GCS inhibitor)
BUC	bladder urothelial cells
B0653	2,3-dihydro-5-hydroxy-2,2-dipentyl-4,6-di-tert-butylbenzofuran
B16-F10 cells	mouse melanoma cells
CAs	chromosome aberrations
CAM	cell adhesion molecule
CAM assay	chorioallantoic membrane assay of angiogenesis
CAT	catalase (decomposes H_2O_2)
Cdc	Cell division cycle
Cdc42	a small GTPase in the Rho/Rac subfamily of Ras-like GTPases
cen+	centromere positive (micronuclei)
cen-	centromere negative (micronuclei)
CFE	Colony-forming efficiency
CFSE	5,6-carboxyfluorescein diacetate succinimidyl ester
CFU	colony-forming units
CGL-2 cells	a cell line derived from a hybrid (ESH5) of the HeLa variant, D98/AH2, and a normal human fibroblast strain, GM77
cGpx	cellular glutathione peroxidase
Chang cells	human cell line thought to be derived from HeLa cells
ChAT	choline acetyltransferase
CHO	Chinese hamster ovary
CI	Confidence interval
CK8	cytokeratin 8

CL3 cells	human lung adenocarcinoma cells (established from a non-small-cell lung carcinoma)
CL3R15 cells	cell line derived from CL3 cells that were maintained in 4 µM As SA
CM-H_2DCFDA	5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate
CML	Chronic myeloid leukemia
Conc	concentration
Contraspin	a serine—or cysteine—proteinase inhibitor isoform
COS-7 cells	African green monkey kidney fibroblast cell line containing 10,000 glucocorticoid receptors per cell that are transcriptionally inactive
CoTr	co-treatment
COX	cytochrome c oxidase; its activity is a measure of mitochondrial function
COX-2	cyclooxygenase-2
CPDs	cyclobutane pyrimidine dimers (UV-induced DNA photoproduct)
Cpp32	caspase-3
CREBP	cAMP response element binding protein
CRL1675 cells	human melanocytes cell line
CRL-1609 cells	chimpanzee transformed skin fibroblast cells
cRNA	RNA derived from complimentary DNA through standard RNA synthesis
CSTP	Clonal survival treat and plate
Cul3	Cullin 3, an Nrf2-finding protein
CV assay	crystal violet assay; it measures cellular protein, which is related to cell number
CYP1A1	cytochrome P450 1A1
Cyp7b1	Cytochrome P450 family 7, subfamily b polypeptide 1
c-Fos	an AP-1 protein
c-Jun or c-jun	an AP-1 protein
c-met	the oncogene that encodes HGF (hepatocyte growth factor) receptor
c-Mos	Proto-oncogene
C-33A cells	a transformed human non-differentiated carcinoma cell line
DA	disodium arsenate
DAP	2,6-diaminopurine
DCF assay	dichlorofluorescein assay
DCFH-DA	2′,7′-dichlorofluorescein diacetate
DCHA	docosahexaenoic acid, a ω-3 polyunsaturated fatty acid vital for the developing nervous system
DEB	diepoxybutane (DNA crosslinking agent)
DENA	Diethylnitrosamine
DES	Diethylstilbestrol
Dex	dexamethasone (synthetic glucocorticoid)
DHA	dehydroascorbic acid
dhfr gene	dihydrofolate reductase gene
DHR123	dihydrorhodamine 123

DIC	dicumarol, and Nqo1 inhibitor
DI-I or II or III	iodothyronine deiodinase-I or II or III (are 3 forms of this selenoenzyme)
DKO	double knock out
dL	Deciliter
DMPO	5,5′-dimethyl-1-pyrroline *N*-oxide (a spin-trap agent)
DMA^{III}	dimethylarsenous acid
DMA^{V}	dimethylarsinic acid
DMA	dimethyl arsenic (used when the oxidative state is unknown or not specified)
$DMA^{III}I$	dimethylarsinous iodide
DMBA	dimethylbenzanthracene
DMN	Dimethylnitrosamine
DMNQ	2,3-dimethoxy-1,4-naphthoquinone
DMPS	2,3-dimercaptopropane-1-sulfonic acid
DMSA	dimercaptosuccinic acid or *meso* 2,3- dimercaptosuccinic acid
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase, which has 3 subunits, of which the Ku70 protein is one
DNMT	DNA methyltransferase
DPC	DNA protein cross-links
DPI	diphenyleneiodonium
DPIC	diphenylene iodonium chloride, an NADPH-oxidase inhibitor
DR	death receptor
DRE-CALUX	dioxin-responsive element (DRE)-mediated Chemical Activated LUciferase eXpression
DSB	double strand break (in DNA)
DTNB	5,5´-dithiobis(2-nitrobenzoic acid)
DTT	Dithiothreitol
DU145 cells	human prostate carcinoma cell line
DW	drinking water
D-NMMA	N^{G}-methyl-D-arginine, the inactive enantiomer of a nitric oxide synthase inhibitor
EA	ethacrynic acid (a GST inhibitor)
EB	ethidium bromide
E. coli	*Escherichia coli*
EDR3 cells	rat hepatoma cell line (glucocorticoid receptor negative, with neither protein nor mRNA detectable)
EGCG	(-)-epigallocatechin gallate
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EGFR ECD	extracellular domain of the epidermal growth factor receptor
EGR	early growth response

eIF	eukaryotic initiation factor
eIF4E	eukaryotic translation initiation factor 4E, which is the mRNA cap binding and rate-limiting factor required for translation
ELISA	Enzyme-Linked ImmunoSorbent Assay
Emodin	(l,3,8-trihydroxy-6-methylanthraquinone)
EMSA	electrophoretic mobility shift assays
EnIII	Endonuclease III
eNOS	endothelial nitric acid synthase
ERCC1	excision repair cross-complement 1 component
ERCC2	excision repair cross-complementing rodent repair deficiency, complementation group 2 (also known as xeroderma pigmentosum group D or XPD)
EROD	Ethoxyresorufin-*O*-deethylase
Erk or ERK	extracellular signal-regulated kinase
ER-α	estrogen receptor-α
ESR	electron spin resonance
ETU	S-ethylisothiourea, a NOS inhibitor
E2N	Ubiquitin-conjugating enzyme
E7 cells	Immortalized human bladder cell line
FACS	fluorescence-activated cell sorting
FADD	Fas-associated death domain protein
FAK	focal adhesion kinase
FBS	fetal bovine serum
FeTMPyP	5,10,15,20-tetrakis (*N*-methyl-4'-pyridyl) porphinato iron(III) chloride ($ONOO^-$ decomposition catalyst)
FGC4 cells	rat hepatoma cells
FGF-2	fibroblast growth factor -2
FGFR1	fibroblast growth factor receptor 1
FISH	fluorescent *in situ* hybridization
FITC	fluorescein isothiocyanate
FLIP	FLICE-inhibitory protein, an antiapoptotic protein controlled by NF-κB
$FLIP_L$	long-splice variant of FLIP
Fox O3a	An oxidative stress inducible forkhead transcription factor
FPG	formamidopyrimidine-DNA glycosylase (digestion of DNA)
GADD	growth arrest and DNA damage-inducible
GCLM	glutamate cysteine ligase modifier, GCLM knockout mice (-/-) have only 9-16% of GSH level of wt littermates
GCR	glucocorticoid receptor
GFP	green fluorescent protein (GFP expressing tumor cells)
GLN	glutamine
GlycoA	glycophorin A
GM04312C cells	SV-40 transformed XPA human fibroblast NER-deficient cell line
GM847 cells	SV-40-transformed human lung fibroblast cell line

GM-CSF	granulocyte-macrophage colony-stimulating factor
GM-Mp	GM-type macrophage
gpt	guanine phosphoribosyltransferase
GPx	glutathione peroxidase
GR	glutathione reductase
GRE	glucocorticoid response elements
GSH	Glutathione
GSSG	Glutathione disulfide
GST	glutathione-*S*-transferase
GTP	guanosine-5'-triphosphate
Gy	gray (unit of ionizing radiation)
G12 cells	pSV2gpt-transformed Chinese hamster V79 (*hprt*$^-$) cell line
G6PDH	glucose-6-phosphate dehydrogenase
G-6-P	glucose 6 phosphatase; the paper that presented data on this chemical called it G-6-PD in the discussion.
HaCat cells	human keratinocyte cell line
HaCaT cells	human epidermal keratinocytes cell line
Hb	Hemoglobin
HCC	hepatocellular carcinoma
HCT116 cells	human colorectal cancer cell line (available in securin-wild-type and securin-null forms)
HCT15 cells	human colon adenocarcinoma cell line
HEC	Hamster embryo cells
HEK 293 cells	adenovirus-transformed human embryonic kidney epithelial cell line (non-tumor cells), also called HEK293 cells
HEK293T cells	human embryonic kidney cells
Hepa-1c1c7 cells	a mouse hepatoma cell line known to respond to a B[α]P or TCCD challenge by activation of the AhR
HepG2 cells	human hepatocellular liver carcinoma cell line (Caucasian)
HeLa cells	human cervical adenocarcinoma cell line
HeLa S3 cells	human cervical carcinoma cell line, derived from the parent HeLa cell line and is adapted to grow in suspension (spinner) culture and has the same virus susceptibility as the parent line
HELF cells	human embryo lung fibroblast cell line
hEp cells	normal human epidermal cells derived from foreskin
HEL cells	an AML cell line that is a cytokine-independent human erythroleukemia cell line that has constitutive STAT3 activity
HFF cells	human foreskin fibroblasts cell line
HFW cells	diploid human fibroblast cell line
HGF	hepatocyte growth factor
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HIF	Hypoxia inducible factor
HK-2 cells	human proximal tubular cell line
HLA	human leukocyte antigen

HLA-DR	human leukocyte antigen DR, which is a major histocompatibility complex class-II antigen
HLF cells	human embryo lung fibroblasts
HLFC cells	an HLF subline that is not Ku70 deficient; it has the null pEGFP-C1 vector transferred into it
HLFK cells	an HLF subline that is Ku70 deficient; it has a recombinant plasmid of Ku70 gene antisense RNA transferred into it; it had 38% as much Ku70 protein content as the HLFC cell line
HL-60 cells	human promyelocytic leukemia cells
HMEC-1 cells	human microvascular endothelial cells
HMOX-1	heme oxygenase 1
HO·	Hydroxyl radicals
HOS cells	human osteogenic sarcoma cell line
HpaII or HPAII	*Haemophilus parainfluenzae* (restriction endonucleases)
HPBM	human peripheral blood monocytes
HPLC	high performance liquid chromatography
HPRT	hypoxanthine phosphoribosyl transferase
HRE	hypoxia response element, the DNA binding element of HIF-mediated transactivation
Hr	hour(s)
HSF1	heat shock transcription factor 1
HSP	heat shock protein
HT1080 cells	human sarcoma cell line
hTER	RNA component of telomerase
hTERT	human telomerase reverse transcriptase
HT1197 cells	human (Caucasian) epithelial bladder cancer cell line
HU	Hydroxyurea
Huh7 cells	human hepatoma cell line
HuR	RNA binding protein
HUVEC cells	human umbilical vein endothelial cell line (or HUVECs)
H1355 cells	human lung adenocarcinoma cell line
H_2O_2	Hydrogen peroxide
H22 cells	hepatocellular carcinoma cell line
H411E cells	rat hepatoma cell line
H460 cells	human non-small-cell lung cancer cell line (also called human lung large cell carcinoma cells)
H9c2 cells	immortalized myoblast cell line derived from fetal rat hearts
IAP	Inhibitor of apoptosis protein family
iAs	Inorganic arsenic
icAA	intracellular ascorbic acid, which is accumulated at up to high concentrations by culturing cells in DHA
ICAM-1	inter-cellular adhesion molecule-1
ICE	interleukin-1β-converting enzyme
IC_{50}	Concentration that causes 50% inhibition of activity
ID1	inhibitor of DNA binding-1

IEC-6 cells	rat intestinal epithelial cell line
IEC cells	primary culture of rat intestinal epithelial cells
IGF	insulin growth factor (system)
IGFBP-1	insulin-like growth factor binding protein 1
IKKβ	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta; also called IkappaB kinase beta subunit
IL	Interleukin
ILK	integrin-linked kinase
Imatinib	imatinib mesylate
IM9 cells	human multiple myeloma cell line
IRE	iron responsive element
IRP-1	iron regulatory protein 1
JAK	Janus kinase
JAR cells	human placental choriocarcinoma cell line
JB6 C141 cells	P^+ mouse epidermal cell line (sometimes called JB6 C1 41 cells)
JB6 C141 PG13 cells	stable p53 luciferase reporter plasmid transfectant of cell line JB6 C141
JB6 C141 P^+1-1 cells	stable activator protein-1 (AP-1) transfectant of cell line JB6 C141
JC-1	voltage-sensitive lipophilic cationic fluorescence probe 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide
JNK	c-Jun N-terminal kinase
Jurkat cells	a transformed human T-lymphocyte cell line (also called lymphoblast cells)
J82 cells	human bladder tumor cells
KCL22 cells	a Bcr-Abl positive CML cell line
KCL22-r cells	KCL22 cells that are resistant to the Bcr-Abl inhibitor imatinib mesylate
KCL22-s cells	KCL22 cells that are sensitive to the Bcr-Abl inhibitor imatinib mesylate
kDa	kilodalton, a unit of mass
Keap1	the cytoplasmic Nrf2-binding protein
KMS12BM	human multiple myeloma cell line
Ku70	one of the 3 subunits of DNA-dependent protein kinase
K1735-SW1 cells	mouse melanoma cell line
K562 cells	a human immortalized myelogenous leukemia cell line that is a bcr:abl positive erythroleukemia line derived from a 53-year-old female CML patient in blast crisis
LAK cells	lymphokine activated killers (effector cells)
LCL-EBV cells	mononuclear cells obtained from healthy donors and transformed by Epstein–Barr virus
LC_{50}	50% lethal concentration
LDH	lactate dehydrogenase
LD_{50}	50% lethal dose
LI	labeling index

LOEC	Lowest observed effect concentration
LOEL	Lowest observed effect level
LOH	loss of heterozygosity
LPO	lipid peroxidation
Luc	the PEPCK-luciferase construct
LU1205 cells	human melanoma cell line
L-NAME	*Nω*-nitro-L-arginine methyl ester (an inhibitor of NOS)
L-NMMA	N^G-methyl-L-arginine, the active enantiomer of a nitric oxide synthase inhibitor
LPS	lipopolysaccharide
LTE_4	leukotriene, a proinflammatory mediator
Lys	Lysine
L-132 cells	human alveolar type II cells
Maf	musculoaponeurotic fibrosarcoma (transcription factor)
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
MCA	20-methylcholanthrene
MC/CAR	Human multiple myeloma cell line
MCF-7 cells	human breast carcinoma cell line
MCR	mineralocorticoid receptor
MDA	Malondialdehyde (the thiobarbituric acid-reactive substance in the brain that reflects extensive lipid peroxidation)
MDAH 2774 cells	human ovarian carcinoma cells
MDA-MB-231 cells	human breast cancer cell line (an invasive estrogen unresponsive cell line)
MDA-MB-435	human metastatic breast cancer cell line
mdm2	murine double minute 2 proto-oncogene
MDR	multidrug resistance gene
MED	minimal erythemic dose
MEF	mouse embryo fibroblasts
MEF cells	mouse embryonic fibroblast cell line
MEK	MAP/ERK kinase (also, a family of related serine-threonine protein kinases that regulate mitogen-activated protein kinase)
MGC-803 cells	human gastric cancer cell line
MI	mitotic index
MiADMSA	monoisoamyl *meso* 2,3- dimercaptosuccinic acid
min	minutes(s)
MK-571	MRP antagonist
MKP-1	MAP kinase phosphatase 1
MMA^{III}	monomethylarsonous acid
MMA^{V}	monomethyl arsonic acid
MMA	monomethyl arsenic (used when oxidative state is unknown or not specified)
$MMA^{III}O$	Methylarsine oxide

MMC	mitomycin C
MMP	mitochondrial membrane potential
MMP-2	matrix metalloproteinase-2
MMP-9	matrix metalloproteinase-9
Mmp13	Matrix metalloproteinase-13
MMS	methyl methanesulfonate
MN	micronuclei
MNNG	1-methyl-3-nitro-1-nitrosoguanidine
mRNA	messenger ribonucleic acid
MnTMPyP	Mn(III)tetrakis(1-methyl-4-pyridyl) porphyrin pentachloride (a cell permeable SOD mimic)
MNU	N-methyl-N-nitrosourea
MRC-5	human lung fibroblast cell line
MRP	multidrug resistance-associated protein
Mrps	efflux transporters encoded by MRP genes
MS	mass spectrometer or mass spectrometry
mtDNA	mitochondrial DNA
MT	metallothionein
MT2A	gene symbol for metallothionein 2A
MT-1	metallothionein-1
MTOC	microtubule-organizing center
MTS assay	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay; in Yi et al. (2004) study this was referred to as the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (MTS) Kit (Promega, Madison, WI)
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
MTX	methotrexate
MW	molecular weight
MYH	MutY homolog, an endonuclease
MYP3 cells	rat epithelial cells line (urinary bladder cells)
M-CSF	macrophage colony-stimulating factor
NAC	*n*-acetyl-cysteine (precursor of GSH; it elevates cellular GSH levels, also an antioxidant), also *N*-acetyl-*L*-cysteine
NADH	reduced form of nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
Namalwa cells	human Burkitt's lymphoma cell line
NB4 cells	human acute promyelocytic leukemia cell line
NB4-As^R	an arsenic resistant subline of NB4 that was made by culturing and maintaining cells in 1μM As_2O_3
NB4-M-AsR2 cells	arsenic resistant human acute promyelocytic leukemia cell line, which is routinely grown in RPMI 1640 media containing 2 μM As_2O_3
NCE	normochromatic erythrocytes
NCI cells	Human myeloma cell line
NE	nuclear extract
NER	nucleotide excision repair (pathway)

NF-κB	nuclear factor-kappa B
NHEK cells	primary normal human epidermal keratinocytes
NIH 3T3 cells	a mouse fibroblast cell line
$NO^{\bullet}$	nitric oxide
NOS	nitric oxide synthase
Nqo1	nicotinamide adenine dinucleotide phosphate-quinone oxidoreductase (or NAD(P)H-quinone oxidoreductase)
NR	Neutral red
Nrf2	cap 'n' collar basic leucine zipper transcription factor (nuclear factor erythroid 2-related factor 2)
NSAID	Non-steroidal anti-inflammatory drug
NSE	no significant effect (often not based on a statistical test but on whether an effect appears likely to be real based on examination of graphs)
NTUB1 cells	a human urothelial carcinoma cell line
NuF	Nuclear fragmentation
N-18 cells	mouse neuroblastoma cell line
OATP-C	organic anion transporting polypeptide-C
ODA	oxidative DNA adducts
OGG1	8-oxoguanine DNA glycosylase
OM431 cells	human melanoma cell line
$ONOO^{-}$	peroxynitrite
OR	Odds ratio
PAEC cells	porcine aortic endothelial cells
PAI-1	plasminogen activator inhibitor-1
PARP	poly(adenosine diphosphate–ribose) polymerase
PBMC	peripheral blood mononuclear cell (human)
PC	protein carbonyl (form of protein oxidation)
PCE	polychromatic erythrocyte
PCI-1 cells	human head and neck squamous cell carcinoma cell line
PCNA	Proliferating cell nuclear antigen
PCR	polymerase chain reaction
PC12 cells	rat sympathetic (neuronal) pheochromocytoma cell line
PDH	pyruvate dehydrogenase
PDT	population doubling time
PD98059	inhibitor of MEK1/2, which are ERK upstream kinases (structurally unrelated to U0126)
PEG	monomethoxypolyethylene glycol (covalent attachment of PEG to CAT or SOD extends their plasma half-lives)
PEPCK	phosphoenolpyruvate carboxykinase gene (a hormone-inducible gene)
pEpREβgeo	β-galactosidase-neomycin-resistance reporter plasmid
PGE_2	prostaglandin E2
PHEN	*o*-phenanthroline (an iron chelator)
PHA	phytohemagglutinin
PI	propidium iodide

PI3K	phosphatidylinositol 3-kinase
PK	proteinase K
PLAP	Placental alkaline phosphatase
PLC/PR/5 cells	human hepatocellular carcinoma cell line
PMA	phorbol 12-myristate 13-acetate
PMN	polymorphonuclear neutrophils (or PMNs)
PMs	Peritoneal macrophages
PNA	peptide nucleic acid
ppb	Parts per billion
ppm	Parts per million
PQ	paraquat (a generator of O_2^-)
PR	progesterone receptor
PRCC	primary renal cortical cell
PSH	protein thiol
pt	pretreatment
PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
p21	a cyclin-dependent kinase inhibitor
p-STAT3	phosphorylated-STAT3
P-gp	P-glycoprotein, the efflux transporter encoded by MDR
P-PKB	phosphorylated protein kinase B
p-XSC	1,4-phenylenebis(methylene)selenocyanate
Rac	a subfamily of the Rho family of GTPases, which are small (~21 kDa) signaling G proteins (more specifically GTPases).
RACs	rapidly adhering cells; epidermal cells with the highest proliferative potential and with properties of stem cells
Raf	a name of a proto-oncogene
RAGE	receptor for advanced glycation end products
RAPD-PCR	random(ly) amplified polymorphic DNA polymerase chain reaction
RANKL	receptor activator of NFκB ligand
Ras	a name of a proto-oncogene
RAW264.7 cells	mouse macrophage cell line, another source described it as mouse macrophage-like cells
RBC	red blood cell, erythrocyte
RFU	Relative Fluorescence Units (units of ROS)
RHMVE cells	rat heart microvessel endothelial cells
RI	replicative index
RKO cells	human colorectal carcinoma cell line that expresses wild-type p53 proteins
ROCK	Rho/kinase, and effector molecule of RhoA
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
RPMI-8226 cells	human myeloma cell line

RT-PCR	reverse transcription-polymerase chain reaction
RWPE-1	Human prostate epithelial cell line
R-3T3 cells	Ras-transformed NIH 3T3 cells, a mouse fibroblast cell line
SA	sodium arsenite
SACs	slowly adhering cells; epidermal cell fraction that contains cells undergoing terminal differentiation, with little ability to form colonies
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCGE	single cell gel electrophoresis (assay)
Se	selenium
SE	standard error of the mean
SEM	scanning electron microscopy
Ser	serine, an amino acid
Se-Met	selenomethionine
SF	sodium formate, an $^{\bullet}OH$ radical scavenger
SFN	sulforaphanem, an activator of transcription factor Nrf2, which plays a critical role in metabolism and excretion of xenobiotics
SHE cells	Syrian hamster ovary cells
SIK cells	spontaneously immortalized human keratinocytes (or epidermal cells)
siRNA	small interfering RNA (ribonucleic acid)
SMC cells	human bladder smooth muscle cells
SLC30A1	gene symbol for the zinc transporter, solute carrier family 30, member 1
SMART	somatic mutation and recombination test
SOCS	suppressors of cytokine signaling
SOD	superoxide dismutase (an antioxidant to $O_2^{\bullet -}$)
SP	shock protein
SRB assay	sulforhodamine B colorimetric assay
Src	first oncogene discovered, the transforming protein of the chicken retrovirus, Rous sarcoma virus
SSB	single strand break (in DNA)
STAT	signal transducer and activator of transcription
StRE site	stress response element recognition site
SU5416	inhibitor of VEGF receptor-2 kinase
SV-40	Simian virus 40
SVEC4-10 cells	C3H/HeN mouse vascular endothelium cell line (also called immortalized mouse endothelial cell line)
SV-HUC-1 cells	SV40 large T-transformed human urothelial cell line (non-tumor cells, derived from urethra, immortalized)
SW13 cells	human adrenal carcinoma cell line
SW480 cells	colorectal adenocarcinoma cell line derived from a Caucasian male that has two base-pair substitution mutations in the p53 gene
SY-5Y cells	human neuroblastoma cell line

T_3	thyroid hormone triiodothyronine
T_4	thyroid hormone thyroxine
TAM	tamoxifen
TAT	tyrosine aminotransferase
TBARS	Thiobarbituric acid reactive substances (a measure of tissue lipid peroxidation)
tBHQ	*t*-butylhydroquinone
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TF	theaflavin
Tg.AC	strain of transgenic mice that contains the fetal beta-globin promoter fused to the v-Ha-*ras* structural gene (with mutations at codons 12 and 59) and linked to a simian virus 40 polyadenylation/splice sequence
TGF	transforming growth factor
THP-1 + A23187	human dendritic cell line; THP-1 cells acquire the characteristics of dendritic cells in the presence of the calcium ionophore A23187
TIG-112 cells	human normal skin diploid cells
TIMP-1	tissue inhibitor of metalloproteinase-1
Tiron	4,5-dihydroxy-*m*-benzenedisulfonic acid, disodium salt
TK6 cells	human lymphoblastoid cells
TM	Tail moment
TM3 cells	immortalized Leydig cells derived from normal mouse testis
TMA^VO	trimethylarsine oxide
TNF-α	tumor necrosis factor α (an inflammatory cytokine)
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TRAIL	TNF-related apoptosis-
TRAIL-R	TRAIL receptor
TRAP	tartrate resistant acid phosphatase (RAW264.7 cells can undergo osteoclast differentiation, which is accompanied by an increase in the number of multinucleate cells expressing TRAP)
TRF	terminal restriction fragment
TRL 1215 cells	nontumorigenic adhesive rat epithelial liver cells originally derived from the liver of 10-day-old Fisher F344 rats
Trolox®	6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid
Trx	thioredoxin
Trx1	cytoplasmic thioredoxin-1
Trx2	mitochondrial thioredoxin-2
TrxR	thioredoxin reductase
TrxR1	cytosolic thioredoxin reductase
TR9-7 cells	a spontaneously immortalized human fibroblast cell line, derived from a Li-Fraumeni patient, and subsequently stably transfected with a tetracycline-regulated p53 expression vector
TUNEL assay	terminal deoxynucleotidyl transferase-mediated deoxyuridine nick-end labeling assay
T47D cells	human mammary adenocarcinoma cell line
Ub	ubiquitin

UROtsa	an SV40-immortalized human urothelium cell line
UV	ultraviolet radiation
UVA	ultraviolet radiation A
UVB	ultraviolet radiation B
UVC	ultraviolet radiation C
U0126	inhibitor of MEK1/2, which are ERK upstream kinases (structurally unrelated to PD98059)
U118MG cells	human glioblastoma cell line, also called U118MG (ATCC HTB-15) cells
U266 cells	human multiple myeloma cell line
U937 cells	human leukemic monocyte lymphoma cell line (also described as a human promonocytic cell line or as a human myeloid leukemia cell line)
U-937 cells	human diffuse histiocytic lymphoma cells, perhaps the same as U937 cells
U-2OS	human osteogenic sarcoma cell line
VEGF	vascular endothelial growth factor or vascular endothelial cell growth factor
VEGFR1	a vascular endothelial cell growth factor receptor (flt-1)
VEGFR2	a vascular endothelial cell growth factor receptor (Flk-1, KDR)
V-FITC	V-fluorescein isothiocyanate
VH16	Human primary fibroblasts
vs	Versus
VSMC	vascular smooth muscle cells
V79 cells	cell line derived from lung fibroblasts of a male Chinese hamster
wk	week(s)
wt	wild-type
WM9 cells	human melanoma cell line
WRL-68	Human hepatic cell line
WT-1	Wilm's tumor protein-1
W138 cells	human diploid lung fibroblast cell line
XIAP	X-linked inhibitor of apoptosis protein, an antiapoptotic protein controlled by NF-κB
XPA (B or F)	xeroderma pigmentosum, complementation group A (B or F)
XRS	X-ray sensitive
XTT	2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide
YC-1	a small molecule inhibitor of HIF signaling
ypt locus	xanthine-guanine phosphoribosyltransferase locus
ZPP	zinc protoporphyrin
Z-DEVD-FMK	benzyloxycarbonyl-L-Asp-Glu-Val-Asp-fluoromethyl ketone, a caspase 3 inhibitor
Z-VAD-FMK	Z-Val-Ala-DL-Asp-fluoromethylketone, a general caspase inhibitor
$1RB_3AN_{27}$ cells	immortalized dopamine-producing rat mesencephalic cell line
1T1 cells	human epithelial cell line

293 cells	cell line derived from adenovirus-transformed human embryonic kidney epithelial cells
2-AAAF	2-acetoxyacetylaminofluorene
2BS cells	human fetal lung fibroblasts
3-NT	3-nitrotyrosine
4HNE	4-hydroxy-2-nonenal
4NQO	4-nitroquinoline 1-oxide
5-aza-dC	5-aza-deoxycytidine, a demethylating agent
6-4 PPs	6-4 photoproducts (UV-induced DNA photoproduct)
7-AAD	7-aminoactinomycin D
8-OHdG	8-hydroxy-2'-deoxyguanosine or 8-hydroxydeoxyguanosine (synonym)
8-oxoG	7,8-dihydro-8-oxoguanine
α7-nAChR	α7-nicotinic acetylcholine receptor
α-Toc	α-tocopherol, an antioxidant
γGCS	γ-glutamylcysteine synthetase
γH2A.X	phosphorylated histone variant H2A.X that is indicative of DNA double strand breaks
ρ^0 cells	AL hybrid cells made highly deficient in mitochondrial DNA by long-term treatment with ditercalinium

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer				
Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
Aberrant Gene or Protein Expression				
Effect of iAs exposure from DW on concentration of RAGE protein in sputum	People in Ajo (high dose) and Tucson (low dose), Arizona, USA	Compared subjects from Ajo (~20 ppb of As in DW) with subjects from Tucson (~5 ppb of As in DW). They also determined total iAs concentrations in urine in individuals.	No difference was seen in concentration of RAGE protein in sputum between cities. Since there was much overlap of total iAs concentrations in urine in individuals in those cities, a comparison was also made using iAs levels in urine. The regression analysis yielded a significant negative association between urinary total iAs concentrations and RAGE concentrations in sputum. Thus iAs exposure caused ⇓ in RAGE level as was seen in mice.	Lantz et al., 2007
Effect of iAs exposure from DW on serum levels of extracellular domain of EGFR (i.e., EGFR ECD)	Araihazar area of Bangladesh	Estimates of iAs exposure level were based on well water As (ranged from 0.1 to 768 ppb), urinary As, and cumulative As index. Such estimates and EGFR ECD protein levels were compared in 574 people.	Found significant positive correlation between EGFR ECD protein levels in serum and all of these measures of iAs exposure, with the association being strongest among individuals with As-induced skin lesions.	Li et al., 2007
Effect of iAs exposure from DW on levels of TGF-α in bladder urothelial cells (BUC)	3 towns in central part of Mexico	Estimates of iAs exposure level were based on levels of different metabolites of As in urine from 72 women who used drinking water that contained 2–378 ppb As	Found significant positive correlation between TGF-α protein levels in exfoliated BUC and each of 6 arsenic species present in urine. Women from areas with high As exposures had significantly higher TGF-α protein levels in BUC than those from areas of low As exposure. BUC cells from people with As-induced skin lesions contained significantly more TGF-α.	Valenzuela et al., 2007
Microarray–based gene expression study comparing groups with and without arsenical skin lesions, both of which were exposed to iAs in DW but to different extents	Bangladesh	Compared subjects with cutaneous signs of arsenicism (mean of 343±258 ppb of As in DW) with asymptomatic individuals (mean of 40±50 ppb of As in DW in one set, and 95±91 ppb in another)	Looked at expression of ~22,000 transcripts in RNA from peripheral blood lymphocytes. When the comparison was restricted to female never-smokers, 312 differentially expressed genes were identified between those with and without As-induced skin lesions, with all of them being down-regulated in the skin-lesion group. Signal transduction through the IL-1 receptor was identified as a significant pathway of differentially expressed genes between the arsenical skin lesion (n = 11) and nonlesion (n = 2) groups. It discriminated between the 2 groups.	Argos et al., 2006
Comparison of expression of several genes between patients with As-related urothelial cancer and non-As-related urothelial cancer	Taiwan, patients with urothelial cancer	All 33 patients with As-related urothelial cancer had been living in the arseniasis endemic area of southwest Taiwan, where people had drunk the As-contaminated artesian well water for at least 10 years. They were compared with 25 patients who had non-As-related urothelial cancer.	Comparisons were made of protein expression of GST-π, p53, Bcl-2, and c-Fos by Western blotting of tumor tissues. A significantly higher proportion of the patients with the As-induced cancer had the proteins present for Bcl-2 (33/33 vs 19/25) and for c-Fos (30/33 vs 16/25), suggesting that up-regulation of these 2 oncoproteins may play important roles in As-mediated urothelial carcinogenesis. Cellular GSH content was down-regulated in both types of tumors, but to a greater extent in the As-induced ones.	Hour et al., 2006

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Topic(s)	Population sampled	Information on exposure levels and durations and on biomarkers	Results	Reference
Comparison of expression of several integrins between people with As-related keratosis and people with normal skin	Taiwan, patients with arsenical keratosis	All 25 arsenical keratosis patients were from arseniasis endemic areas of southwest Taiwan, where water is contaminated by high concentrations of iAs. Control specimens were obtained from the non-sun-exposed skin of 8 age-comparable patients who did not live in the endemic areas.	Immunohistochemical staining patterns of integrin β_1, $\alpha_2\beta_1$, and $\alpha_3\beta_1$ were observed. The various patterns of staining among the patients in comparison to the controls showed decreased expression of all 3 integrins in both arsenical keratosis and in perilesional skin. None showed the normal expression pattern of all 3 integrins. However, there was no association with the occurrence of basal cell carcinoma or squamous cell carcinoma and the expression pattern of any of the 3 integrins.	Lee et al., 2006b
Apoptosis				
Possible association of specific p53 polymorphisms with As-related keratosis in individuals exposed to As in DW	West Bengal, India	Compared 177 As-exposed subjects with keratosis (mean of 177 ppb of As in DW) with 189 As-exposed subjects without such skin lesions (mean of 180 ppb of As in DW), and looked for association of keratosis with 3 specific p53 polymorphisms. Used As concentration comparisons in DW, urine, nails, and hair.	Homozygotes for alleles at 2 of the polymorphisms were significantly over represented in the individuals with keratosis. Results suggest that individuals carrying the arginine homozygous genotype at codon 72 and/or the no duplication homozygous genotype at intron 3 are at higher risk for the development of As-induced keratosis. In both cases the OR was 2.086 and the 95% CI did not overlap 1. Urinary excretion of As was slightly lower (NSE) in the group with keratosis suggesting higher retention of As in the body, which was reflected in significantly higher As content in nails and hair.	De Chaudhuri et al., 2006
Chromosomal Aberrations and/or Genetic Instability				
Nested case-control study/ CAs and/or SCEs as biomarkers for the prediction of cancer development	Blackfoot endemic area in Taiwan	Looked at CAs and SCEs in lymphocytes from venous blood samples	Chromosome-type CAs, but not chromatid-type CAs or SCEs, were significantly higher in the cases than in the controls. The cancer risk OR for subjects with > 0 chromosome-type breaks was 5.0 (95% CI = 1.09–22.82). The OR became even higher with more refinements. Thus chromosome-type CAs (but not chromatid-type CAs or SCEs) can serve as useful biomarkers for prediction of cancer development.	Liou et al., 1999
Induction of MN	West Bengal, India	Compared subjects with cutaneous signs of arsenicism (368 ppb of As in DW) with asymptomatic individuals (5.5 ppb of As in DW). Also used As concentration comparisons in urine, nails, and hair.	In the exposed group, the frequencies of MN per 1,000 cells were highly elevated over those of the control group (# per 1000 cells): 5.15 vs 0.77 in the oral mucosa, 5.74 vs 0.56 in urothelial cells, and 6.39 vs 0.53 in peripheral lymphocytes, respectively.	Basu et al., 2002
Induction of MN and CAs (relationship to presence of arsenicism and GST polymorphisms)	West Bengal, India	Compared As-exposed subjects with cutaneous signs of arsenicism (mean of 242 ppb of As in DW), As-exposed subjects without cutaneous signs of arsenicism (mean of 202 ppb of As in DW), and As-unexposed subjects (mean of 7.2 ppb of As in DW), and looked for association of effects with different GSTT1 and GSTM1 genotypes. Used As concentration comparisons in DW, urine, nails, and hair.	As-exposed groups showed ⇑⇑ in MN in the lymphocytes, oral mucosa, and urothelial cells and ⇑ in frequencies of CAs in lymphocytes. The symptomatic (i.e., with cutaneous signs of arsenicism) exposed group had more of all types of cytogenetic damage than the asymptomatic exposed group, and the asymptomatic exposed group had more of all types of cytogenetic damage than the unexposed group. Asymptomatic and symptomatic exposed groups demonstrated rather similar concentrations in the urine, nails, and hair. Individuals carrying at least one GSTM1-positive allele had a significantly higher risk of developing cutaneous signs of arsenicism.	Ghosh et al., 2006

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Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
Association between a polymorphism in *ERCC2* codon 751 that probably improves NER and (a) the incidence of CAs and (b) the presence of iAs-induced hyperkeratosis	West Bengal, India	Comparisons were made between people with hyperkeratosis and individuals with no skin lesions that were drinking similar iAs contaminated water. Groups with and without hyperkeratosis had means of 195 and 185 ppb As in DW, respectively, with large standard deviations.	The polymorphism resulted from a base pair change from A to C at codon 751 that resulted in an amino acid substitution from lysine to glutamine. The A/A (i.e., Lys/Lys) genotype was compared with the A/C and C/C genotypes combined. In the study population, the allele frequencies of A and C were 0.4 and 0.6, respectively. A/A individuals were shown to be at significantly higher risk of having hyperkeratosis and also to have a higher frequency of CAs in their lymphocytes, as follows: A/A individuals were over-represented among individuals with iAs-induced hyperkeratosis (OR = 4.77, 95% CI = 2.75-8.23). There was a higher percentage of cells with CAs in A/A individuals than in (A/C and C/C) individuals: 43% more in those exposed to iAs but not having hyperkeratosis, 18% more in those exposed to iAs and having hyperkeratosis, and 31% in both groups combined. Also, CAs were significantly more frequent in iAs-exposed people with hyperkeratosis.	Banerjee et al., 2007
Induction of MN (bladder cells)	Chile, men	Compared subjects having high (average 600 ppb of As in DW) and low (average 15 ppb of As in DW) exposures.	Used a fluorescent version of exfoliated bladder cell MN assay to identify presence or absence of whole chromosomes within MN. Significant ⇑ in induction of MN by As was found, and chromosome breakage appeared to be its major cause. 4th highest quintile of exposure groups gave the highest response, but there was a significant ⇑ in each of quintiles 2-4. Highest (5th) quintile (729-1894 ppb) returned to baseline MN level, perhaps because of cytostasis or cytotoxicity.	Moore et al., 1997b
Induction of SCEs (Fowler's solution, (lymphocytes)	6 patients treated with Fowler's solution who developed arsenicism and biopsy-proven skin cancers	Nothing is known about doses; duration of treatment with iAs ranged from 4 months to 27 years, and in most cases treatment ceased decades before this cytogenetic analysis.	Patients treated with Fowler's solution had mean of 14.0 SCE/mitotic cell, while 44 normal controls had mean of 5.8 SCEs/mitotic cell. They saw no difference in chromosome breakage between the groups.	Burgdorf et al., 1977
Induction of CAs and SCEs (Fowler's solution, lymphocytes)	8 psoriasis patients treated with Fowler's solution were compared with 8 psoriasis patients not treated with iAs (7 men in each group)	The total doses of iAs were from 300 to 1200 mg for the 7 with known doses. iAs treatments ceased many years before this study. Comparisons were also made to 30 apparently healthy untreated males.	⇑ in frequency of chromosomal breaks (i.e., chromatid and chromosome aberrations together) in psoriasis patients with iAs treatment and an even bigger ⇑ in comparison to healthy untreated males. iAs treatment had NSE on SCE frequency.	Nordenson et al., 1979
Induction of CAs (mostly airborne iAs, lymphocytes)	9 workers exposed to iAs at smelter in northern Sweden	Little information was presented except to say that there was no obvious relationship between exposure and CA frequencies.	87 CAs/819 mitotic cells among smelter workers and 13 CAs/1012 mitotic cells in controls. Person with highest CA frequency had also been exposed to lead and selenium.	Beckman et al., 1977

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Topic(s)	Population sampled	Information on exposure levels and durations and on biomarkers	Results	Reference
Induction of CAs and SCEs (lymphocytes)	People in Fallon (exposed) and Reno (control), Nevada, USA	The exposed sample of 104 used DW containing >50 ppb As (mostly > 100 ppb As) for at least 5 years and the control sample of 86 used DW containing <50 ppb As (and often much less) for the same period.	No hint of any effect of iAs on CA or SCE frequencies was seen even though there was an approximately 9-fold difference in the mean iAs concentrations in DW between the 2 groups.	Vig et al., 1984
Induction of CAs and MN (lymphocytes for CAs)	People in Santa Ana (high dose) and Nazareno (control), Mexico	The high-dose group used DW containing a mean of 408 ppb As, and the control (i.e., low dose) group used DW containing a mean of 30 ppb As. They also considered As concentrations in urine and blood and concentrations of As metabolites.	iAs caused ⇑ in CA (chromatid and isochromatid deletions) frequency in lymphocytes and an ⇑ in MN frequency in exfoliated epithelial cells obtained from the oral mucosa and from urine samples. MN frequencies were higher in people with skin lesions, by factor of 2.3 times in oral mucosa and by 4.3 times in urothelial cells. There was also much more induction of MN in males than in females for both cell types.	Gonsebatt et al., 1997
Induction of MN	People in Nevada, USA, with either very high or low exposure to iAs in DW	The high-dose group of 18 used DW containing a mean of 1312 ppb As, and the individually matched control (i.e., low-dose) group used DW containing a mean of 16 ppb As. They also considered the concentration of iAs and methylated metabolites in urine.	iAs caused ⇑ in MN frequency in exfoliated bladder cells to 1.8x (90% CI, 1.06x to 2.99x). The MN frequency was positively correlated with the urinary concentration of iAs plus methylated metabolites. In contrast, iAs had no effect on the MN frequency in epithelial cells obtained from the buccal mucosa.	Warner et al., 1994
Induction of MN (chromosome breakage and/or aneuploidy)	People in Nevada, USA, with either very high or low exposure to iAs in DW	The high dose group of 18 used DW containing a mean of 1,312 ppb As, and the individually matched control (i.e., low dose) group used DW containing a mean of 16 ppb As. They also considered the concentration of iAs and methylated metabolites in urine.	The exfoliated cell MN assay using FISH with a centromeric probe was applied: frequencies of MN containing acentric fragments (MN-) and those containing whole chromosomes (MN+) both showed ⇑, to 1.65x (statistically significant) and 1.37x ($P = 0.15$), respectively, suggesting that As has clastogenic and possibly even aneuploidogenic properties. Effect was stronger in males than in females. Thus, in males the increases were 2.06x ($P = 0.07$) and 1.86x ($P = 0.08$), respectively. The frequencies of MN- and MN+ were both positively correlated with urinary As and its metabolites.	Moore et al., 1996

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Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
Induction of CAs and SCEs (lymphocytes for CAs)	People in Santa Ana (high dose) and Nuevo Leon (low exposure group), Mexico	The high exposure group of 11 used DW containing a mean of 390 ppb As (98% as As^{V}), and the low exposure group of 13 used DW that ranged from 19 to 60 ppb As. They also considered As concentrations in urine.	Examined the levels of CAs and SCEs in peripheral blood lymphocytes. There were no skin lesions in the control subjects, but 4 of the 11 exposed subjects had cutaneous signs of arsenicism. The percentages of total CAs and SCEs were similar in the two groups; however, the finding of a higher point estimate of the frequency of complex CAs (i.e., dicentrics, rings, and translocations) in the high exposure group was considered suggestive of a possible effect of iAs. Average generation times (AGT) of lymphocytes were 19.02 hr in the laboratory control, 19.90 hr in the low exposure group, and 28.70 hr in the high-exposure group, with this difference being statistically significant. It was suggested that this effect might suggest an impairment of the immune response.	Ostrosky-Wegman et al., 1991
DNA Damage				
DNA damage detected using SCGE (comet) assay (lymphocytes)	New Hampshire, USA	Low-exposure (control) group had < 0.7 ppb As in DW and high-exposure group had ≥13 (and up to 93) ppb As in DW.	Using the SCGE (comet) assay, baseline DNA damage as well as the capacity of the lymphocytes from these subjects to repair damage induced by an *in vitro* challenge with 2-AAAF were assessed. 2-AAAF was used because its adducts are primarily repaired through the NER pathway. High-exposure group had ⇑ in baseline damage (i.e., damage resulting from iAs exposure only) to ~1.8x. Two hours after identical *in vitro* 2-AAAF treatments to cells from both high- and low-iAs-exposure groups, cells from both groups showed big ⇑ in DNA damage, with iAs-high-exposure group showing ~15% more DNA damage than control (NSE). After 4-hr repair period, significantly more DNA damage remained in lymphocytes from individuals in high exposure group (~1.54x), and essentially all 2-AAAF-induced DNA damage had been repaired in the control cells.	Andrew et al., 2006

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer				
Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
Oxidative DNA damage	Residents of Bayingnormen (Ba Men), Inner Mongolia, China, with exposures to a wide range of concentrations of iAs in DW	Concentrations of iAs in DW were determined for individuals; ~70% of subjects used DW containing nondetectable As through 200 ppb As, with the rest using DW containing up to ~830 ppb As, with all exposures lasting at least 5 years. They also determined As levels in toenail clippings as a biomarker of exposure.	OGG1 expression was used as an indicator of oxidative stress. OGG1 was selected because it codes for the enzyme 8-oxoguanine DNA glycosylase, which is involved in base excision repair of 8-oxoguanine residues that result from oxidative damage to DNA. The study found that OGG1 expression was closely linked to the levels of arsenic in the drinking water and toenails of the individuals examined, indicating a link between ROS damage to DNA and arsenic exposure in humans. There were no significant differences in As-induced expression due to gender, smoking, or age. OGG1 expression was also associated with skin hyperkeratosis in males, and there was a hint of the same in females. There was an inverse relationship between OGG1 expression and Se levels in toenails indicating possible protective effects of Se against arsenic-induced oxidative stress. The maximal OGG1 response appeared to be at a water As concentration of 149 ppb, after which its expression leveled off and was gradually down-regulated.	Mo et al., 2006
Correlation of urinary 8-OHdG with urinary metal elements and many other substances	6 regions of Japan	128 men and 120 women from Japan who did not live within several kilometers of large chemical factories or garbage incinerator facilities	The association was investigated between urinary concentrations of 8-OHdG and urinary concentrations of As, Al, Cr, Ni, Hg, Zn, Cu, Pb (in ng of element/mg creatinine) as well as with 5 antioxidants and several other substances. Statistically significant positive correlations were found with As, Cr, and Ni and not with any other substances. (The correlation coefficient for As was 0.25.) It thus appears that exposure of healthy people to these 3 metals under normal conditions may increase oxidative DNA damage. Urinary As levels ranged from ~0 to ~230 ng As/mg creatinine.	Kimura et al., 2006
Levels of urinary 8-OHdG following acute arsenic poisoning incident	Wakayama, Japan	63 people were poisoned by eating food contaminated with arsenic trioxide, with 4 dying about 12 hours after eating. Doses in individuals were poorly known.	Some interesting observations were made among the 52 poisoned individuals who were tested for 8-OHdG levels in urine following acute poisoning. After 30 days, urinary 8-OHdG levels were maximal with a mean for all patients of ~1.5x the normal level in Japanese people. By 180 days after the poisoning, levels returned to normal. About 37% of the patients never showed any increase in the concentration of 8-OHdG in urine. The same paper documented a significant increase in urinary 8-OHdG in people from Outer Mongolia, China, who drank water contaminated with about 130 ppb As. The increase in urinary 8-OHdG disappeared after they drank "low-arsenic" water for 1 year.	Yamauchi et al., 2004

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer				
Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
DNA damage in peripheral blood lymphocytes detected by alkaline comet assay	West Bengal, India	Low-exposure (control) group had 7.7±0.5 ppb As in DW. High-exposure group had 247±19 ppb As in DW. They also considered As levels in nails, hair, and urine.	Used SCGE (comet) assay with DNA denaturation at pH >13. High exposure group had significantly more DNA damage in lymphocytes. Assay was also combined with FPG enzyme digestion to demonstrate that As induced oxidative base damage.	Basu et al., 2005
DNA Repair Inhibition or Stimulation				
Decreased DNA repair (lymphocytes)	New Hampshire, USA, and the towns of Esperanza and Colonia Allende, Mexico	Subjects from New Hampshire were from an ongoing epidemiological study of bladder cancer. Low-exposure (control) group had 0.007-5.3 ppb (average of 0.7) As in DW. High-exposure group had 10.4-74.7 ppb (average of 32) As in DW. Subjects from Colonia Allende had 5.5 ± 0.20 ppb As in DW, and those from Esperanza had 43.3 ± 8.4 ppb As in DW. Comparisons between the low (i.e., control) and high exposure groups used either 5 (for protein analysis) or 6 ppb (for mRNA analysis) as the dividing line between low and high. They also considered As levels in urine and toenails.	Earlier work suggested that iAs exposure was correlated with decreased expression of the nucleotide excision repair genes ERCC1, XPB, and XPF. This study focused on ERCC1 and, besides considering gene expression, it looked at both the protein and DNA repair functional levels (for latter, see part of study described in DNA damage part of this table). iAs exposure was associated with ⇓ in expression of ERCC1 in isolated lymphocytes both at the mRNA and protein levels. In combined data, there was a ⇓ to ~0.71x, with a significant effect in New Hampshire alone and in the total data. Estimate of effect in Mexico was ⇓ to ~0.84x (NSE). ⇓ in ERCC1 protein level to ~0.28x was also demonstrated in high exposure group in New Hampshire.	Andrew et al., 2006
Decreased DNA repair (lymphocytes)	New Hampshire, USA	Subjects from New Hampshire were from an ongoing epidemiological study of bladder cancer. They compared levels of expression of 5 NER genes in 6 cases and 10 controls with the iAs levels in their DW and in their toenails.	Toenail and DW As levels were inversely correlated with expression of ERCC1, XPB, and XPF. The As levels in toenails were more strongly negatively correlated with the changes in gene expression that the As concentrations in DW. In these comparisons, expression levels were compared between high and low levels of As exposure. By definition a high level in DW was anything ≥2 ppb As and a high level in toenails was anything ≥2 ppm As.	Andrew et al., 2003
Effects Related to Oxidative Stress (ROS)				
Evidence of oxidative damage to DNA caused by As, but not necessarily from iAs in DW	Taichung County, Taiwan	School children ages 10-12, with attention being given to possibility of oxidative stress to DNA from exposure to environmental pollutants As, Cr, and Ni. No information given on concentrations of iAs in DW.	When oxidative damage occurs in DNA, the excised 8-OHdG adduct is excreted into urine and is a biomarker of oxidative stress. In this cross-sectional study, subjects with higher urinary As tended to have more (19% more, $p = 0.09$) urinary 8-OHdG than those with lower urinary As. Cr was also on the borderline of showing a significant ⇑; when both As and Cr were at a higher level in urine, there was a highly significant ⇑ of 39% in urinary 8-OHdG.	Wong et al., 2005

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Topic(s)	Population sampled	Information on exposure levels and durations and on biomarkers	Results	Reference
Evidence of oxidative damage to DNA caused by iAs in DW, and the relationship of that DNA damage to As-related skin lesions	2 villages in Wuyuan prefecture in Hetao Plain, Inner Mongolia, China	Adults from low-As-exposure village (mean of 5.3 ppb As in DW) and from high-As-exposure village (mean of 158.3 ppb As in DW). They also measured levels of MMA and DMA in the urine, and the levels of those metabolites in the urine in the high-As-exposure village were at least 17x higher than they were in the low-As-exposure village.	When oxidative damage occurs in DNA, the excised 8-OHdG adduct is excreted into urine and is a biomarker of oxidative stress. For subjects without As-related skin lesions in the high-As-exposure village, there was no statistically significant correlation found between iAs, MMA, or DMA and 8-OHdG adducts in the urine. However, for subjects with As-related skin lesions in the high-As-exposure village, there was a significant positive correlation in urine between levels of each those 3 types of As and the level of 8-OHdG adducts. There was so much individual variability that overall there was no excess of 8-OHdG adducts in urine in the high-As village compared to the low-As village, even if restricted to only those with As-related lesions. An overall comparison did, however, show an excess of 8-OHdG adducts in urine in the high-arsenic village among those who had been drinking well water for more than 12 years when compared to those who had been drinking it for less than 12 years, regardless of whether they had skin lesions.	Fujino et al, 2005
Gene Mutations				
Induction of HGPRT mutations (isolated mononuclear cells)	People in Santa Ana (high dose) and Nuevo Leon (low exposure group), Mexico	The high-exposure group of 11 used DW containing a mean of 390 ppb As (98% as As^{V}), and the low exposure group of 13 used DW that ranged from 19 to 60 ppb As. They also considered As concentrations in urine.	The frequency of monocytes resistant to thioguanine (i.e., mutants) was twice as high in the high exposure group, but this suggestion of an ⇑ was not statistically significant.	Ostrosky-Wegman et al., 1991
Hypermethylation of DNA				
Extent of methylation of the promoters of tumor suppressor genes p53 and p16 (relationship to arsenicosis)	West Bengal, India	Criteria for diagnosis of arsenicosis included a history of using DW containing > 50 ppb As for more than 6 months and presence of skin lesions characteristic of chronic arsenic toxicity. Comparisons were made to individuals without skin lesions or those who live in non-arsenic affected areas.	Methylation of the p53 promoter region of DNA obtained from blood samples was studied using methyl-sensitive restriction endonuclease HPAII. Methylation of p16 was studied using bisulfite modification of the DNA followed by methyl sensitive PCR. Hypermethylation of the promoter region of both genes was observed in people with arsenicosis, and there was a positive dose response for this hypermethylation. There was a strong suggestion that the promoter region of p53 is hypermethylated in individuals with As-induced skin cancer in comparison to those with skin cancer unrelated to iAs exposure, but this comparison did not reach statistical significance ($p < 0.2$)	Chanda et al., 2006
Relationship between epigenetic silencing of 3 tumor suppressor genes and exposure to As in patients with bladder cancer	New Hampshire patients with bladder cancer	Estimated internal dose of arsenic exposure from toenail measurements. 18 patients with bladder cancer had ≥ 0.26 ppm As in their toenails, and 318 had < 0.26 ppm As in their toenails. 0.26 ppm was the 95th percentile of As exposure in this population.	They applied methylation-specific PCR. A significant relationship was identified between As exposure and promoter methylation of RASSF1A and PRSS3 but not $p16^{INK4A}$. The promoter hypermethylation was associated with advanced tumor state. Thus the data provide a potential link between arsenic exposure and epigenetic alterations in patients with bladder cancer.	Marsit et al., 2006
Hypomethylation of DNA				

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer				
Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
Extent of methylation of the promoters of tumor suppressor genes p53 and p16 (relationship to arsenicosis)	West Bengal, India	Criteria for diagnosis of arsenicosis included a history of using DW containing > 50 ppb As for more than 6 months and presence of skin lesions characteristic of chronic arsenic toxicity. Comparisons were made to individuals without skin lesions or those who live in non-arsenic affected areas.	Methylation of the p53 promoter region of DNA obtained from blood samples was studied using methyl-sensitive restriction endonuclease HPAII. Methylation of p16 was studied using bisulfite modification of the DNA followed by methyl sensitive PCR. In the study described in the row above, a small number of people with high As exposure showed hypomethylation. Hypomethylation occurs only after prolonged As exposure at higher doses. The authors noted that cases of both hyper- and hypomethylation leading to silencing of tumor suppressor genes and activation of oncogenes have been documented in different types of cancers.	Chanda et al., 2006
Immune System Response				
Association between biomarkers of lung inflammation and level of iAs exposure from DW	Ajo and Tucson, Arizona, USA	40 subjects were from the high-As-exposure town of Ajo (20.3 ± 3.7 ppb As in DW), and 33 were from the low-As-exposure town of Tucson (4.0 ± 2.3 ppb As in DW). They also measured iAs levels in urine, with the mean in Ajo being 2.6 times higher than that in Tucson.	Proteolytic enzymes including MMP-2 and MMP-9 are continually secreted in the airways, and their activities are regulated mainly by TIMP-1. The log-normalized concentrations of these 3 substances in induced sputum were not significantly different between these towns. However, after adjusting for town, asthma, diabetes, urinary MMA/iAs, and smoking history, total urinary As was negatively associated with MMP-2 and TIMP-1 levels and positively associated with the ratio of MMP-2/TIMP-1 and MMP-9/TIMP-1. This suggests an association between changes in sensitive markers of lung inflammation and levels of iAs of only ~20 ppb in DW. It appears that iAs levels in DW and the extent of As methylation may be important predictors of lung metalloproteinase concentrations.	Josyula et al., 2006
Signal Transduction				

Table C-1. *In Vivo* Human Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer				
Topic(s)	**Population sampled**	**Information on exposure levels and durations and on biomarkers**	**Results**	**Reference**
Association between TGF-α and/or EGFR and cumulative iAs exposure from DW	Taiwan	150 persons were selected from the arseniasis endemic area in Ilan county in northeast Taiwan, with 30 each coming from those having residential well water in the following ranges (all in ppb of As): 0-50, >50–100, >100–300, >300–600, and > 600. Of them, the 66 who agreed to participate in medical surveillance were compared to 35 healthy individuals with no known As exposure. Those with As exposure were further divided on the basis of cumulative As dose (i.e., total DW iAs levels x years of exposure) into the following 2 groups: 32 with ≤6 ppm-years and 34 with > 6 ppm-years.	Blood plasma was collected and tested for TGF-α and EGFR levels using immunoassays. No relationship between As exposure and EGFR protein levels was found. However, both levels of plasma TGF-α and the proportion of individuals with TGF-α overexpression were significantly higher in the high cumulative As exposure group than in the control group. After adjusting for age and sex, there was also a significant linear trend between cumulative As exposure and the prevalence of plasma TGF-α overexpression.	Hsu et al., 2006

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Aberrant Gene or Protein Expression						
Lung/mouse (C57BL/6)	As^{III} SA	* 5.8, 28.8 ppm (DW)	8 wk	28.8 ppm	mRNA levels were determined in a microchip analysis and validated using real-time PCR: 29 genes were up-regulated and 42 down-regulated. 15% of affected genes were associated with inflammation, including HSP27 and HSP90 (both up-regulated). Numerous extracellular matrix genes were affected, as reflected in phenotypic lung changes related to the organization of elastin and collagen. Protein levels were determined by a Western blot assay: ⇑ for 4 genes, ⇓ for 14. No correlation was found between altered genes and altered proteins.	Lantz and Hays, 2006
Lung/mouse (C57B16, male, 21 days of age at start of exposure)	As^{III} SA	10, 50 ppb (DW) Note in ppb!	4 wk	50 ppb	Protein levels in BALF determined by proteomic analysis: it is unclear if samples from 10 ppb were examined. ⇑ after dose of 50 ppb: peroxiredoxin-6 and enolase 1. ⇓ after dose of 50: GST-omega-1, RAGE, contraspin, and apolipoproteins A-I and A-IV.	Lantz et al., 2007
Urothelial cells/rat (F344, female)	DMA^{V} sodium cacodylate-trihydrate	* 0.35, 1.4, 14, 35 ppm (DW)	28 days	0.35 ppm	Microarray analysis using chip for 4395 genes: gene trees generated by hierarchical clustering of the 510 responsive genes showed marked changes at every dose in comparison to the dose (or dose of 0) below it. Of the 510 genes, 38% were up-regulated and 9% down-regulated by ≥3-fold. Most affected genes related to the functional categories of apoptosis, cell cycle regulation, adhesion, signal transduction, stress response, or growth factor and hormone receptors. There was a change in the types of genes affected at the different doses, particularly when comparing the higher 2 doses (both cytotoxic) with the 2 non-cytotoxic doses. The dose with most genes affected was 14 ppm. At the lowest dose, 503 genes (11%) were significantly affected, of which 41% were up-regulated and 6% down-regulated by ≥3-fold.	Sen et al., 2005
Liver cells/mouse (129/SvJ)	As^{III} SA	45 ppm (DW)	48 wk	45 ppm	Microarray analysis, RT-PCR, and immunochemistry: big ⇑ in ER-α and cyclin D1 mRNA and protein levels. Of 588 genes tested in microarray analysis, 30 showed aberrant expression, including steroid-related genes, cytokines, apoptosis-related genes, cell cycle-related genes, and genes encoding for growth factors and hormone receptors.	Chen et al., 2004b
Brain, liver, placenta/mouse (only pregnant ICR females drank the water)	As^{III} SA	* 4.35 mg/kg (gavage)	1 time only on each of 9 days, gestation days 7 to 16	4.35 mg/kg	Activities of selenoenzymes GPx, TrxR, DI-I, DI-II, and DI-III in maternal tissues when examined on gestation day 17 of their litter: liver: ⇓ of DI-I to ~0.61x when Se-adequate diet; liver: ⇓ of DI-I to ~0.30x when Se-deficient diet; All other comparisons were either slight or NSE.	Miyazaki et al., 2005

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Fetal brain, fetal liver/mouse (only pregnant ICR females drank the water)	As^{III} SA	* 4.35 mg/kg (gavage)	1 time only on each of 9 days, gestation days 7 to 16	4.35 mg/kg	Activities of selenoenzymes GPx, TrxR, DI-I, DI-II, and DI-III in fetal tissues when examined on gestation day 17: brain: ⇑ of DI-II to ~4.1x when Se-deficient diet liver: ⇓ of TrxR to ~0.78x when Se-deficient diet; All other comparisons were either slight or NSE.	Miyazaki et al., 2005
Liver/mouse (BALB/c, male)	As^{III} SA	50, 100, 150 µg/mouse/day for 6 days/week (gavage)	3, 6, 9, 12 months	50 at 9 and 12 months only	Levels of TNF-α and IL-6: NSE on either one at any dose in first 6 months. At 9 months: TNF-α: 50, ~1.2x; 100, ~1.2x; 150, ~1.4x; IL-6: 50, ~2.0x; 100, ~2.5x; 150, ~2.7x; At 12 months: TNF-α: 50, ~1.9x; 100, ~2.3x; 150, ~3.0x; IL-6: 50, ~2.8x; 100, ~5.7x; 150, ~9.5x	Das et al., 2005
Liver/mouse (BALB/c, male)	As^{III} SA	50, 100, 150 µg/mouse/day for 6 days/week (gavage)	3, 6, 9, 12 months	50 at 9 and 12 months	Concentration of total collagen: At 3 months: NSE at all doses, but hint of ⇑ at 100 (~1.2x) and 150 (~1.3x); At 6 months: NSE at all doses, but hint of ⇑ at 100 (~1.3x) and 150 (~1.4x); At 9 months: 50, ~1.3x; 100, ~1.4x; 150, ~1.6x; At 12 months: 50, ~1.5x; 100, ~1.9x; 150, ~2.1x	Das et al., 2005
Liver, kidney & lung/mouse (B6C3F1, female)	As^{III} SA As^{V} sodium arsenate DMA^{V}	In all cases, dissolved in water and administered once by gavage: * 9.58 mg/kg for all * 9.58 mg/kg for all *391 mg/kg for all	One dose for all	9.58 mg/kg 9.58 mg/kg None	HMOX-1 activity 6 hr after the single oral dose was administered by gavage: Liver: As^{III}, ~7.5x; As^{V}, ~5.1x, DMA^{V}, ~0.96x (NSE); Kidney: As^{III}, ~7.6x; As^{V}, ~3.2x, DMA^{V}, ~1.03x (NSE); Lung: none of the arsenicals induced HMOX-1 activity.	Kenyon et al., 2005b

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver & kidney/mouse (B6C3F1, female)	As^{III} SA	In all cases, dissolved in water and administered once by gavage: * 0.0749, 0.749, 2.25, 7.49 mg/kg for both	One dose	2.25 mg/kg in liver 7.49 mg/kg in kidney	HMOX-1 activity in liver 6 hr after the single oral dose was administered by gavage: at 2 lower doses, NSE; 2.25, ~2.5x; 7.49, ~7.5x; HMOX-1 activity in kidney 4 hr after the single oral dose was administered by gavage: at 3 lower doses, NSE; 7.49, ~3.5x	Kenyon et al., 2005b
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW); report did not state if molecular analysis was done on one or both of these doses combined.	10 days, gestation days 8 to 18	42.5 or 85 ppm	Comparisons of gene expression based on microarray analysis, with comparisons being made between HCC tumors from offspring of exposed dams and normal liver tissue from offspring of unexposed dams: ⇑ of AFP to ~18.5x; ⇓ of IGF-1 to 0.78x; ⇑ of IGFBP-1 to ~8.8x; ⇑ of CK8 to ~2.4x; ⇑ of CK18 to ~8.8x; ⇓ of BHMT to ~0.33x.	Waalkes et al., 2004b
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW); report did not state if molecular analysis was done on one or both of these doses combined.	10 days, gestation days 8 to 18	42.5 or 85 ppm	Comparisons of gene expression based on microarray analysis, with comparisons being made between HCC tumors of offspring of exposed dams and spontaneous liver tumors of offspring of unexposed dams: ⇑ of AFP to ~6.2x; NSE for IGF-1; ⇑ of IGFBP-1 to ~1.7x; ⇑ of CK8 to ~1.4x; ⇑ of CK18 to ~5.8x; ⇓ of BHMT to ~0.36x.	Waalkes et al., 2004b
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW); report did not state if molecular analysis was done on one or both of these doses combined.	10 days, gestation days 8 to 18	42.5 or 85 ppm	Comparisons of gene expression based on microarray analysis, with comparisons being made between HCC tumors and normal-appearing liver cells of offspring of exposed dams: ⇑ of AFP to ~7.4x; ⇓ of IGF-1 to ~0.68x; ⇑ of IGFBP-1 to ~3.7x; ⇑ of CK8 to ~1.3x; ⇑ of CK18 to ~7.0 x; ⇓ of BHMT to ~0.32x.	Waalkes et al., 2004b
Liver cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW); report did not state if molecular analysis was done on one or both of these doses combined.	10 days, gestation days 8 to 18	42.5 or 85 ppm	Comparisons of gene expression based on microarray analysis, with comparisons being made between normal-appearing liver cells in both offspring of exposed dams and unexposed dams: ⇑ of AFP to ~2.5x; ⇑ of IGF-1 to ~1.1x; ⇑ of IGFBP-1 to ~2.4x; ⇑ of CK8 to ~1.8x; NSE for CK18 or BHMT.	Waalkes et al., 2004b
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW); report did not state if molecular analysis was done on one or both of these doses combined.	10 days, gestation days 8 to 18	42.5 or 85 ppm	In general, the results in the 4 previous rows were confirmed by real-time RT-PCR analysis. Aberrant gene expression was also noted in the microarray analysis for numerous other genes including those related to cell proliferation, oncogenes, stress, and metabolism.	Waalkes et al., 2004b

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Uterus/mouse (only pregnant CD1 females drank the water, female offspring only)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm if also treated with DES or TAM	Expression (by real-time RT-PCR) of various estrogen-related genes in uteri at 11 days of age: ⇑ in ER-α to 1.56x. Some female offspring were also exposed by subcutaneous injection to DES on the first 5 days after birth. DES alone or (iAs + DES) did not significantly increase ER-α expression. iAs alone did not ⇑ expression of pS2, CYP2A4, or lactoferrin. However, DES alone caused large ⇑ in expression of all 3 of these genes, and (iAs + DES) caused a further ⇑ to 3.0 times, 7.8 times, and 1.47 times that of DES alone, respectively. These and other results showed that iAs acts with estrogens to enhance production of urogenital cancers in female mice.	Waalkes et al., 2006a
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW)	10 days, gestation days 8 to 18	42.5 ppm	Comparisons of gene expression based on microarray analysis of RNA, with comparisons being made between HCC tumors from offspring of exposed dams and normal (i.e., non-tumorous) liver tissue from offspring of unexposed dams: 13.7% of 600 genes were significantly up-regulated or down-regulated. Only 7.7% of those 600 genes were similarly affected in spontaneous tumors in liver tissue from offspring of unexposed dams. The 600 genes studied included oncogenes and genes associated with cell proliferation, differentiation, or otherwise related to cancer outcome.	Liu et al., 2004
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW)	10 days, gestation days 8 to 18	42.5 ppm	Comparisons of gene expression based on microarray analysis of RNA (see row above): Up-regulated genes included oncogene/tumor suppressor genes and genes related to cell proliferation, hormone receptors, metabolism, stress, apoptosis, growth arrest, and DNA damage. A wide array of different types of genes was also down-regulated. Real-time RT-PCR analysis largely confirmed the findings of microarray analysis. The higher dose tended to yield more significant differences, but a positive dose response was not always evident.	Liu et al., 2004
Liver cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	42.5, 85 ppm (DW)	10 days, gestation days 8 to 18	42.5 ppm	Comparisons of gene expression based on microarray analysis of RNA, with comparisons being made between non-tumorous liver cells in both offspring of exposed dams and unexposed dams: ~10% of 600 genes were significantly up-regulated or down-regulated. The 600 genes studied included oncogenes and genes associated with cell proliferation, differentiation, or otherwise related to cancer outcome.	Liu et al., 2004

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
HCC cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Comparisons of gene expression based on microarray analysis of RNA, with comparisons being made between HCC tumors from offspring of exposed dams and normal liver tissue from offspring of unexposed dams: statistically significant alterations in expression were seen for 2,540 genes. Real-time RT-PCR and Western blot analyses of selected genes or proteins showed >90% concordance. Affected gene expression included oncogenes, HCC biomarkers, cell proliferation-related genes, stress proteins, insulin-like growth factors, estrogen-linked genes, and genes involved in cell-cell communication.	Liu et al., 2006c
Liver cells/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Comparisons of gene expression based on microarray analysis of RNA, with comparisons being made between non-tumorous liver cells in both offspring of exposed dams and unexposed dams: statistically significant alterations in expression were seen for 2010 genes. See row above for results in HCC cells.	Liu et al., 2006c
Fetal livers/mouse (only pregnant C3H females drank the water, male offspring)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Comparisons of gene expression based on microarray analysis of RNA from fetal livers just after treatment ended, with confirmation by real-time RT-PCR: alteration of expression of 187 genes (of 22,000 in array) was demonstrated, with ~25% of them being related to either estrogen signaling or steroid metabolism—some with dramatic (here meaning >>100x) up-regulation. Expression of some genes important in methionine metabolism was suppressed.	Liu et al., 2007a
Livers of newborn males/mouse (only pregnant C3H females drank the water)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Comparisons of gene expression based on microarray analysis of RNA from livers of newborn males, with confirmation by real-time RT-PCR: among 600 genes examined, marked alteration of expression of 40 genes was demonstrated. Affected genes included genes related to stress (several in the glutathione system), metabolism (several cytochrome P450 genes), growth factors (several insulin-like growth factor genes), and hormone metabolism.	Xie et al., 2007

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver and liver tumors/mouse (only pregnant C3H females drank the water)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	Samples from adults of both sexes were tested. Some had had a post-weaning 21-wk dermal treatment with TPA. Comparisons with the TPA-treatment-only control were made regarding gene expression based on microarray analysis of RNA, with confirmation by real-time RT-PCR. Alteration of expression of ~70 genes (of 588 in array) was demonstrated. There were generally similar gene alteration patterns in both sexes both in iAs/TPA exposed non-tumorous livers and in iAs/TPA-induced tumors. The tumors themselves generally had more pronounced alterations in gene expression than the normal tissue around them. In general, the iAs/TPA induced gene expression alterations were similar to those seen in liver samples from male mice exposed only to iAs *in utero.* It should be noted that while *in utero* iAs-exposed males developed hepatocellular carcinoma without the TPA treatment, *in utero* iAs-exposed females only developed those tumors after TPA treatment.		Liu et al., 2006b
Bladder and liver/rat (Fisher 344, male)	MMA^V DMA^V TMA^VO	* 121 ppm (DW)[3] * 109 ppm (DW)[3] * 110 ppm (DW)[3]	20 days for all	Changes in gene expression observed in cDNA microarray analysis: MMA^V caused ⇑ for 20 genes and ⇓ for 1 gene in liver and ⇑ for 5 genes and ⇓ for 5 genes in bladder. DMA^V caused ⇑ for 15 genes and ⇓ for 2 genes in liver and ⇑ for 13 genes and ⇓ for 4 genes in bladder. TMA^VO caused ⇑ for 23 genes and ⇓ for 2 genes in liver and ⇑ for 6 genes and ⇓ for 7 genes in bladder. Groups of genes affected by all arsenicals in both tissues included genes related to xenobiotic metabolism, growth factor receptors, and energy metabolism. In the liver, phase I and II metabolizing enzymes were induced to a lesser extent by MMA^V and DMA^V than by TMA^VO, and in the bladder they were induced only by DMA^V. CYP1A1 was only overexpressed by TMA^VO and in liver.		Kinoshita et al., 2007a
Lung/mice ($C57BL/6J\ Ogg1^{+/+}$ wt mice and $Ogg1^{-/-}$ knockout mice, both sexes, 14 weeks old at start of treatment)	DMA^V	* 115.3 ppm (DW)	4 weeks	Results of an Affymetrix oligonucleotide microarray analysis: a change in expression was found for 165 and 182 genes in male and female knockout $Ogg1^{-/-}$ mice, respectively. In DMA^V-treated knockout $Ogg1^{-/-}$ mice, there was marked induction of Pola1, Cyp7b1, Ndfua3, Mmp13 and other genes specific to cell proliferation, cell signaling, and xenobiotic metabolism.		Kinoshita et al., 2007b
Liver cells/rat (Sprague Dawley)	As^V as $Na_2HAsO_4 \cdot 7H_2O$	* 0.24, 2.4, 24 ppm (DW)	1 month	Various	Determination of mRNA levels of cancer-related genes using real-time quantitative RT-PCR: ⇑ cyclin D1 at 2.4 only; ⇑ $p27^{Kip1}$ at 2.4 only; ⇑ ILK at 0.24 only; ⇓ PTEN at 0.24 only; ⇓ β-catenin at 24 only.	Cui et al., 2004b

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver cells/rat (Sprague Dawley)	As^V as $Na_2HAsO_4 \cdot 7H_2O$	* 0.24, 2.4, 24 ppm (DW)	4 months	Various	Determination of mRNA levels of cancer-related genes using real-time quantitative RT-PCR: ⇑ cyclin D1 at 24 only; ⇑ ILK at 0.24 and 2.4; ⇑ $p27^{Kip1}$ at 0.24 only; ⇓ PTEN at all doses; ⇓ β-catenin at all doses. Results were confirmed by protein levels. (Histograms were assumed to be correct for ILK and p27; the descriptions for them appear to have become reversed in the text.)	Cui et al., 2004b
Skin/mouse (homozygous, strain Tg.AC, female)	As^{III} SA	200 ppm (DW)	4, 10 wk	200 ppm	Kinetics of mRNA expression based on RT-PCR: EGFR and TNF-α: ⇑ by week 10; GM-CSF and TGF-α: ⇑ by week 4; big ⇑ by week 10; c-myc: NSE.	Germolec et al., 1998
Heart/mouse (C57BL/6NCr, male)	As^{III} SA	* 28.8, 144, 288 ppb (DW) Note in ppb!	5, 10, 20 wk	Various	mRNA levels determined by RT-PCR: $VEGF_{165}$: ⇑ at 144 and 288 at wk 5; ⇑ at all doses at wk 10; NSE at wk 20; VEGFR1: NSE at wk 5 and 10; big ⇑ at 144 and big ⇓ at 288 at wk 20; VEGFR2: ⇑ at 288 at wk 5; NSE at wk 10; ⇑ at 28.8 and 144 and ⇓ at 288 at wk 20; PAI-1: NSE at wk 5; ⇑ at 288 at wk 10; ⇑ at 144 and 288 at wk 20; Endothelin-1: NSE at wk 5 and 10; ⇑ at 28.8 and big ⇑ at 144 at wk 20; MMP-9: NSE at wk 5; ⇑ at 288 at wk 10; ⇑ at all doses at wk 20	Soucy et al., 2005
Blood plasma/mouse (C57BL/6NCr, male)	As^{III} SA	* 288 ppb (DW) Note in ppb!	20 wk	288 ppb	PAI-1 protein levels determined by ELISA assay: ⇑ to ~1.33x	Soucy et al., 2005
Tumors that developed from B16-F10 (GFP) melanoma tumor cells/ mice (NCr nu/nu, male)	As^{III} SA	* 5.8, 28.8, 115 ppb (DW) Note in ppb!	9 wk	5.8 ppb	Protein levels in primary melanoma tumors determined by immunohistochemical staining: ⇑ HIF-1α at 5.8 and 28.8 only; ⇑ VEGF at 5.8 and 115 only. ⇑ for both proteins was just locally around tumor blood vessels. Western blot assay of whole tumor lysates showed no more than barely detectable ⇑ of HIF-1α at any dose.	Kamat et al., 2005
Apoptosis						
Bladder and liver/rat (Fisher 344, male)	MMA^V DMA^V TMA^VO	* 121 ppm (DW)[3] * 109 ppm (DW)[3] * 110 ppm (DW)[3]	5, 10, 15, and 20 days for all	None Various Various	Apoptosis labeling index based on an immunochemistry method of staining single-stranded DNA: Bladder: ⇑ on day 20 to ~1.5x for DMA^V only; Liver: ⇑ on day 20 to ~3.3x for TMA^VO only	Kinoshita et al., 2007a

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver/rat (Wistar, male)	As^{III} SA	* 0.03, 1.4, 2.9 ppm (DW)	60 days	1.4	Induced apoptosis (experimental – control) based on TUNEL assay with PI staining and analysis using fluorescence microscopy: 0.03, 5.0; (NSE); 1.4, 14.9; 2.9, 22.3; these results were consistent with DNA ladder formation found by agarose gel electrophoresis for which there was an ⇑ at 1.4; bigger ⇑ at 2.9. There was also microscopic evidence of cell death by necrosis.	Bashir et al., 2006a
Kidney, leukocytes and liver/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	30 days	57.7 ppm	TNF-α levels: kidney, ⇑ ~1.6x; leuko., ⇑ ~2.2x; liver, ⇑ ~1.9x; caspase-3 levels: kidney, ⇑ ~3.2x; leuko., ⇑ ~2.8x; liver, ⇑ ~3.5x; effects on both endpoints in all 3 tissues were markedly reduced by co-treatment with AA and/or α-Toc.	Ramanathan et al., 2005
Kidney, leukocytes and liver/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	30 days	57.7 ppm	Induced percentage of DNA that was fragmented (experimental – control): kidney, ⇑ ~17.6%; leuko., ⇑ ~17.4%; liver, ⇑ ~21.8%; Induced percentage of TUNEL positive cells (experimental – control): kidney, ⇑ ~6.7%; leuko., ⇑ ~5.1%; liver, ⇑ ~8.1%; effects on both endpoints in all 3 tissues were markedly reduced by co-treatment with AA and/or α-Toc. Confirmation of induced apoptosis in leukocytes shown by finding typical DNA ladders after agarose gel electro-phoresis; co-treatment with AA and/or α-Toc abolished that effect.	Ramanathan et al., 2005
Splenocytes and thymocytes/mouse (C57BL/6, female)	As^{V} as $Na_2HAsO_4 \cdot 7H_2O$	0.5, 5, 50 ppm (DW)	8, 12 wk	50 at 8 wk for both cell types	Induced apoptosis (experimental – control) determined by TUNEL method: splenocytes: 8 wk: 0.14% of cells at dose of 50 (or 6.6x); 12 wk: 0.22% of cells at dose of 50 (or 5.4x). Thymocytes: 8 wk: 0.40% of cells at dose of 50 (or 4.0x); 12 wk: 0.28% (NSE) of cells at dose of 50 (or 2.5x). For both cell types, the data suggested a positive dose response across all doses; however, the other results showed much variability.	Stepnik et al., 2005

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Brain and liver/rat (Wistar, male)	As^{III} SA	* 3.6, 6.1, 7.3 mg/kg (gavage, with animals being killed 24 hr later for sample collection)	One dose	Various	Brain: caspase-3 activity: ⇑ to ~1.4x (NSE) at 3.6, to ~2.0x at 6.1, and to ~2.6x at 7.3; Liver: caspase-3 activity: ⇑ to ~1.8x at 3.6, to ~2.5x at 6.1, and to ~3.0x at 7.3. Both brain and liver: agarose gel electrophoresis showed DNA "nucleosomal ladder", suggesting induction of apoptosis; results were not quantified. Histopathological examination also showed evidence of cellular necrosis.	Bashir et al., 2006b
Cancer Promotion						
Skin/mouse (homozygous, strain Tg.AC, female)	As^{III} SA	200 ppm (DW)	14 wk	200 ppm	After low-dose application of TPA on 4 occasions over 2 weeks starting after 31 days of iAs exposure, there was a marked ⇑ in the number of skin papillomas compared to single treatments, whereas no papillomas developed in iAs-treated Tg.AC mice without TPA treatment or in FVB/N mice with the combined treatment. Injection of neutralizing antibodies to GM-CSF after TPA application reduced the number of papillomas in Tg.AC mice. iAs acted like a co-promoter.	Germolec et al., 1998
Skin/mouse (hairless swiss-bald strain, male)	As^{V} sodium arsenate	* 11.4 ppm (DW)	25 wk	None, but 11.4 ppm if also treated with DMBA	PCNA protein levels determined by Western blotting: no PCNA was present following the iAs treatment alone, compared to the baseline of 22 units of PCNA in the control (set equal to x). When mice were given 4 DMBA treatments (as an initiating carcinogen) during the first 2 weeks of the iAs treatment, there was PCNA ⇑ to ~5.3x. DMBA treatment alone caused ⇑ to only 2.9x. Mice that were untreated or treated with iAs alone developed no papillomas or skin tumors. DMBA treatment alone induced development of squamous cell papillomas. Combined iAs and DMBA treatment caused development of well differentiated squamous cell carcinomas. iAs acted as a skin tumor promoter by promoting abnormal cell proliferation. Findings suggest that iAs is toxic to normal skin cells and that preneoplastic cells are more resistant to iAs.	Motiwale et al., 2005

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Lung/mouse (ddY, male)	DMA^V assumed to be dimethylarsinic acid	* 217 ppm (DW)	25 wk	217 ppm, but only following 4NQO treatment	Some of the mice were subcutaneously injected with 10 mg/kg of 4NQO just before the 25-wk DMA treatment began. Some of the mice ate only feed containing 0.05% of the antioxidant EGCG. Number out of 10 mice in each group bearing tumors: control, 0; DMA alone, 0; 4NQO alone, 7; EGCG alone, 0; (4NQO + DMA), 10; (4NQO + DMA + EGCG), 7. That last group had only 0.89 tumor/mouse compared to 3.10 tumors/mouse in 4NQO group and 4.00 tumors/mouse in the (4NQO + DMA) group.	Mizoi et al., 2005
Cell Cycle Arrest or Reduced Proliferation						
Heart/mouse (C57BL/6NCr, male)	As^{III} SA	* 288 ppb (DW) Note in ppb!	5, 10, 20 wk	288 ppb at 20 wk	Density of microvessels of < 12 µm diameter using histopathology and a digital-imaging subroutine: ⇓ to ~0.82x at 20 wk; hint of a ⇓ at 10 wk	Soucy et al., 2005
Cell Proliferation Stimulation						
Bladder/rat (F344, female)	DMA^V	* 54.3 ppm (food) (assumes MW of chemical used was 138.0)	2 wk	54.3 ppm	Stimulation of proliferation determined by BrdU labeling assay: ⇑ to 3.9x; co-treatment with DMPS (a chelator of trivalent arsenicals) completely eliminated the effect.	Cohen et al., 2002
Bladder/rat (F344, female)	DMA^V	* 54.3 ppm (food) (assumes MW of chemical used was 138.0)	26 wk	54.3 ppm	Stimulation of proliferation determined by BrdU labeling assay: ⇑ to 1.6x; co-treatment with DMPS (a chelator of trivalent arsenicals) completely eliminated the effect. Histological examination showed simple hyperplasia in 4 of 9 rats; compared to in 0 of 10 rats in control and in 1 of 10 rats with co-treatment with DMPS.	Cohen et al., 2002
Liver/rat (Fischer 344, male) They used normal-appearing tissue.	TMA^VO	* 27.5, 110.2 ppm (DW) Estimated total intakes: 351 and 1363 mg As/rat.	104 wk	110.2 ppm	Livers were stained for the analysis of PCNA by an immunohistochemical method, with the PCNA index being the number of positive cells/100 cells : ⇑ in PCNA index to 2.0x, thereby suggesting that cell proliferation in the normal-appearing parenchyma was elevated. The point estimate of the index was also ⇑ at lower dose, but the SE for it was large.	Shen et al., 2003
Bladder and liver/rat (Fisher 344, male)	MMA^V DMA^V TMA^VO	* 121 ppm (DW)[3] * 109 ppm (DW)[3] * 110 ppm (DW)[3]	5, 10, 15, and 20 days for all	None Various Various	PCNA labeling index based on an immunochemistry method: Bladder: ⇑ on day 20 to ~1.8x for DMA^V only; Liver: ⇑ on day 20 to ~1.8x for TMA^VO only	Kinoshita et al., 2007a

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Bladder/mouse (only pregnant CD1 females drank the water, male offspring only)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm if also treated with DES or TAM	Some male offspring were also exposed by subcutaneous injection to DES or TAM on the first 5 days after birth; all male offspring were held for 90 wk before examination. Induced (i.e., experimental – control) % of mice with bladder hyperplasia: iAs alone, 9% (NSE); DES alone, 12% (NSE); TAM alone, 10% (NSE); (iAs + DES), 45%; (iAs + TAM), 30%. All induced percentages were the same for total proliferative lesions, except for (iAs + TAM), which was 40%. The lesions induced by iAs with either DES or TAM overexpressed ER-α.	Waalkes et al., 2006b
Kidney/mouse (only pregnant CD1 females drank the water, male offspring only)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Some male offspring were also exposed by subcutaneous injection to DES or TAM on the first 5 days after birth; all male offspring were held for 90 weeks before examination. Induced (i.e., experimental – control) % of mice with cystic tubular hyperplasia: iAs alone, 23%; DES alone, 0%; TAM alone, 0%; (iAs + DES), 24%; (iAs + TAM), 7%.	Waalkes et al., 2006b
Bladder/mouse (only pregnant CD1 females drank the water, female offspring only)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm if also treated with DES or TAM	Some female offspring were also exposed by subcutaneous injection to DES or TAM on the first 5 days after birth; all female offspring were held for 90 wk before examination. Induced (i.e., experimental – control) % of mice with bladder hyperplasia: iAs alone, 12% (NSE); DES alone, 0% (NSE); TAM alone, -3% (NSE); (iAs + DES), 26%; (iAs + TAM), 23%. All induced percentages were the same for total proliferative lesions, except for (iAs + DES), which was 35%, and (iAs + TAM), which was 26%. Unlike in the male offspring, iAs did not induce hyperplasia in kidneys.	Waalkes et al., 2006a
Lung/mice ($C57BL/6J\ Ogg1^{+/+}$ wt mice, both sexes, 14 weeks old at start of treatment) Lung/mice ($C57BL/6J\ Ogg1^{-/-}$ knockout mice, both sexes, 14 weeks old at start of treatment)	DMA^{V}	* 115.3 ppm (DW)	72 weeks	None 115.3	PCNA labeling index based on an immunochemistry method, x = wt control level: wt with iAs treatment: ⇑ to ~3x (NSE); knockout $Ogg1^{-/-}$ without iAs: ⇑ to ~6x; knockout $Ogg1^{-/-}$ with iAs treatment: ⇑ to ~17x. Results were confirmed in a study with only a 4 week exposure.	Kinoshita et al., 2007b
Bladder/mouse (C57BL/6, female)	As^{III} SA	* 57.7 ppm (DW)	4 wk	57.7 ppm	All experimental mice developed mild hyperplasia of the urinary bladder epithelium, that being a 3- to 4-fold ⇑ in the thickness of the transitional cell layer.	Simeonova et al., 2000

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Bladder/mouse (C57BL/6, female)	As^{III} SA	* 57.7 ppm (DW)	16 wk	57.7 ppm	⇑ in PCNA-stained nuclei in the bladder epithelium from 2% in control to 31% in experimental group, an indication of big ⇑ in cell proliferation. Similar ⇑ also seen at 4 weeks.	Simeonova et al., 2000
Bladder/mouse (C57BL/6, female)	As^{III} SA	* 11.5, 57.7 ppm (DW)	16 wk	11.5 ppm	Also consistent with ⇑ in proliferation: ⇑ in DNA binding of the AP-1 transcription factor to ~1.9x and ~4.7x at the 2 doses, respectively. At one or both doses (not specified): 38% and 76% of the bladder cells stained positive for the c-jun and c-fos immunoreactive proteins, respectively, compared to only 2% in control mice.	Simeonova et al., 2000
Blood vessels/chicken (Leghorn, chorioallantoic membranes of 10-day-old chicken embryos)	As^{III} SA	0.00033, 0.001, 0.0033, 0.01, 0.033, 0.1, 0.33, 1.0, 3.3, 10 µM	24 hr	0.033 µM	CAM assay to determine vascularity (i.e, blood vessel density): ⇑ to ~2.2x and remained at about that level to dose of 1; ⇓ to ~0.28x at dose of 3.3 and remained at about that level to dose of 10.	Soucy et al., 2003
Matrigel implants/mouse (C57BL/6NCr, male)	As^{III} SA	* 0.6, 2.9, 5.8, 28.8 ppb (DW) Note in ppb!	5 wk	0.6 ppb	Blood vessel no. determined in Matrigel implants surgically inserted during last 2 wk of iAs treatment: probable ⇑ to ~1.8x at dose of 0.6; statistically significant ⇑ to ~2.4x at the higher 3 doses. Implants were supplemented with recombinant FGF-2; iAs-enhanced neovascular-ization did not occur without FGF-2. Data suggest that iAs potentiates, but does not directly cause, neovascularization in Matrigel implants.	Soucy et al., 2005
Matrigel implants/mouse (C57BL/6NCr, male)	As^{III} SA	* 28.8, 144, 288 ppb (DW) Note in ppb!	5, 10, 20 wk	28.8 ppb for each duration	Blood vessel number determined in Matrigel implants surgically inserted during last 2 wk of iAs treatment: at 5 wk: ⇑ to ~2.6x, ~4.4x, and ~5.5x at the 3 doses in ascending order. For each longer duration treatment, there was still a strong ⇑ at dose of 28.8 but a somewhat diminished ⇑ at 2 higher doses.	Soucy et al., 2005
Tumors that developed from B16-F10 (GFP) melanoma tumor cells/mice (NCr nu/nu, male)	As^{III} SA	* 5.8, 28.8, 115 ppb (DW) Note in ppb!	8 wk	5.8 ppb	Tumor volume and tumor growth rate, after implantation of tumor cells (into external surface at the base of right ear) 5 wk after iAs treatment began: Volume: 5.8, ~1.4x (NSE); 28.8, ~2.2x; 115, ~3.0x; Rate: 5.8, ~1.9x (NSE); 28.8, ~2.2x; 115, ~3.2x.	Kamat et al., 2005
Tumors that developed from B16-F10 (GFP) melanoma tumor cells/mice (NCr nu/nu, male)	As^{III} SA	* 5.8, 28.8, 115 ppb (DW) Note in ppb!	8 wk	5.8 ppb	Mean no. of lung metastases/lobe, after implantation of tumor cells (into external surface at the base of right ear) 5 wk after iAs treatment began: 5.8, ~1.6x; 28.8, ~2.0x; 115, ~2.0x (statistically significant at 5.8 and 115); the metastases were significantly larger at the 2 lower doses.	Kamat et al., 2005

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Blood vessels/chicken (Leghorn, chorioallantoic membranes of 10-day-old chicken embryos)	As^{III} SA	0.33, 10 µM	48 hr	0.33 µM	CAM assay to determine vascularity (i.e, blood vessel density): ⇑ to ~1.8x at 0.33 but big ⇓ at dose of 10. At dose of 0.33, co-treatment with YC-1 or SU5416 (inhibitors of HIF and VEGF receptor-2 kinase) eliminated iAs effect. 10µM iAs + YC-1 caused no change from control, but iAs alone, or in addition to SU5416, resulted in ⇓ to ~0.28x.	Kamat et al., 2005
Skin/mouse (homozygous, strain Tg.AC, female)	As^{III} SA	200 ppm (DW)	10 wk	200 ppm	By 10 weeks the skin showed hyperkeratosis as well as ⇑ in numbers of proliferating cells. A kinetic study with samples at weekly intervals demonstrated ⇑ in number of BrdU-positive nuclei in skin after 4 weeks and number remained elevated through 10 weeks.	Germolec et al., 1998
Chromosomal Aberrations and/or Genetic Instability						
Bone marrow/rat (*Rattus norvegicus*, Charles foster strain)	As^{V} as disodium hydrogen arsenate	* 4.0 mg As/kg bw (unspecified route of administration)	15, 21 days	4.0 mg/kg	Chromosomal analysis of Giemsa-stained cells, with few details provided: induction of gross CAs for both periods of treatment; induction of hyperploidy detected as aneuploids for longer treatment	Datta et al., 1986
Bone marrow/mouse (albino Swiss, male)	As^{III} SA	* 1.44 mg/kg x 4, 5, and 6 times at weekly intervals, (gavage)	Single dose each week	1.44 x 4	Significant ⇑ in CA and probably also in polyploidy after 4, 5, and 6 gavage treatments. CA frequencies were significantly higher than control in all 3 comparisons at 2.5x, 2.7x, and 4.4x, respectively. Similar experiments with 7 and 8 exposures killed the mice. Daily treatments by gavage with a black tea infusion for one week before every iAs treatment caused a significant reduction in the frequency of CAs after 4 and 6 iAs treatments.	Patra et al., 2005
Bone marrow/mouse (C57BL/6J/Han, female)	As^{V} as $Na_2HAsO_4 \cdot 7H_2O$	50, 200, 500 ppb (DW) Note in ppb!	3, 6, 12 months	None	Half of the mice were maintained on a low-Se diet. Mouse erythrocyte MN test: iAs caused no induction of MN in PCEs and no change in the PCE:NCE ratio at any dose at any interval, with or without the low-Se diet.	Palus et al., 2006
Co-carcinogenesis						
Skin/mouse (Hairless mice, strain Skh1)	As^{III} SA	* 0.7, 1.4, 2.9, 5.8 ppm (DW)	161 days beginning at 21 days of age	0.7 ppm	Starting 21 days after the As^{III} treatments began, mice had their dorsal skin exposed to 1.0 kJ/m^2 of solar spectrum UV (a low nonerythemic dose) 3 times weekly. Untreated control mice and iAs-treated mice unexposed to UV developed no skin tumors. Of mice exposed to UV, skin tumor yields per mouse at the different doses of iAs were as follows: 0, 2.40; 0.7, 5.40; 1.4, 7.21; 2.9, 11.10; 5.8, 6.80. More than 95% of tumors were squamous cell carcinomas. Mice in all dose groups exposed to UV and iAs showed a 2.5-3x ⇑ in epidermal hyperplasia above that caused by UV alone, with the highest point estimate at 0.7.	Burns et al., 2004

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Skin/mouse (Hairless CrL:SK1-hrBD, female, weanling) Starting 3 wk after iAs treatment began; mice were irradiated thrice weekly with UV at a dose of 1.0 kJ/m^2 (i.e., ~30% of MED)	As^{III} SA	* 2.9 ppm (DW)	29 wk	2.9 ppm	Immunohistological determination of oxidative DNA damage shown by staining of 8-oxo-dG: Control: no effect; UV alone: very slight ⇑; iAs alone at 5.8 ppm (earlier experiment): ⇑; iAs + UV (this experiment): huge ⇑; Co-treatment with vitamin E or p-XSC: ⇑ (i.e., a significant reduction in iAs + UV effect). Above effects roughly paralleled those for SCC induction, except that no tumors were caused by As alone.	Uddin et al., 2005
Co-mutagenesis						
Skin/mouse (F_1 offspring from cross of FVB/N carrying G11 PLAP transgene x C57BL/6J, both sexes)	As^{III} SA	* 5.8 ppm (DW)	10 wk	None, but 5.8 ppm if co-treatment with B[α]P	Frequencies of induction of $PLAP^+$ cells (result from frameshift mutations) in (A) untreated control, (B) group with iAs treatment alone, (C) group with skin painting with B[α]P 5 days/week during weeks 3-10 after start of experiment, and (D) group with both B & C: A = x; B, ~1.9x, was a NSE; C, ~3.2x, was a NSE; D, ~10.7x. Also, significantly more of the individual mutations arose as clusters in group D, which suggests that more mutations arose in stem cells. This assay in bladder, spleen, lung, kidney, and liver yielded no obvious effect. Oxidation of guanosines in poly G tracts of G:C basepairs is thought to be one cause of these frameshift mutations.	Fischer et al., 2005
Cytotoxicity						
Bladder/rat (F344, female)	DMA^V	* 54.3 ppm (food) (assumes MW of chemical used was 138.0)	2 wk	54.3 ppm	Evidence of cytotoxicity by SEM as frequency of class-5 bladders, which showed necrosis and piling up of rounded urothelial cells: 6 of 10 rats, compared to 0 of 10 in control. In group with co-treatment with DMPS (a chelator of trivalent arsenicals), only 1 in 10 rats had a class-1 bladder. In another experiment with the same dose for 26 weeks, none of the rats had class-5 bladders.	Cohen et al., 2002

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Urothelium/rat (F344, female)	DMA^V as sodium cacodylate-trihydrate	* 0.35, 1.4, 14, 35 ppm (DW)	28 days	14 ppm	By light and transmission electron microscopy, no alterations were detected at lower 2 doses. At higher 2 doses, urothelial cells showed signs of swelling, appearance of cytoplasmic vacuoles and a decreased number of mitochondria (all being signs of cytotoxicity), with a positive dose response.	Sen et al., 2005
DNA Damage						
Liver/rat (Fischer 344, male) They used normal-appearing tissue.	TMA^VO	* 27.5, 110.2 ppm (DW) Estimated total intakes: 351 and 1363 mg As/rat.	104 wk	110.2 ppm	8-OHdG formation assessed by HPLC: ⇑ to ~1.22x; point estimate was also ⇑ at lower dose, but the SE for it was large.	Shen et al., 2003
Lung/mice ($C57BL/6J\ Ogg1^{+/+}$ wt mice, both sexes, 14 weeks old at start of treatment) Lung/mice ($C57BL/6J\ Ogg1^{-/-}$ knockout mice, both sexes, 14 weeks old at start of treatment)	DMA^V	* 115.3 ppm (DW)	72 weeks	None 115.3	8-OHdG formation assessed by HPLC, x = level of wt control: wt with iAs treatment: ⇑ to ~1.6x (NSE); knockout $Ogg1^{-/-}$ without iAs: ⇑ to ~7.8x; knockout $Ogg1^{-/-}$ with iAs treatment: ⇑ to ~13.1x;	Kinoshita et al., 2007b
Peripheral blood leukocytes/mouse (C57BL/6J/Han, female)	As^V as $Na_2HAsO_4 \cdot 7H_2O$	50, 200, 500 ppb (DW) Note in ppb!	3, 6, 12 months	50 ppb	Half of the mice were maintained on a low Se diet. Alkaline SCGE (comet assay) was used to detect DNA fragmentation (SSBs) and alkaline labile sites as well as oxidative DNA base damage identified by using FPG and EnIII enzymes. The only significant iAs effects were seen at 3 months, perhaps because water consumption (and thus iAs consumption) was lower at the last 2 times sampled. An ⇑ in DNA fragmentation was observed only in the mice with the low-Se diet, but there was no positive dose response. An ⇑ in oxidative DNA damage was observed only in the mice with the normal-Se diet, and again there was no positive dose response.	Palus et al., 2006
Lung/mouse (ddY, male)	DMA^V assumed to be dimethylar-sinic acid	* 217 ppm (DW)	4 wk	217 ppm	8-oxo-dG levels: ⇑ to 1.42x; subcutaneous injection of 10 mg/kg 4NQO just before 4-wk DMA treatment had no significant effect on this level; it was 1.38x. Use of feed containing 0.05% of the antioxidant EGCG was tested. 8-oxo-dG level in the (4NQO + DMA + EGCG) group was only 1.09x.	Mizoi et al., 2005

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver/rat (Fisher 344, male)	MMA^V DMA^V TMA^VO	* 121 ppm (DW)[3] * 109 ppm (DW)[3] * 110 ppm (DW)[3]	5, 10, 15, and 20 days for all	None None 110 ppm	8-OHdG formation assessed by HPLC: TMA^VO: ⇑ on day 15 to ~1.5x and on day 20 to ~1.82x	Kinoshita et al., 2007a
Bladder/rat (Fisher 344, male)	MMA^V DMA^V TMA^VO	* 121 ppm (DW)[3] * 109 ppm (DW)[3] * 110 ppm (DW)[3]	20 days for all	None 109 ppm None	8-OHdG formation assessed by HPLC: DMA^V: ⇑ to ~1.62x	Kinoshita et al., 2007a
Effects Related to Oxidative Stress (ROS)						
Brain, liver, RBCs/rat (Wistar, male)	As^{III} as SA	* 57.7 ppm (DW)	12 wk	57.7 ppm	In liver and brain: ⇓ GSH levels; ⇑ GSSG levels; ⇑ MDA levels; In RBCs: ⇓ GSH levels; ⇓ ALAD levels; ⇑ MDA levels; Some, but not all, of these effects were mitigated by oral post-treatment with NAC and/or DMSA.	Flora, 1999
Liver/rat (Fisher 344, male)	MMA^V DMA^V TMA^VO	* 121 ppm (DW)[3] * 109 ppm (DW)[3] * 110 ppm (DW)[3]	5, 10, 15, and 20 days for all	None 109 ppm 110 ppm	Oxidative stress in microsomes shown by elevation of total cytochrome P450 content and/or by ⇑ in hydroxyl radical levels: DMA^V for P450: ⇑ on day 10 only to ~1.14x; DMA^V for OH radicals: ⇑ on day 15 only to ~1.18x; TMA^VO for P450: ⇑ on days 10-20, maximum ⇑ on day 15 to ~1.25x; TMA^VO for OH radicals: ⇑ on days 15 & 20, maximum ⇑ on day 20 to ~1.33x	Kinoshita et al., 2007a
Kidney and liver/rat (Wistar, female)	As^{III} ATO	* 30.3 mg/kg, 15 times (gavage)	Every other day for 30 days	30.3 mg/kg x 15	Kidney: MDA level ⇑ to 3.8x; GSH level ⇓ to 0.78x; GSSG level ⇑ to 7.5x; GST activity ⇓ to 0.44x; Liver: MDA level ⇑ to 2.0x; GSSG level ⇑ to 5.3x; GST activity ⇓ to 0.52x; co-treatment with L-ascorbate reduced the size of the iAs-induced effects (either ⇑ or ⇓) on all 4 endpoints in kidneys and on all but GSH in livers.	Sohini and Rana, 2007

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Kidney and liver/rat (Wistar, male)	As^{III} ATO	* 30.3 mg/kg, 15 times (gavage)	Every other day for 30 days	30.3 mg/kg x 15	Kidney: MDA level ⇑ to 3.4x; GSH level ⇓ to 0.62x; GSSG level ⇑ to 8.5x; GST activity ⇓ to 0.49x; Liver: MDA level ⇑ to 2.7x; GSH level ⇓ to 0.82x; GSSG level ⇑ to 5.9x; GST activity ⇓ to 0.49x; co-treatment with L-ascorbate reduced the size of the iAs-induced effects (either ⇑ or ⇓) on all 4 endpoints in kidneys and on all but GSH in livers.	Sohini and Rana, 2007
Blood, kidney, liver/mouse (albino Swiss, male)	As^{III} SA	* 57.7 ppm (DW)	8 wk	57.7	Blood: ALAD activity ⇓ to 0.32x; GSH level ⇓ to 0.78x; ROS level ⇑ to 2.82x; Kidney: SOD activity ⇓ to 0.38x; CAT activity ⇓ to 0.34x; TBARS level ⇑ to 1.17x; GSH level ⇓ to ~0.39x; GSSG level ⇑ to ~2.5x; GPx activity ⇓ 0.94x (NSE); Liver: SOD activity ⇓ to 0.33x; CAT activity ⇓ to 0.54x; TBARS level ⇑ to 1.25x; GSH level ⇓ to ~0.44x; GSSG level ⇑ to ~3.1x; GPx activity ⇓ 0.76x (NSE); G-6-P activity ⇓ to ~0.73x	Mittal and Flora, 2006
Liver/rat (Wistar, male)	As^{III} SA	* 0.03, 1.4, 2.9 ppm (DW)	60 days	Various	Cytochrome P450 activity: ⇑ to 1.41x and 1.51x at 1.4 and 2.9, respectively; MDA level: ⇑ to 1.39x and 1.55x at 1.4 and 2.9, respectively; GSH level: ⇓ to 0.59x, 0.47x, and 0.42x at 3 doses in ascending order; SOD activity: ⇓ to 0.76x, 0.60x, and 0.55x at 3 doses in ascending order; ⇓ in activities of CAT, GPx, GR, G-6-P, and GST, respectively, to 0.90x, 0.75x, 0.50x, 0.76x, and 0.61x at 1.4 ppm and to 0.54x, 0.66x, 0.42x, 0.64x, and 0.45x at 2.9 ppm.	Bashir et al., 2006a
Liver/mouse (BALB/c, male)	As^{III} SA	50, 100, 150 µg/mouse/day for 6 days/week (gavage)	3 months	50 for ⇑ None None None 50 for ⇑ 50 for ⇑	Changes in various components of antioxidant defense system: GSH level: 50, 1.14x; 100, 1.17x; 150, 1.25x; MDA level: NSE at any dose PSH level: NSE at any dose PC level: NSE at any dose GPx activity: 50, 1.12x; 100, 1.15x; 150, 1.24x; CAT activity: 50, 1.06x; 100, 1.08x; 150, 1.10x.	Das et al., 2005

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver/mouse (BALB/c, male)	As^{III} SA	50, 100, 150 µg/mouse/day for 6 days/week (gavage)	6 months	None 100 for ⇑ 100 for ⇓ 100 for ⇑ 100 for ⇓ 100 for ⇓	Changes in various components of antioxidant defense system: GSH level: NSE at any dose MDA level: 50, NSE; 100, 1.39x; 150, 1.44x; PSH level: 50, NSE; 100, 0.81x; 150, 0.75x; PC level: 50, NSE; 100, 1.16x; 150, 1.30x; GPx activity: 50, NSE; 100, 0.91x; 150, 0.90x; CAT activity: 50, NSE; 100, 0.94x; 150, 0.92x.	Das et al., 2005
Liver/mouse (BALB/c, male)	As^{III} SA	50, 100, 150 µg/mouse/day for 6 days/week (gavage)	9 months	50 for ⇓ 50 for ⇑ 50 for ⇓ 50 for ⇑ 50 for ⇓ 50 for ⇓	Changes in various components of antioxidant defense system: GSH level: 50, 0.80x; 100, 0.77x; 150, 0.66x; MDA level: 50, 1.97x; 100, 2.06x; 150, 2.16x; PSH level: 50, 0.80x; 100, 0.75x; 150, 0.71x; PC level: 50, 1.64x; 100, 1.78x; 150, 1.94x; GPx activity: 50, 0.95x; 100, 0.91x; 150, 0.87x; CAT activity: 50, 0.95x; 100, 0.93x; 150, 0.92x.	Das et al., 2005
Liver/mouse (BALB/c, male)	As^{III} SA	50, 100, 150 µg/mouse/day for 6 days/week (gavage)	12 months	50 for ⇓ 50 for ⇑ 50 for ⇓ 50 for ⇑ 50 for ⇓ 50 for ⇓	Changes in various components of antioxidant defense system: GSH level: 50, 0.76x; 100, 0.72x; 150, 0.63x; MDA level: 50, 2.20x; 100, 3.03x; 150, 3.97x; PSH level: 50, 0.73x; 100, 0.63x; 150, 0.56x; PC level: 50, 2.09x; 100, 2.91x; 150, 3.46x; GPx activity: 50, 0.87x; 100, 0.84x; 150, 0.75x; CAT activity: 50, 0.93x; 100, 0.92x; 150, 0.88x.	Das et al., 2005
Blood/rat (Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	6 weeks	57.7 ppm	Effects on levels of biochemical variables indicative of disturbances in the heme synthesis pathway and oxidative stress: ALAD ⇓ to 0.12x; GSH ⇓ to 0.73x; RBC ROS ⇑ to 1.35x; GPx showed NSE.	Kalia et al., 2007
Liver/rat (Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	6 weeks	57.7 ppm	Effects on levels of biochemical variables indicative of oxidative stress: GSH ⇓ to 0.69x; GSSG ⇑ to 1.41x; TBARS ⇑ to 1.16x; catalase showed NSE. There was NSE for any of these parameters in the kidney.	Kalia et al., 2007
Blood, kidney, liver/rat (Wistar, male)	As^{III} SA	*1.15 mg/kg/day (gavage)	3 weeks	Various	ALAD activity: blood, 0.45x; CAT activity; kidney, 1.12x (NSE); liver, 1.16x; GSH level; blood and kidney, NSE; liver, 0.79x; TBARS level; kidney, NSE; liver, 1.28x; co-treatment with NAC (i.p. injection) and/or zinc sulfate (oral) reduced some effects, especially when used together	Modi et al., 2006

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Brain/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	60 days	57.7 ppm	Effects on levels of chemicals indicative of oxidative stress in 5 regions of the brain (hippocampus, cortex, striatum, hypothalamus, and cerebellum): MDA ⇑ to from 1.64x to 2.21x; GSH ⇓ to from 0.43x to 0.58x; GPx ⇓ to from 0.77x to 0.81x; GR ⇓ to from 0.73x to 0.78x; G6PDH ⇓ to from 0.70x to 0.84x; simultaneous treatment with DL-α-lipoic acid markedly reduced all of these effects.	Shila et al., 2005a
Brain/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	60 days	57.7 ppm	Effects on levels of chemicals indicative of oxidative stress in 5 regions of the brain (hippocampus, cortex, striatum, hypothalamus, and cerebellum): (This is the same experiment as in the previous row; findings not already listed in that row are listed here.) ROS based on DCF assay ⇑ to from 1.62x to 2.18x; Total SOD ⇓ to from 0.56x to 0.77x; Mn SOD ⇓ to from 0.36x to 0.55x; Cu/Zn SOD ⇓ to from 0.53x to 0.62x; CAT ⇓ to from 0.67x to 0..80x; simultaneous treatment with DL-α-lipoic acid markedly reduced all of these effects.	Shila et al., 2005b
Brain/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	60 days	57.7 ppm	Measures of protein oxidation: ⇑ in protein carbonyl level: cerebellum, 1.23x; cortex, 1.32x; hippocampus, 1.48x; hypothalamus, 1.25x; striatum, 1.49x; ⇓ in membrane protein sulfhydryl content: cerebellum, 0.71x; cortex, 0.55x; hippocampus, 0.50x; hypothalamus, 0.79x; striatum, 0.61x; essentially the same regional pattern of iAs-induced loss occurred with total protein-bound sulfhydryls. Co-treatment with DL-α-lipoic acid mostly or completely abolished all of the above effects.	Samuel et al., 2005
Kidney, liver, RBCs/rat (albino Wistar, male)	As^{III} SA	* 5.8 ppm (DW)	12 weeks	5.8 ppm, but for only some effects	MDA level: ⇑ in kidney to ~2.1x, in liver to ~1.7x, and in RBCs to ~1.4x; CAT activity: ⇓ in kidney to ~0.73x, in liver to ~0.91x (NSE), and in RBCs to ~0.78. SOD activities were measured but with NSE. Co-treatment with cysteine, methionine, AA, or thiamine usually decreased tissue As concentrations (especially in kidney and liver) and blocked oxidative damage to variable degrees.	Nandi et al., 2005

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Kidney /rat (albino Wistar, male)	As^{III} SA	* 5.8 ppm (DW)	4, 8, 12 weeks	Various for ⇑ and ⇓	MDA level: ⇑ at 4 wk to ~1.27x (NSE), at 8 wk to ~1.54x, and at 12 wk to ~2.11x; CAT activity: ⇑ at 4 wk to ~1.72x, at 8 wk to ~1.18x (NSE) but ⇓ at 12 wk to ~0.75x; SOD activity: ⇑ at 4 wk to ~1.84x, at 8 wk to ~1.23x, but ⇓ at 12 wk to 0.91x (NSE)	Nandi et al., 2006
Liver/rat (albino Wistar, male)	As^{III} SA	* 5.8 ppm (DW)	4, 8, 12 weeks	Various for ⇑ and ⇓	MDA level: ⇑ at 4 wk to ~1.07x (NSE), at 8 wk to ~1.46x, and at 12 wk to ~1.49x; CAT activity: ⇑ at 4 wk to ~1.19x (NSE), at 8 wk to ~1.52x but ⇓ at 12 wk to ~0.91x (NSE); SOD activity: ⇑ at 4 wk to ~1.52x, at 8 wk to ~1.16x, but NSE at 12 wk	Nandi et al., 2006
RBCs/rat (albino Wistar, male)	As^{III} SA	* 5.8 ppm (DW)	4, 8, 12 weeks	Various for ⇑ and ⇓	MDA level: ⇑ at 4 wk to ~1.13x (NSE), at 8 wk to ~1.28x, and at 12 wk to ~1.41x; CAT activity: ⇑ at 4 wk to ~1.36x, NSE at 8 wk, and ⇓ at 12 wk to ~0.71x; SOD activity: ⇑ at 4 wk to ~1.81x, at 8 wk to ~1.59x, but NSE at 12 wk	Nandi et al., 2006
Liver & kidney/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	30 days	57.7 ppm	Level of ROS determined by DCFH assay: ⇑ in liver to ~3.6x and in kidney to ~3.5x. Level of MDA released per mg protein: ⇑ in liver to ~1.5x and in kidney to ~1.6x. Co-treatment with both DL-α-lipoic acid and DMSA markedly reduced all of these effects.	Kokilavani et al., 2005
Liver & kidney/rat (albino Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	30 days	57.7 ppm	Activities of antioxidant enzymes: ⇓ of SOD in liver to ~0.51x and in kidney to ~0.55x; ⇓ of CAT in liver to ~0.59x and in kidney to ~0.58x; ⇓ of GPx in liver to ~0.53x and in kidney to ~0.56x; Levels of non-enzymatic antioxidants: ⇓ of GSH in liver to ~0.56x and in kidney to ~0.67x; ⇓ of AA in liver to ~0.48x and in kidney to ~0.50x; ⇓ of α-Toc in liver to ~0.49x and in kidney to ~0.58x; ⇓ of total sulfhydryls in liver to ~0.53x and in kidney to ~0.59x. Co-treatment with both DL-α-lipoic acid and DMSA markedly reduced all of these effects.	Kokilavani et al., 2005

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Blood (whole), brain, kidney, liver/mice (Swiss albino, male)	As^{III} SA	* 14.4 ppm (DW)	3 months	14.4 ppm	Whole blood: ⇓ of ALAD activity to 0.37x; ⇓ of GSH level to 0.93x; Brain: ⇑ in TBARS level to ~2.2x; ⇓ in GSH/GSSG ratio to ~0.96x. Kidney: ⇑ in TBARS level to 1.65x. Liver: ⇑ in TBARS level to 1.21x; ⇓ in SOD activity to 0.76x; ⇓ in CAT activity to 0.89x; ⇓ in GSH/GSSG ratio to 0.89x; post-treatments with 3 different extracts of *Hippophae rhamnoides* L. (thought to have antioxidant properties) showed various levels of effectiveness in reducing some of the above effects in all but the kidney.	Gupta and Flora, 2005
Blood (whole), brain, kidney, liver/rat (Wistar, male)	As^{III} SA	* 11.5 ppm (DW)	4 wk	11.5 ppm	Whole blood: ⇓ of ALAD activity to 0.24x; ⇓ of GSH level to 0.86x; ⇑ of ZPP level to 1.30x; Brain: ⇑ in TBARS level to 1.89x; ⇓ in GSH level to 0.85x; NSE on GSSG level; ⇓ in SOD activity to 0.75x; ⇓ in CAT activity to 0.75x; Kidney: ⇑ in TBARS level to 1.39x; ⇓ in GSH level to 0.55x; ⇑ in GSSG level to 1.59x; Liver: ⇑ in TBARS level to 1.96x; ⇓ in GSH level to 0.61x; ⇑ in GSSG level to 2.00x; oral co-treatment with *Centella asiatica* (thought to have antioxidant properties) showed various levels of effectiveness in reducing some of the above effects.	Gupta and Flora, 2006
Blood (whole), brain/rat (Wistar, male)	As^{III} SA	* 57.7 ppm (DW)	10 wk	57.7 ppm	Whole blood: ⇑ of ROS level to 2.63x; ⇓ of ALAD activity to 0.46x; ⇓ of GSH level to 0.85x; ⇓ of Hb as grams/dL to 0.79x; Brain: ⇑ of ROS level to 4.03x; ⇑ in TBARS level to 1.50x; ⇓ in GSH level to 0.82x; ⇓ in SOD activity to 0.92x (NSE); ⇓ of ALAD activity to 0.58x; ⇑ of ALAS activity to 1.21x; ⇓ of GPx activity to 0.84x (NSE); ⇑ of GST activity to 1.08x (NSE); "considerable" but unquantified ⇑ in DNA fragmentation (single-strand breaks) was detected by polyacrylamide gel electrophoresis; postreatment with the thiol chelating agents DMSA, DMPS, and MiADMSA showed various levels of effectiveness in reducing some of the above effects.	Flora et al., 2005

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Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Liver/mouse (BALB/c, male)	Unspecified arsenical, but from discussion assumed to be As^{III} SA	* 1.8 ppm (DW)	3, 6, 9, 12, 15 months	1.8 at ≥9 months for MDA 1.8 at ≥6 months for GSH	MDA conc: ⇑ to ~1.7x at 9, ~1.9x at 12, and ~2.2x at 15; GSH content: ⇓ to ~0.84x at 6, ~0.78x at 9, ~0.67x at 12, and ~0.58x at 15. ⇓ in activities were also noted for G6PDH, GPx, and plasma membrane Na^+/K^+ ATPase at 6 months, for CAT at 9 months, and for GST and GR at 12 and 15 months. It seems likely that the activities remained lower at later times than when each ⇓ was noted, but that was not stated.	Mazumder, 2005
Lung/mouse (ddY, male)	DMA^V	* 217.2 ppm (DW)	2, 4, 8, 15, 25 wk	217.2 ppm at 8 wk or longer	Immunohistochemical analysis of 4HNE adducts showed that lipid peroxidation occurred in 48.8%, 72.9%, and 77.6% of terminal bronchiolar Clara cells by 8, 15, and 25 weeks, respectively. (None before that.) The modified proteins were specifically in the secretory granules of those cells. 8-OHdG adducts (showing oxidative DNA) damage were also demonstrated in the same cells. Clara cells are the major target cell for DMA-induced oxidative stress, and the authors suggested that lipid peroxidation via the formation of ROS is involved in promotion of lung tumor (malignant adenocarcinoma) formation following initiation by 4NQO.	An et al., 2005
Liver/rat (Wistar, male)	As^{III} SA	* 3.6, 6.1, 7.3 mg/kg (gavage, with animals being killed 24 hr later for sample collection)	One dose	Various	Significant dose-related ⇑ in total As conc at all doses; conc in liver at highest dose was ~22 times that in brain. MDA conc: ⇑ to 1.43x at 6.1 and 1.52x at 7.3; GSH level: ⇓ to 0.57x at 3.6, to 0.41x at 6.1, and to 0.39x at 7.3; Total cytochrome P450 activity: ⇑ to 1.46x at 6.1 and 1.54x at 7.3; SOD level: ⇓ to 0.67x at both 6.1 and 7.3; CAT activity: ⇓ to 0.54x at 6.1 and 0.49x at 7.3; GPx activity ⇑ to 1.15x at 3.6, 1.21x at 6.1, and 1.27x at 7.3; GST activity: ⇓ to 0.72x at 6.1 and 0.62x at 7.3; NSE on either GR or G6PD activity	Bashir et al., 2006b

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Brain/rat (Wistar, male)	As^{III} SA	* 3.6, 6.1, 7.3 mg/kg (gavage, with animals being killed 24 hr later for sample collection)	One dose	Various	Significant ⇑ in total As conc at both higher doses. MDA conc: ⇑ to 1.48x at 6.1 and 1.56x at 7.3; GSH level: ⇓ to 0.79x at 3.6, to 0.60x at 6.1, and to 0.51x at 7.3; SOD level: ⇓ to 0.73x at 6.1 and 0.70x at 7.3; CAT activity: ⇓ to 0.58x at 6.1 and 0.51x at 7.3; GPx activity ⇑ to 1.17x at 6.1, and 1.26x at 7.3; GST activity: ⇓ to 0.71x at 6.1 and 0.69x at 7.3; NSE on either GR or G6PD activity	Bashir et al., 2006b
Kidney, rat (Wistar, male)	As^{III} ATO	* 30.3 mg/kg, 15 times (gavage)	Every other day for 30 days	30.3 x 15	GSH content ⇓ to ~0.59x; GST activity: NSE	Rana and Allen, 2006
Gene Mutations						
Skin/mouse ($Aprt^{+/-}$ hybrid mice of complex genotype needed for assay: see paper)	As^{III} SA	* 5.7 ppm (DW)	10 wk	None	Starting 2 wk after consumption of iAs-contaminated water began, half of the mice were also exposed to B[α]P for 8 wk by skin painting. Skin was assayed for DAP-resistant (DAP^r) colonies indicative of cells lacking Aprt activity as the result of loss of heterozygosity (LOH) at Aprt because of malsegregation or mitotic recombination *in vivo*. No significant differences were found because of iAs and/or B[α]P exposure, and thus there was no evidence that iAs alone, or by enhancement of a known mutagen (but not one + in this assay), caused such genetic changes. Curiously, the point estimate for most LOH was in the control (45%); it was 38% for B[α]P alone, 8% for iAs alone, and 30% for them together. Because there was much variability, these seemingly large differences were not statistically significant.	Fischer et al., 2006
Wing/*Drosophila melanogaster*	DMA^V	0.05, 0.1, 0.25, 0.5 mM (in medium)	72 hr	0.25 mM, regarding total spots	SMART (somatic mutation and recombination test) wing spot assay: positive dose response was found, but nature of induced mutations was uncertain. Was earlier shown that iAs is inactive in this assay. They showed no biomethylation occurs in larvae or in growth medium. Results suggest importance of biomethylation as a determinant of genotoxicity of arsenic compounds, at least in *Drosophila*.	Rizki et al., 2006

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Hypermethylation of DNA						
Lung/mice (A/J, male)	As^V as $Na_2HAsO_4 \cdot 7H_2O$	* 0.24, 2.4, 24 ppm (DW)	18 months	The LOEL was 0.24 ppm. Extent of hypermethylation of promoter regions of tumor suppressor genes $p16^{INK4a}$ and RASSF1A in lung adenocarcinomas from iAs exposed mice compared to the control, based on methylation-specific PCR: percentages of methylated promoters of $p16^{INK4a}$ in lung tumors of 0, 0.24, 2.4, and 24 ppm dose groups were 11%, 30%, 36%, and 42%, respectively. Percentages of methylated promoters of RASSF1A in lung tumors of the same dose groups were 33%, 70%, 82%, and 89%, respectively. Reduced expression, or lack of expression, of these 2 genes was correlated with the extent of hypermethylation. There was constant expression of these genes in lungs without tumors in both control and iAs-treated mice. They concluded that epigenetic changes of tumor suppressor genes are involved in iAs-induced lung carcinogenesis.	Cui et al., 2006
Hypomethylation of DNA						
Liver cells/mouse (129/SvJ)	As^{III} SA	45 ppm (DW)	48 wk	45 ppm	There was global DNA hypomethylation, as shown by 5-methylcytosine content of DNA and by using the methyl acceptance assay. In particular, there was a marked ⇓ in methylation within the ER-α gene promoter region, which was statistically significant in 8 of 13 CpG sites. Control had 28.3% of ER-α sites methylated, but experimental group had 2.9%.	Chen et al., 2004b
Livers of newborn males/mouse (only pregnant C3H females drank the water)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Global DNA methylation status was not significantly altered based on methyl acceptance assay, which measures methylation in both quiescent and active areas of DNA. However, another assay showed that GC-rich regions globally were less methylated if they were from livers of newborn males exposed *in utero* to iAs. Band intensity showing the extent of methylation was 0.20x after RsaI + MspI digestion and 0.40x after RsaI + HpaII digestion. MspI and HpaII are methylation sensitive enzymes.	Xie et al., 2007
Interference with Hormone Function						

Table C-2. *In Vivo* Experiments on Laboratory Animals Related to Possible Modes of Action of Arsenic in the Development of Cancer— Only Oral Exposures

Tissue or cell type/Species	Arsenic species	Dose in elemental As[1] (in units stated)	Duration of treatment	LOEL[2]	Results	Reference
Kidney, rat (Wistar, male)	As^{III} ATO	* 30.3 mg/kg, 15 times (gavage)	Every other day for 30 days	30.3 x 15	T_3 and T_4 levels in serum: triodothyronine (T_3) ⇑ to ~4.8x; thyroxine (T_4) ⇑ to ~1.7x	Rana and Allen, 2006
Signal Transduction						
Fetal lungs/mouse (only pregnant C3H females drank the water, female offspring only)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	⇑ in ER-α transcript (5.3x) and protein levels; ⇑ in expression of the following estrogen-related genes: trefoil factor-3 (9.66x), anterior gradient-2 (3.21x); ⇑ in expression of the following steroid metabolism genes: 17-β-hydroxysteroid dehydrogenase type 5 (3.55x) and aromatase (2.53x). (Expression of ER-α and the ER-linked genes was unchanged in male fetal lung as compared to control.) The insulin growth factor system was also activated, with transcripts for IGF-1, IGF-2, IGF-R1, IGF-R2, IGF-BP1, and IGF-BP5 all being increased to 1.6-2.5x. Also, there was overexpression of the following genes that have been associated with lung cancer: AFP (6.9x), EGFR (3.2x), L-myc (1.9x), and metallothionein-1 (2.1x).	Shen et al., 2007
Adenomas and adeno-carcinomas from lungs of adults exposed *in utero*/mouse (only pregnant C3H females drank the water, female offspring only)	As^{III} SA	85 ppm (DW)	10 days, gestation days 8 to 18	85 ppm	Based on immunohistochemical analysis: intense and widespread ⇑ in nuclear ER-α expression; in contrast, normal adult lung and DENA-induced lung adenocarcinoma showed little evidence of ER-α expression.	Shen et al., 2007

[1]When doses were reported in mg arsenic/L or in ppm As, it was assumed that the doses included adjustment for the amount of As in solution. Because it was sometimes unclear from the papers whether a correction was needed, a "*" was put front of the doses listed in the table if those doses were corrected to the amount of As in the dose.
[2]Lowest Observed Effect Level
[3]Estimates were based on the reported concentrations of MMA^V, DMA^V, and TMA^VO in DW of 1.62, 1.45, and 1.47 mM, respectively, and on their molecular weights (MWs) of 139.969, 137.997, and 136.025 g and on the atomic weight of As of 74.926 g. The paper stated that the concentrations of all arsenicals were 0.02% (or 200 ppm). For the arsenicals themselves, the concentrations were actually 226, 200, and 200 ppm, respectively, if based on the MWs just listed.

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Aberrant Gene or Protein Expression						
HaCaT cells	As^{III} SA	0.5, 1.0	20 passages	0.5	⇑ intracellular GSH quantities ⇓ keratins 5, 6, 7, 8, 10, and 17	Chien et al., 2004
TRL 1215 cells (normal rat liver)	As^{III} SA	0.125, 0.250, 0.500	24 wk	0.500 for effects noted here	Using Atlas Rat cDNA expression microarrays, ~80 of the 588 genes assayed were aberrantly expressed—including genes related to stress and DNA damage, signal transduction modulators and effectors, apoptosis related proteins, cytokines and cytokine-related components, and growth factors and hormone receptors	Chen et al., 2001
Hepa-1 cells (mouse hepatoma)	As^{III} SA	1, 3, 10, 30	30 min before 4 hr co-treatment with 1 nM TCDD	1	Results of northern blot analysis of mRNA: ⇑ TCDD-inducible levels of Nqo1 mRNA; response was much higher at 3 and 10, but decreased markedly at 30 to slightly more than was present at 1	Maier et al., 2000
Huh7 cells	As^{III} SA	0.5, 1, 3, 5, 10, 20	24 hr	3	Following co-treatment with 10 nM TCDD: ⇓ TCDD-inducible level of CYP1A1 activation to ~45% of level without iAs, then reached plateau of ~18% at doses of 5-15 (based on EROD assay); iAs did not affect CYP1A1 activation by itself.	Chao et al., 2006b
Huh7 cells, transfected for use in the DRE-CALUX bioassay	As^{III} SA	0.5, 1, 3, 5, 10, 20	24 hr	3	Following co-treatment with 10 nM TCDD: ⇓ TCDD-inducible luciferase activity in the DRE-CALUX bioassay to ~80% of level without iAs, followed by a dose-related ⇓ to 42% at dose of 20	Chao et al., 2006b
$PARP\text{-}1^{+/+}$ MEF cells $PARP\text{-}1^{-/-}$ MEF cells	As^{III} SA for both	11.5 for both	24 hr for both	In a microarray gene chip analysis that analyzed the expression pattern of more than 34,000 genes, ~311 genes were found to be differentially expressed among the different groups (i.e., control versus iAs treatment or in comparisons between the 2 genotypes). Many of those genes belonged to the following groups: responders to stress and external stimuli, genes related to cell growth and maintenance, cell death, or DNA metabolism. While some genes were markedly up-regulated in both genotypes (sometimes to widely different amounts), other genes were up-regulated for one genotype and down-regulated for the other, and vice-versa.		Poonepalli et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NB4 cells	As^{III} ATO	0.5	6, 12, 24, 48, and 72 hr for transcriptome analysis; 12 and 48 hr for proteomic analysis	In a microarray and 2-dimensional gel electrophoresis (with mass spectrometry) study aimed at understanding effects of therapies with ATO alone, retinoic acid alone, and their combined therapy, the main findings for ATO were as follows. At the transcriptome level, ATO affected regulation of 487 genes, many of which were probably related to essential aspects of cell-activity control such as induction of differentiation antigens, modulation of apoptosis regulators, and regulation of genes involved in cell-cycle and growth control. Other groups of affected genes included those involved with protein degradation, cell defense, stress response, protein modification and synthesis, and a group of 5 down-regulated HLA-class I genes. At the proteome level, ATO affected 982 protein spots, and there was often a time-dependent pattern of regulation, with much lower protein levels at 48 hr than at 12 hr after treatment. A group of enzymes involved in biochemical metabolism was found to be significantly down-regulated, and there was a strong reduction of cytoskeleton proteins, implying a considerable reorganization of the cell nucleus and cytoplasmic structures. By comparison with relatively minor changes at many of the corresponding genes at the transcriptome level, the significant changes found at the proteomic level suggest that ATO particularly enhances mechanisms of post-transcriptional/translational modification.		Zheng et al., 2005
PRCCs HEK293 cells	As^{III} ATO for both	0.1 1	10 min, 1, 6, 24 hr for both	0.1 at 6 hr 1 at 6 hr	HMOX1 gene expression (mRNA levels measured by quantitative PCR): In PRCCs: NSE at 10 min or 1 hr; ~2.3x at 6 hr, ~2.8x at 24 hr; HEK293: NSE at 10 min or 1 hr; ~40x at 6 hr, ~54x at 24 hr.	Sasaki et al., 2007
PRCCs HEK293 cells	As^{III} ATO for both	0.1, 0.5, 2 for both	24 hr for both	0.1 0.5	HMOX1 gene expression (mRNA levels measured by quantitative PCR): In PRCCs: 0.1, 2.2x, 0.5, 11.7x; 2, 33.5x; In HEK293: 0.1, 1.2x, 0.5, 8.3x; 2, 224.9x. Western blot analysis for heme oxygenase 1 protein for dose of 1 for 24 hr: huge ⇑ in PRCCs and big ⇑ in HEK293.	Sasaki et al., 2007
PRCCs HEK293 cells	As^{III} ATO for both	0.1 for both	10 min, 1, 6, 24 hr for both	Microarray analysis identified 73 genes whose expression changed in both types of cells, and for many expression increased in a time dependent manner. These included HMOX1, Bax (involved in induction of apoptosis), and genes involved in many other biological processes including intracellular protein transport, signal transduction, differentiation, GSH metabolism, and protein complex assembly among others. Data were presented that suggest that heme oxygenase 1 protein confers a cytoprotective effect against iAs treatment.		Sasaki et al., 2007

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HCT15 cells	As^{III} SA for all	278.33, the LC_{50}	24 hr for all	278.33	Western blot assay to determine eIF4E protein levels: For all cell lines, there was a reduction in the protein level to roughly 50-60% of the corresponding control level. There was also a statistically significant, but smaller, ⇓ after 16 hr for all lines.	Othumpangat et al., 2005
HeLa cells		200.33, the LC_{50}		200.33		
PLC/PR/5 cells		376.66, the LC_{50}		376.66		
Chang cells		328.33, the LC_{50}		328.33		
HCT15 cells	As^{III} SA for all	278.33, the LC_{50}	24 hr for all	278.33	Quantitative real-time PCR to determine eIF4E mRNA levels: there was a statistically significant ⇓ only in lines HCT15 and HeLa. Actual data on gene expression, in arbitrary units: HCT15: no iAs, 0.099, with iAs, 0.049; HeLa: no iAs, 0.041, with iAs, 0.029; PLC/PR/5: no iAs, 0.051, with iAs, 0.028; Chang: no iAs, 0.018, with iAs, 0.019 (Judging from their SEs, the result for PLC/PR/5 must have been of borderline significance.)	Othumpangat et al., 2005
HeLa cells		200.33, the LC_{50}		200.33		
PLC/PR/5 cells		376.66, the LC_{50}		376.66		
Chang cells		328.33, the LC_{50}		None		
HeLa cells	As^{III} SA	200	24 hr	200	Western blot assay to determine protein levels: big ⇓ in cyclin D1; ⇑ in cellular levels of ubiquitin and in the process of ubiquitination	Othumpangat et al., 2005
HeLa cells, HCT15 cells, CHO-K1 cells	As^{III} SA for all	Various	Additional experiments involving a genetically modified cell line, an siRNA that targeted expression of eIF4E, and proteasome inhibitors suggested (1) that the changes seen in eIF4E protein levels played a role in the observed cytotoxicity, (2) and that the inhibition of cyclin D1 is mediated through the inhibition of eIF4E, and (3) that the iAs stimulated ubiquitination and the resulting proteolysis play an important role in reducing eIF4E protein levels.			Othumpangat et al., 2005
TR9-7 cells that were released from being mostly synchronized in G2 (using Hoechst 33342) shortly before iAs treatment began	As^{III} SA	5	3-24 hr	Conclusions based on determining protein levels using Western blot assays until 24-hr of iAs treatment in cells made $p53^{(+)}$ or $p53^{(-)}$ by controlling tetracycline levels: big ⇑ in ID1, but it occurred only when there was p53 protein present. As p53 protein level decreased, ID1 protein level decreased. The general finding was confirmed by microarray analysis. Work by others showed that ID1 protects against apoptosis through activation of the NF-κB signaling pathway.		McNeely et al, 2006
TR9-7 cells that were released from being mostly synchronized in G2 (using Hoechst 33342) shortly before iAs treatment began	As^{III} SA	5	3 hr	Conclusions based on microarray analysis (done by hybridizing fragmented cRNAs to U95Av2 GeneChips) in cells made $p53^{(+)}$ or $p53^{(-)}$ by controlling tetracycline levels: several genes were induced by iAs independently of p53 status, of which some of the biggest effects were as follows (at both p53 conditions): HMOX1: huge ⇑ by >25x; MT2A: ⇑ by >3x; SLC30A1: ⇑ by >3x. MKP-1 was induced only in $p53^{(+)}$ cells, and ubiquitin-conjugating enzyme E2N was induced only in $p53^{(-)}$ cells.		McNeely et al, 2006

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer						
Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
HeLa cells	As^{III} ATO	2	6 and 24 hr	In a cDNA microarray-based global transcription profiling experiment that compared the iAs treatment with a co-treatment of the same iAs dose with 30 μM emodin, the numbers of genes with an expression level that differed between the two treatments by more than a factor of 2 at the 2 time points were 793 and 480, respectively. The affected genes included genes involved in such things as cell signaling, organelle functions, cell-cycle control, redox regulation, and apoptosis. The manner of data presentation did not permit identification of genes affected exclusively by iAs.		Wang et al., 2005
TRL 1215 cells	As^{III} SA	0.125, 0.250, 0.500	24 weeks	Various	mRNA levels determined by real time RT-PCR: effects on oncogenes AFP: ⇑ at 0.250, big ⇑ at 0.500; WT-1: ⇑ at 0.125, big ⇑ at 0.250 and 0.500; c-jun: ⇑ at 0.250, big ⇑ at 0.500; H-ras: ⇑ at 0.125, big ⇑ at 0.250 and 0.500. (By 24 weeks of exposure at the higher 2 doses, these cells had undergone malignant transformation and were called CAsE cells.)	Liu et al., 2006d
TRL 1215 cells	As^{III} SA	0.125, 0.250, 0.500	24 weeks	Various	mRNA levels determined by real time RT-PCR: effects on stress-related genes HMOX-1: ⇑ at 0.125 and 0.250, big ⇑ at 0.500; SOD: ⇑ at 0.250, big ⇑ at 0.500; MT-1: big ⇑ at 0.250, ⇑ at 0.500; GSTπ: ⇑ at 0.125, big ⇑ at 0.250 and 0.500. (By 24 weeks of exposure at the higher 2 doses, these cells had undergone malignant transformation and were called CAsE cells.)	Liu et al., 2006d
TRL 1215 cells	As^{III} SA	0.125, 0.250, 0.500	24 weeks	Various	mRNA levels determined by real time RT-PCR: effects on cell cycle regulators Cyclin D1: ⇑ at 0.125, then ⇑ with dose to 0.500; PCNA: ⇑ at 0.250, big ⇑ at 0.500; p21: big ⇓ at 0.125, then ⇓ with dose to 0.500; p16: ⇓ at 0.125, big ⇓ to ~0% at 0.250 and 0.500. (By 24 weeks of exposure at the higher 2 doses, these cells had undergone malignant transformation and were called CAsE cells.)	Liu et al., 2006d
TRL 1215 cells	As^{III} SA	0.125, 0.250, 0.500	24 weeks	Various	mRNA levels determined by real time RT-PCR: effects on growth factor genes c-met: big ⇑ at 0.125, then ⇑ with dose to 0.500; HGF: ⇑ at 0.125, big ⇑ at 0.250 and 0.500; FGFR1: huge ⇓ at 0.250, then ⇓ to ~0% at 0.500; IGF-II: huge ⇓ to ~0% at all doses. (By 24 weeks of exposure at the higher 2 doses, these cells had undergone malignant transformation and were called CAsE cells.)	Liu et al., 2006d

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer						
Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
TRL 1215 cells	As^{III} SA	0.125, 0.250, 0.500	24 weeks	Various	Protein levels determined using Western blots: AFP: slight ⇑ at 0.125 through 0.500; WT-1: huge ⇑ at 0.125 through 0.500; Cyclin D1: ⇑ at 0.125 through 0.500; p16: huge ⇓ at all doses; p21: ⇓ at 0.125, then ⇓ with dose to 0.500. (By 24 weeks of exposure at the higher 2 doses, these cells had undergone malignant transformation and were called CAsE cells.)	Liu et al., 2006d
TRL 1215 cells	As^{III} SA	0.500	24 weeks	Various	mRNA levels determined by real time RT-PCR: effects of 72-hr post-treatment with 5 μM 5-aza-dC, (Results were compared to cells with iAs treatment alone.): MT-1: ⇑ 19x over already elevated level; p21: ⇑ 15x over what was a greatly reduced level, and level then far above that with no iAs exposure; p16 and IGF-II: NSE. (By 24 weeks of exposure at the higher 2 doses, these cells had undergone malignant transformation and were called CAsE cells.)	Liu et al., 2006d
CL3 cells	As^{III} SA	2	24 hr	2	⇑ Nqo1 mRNA level to 1.7x control; ⇑ Nqo1 protein level to 6.4x control. Cells given this iAs pretreatment became more sensitive to MMC-induced cytotoxicity and less sensitive to ADM-induced cytotoxicity. Co-treatment with MMC and the Nqo1 inhibitor DIC resulted in big ⇑ in cell survival (even higher than after MMC treatment without an iAs pretreatment). CL3R15 cells, which have much higher levels of Nqo1 activity than CL3 cells, are also much more sensitive to MMC-induced cytotoxicity than CL3 cells.	Lin et al., 2006
H460 cells CL3 cells	As^{III} SA for both	2.5, 5, 10, 20 1, 2.5, 5, 10	72 hr for both	2.5 1	Cell survival determined by SRB assay: LC_{50}s: H460, 9.0; CL3, 3.7; H460 cell have ~30x higher endogenous Nqo1 activity than CL3 cells, and unlike CL3 cells they showed no statistically significant induction of Nqo1 after 24-hr treatments with iAs at doses of 2, 5, or 10. (Even at the highest level of induction in CL3 cells, the endogenous level of Nqo1 activity in H460 cells was still ~15x higher.) These findings raised question whether Nqo1 plays a role in iAs resistance.	Lin et al., 2006
CL3R15 cells CL3R15 cells co-treated with 200 μM DIC for 6 hr to inhibit >95% of the high endogenous level of Nqo1 activity	As^{III} SA for both	50, 100, 200 for both	6 hr for both	100 50	Cell survival determined by colony-forming assay: LC_{50}s: with DIC, ~35; without DIC, 120	Lin et al., 2006

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
SIK cells	As^{III} SA	2	1, 3, 5, 7, 9 days	Changes in protein levels detected at each of the 5 times using 2-dimensional gel electrophoresis of soluble proteins, with proteins identified by peptide mass mapping and other methods: ~300 distinct protein spots were monitored with ~40% showing ≥2-fold ⇑ or ⇓ in silver staining intensity at every time point, about as many ⇑ as ⇓, with at least as many changes on day 1 as on other days. There were some changes as to the proteins affected over time. Of 10 proteins identified as showing prominent changes within first few days of iAs treatment, enzymes of the glycolytic pathway were seen to be substantially elevated. This dose of iAs suppressed differentiation but did not cause cell loss.		Lee et al., 2005
TRL 1215 cells	MMA^{V} DMA^{V} $TMA^{V}O$	1300 700 10000	20 weeks for all	1300 700 10000	⇑ GST activity to 2.6x control, ⇑ cellular GSH protein level to 2.2x control; ⇑ GST activity to 1.7x control, ⇓ cellular GSH protein level to 43% of control; ⇑ GST activity to 1.8x control, ⇑ cellular GSH protein level to 2.4x control. All 3 treatments increased GST, MRP and MDR at the mRNA level, and all 3 treatments increased GST, Mrps, and P-gp at the protein level. GST and MRP have several forms. While not all forms responded in the same way, the overall responses were as noted. Experiments with inhibitors of GSH, Mrps, and P-gp led to the conclusion that increased arsenic excretion caused the resistance to arsenic-induced cytotoxicity that resulted from these treatments.	Kojima et al., 2006
$Ahr^{+/+}$ MEFs	As^{III} SA	1, 2, 5	6 hr	2 for Nqo1 only None for Cyp1b1	mRNA levels measured by real-time RT-PCR: ⇑ Nqo1 mRNA to 4x control; 5 μM B[α]P increased Nqo1 mRNA to 8x control; there was a synergistic interaction between them such that the dose of 2 of iAs plus the dose of 5 of B[α]P increased Nqo1 mRNA to 27x control. A synergistic interaction to 20x control also occurred with a dose of 1 of iAs. At a dose of 5 of iAs, the interaction became only additive. The interaction between iAs and B[α]P regarding Cyp1b1 mRNA was never more than additive. In $Ahr^{-/-}$ MEFs, there was no interaction of iAs and B(α)P regarding Nqo1 mRNA; the combined treatment did not ⇑ Nqo1 mRNA levels. Thus the synergistic interaction requires the wt Ahr gene.	Kann et al., 2005a
$Ahr^{+/+}$ MEFs	As^{III} SA	Following treatment with 2 μM iAs, 5 μM B[α]P, or both, for an unspecified time, oligonucleotide microarray analysis of 13,332 sequences from annotated mouse genes: they identified 64 genes that were up-regulated or down-regulated by iAs, B[α]P, or both; of these, 13 showed at least a 2x up-regulation and 12 caused at least a 2-fold down-regulation in gene expression because of the iAs treatment alone. Many different types of genes were affected. One of the major consequences of exposure to these mixtures was the up-regulation of oxidative stress and protein chaperone responses and the down-regulation of the TGF-β pathway. Exposure to iAs/B[α]P mixtures caused regulatory changes in the expression of detoxification genes that ultimately affect the metabolic activation and disposition of toxicants.				Kann et al., 2005a

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
AG06 cells	As^{III} SA	0.2, 1, 3, 10	24 hr 48 hr	1 0.2	⇑ GSH concentration	Snow et al., 1999
AG06 cells	As^{III} SA	3	48 hr	3	Specific activities: GSTπ ⇑ to ~1.6x and γGCS to ~2.2x at dose of 3	Snow et al., 1999
AG06 cells GM847 cells	As^{III} SA	0.1, 0.25, 0.5, 1.0, 5, 10, 25	24 hr	1.0 0.25	⇑ GR protein level to 2.9x at 1 ⇑ GR mRNA level to 1.3x and enzyme activity to 2.0x at 0.25	Snow et al., 2001
GM847 cells	As^{III} SA	0.5, 1.0, 10, 25	24 hr	0.5 for ⇑ 0.5 for ⇓	⇑ Trx, TrxR, GR mRNA levels; for TrxR and GR: ⇑ to ~2.7x by 10 and and then ⇓ to ~1.5x by 25). ⇓ GPx mRNA level to ~0.5x by 1 and ~0.2x by 25.	Snow et al., 2001
AG06 cells	As^{III} SA	0.2, 4, 20	3 hr 24 hr	0.2 0.2	APE/Ref-1 mRNA levels: At 3 hr: ⇑ to ~2.7x at 0.2 and then only slight ⇑ to ~3.0x at 20. At 24 hr: ⇑ to ~3.0x at 0.2 but ⇓ to ~0.9x at 20; (APE/Ref-1 is required for BER.)	Snow et al., 2001
WI38 cells	As^{III} SA	0.3, 1.4, 5.7, 29	Not reported	0.3	⇑ DNA Poly β level (both cytoplasmic and nuclear) to ~2x by 1.4 but ⇓ to ~0.8x by 29. (DNA Poly β is required for BER.)	Snow et al., 2001
HaCaT cells	As^{III} SA	0.001, 0.01, 0.05, 0.1, 0.5, 1.0	2 days 14 days	0.1 0.01	⇓ p53 protein; ⇑ mdm2 protein ⇓ p53 protein; ⇑ mdm2 protein	Hamadeh et al., 1999
HaCaT cells	As^{III} SA, As^{V} MMA^{V}, DMA^{V}	1.0 1.0	2 days for all	1.0 None	⇓ p53 protein; ⇑ mdm2 protein; (Much bigger effect for As^{III}) No significant change	Hamadeh et al., 1999
JB6 Cl41 cells	As^{III} SA	0.05, 0.2, 0.8, 3.125, 12.5, 50, 200	15 min	0.8	⇑ Erk activation resulting from Erk phosphorylation; another experiment showed that overexpression of dominant negative Erk2 blocks arsenite-induced activation of Erk	Huang et al., 1999a
K562 cells	As^{III} ATO	2.5	6 hr	2.5	⇑ GlycoA, HLA-DR, CD33, and CD34 on the cell surface, indicating maturation of myeloid cells	Li and Broome, 1999
MCF-7 cells	As^{III} ATO	3	12 hr	3	Microtubule polymerization, with a major effect on the organization of the cellular microtubule network, resulting in the formation of long polymerized microtubule bundles; ⇑ $p34^{cdc2}$/cyclin B complex (both activation and accumulation); ⇑ Bcl-2 phosphorylation	Ling et al., 2002

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
H460 cells	As^{III} ATO	10	24 hr	10	The following changes occurred only in mitotic cells (definitely not in interphase cells): ⇑ caspase-3 activation, ⇑ caspase-7 activation, cleavage of PARP and β-catenin. These findings suggest that arsenic-induced mitotic arrest may be a requirement for the activation of apoptotic pathways.	Ling et al., 2002
Primary cultures of rat cerebellar neurons	As^{III} SA	10	24 hr	10	⇑ caspase activity (apoptosis is blocked in these cells if caspase is inhibited; there was a much bigger effect with a 48-hr treatment)	Namgung and Xia, 2001
MC/CAR (human multiple myeloma cell line)	As^{III} ATO	2	72 hr	2	⇑ caspase-3 activity, p21, and CDK1; up-regulation of cdc2 phosphorylation; ⇓ in CDK6, cdc2, cyclin A, and Bcl-2 levels; ⇑ binding of p21 with CDK6, cdc2, and cyclins A and E; ⇓ activity of CDK6-associated kinase and cdc2-associated kinase; loss of mitochondrial transmembrane potential ($\Delta\psi_m$); no change in p27, CDK2, CDK4, or cyclins B1, D1, or E levels	Park et al., 2000
PCI-1 (human head and neck squamous cell carcinoma cell line)	As^{III} ATO	2	3 days	2	⇑ p21 and its binding with cdc2; ⇓ protein levels of cdc2 and cyclin B1; ⇓ activity of cdc2 kinase; no change in CDK2, CDK4, CDK6 and cyclins A, D1, E	Seol et al., 1999
(Human myeloma-like cell lines) RPMI 8226 Karpas 707 U266	As^{III} ATO	0.5	72 hr	0.5	⇑ CD38 and CD54 (molecules involved in cell-cell interactions)	Deaglio et al., 2001
LAK effector cells	As^{III} ATO	0.5	72 hr	0.5	⇑ CD11a and CD31 (molecules involved in cell-cell interactions, and the ligands [i.e., counter-receptors] of CD54 and CD38, respectively)	Deaglio et al., 2001
WRL-68 (human hepatic cell line)	As^{III} SA	0.001, 0.01, 0.1, 10	16 hr	0.1 0.001	⇑ GSH ⇑ CK18	Ramírez et al., 2000
Human aorta VSMCs (vascular smooth muscle cells)	As^{III} SA	2.5, 5, 10	4 hr	~5	⇑ p22phox mRNA expression (p22phox is 1 of at least 7 subunits of NADH oxidase.) ⇓ α-actin mRNA expression	Lynn et al., 2000
Human aorta VSMCs (vascular smooth muscle cells)	As^{III} SA	2.5, 5, 10, 20	4 hr	~5	⇑ NADH oxidase activity. The effect was even stronger, with a LOEC of 1, in nonproliferating VSMCs.	Lynn et al., 2000
WI38 cells	As^{III} SA	0.1 10, 20, 50	14 days 18 hr	0.1 50	⇑ p53 (3 fold increase) ⇑ p53 (large increase)	Vogt and Rossman, 2001

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
WI38 cells	As^{III} SA	0.1 50	14 days 18 hr	0.1 50	⇑ cyclin D1; also treatment blocks ⇑ in p21 that occurs follow exposure to 6 Gy of ionizing radiation ⇓ cyclin D1; also treatment mostly blocks ⇑ in p21 that occurs follow exposure to 6 Gy of ionizing radiation	Vogt and Rossman, 2001
Untransformed and immortalized RWPE-1 cells (human prostate epithelial cell line)	As^{III} SA	5	Up to 30 wk	5	⇑ MMP-9 activity (likely biomarker of when malignant transformation occurred); ⇓ in DNA methyltransferase activity but no change in DNA methyltransferase mRNA levels; ⇑ K-ras mRNA and protein levels. Time course study suggested over-expression of K-ras preceded malignant transformation. There was no indication of mutations being induced in K-ras gene and no indication that hypomethylation of K-ras promoter region caused K-ras changes. The cells became tumorigenic after 29 weeks of treatment and were then called the CAsE-PE cell line.	Benbrahim-Tallaa et al., 2005
PAEC from freshly harvested vessels	As^{III} probably ATO, but called arsenite	5	15 min to 3 hr depending on endpoint	5	⇑ NF-κB dependent transcription, ⇑ H_2O_2-dependent tyrosine phosphorylation (which was blocked by CAT), ⇑cSrc activation. MAP kinases, extracellular signal-regulated kinase, and p38 were only activated at a dose of 100, which causes cell death.	Barchowsky et al., 1999a
HeLa S3 cells	As^{III} SA	5	24 hr	5	Changes in cells that were arrested in mitosis by As^{III}: c-Mos was hyperphosphorylated, cyclin A was degraded, cyclin B accumulated; ⇑⇑ $p34^{cdc2}$/cyclin B kinase activity. These and numerous other changes in mitotic proteins were similar to changes seen in cells arrested in mitosis by nocodazole, which is a known microtubule disassembly agent.	Huang and Lee, 1998
TM3 cells	As^{III} SA	0.008, 0.77, 7.7	70 days	Various	Changes in expression of cell-cycle related genes: ⇓ at 7.7 for Cyclin D1; for PCNA: ⇑ at 0.008, ⇓ at 0.77 and 7.7. Changes in expression of DNA repair genes: ⇓ at 0.77 and higher for ERCC6 and OGG1; ⇓ at 7.7 for XPC, MYH, and DNA polymerase-β; Changes in expression of other genes: ⇓ at 7.7 for, MnSoD, and Bax; for DNMT1: ⇑ at 0.008, NSE at 0.77, ⇓ at 7.7.	DuMond and Singh, 2007
E7 cells	As^{III} ATO	0.025, 0.05, 0.1, 0.25, 0.51	4 weeks	0.005	⇑ Aurora-A protein expression level, with a positive dose response, reaching 4.2x control at dose of 0.1; unreported data showed ⇑ Aurora-A mRNA.	Tseng et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
BEAS-2B cells	As^{III} AC	1.25, 2.5, 5, 10, 20	12 hr	1.25	⇑ GADD45α protein expression level, with a positive dose response; however, only a marginal ⇑ in GADD45α transcription; pretreatment with NAC completely blocked the ⇑ of GADD45α. After iAs dose of 20 for 4-20 hr: transitory activation of Akt & transitory ⇑ phosphorylation of FoxO3a. iAs induced accumulation of GADD45α mRNA and did not affect the degradation of GADD45α protein. iAs stabilized GADD45α mRNA through nucleolin; it induced the binding of mRNA stabilizing proteins, nucleolin and less potently, HuR, to GADD45α mRNA. iAs did not affect the expression of nucleolin; iAs treatment resulted in redistribution of nucleolin from nucleoli to nucleoplasm. Silencing of nucleolin reversed iAs-induced stabilization of the GADD45α mRNA.	Zhang et al., 2006
$Gclm^{+/+}$ MEF cells and $Gclm^{-/-}$ MEF cells, from GCLM knockout mice	As^{III} SA for all	See rows under Apoptosis and Cytotoxicity for this citation for experimental conditions. Analysis of global gene expression profiles revealed up-regulation or down-regulation of vast numbers of genes by iAs. Significant changes were largely consistent with changes in the expression of DNA damage and repair genes, the suppression of TGF-β signals, inhibition of integrin-mediated cell adhesion, induction of multiple transcription factors, repression of co-repressors, and the derailment of cell cycle regulatory functions. iAs exposure also caused profound changes in protein levels in what appear to be conflicting regulatory changes. These changes go hand in hand with massive up-regulation of HSPs, metalloproteinases, and proteasome components, and the authors suggested that iAs induces critical changes in protein folding and structure and that the cells mount a major effort to properly refold misfolded proteins or to eliminate them altogether. Global gene expression profiles also indicated that tBHQ is significantly effective in reversing iAs-induced gene deregulation in $Gclm^{+/+}$ but not in $Gclm^{-/-}$ MEFs. These results suggested that regulation of GSH levels by GCLM determines the sensitivity to iAs-induced apoptosis and cytotoxicity by setting the overall ability of the cells to mount an effective antioxidant response.				Kann et al., 2005b
NB4 cells NB4-M-AsR2 cells	As^{III} ATO for both	0.5, 1 2, 4	16 hrs for both	0.5 2	JNK activation leading to phosphorylation of c-jun, after treatment with ATO alone and co-treatment with 100 μM Trolox: At 0.5: slight ⇑ alone, ⇑ with Trolox,; at 1: big ⇑ alone, huge ⇑ with Trolox; At 2: slight ⇑ alone, ⇑ with Trolox; at 4: big ⇑ alone, huge ⇑ with Trolox	Diaz et al., 2005
JB6 C141 PG13 cells JB6 C141 PG13 cells exposed to 4 kJ/m^2 of UVB at end of iAs treatment	As^{III} SA for both	1, 5, 10, 20 for both	24 hrs for both	5 5	⇓ in p53 activity with dose, reaching ~30% of control at dose of 20. ⇓ in p53 activity with dose, reaching ~5% of that with the UVB treatment alone at dose of 20. The UVB exposure strongly stimulated p53 activation (to ~9x the control level), and the iAs treatment inhibited that increase, reducing it to a point estimate less than that of the untreated control at the dose of 20.	Tang et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
JB6 C141 P^+1-1 cells JB6 C141 P^+1-1 cells exposed to 4 kJ/m^2 of UVB at end of iAs treatment	As^{III} SA for both	1, 5, 10, 20 0.1, 1, 5, 10	24 hrs for both	5 5	⇑ in AP-1 activity to 2x control at 5 and to 5x control at 10, back to control level at 20; ⇑ in AP-1 activity to 1.5x and 1.7x that with the UVB treatment alone at doses of 5 and 10, respectively. It should be noted that the UVB exposure strongly stimulated AP-1 activation (to ~6x the control level).	Tang et al., 2006
JB6 C141 cells exposed to 4 kJ/m^2 of UVB at end of iAs treatment	As^{III} SA for both	5, 10 1, 5, 10	24 hrs for both	5 5	⇓ UVB-induced p53 phophorylation (at serines 15 and 392); bigger ⇓ at 10; ⇓ UVB-induced p53 DNA binding activity; bigger ⇓ at 10; Other experiments not involving UVB showed that iAs inhibited casein kinase 2α activity and decreased p53-regulated p21 protein expression.	Tang et al., 2006
SV-HUC-1 cells	As^{III} SA MMA^{III} DMA^{III}	0.5 0.05, 0.1, 0.2 0.2, 0.5	Subcultured twice weekly for 25 passages	0.5 0.05 0.2	Results of cDNA microarray analysis of ~2000 genes: 114 genes were differentially expressed among the 6 groups; DMA^{III} had a substantially different gene profile from other 2. Gene coding for IL-1 receptor, type II, was the only gene with ⇑ expression by all arsenicals. 11 genes had ⇓ expression by all arsenicals. For 2 of those 11, transcription was partially restored by treatment with 5-aza-dC, which suggests that the suppression resulted from epigenetic DNA hypermethylation. The treatments also caused differential morphological changes affecting cell size, extent of aggregation, and adhesion ability.	Su et al., 2006
SVEC4-10 cells	As^{III} SA	5, 10, 20	24 hr	5	Protein levels: α7-nAChR: slight ⇓ at 5, huge ⇓ at 10 and 20, with only a trace present at 20; eNOS: slight ⇓ at 5, huge ⇓ at 10 and 20, with none present at 20; ChAT: NSE	Hsu et al., 2005
BEAS-2B cells	As^{III} ATO	10, 20, 50	12 hr	10	HSP70 protein ⇑: fold increases over control by Western blotting after 12-hr recovery period: 2.6x, 2.5x, and 7.9x at doses of 10, 20, and 50, respectively; alternative ELISA analysis gave similar response but with much higher fold increases over the control. Co-treatments with large doses of antioxidants CAT, SOD, NAC, or SF considerably reduced the arsenic effect, with the NAC treatment completely eliminating it.	Han et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
BEAS-2B cells	As^{III} ATO	10, 20, 50	6 hr	10 for all	mRNA levels determined by RT-PCR, with no recovery time after exposure, fold ⇑ over control: at 10: HSP70A, 4.4x; HSP70B, 4.3x; HSP70C, 3.6x. After 4, 8 & 12 hr recovery periods, mRNA levels usually ⇓ to levels closer to control and often NSE; however, all increases remained significantly higher than control at dose of 50.	Han et al., 2005
BEAS-2B cells	As^{III} ATO	10, 20, 50	6 hr	10	Intracellular GSH levels: ⇓ to 80% of control at 10, followed by dose-related decrease to 70% of control at dose of 50; co-treatment with NAC blocked this effect of iAs.	Han et al., 2005
HT1197 cells	As^{III} SA	10	8 hr	10	p53 protein levels: slight ⇑; at 24 hr at this dose: big ⇑ to 4x control; p21 protein levels: ⇑ to 7.5x control; also at this dose: at 12-20 hr, much smaller increases; at 24 hr, big ⇓; at 4 hr, 2.4x control	Hernández-Zavala et al., 2005
SVEC4-10 cells	As^{III} SA	4, 8, 12, 16 Separase was tested only at the highest dose.	24 hr	Various	Effects on protein levels: securin: ⇓ at 12 to 23%, ⇓ at 16 to 5%; separase: ⇑ to 1.2x control (of ?-able significance); phospho-CDC2 (threonine-161): ⇓ at 16 to 34%; CDC2: ⇓ at 12 to 73%, ⇓ at 16 to 38%. cyclin B1: ⇓ at 16 to 11%; p53 (DO-1): ⇑ at 4 to 2x control with positive dose response reaching 8x control at dose of 16;	Chao et al, 2006a
RAW264.7 cells	As^{III} SA	2.5, 5	24 hr	2.5	TRAP histochemistry was done 3 days after the end of the iAs treatment: huge ⇑ in TRAP activity at both doses; this increased activity accompanied multinucleated cell formation and the beginning of osteoclast differentiation; the level of effect at both doses was comparable to (and, at the dose of 2.5, probably higher than) that caused by a RANKL treatment; co-treatment with CAT blocked most of the iAs-induced effect.	Szymczyk et al., 2006
HCT116 cells (securin +/+) HCT116 cells (securin -/-)	As^{III} SA for both	4, 8, 12, 16 for both	24 hr for both	Various Various	Effects on protein levels: securin: ⇓ at 4, then ⇓ with dose to ~30% at 16; phospho-p53 (serine 15): ⇑ to 2x control at 4 and then ⇑ with dose to 6x control at 16. p53 (DO-1): ⇑ to 2x control at 12 and ⇑ to 3.4x control at 16. No securin present at any dose in -/- mutant; phospho-p53 (serine 15): ⇑ to 3.5x control at 4 and then ⇑ with dose to 7x control at 16; p53 (DO-1): ⇑ to 1.8x control at 4 and then ⇑ with dose to 3.2x control at 16.	Chao et al., 2006a
RKO cells (p53 wt) SW480 cells (p53 mutant)	As^{III} SA for both	8, 16 for both	24 hr for both	16 16	Effects on protein levels of securin: rather similar ⇓ in both, reaching 27% and 13% of control in RKO and SW480, respectively.	Chao et al., 2006a

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
FGC4 cells	As^{III} SA	50, 65 Equivalent to ≤5% and 20-25% cytotoxicity	24 hr	Various	Effects on protein levels of SPs: MT, HSP60 & HSP90: NSE at either dose; HSP25: big ⇑ at 50, big ⇑ at 65; HSP40: big ⇑ at 50, big ⇑ at 65; HSP70: big ⇑ at 50, huge ⇑ at 65.	Gottschalg et al., 2006
HepG2 cells	As^{III} SA	15, 55 Equivalent to ≤5% and 20-25% cytotoxicity	24 hr	Various	Effects on protein levels of SPs: MT: NSE at 15, very slight ⇑ at 55. HSP60 & HSP90: NSE at either dose. HSP27: slight ⇑ at 15, ⇑ at 55. HSP40: slight ⇑ at 15, big ⇑ at 55. HSP70: ⇑ at 15, big ⇑ at 55	Gottschalg et al., 2006
Rat hepatocytes	As^{III} SA	10, 20 Equivalent to ≤5% and 20-25% cytotoxicity	24 hr	Various	Effects on protein levels of SPs: MT, HSP60 & HSP90: NSE at either dose. HSP25: ⇑ at 10, ⇑ at 20. HSP40: NSE at 10, ⇑ at 20; HSP70: NSE at 10, big ⇑ at 20	Gottschalg et al., 2006
HELF cells	As^{III} SA	0.1, 0.5, 1, 5, 10	3, 6, 12, 24, or 48 hr	Various	HSP27 protein: ⇑ at 0.5 and 1 after 12-hr treatment, but ⇓ at 5 and 10 after 48-hr treatment; HSP27 was said to be a chaperone whose expression protects against oxidative stress and is anti-apoptotic. HSP70 protein: ⇓ at 1 and 5 after 12-hr treatment, but ⇑ at 5 and 10 after 24-hr treatment; an inducible form of HSP70 was said to be expressed at a high level in various malignant human tumors.	Yang et al., 2007
MDAH 2774 cells	As^{III} ATO	1, 2, 5, 8	probably 72 hr or 96 hr	1 or 2	⇓ topoisomerase IIα to about half of control value at dose of 5 (paralleling degree of cytotoxicity)—there is some question about this result because band densities were not normalized to another protein; decrease possibly resulted from ⇓ in cell number	Askar et al., 2006
UROtsa cells	As^{III} SA	0.5, 5, 10, 25	24 hr	5	⇑ accumulation of high-molecular-weight Ub-conjugated proteins; co-treatment with BSO: ⇑⇑ in the same effect, which was then seen even at dose of 0.5.	Bredfeldt et al. 2004
UROtsa cells	MMA^{III}	0.05	12 weeks	0.05	Huge ⇑ COX-2 protein, with an even higher level after 24 weeks and still high level after 52 weeks	Eblin et al., 2007
UROtsa cells	As^{III} SA MMA^{III}	1, 10 0.01, 0.05, 0.1	4 hr for both	1 0.01	Big ⇑ COX-2 protein level at both doses; Regarding COX-2 protein level: huge ⇑ at 0.01, big ⇑ over control at 0.05, ⇑ over control at 0.1. Various experiments, including some with pharmacological inhibitors of various signal transduction pathways, led to the conclusion that MMA^{III} appears to stimulate ligand-independent activation of EGFR, subsequent ERK-1 and -2 phosphorylation via MEK-1 and -2, as well as activation of PI3K, which leads to elevated levels of COX-2 protein.	Eblin et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
UROtsa cells	As^{III} SA	1, 10	30 min for both	1	⇑ HSP70 protein (similar response at both doses; with lower dose, the level decreases from 60 to 240 min); ⇑ MT protein (much bigger ⇑ at higher dose).	Eblin et al., 2006
	MMA^{III}	0.05, 0.5, 5		0.05	⇑ HSP70 protein (strong response at all doses); ⇑ MT protein (much bigger ⇑ at higher doses).	
HaCaT cells	As^{III} SA	2, 6, 10	24 hr for all	6, 2	Extent of selenium incorporation into selenoproteins determined using ^{75}Se-selenite: LOECs of 6 and 2 for ⇓ TrxR1 and ⇓ cGpx, respectively; big ⇓ at higher dose(s);	Ganyc et al., 2007
	As^{V}	1, 5, 10		None	NSE	
	MMA^{III}	1, 2, 3		2, 3	LOECs of 2 and 3 for ⇑ TrxR1 and ⇓ cGpx, respectively;	
	DMA^{III}	1, 4, 7		4	⇑ of TrxR1 and cGpx at dose of 4 and decrease for both proteins to near control levels at higher dose.	
MEF cells	As^{III} SA	0.01, 0.1, 5, 10, 20, 40	5 hr	5	⇑ eIF2α phosphorylation; ⇑ ATF4 protein; ⇑ ATF3 protein; at doses ≥ 10: ⇑ GADD45a protein and ⇑ CHOP protein; all effects showed substantial dose-related increases; effects were mostly blocked by NAC pretreatment (ATF3 was not tested.)	Jiang et al., 2007
MEF cells	As^{III} SA	20 in most assays	GADD45a is a small protein implicated in the regulation of the cell cycle, DNA repair, genome stability, innate immunity, and apoptosis. Additional tests with modulators and genetic variants of MEF cells showed the following: ATF4 is required for an increase in GADD45a mRNA following iAs exposure, and its induction is independent of p53. ATF4 binds to a GADD45a promoter element in response to iAs stress. Exposure to iAs reduces proteasome activity, which permits the increase in transcription of GADD45a to actually result in an increase in the protein level of GADD45a, which is labile.			Jiang et al., 2007
Protein extracts (membrane fraction) derived from BAEC cells	MMA^{III}	1, 2.5, 5, 7.5, 10, 15	5 min for all	1	For MMA^{III} only: ⇓ eNOS activity, IC_{50} = 2.1 and a 5-min treatment at dose of 10 caused ~90% ⇓; co-treatment with DTT substantially blocked the MMA^{III} effect resulting in only ~50% ⇓	Sumi et al, 2005
	As^{III} SA, As^{V}, MMA^{V} or DMA^{V}	10		None		

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N-18 cells	As^{III} SA	5, 10, 20, 50	6 hr	5 for first effect noted	⇑ synthesis of HSP proteins of 50, 73, 78, 89, 98, and 104 kDa; other experiments demonstrated: ⇑ activation of HSF1 DNA-binding (detected by EMSA) by dose of 20 (lowest dose tested) in 2 hr; ⇑ induction of HSP70-luciferase reporter gene expression by dose of 20 (lowest dose tested) in 6 hr; an ⇑ induction of HSP70 mRNA by dose of 50 (lowest dose tested) in 1 hr	Khalil et al., 2006
N-18 cells	As^{V} potas-sium arsenate	20	6 hr	20	⇑ induction of HSP70-luciferase reporter gene expression (point estimates suggests weaker response from As^{V} than from same dose of As^{III} SA)	Khalil et al., 2006
N-18 cells	As^{III}	2, 5, 10, 20, 50, 100, 200, 500	0.5, 1, 2, 3, 6, or 12 hr	Various	⇑ induction of HSP70-luciferase reporter gene expression, with bell-shaped dose-response curves for each duration of treatment; e.g. for 1-hr treatment, the peak occurred at dose of 200 (highest peak seen); for 6-hr treatment, the peak occurred at dose of 20; the bell-shaped curves shifted to the left as the duration increased. Results on HSP70-firefly luciferase activity were normalized against that of Renilla luciferase to correct for differences in transfection efficiency and/or toxic and non-specific effects of the experimental treatment conditions.	Khalil et al., 2006
$hsf^{+/+}$ immortalized MEF cells $hsf^{-/-}$ immortalized MEF cells $hsf^{-/-}$ immortalized MEF cells transfected with HSF1 expression vector	As^{III} SA for all	5, 10, 20, 50, 100, 200, 500 for all	1 hr for all	50 None 50	⇑ induction of HSP70-luciferase reporter gene expression: ⇑ with dose up to peak at 200; still big ⇑ at 500 No effect; clearly iAs requires a functional HSF1 gene to induce HSP70-luciferase reporter gene expression. ⇑ with dose up to peak at 200; still big ⇑ at 500. Generally similar results were also found with treatment durations of 0.5 and 2 hr.	Khalil et al., 2006
H1355 cells	As^{III} ATO	5, 25, 50, 100, 200	24 hr	Various	Phosphorylation of ERK 1/2: ⇑ at 50, huge ⇑ at 100 and 200; Phosphorylation of JNK: slight ⇑ at 50, huge ⇑ at 100 and 200; Phosphorylation of p38: slight ⇑ at 100, big ⇑ at 200; PARP cleavage: ⇑ at 100 and 200; survivin protein level: ⇓ at 100 and 200; ubiquitination in total cell lysate: big ⇑ at 100 (the only dose tested for it)	Cheng et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
H1355 cells	As^{III} ATO	100	24 hr	Various	Effects of pretreatments with specific inhibitors of p38, JNK, MEK 1/2 (upstream of ERK 1/2) or ubiquitin-proteasome showed that blockage of either p38 or JNK phosphorylation attenuated the ATO-induced down-regulation of survivin and increase of PARP cleavage; however, blockage of ERK 1/2 or ubiquitin-proteasome did not attenuate those same effects. Also, only inhibitors of p38 and JNK affected ATO-induced cytotoxicity, which was just slightly reduced (i.e., there was ~5-8% more cell survival). The specific inhibitors of p38, JNK, and MEK 1/2 did block the phosphorylations of p38, JNK, and ERK 1/2, respectively.	Cheng et al., 2006
A549 cells	As^{III} ATO	2	48 hr	Protein levels and mRNA levels: 2 μM iAs: NSE on survivin. 200 μM sulindac: NSE on survivin. (Sulindac is a NSAID that inhibits COX-2.) (2 μM iAs + 200 μM sulindac): big ⇓ in survivin (by 72 hr almost no survivin was protein present). Protein levels only for combined treatment: big ⇑ for p53 but NSE for XIAP, cIAP-1, cIAP-2, and Bcl-2. Inhibition of p53 ⇑ by siRNA blocked the down-regulation of survivin by the (2 μM iAs + 200 μM sulindac) treatment. (It is known that p53 binds to the survivin promoter and suppresses its transcription.) Transfected cells with a survivin-luciferase reporter also showed the big ⇓ in survivin for the combined treatment and NSE for single treatments. Pretreatment with NAC mostly (or entirely) blocked the synergistic effect of a ⇓ of survivin protein (was shown both by Western-blot and luciferase reporter assays)		Jin et al., 2006b
A549 cells	As^{III} ATO	2	48 hr	More about the synergistic effect between 2 μM iAs and 200 μM sulindac: evidence that changes in survivin levels are related to synergistic big ⇑ in cytotoxicity: (1) if marked overexpression of survivin by transfection, then ⇓ in cytotoxicity by 1/3, (2): if inhibition of survivin level by siRNA, then ⇑ in cytotoxicity. (Sulindac is a NSAID that inhibits COX-2.)		Jin et al., 2006b
N-18 cells	As^{III} SA	2, 5, 10, 20, 50, 100, 200, 500	6 hr	10	Induction of HSP70-luciferase reporter gene expression: big ⇑ at 10, huge ⇑ (peak) at 20, big ⇑ at 50, then NSE. Effects of pretreatment + co-treatment with modulators: DTT: almost entirely blocked iAs effect: slight ⇑ at 20 and 50, questionable ⇑ at 10 and 100; NAC and GSH (individually): ⇑ at 10, big ⇑ at 20, huge ⇑ (peak) at 50, ⇑ at 100, then NSE	Khalil et al., 2006
NHEK cells	As^{III} SA	0.1, 1, 5, 10	72 hr	0.1 for ⇑ 1 for ⇓	Level of β_1-integrin protein: After a possible slight ⇑ at 0.1, there was a ⇓ to 61-63% of control level at other 3 doses.	Lee et al., 2006b
NHEK cells	As^{III} SA	0.1, 1, 5, 10	7 days	0.1 for ⇑ 1 for ⇓	Level of β_1-integrin mRNA: After a possible slight ⇑ at 0.1, a dose-related ⇓ at other 3 doses reaching 47% of control at dose of 10.	Lee et al., 2006b

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NHEK cells	As^{III} SA	1	24, 48, 72 hr	1	Level of FAK protein based on immunofluorescence: ⇑ at 24 hr followed by ⇓ below control level at later times, with almost none present at 72 hr	Lee et al., 2006b
Normal human mammary epidermal keratinocytes	As^{III} SA for all	0.005, 0.5, 1. 2.5 1, 2.5, 5	4 hr 8 hr	0.005 2.5	⇑ COX-2 mRNA (also at 8 and 24 hr) ⇑ COX- protein (also at 12 hr), also under the same or similar conditions: ⇑ PGE_2 secretion, phophorylation of p42/44 MAPK, and DNA synthesis. Tests with various modulators showed that iAs^{III} elevates COX-2 at the transcriptional and translational levels.	Trouba and Germolec, 2004
Swiss 3T3 mouse cells	As^{III} SA	1, 2.5, 5, 10, 20, 40	16 hr	1	⇑ GSH synthesis; starting at 2.5: cell retraction and loss of thick cables of actin filaments, ⇓ cytoskeletal protein synthesis; starting at 20: ⇑ in protein sulfhydryl content of both cytoskeletal and cytosolic protein fractions, with the time course showing a slight decrease before the increase. There was also severe loss of microtubules.	Li and Chou, 1992
UROtsa cells	As^{III} SA MMA^{V} DMA^{V}	5, 50 for all	2 hr for all	5 for all	Increased DNA binding of the AP-1 transcription factor, which is often associated with the regulation of genes involved in cell proliferation. For all 3 chemicals the response was higher at dose of 50; the highest amount of binding was with SA.	Simeonova et al., 2000
UROtsa cells	As^{III} SA	10, 50	2 hr	10	Use of a cDNA array consisting of 588 human genes, and other methods: At 10: ⇑ activity of 7 genes; ⇓ activity in 6 genes At 50: ⇑ activity of 15 genes; ⇓ activity in 6 genes. Specifics: Genes affecting cell growth: ⇑ for c-fos, c-jun, Pig 7, EGR-1, and Rho 8 Genes affecting cell growth arrest: ⇑ for GADD45 and GADD153	Simeonova et al., 2000
C-33A cells HeLa cells Jurkat cells LCL-EBV cells	As^{III} SA for all	1, 10, 25, 50 for all	24 hr for all	None 10 1 1	p53 protein expression: No ⇑, slight ⇓ at high doses, very high basal level; ⇑, peak at 25, low basal level; ⇑, peak at 10, moderate basal level; ⇑, peak at 10, very low basal level. Decreases above peak may result from cell death.	Salazar et al., 1997

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HeLa cells	As^{III} SA	100, 200, 400	30 min	100	⇑ GADD153 mRNA expression (harvested for RNA isolation after 4 hours of incubation following the arsenite treatment). This effect was increased by pretreatment with BSO, PHEN (slight increase), BCS, or mannitol (an HO^- scavenger). Effect was completely blocked by pretreatment with NAC.	Guyton et al., 1996
WI38 cells Simian virus 40 (SV40)-transformed subline of the above parental W138 line with twice the GPx specific activity of parental cells	As^{III} SA for both	100, 200, 400 for both	30 min	100 for both	⇑ GADD153 mRNA expression (harvested for RNA isolation after 4 hours of incubation following the arsenite treatment). The increase was cut approximately in half (i.e., half the slope) in the transformed cell line. Other parts of this study showed that AP-1 is critical to oxidative regulation of GADD153.	Guyton et al., 1996
JB6 Cl41 cells	As^{III} SA As^V	3.125, 12.5, 50, 200 3.125, 12.5, 50, 200	3 hr	50 50	⇑ activity of JNKs: Stronger response at 50 for As^V (sodium arsenate); both forms shown some response by 1 hr at dose of 200; arsenic did not induce p53-dependent transactivation.	Huang et al., 1999b
JB6 Cl41 cells	As^{III} SA As^V	200 200	0 min 60 min	200 200	⇑ phosphorylation of JNKs: stronger response for As^V (sodium arsenate)	Huang et al., 1999b
HFW cells (diploid human fibroblasts)	As^{III} SA	5, 10, 20	24 hr	5	⇑ heme oxygenase activity (arsenic-induced synthesis of this enzyme was blocked by co-treatment with antioxidants sodium azide or DMSO); ⇑ ferritin	Lee and Ho, 1995
HFW cells (diploid human fibroblasts)	As^{III} SA	1, 2.5, 5, 10, 20	24 hr	1	⇑ GSH (by 20 level drops to control level)	Lee and Ho, 1995
HFW cells (diploid human fibroblasts)	As^{III} SA	0.5. 2, 10	24 hr	See next column	⇑ SOD activities, ⇓ catalase and GPx activities, with LOECs being 0.5, 2, and 10, respectively	Lee and Ho, 1995
Both HL-60 cells and HaCaT cells	As^{III} SA	0.5, 20	3 days	0.5	⇑ hTERT protein expression; however ⇓ hTERT protein expression at 20 (i.e., significantly inhibited at higher concentration)	Zhang et al., 2003
HaCaT cells	As^{III} SA	0.5, 10, 20	3 days	0.5	⇑ telomerase activity; however; telomerase activity was below control level at 10 and even lower at 20	Zhang et al., 2003

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HL-60 cells	As^{III} SA	0.1, 0.5, 1, 10, 20	3 days	0.1	⇑ telomerase activity; however; telomerase activity was below control level at 10 and even lower at 20	Zhang et al., 2003
NB4 cells	As^{III} ATO	0.75	8 days	0.75	⇓ telomerase activity; ⇓ hTERT mRNA and protein levels; ⇓ c-myc mRNA and protein levels; ⇑ hTER mRNA level; no change in p53 mRNA or protein level; no change in Sp1 mRNA or protein levels. Further experiments showed that arsenic inhibits transcription of hTERT and inhibits the function of Sp1 in hTERT transcription.	Chou et al., 2001
NB4 cells	As^{III} ATO	0.75	2 days	0.75	⇓ hTERT mRNA	Chou et al., 2001
NB4 cells	As^{III} ATO	0.1, 0.25	12 days	0.1	⇓ hTERT mRNA	Chou et al., 2001
HeLa cells LoVo cells MCF7 cells	As^{III} ATO for all	2 for all	14 days for all	2 for all	⇓ hTERT mRNA and ⇓ c-myc mRNA for all	Chou et al., 2001
Normal human keratinocytes treated with 50 mJ/cm^2 UVB before or after iAs treatment	As^{III} SA for both	1, as pretreatment 1, as post-treatment begun 24 hr after irradiation	24 hr for both	1 None	No change from control in procaspase-8 and procaspase-9 protein levels or in caspase-3, caspase-8, and caspase-9 enzyme activities; this is considered an LOEC because the iAs-pretreatment blocked the effects of UVB described below. ⇓ procaspase-8 protein level, slight ⇓ procaspase-9 protein level; ⇑ caspase-8 enzyme activity; ⇑ caspase-9 enzyme activity; ⇑ caspase-9 enzyme activity; effects similar to with UVB alone	Chen et al., 2005b
NB4 cells	As^{III} ATO for both	1 0.5, 1.0, 1.5, 2.0	2 days 3 days	1 1.0	As a result of permeability changes in the outer mitochondrial membrane: Slight release of cytochrome c into cytoplasm; complete release by 3 days of treatment; ⇑ Cpp32 (was activated) as shown by ⇓ of its precursor	Jing et al., 1999
SHE cells	As^{III} SA As^{V}	6, 8 50, 100, 150	48 hr for both	-	From among these treatment groups, 5 neoplastic transformed cell lines were produced that were shown to be tumorigenic, of these: All had ⇑ c-Ha-ras (oncogene) mRNA expression; 4 had ⇑ c-myc (oncogene) mRNA expression; A few other arsenic-treated cell lines also showed the same effects.	Takahashi et al., 2002

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Peritoneal macrophages (PMs) from CDF_1 mice	As^{III} SA	1.25, 2.5, 5, 10	48 hr for all	1.25	Changes in release of TNF-α from macrophages in the presence of both lipopolysaccharide and recombinant murine interferon γ, which are two compounds known to increase secretory functions of PMs: ⇓ at 1.25, no change from control at 5; big ⇑ at 10;	Sakurai et al., 1998
	As^{V}	125, 250, 500, 1000		500	Big ⇑ at 500 and much bigger ⇑ at 1000;	
	MMA^{V}	1.25, 2.5, 5, 10 mM		None	No effect;	
	DMA^{V}	1.25, 2.5, 5, 10 mM		2.5 mM	⇓ at 2.5, 5 and 10 mM;	
	TMA^{V}	1.25, 2.5, 5, 10 mM		5 mM	⇓ at 5 and 10 mM.	
U118MG cells	As^{III} ATO	1, 5, 10, 25	24 hr	1 or 5	Changes in protein expression: p53: ⇑ at 1, ⇓ at 5 or higher; Bcl-2: ⇑ at 1 or higher; Bax: ⇓ at 1 or higher; HSP_{70}: ⇑ at 5 or higher; co-treatment with lipoic acid blocked all of these effects at an iAs^{III} dose of 5.	Cheng et al., 2007
HaCaT cells (immortalized, non-tumorigenic human keratinocyte cell line)	As^{III} SA for both	20 for both	6 hr for both	20 for both	⇑ caspase-3 activation	Pi et al., 2005
As-TL cells (arsenic-tolerant cells, which are HaCaT cells that were cultured for 28 weeks in 100 nM As^{III} SA)					Much smaller ⇑ in caspase-3 activation than in HaCaT cells	
HUVEC cells	As^{III} ATO	20	2 hr	20	⇑ expression of ICAM-1; effect was similarly strong after 24-hr treatment but weaker after 4- or 8-hr treatment (yet still ⇑ above control level). Effect was completely blocked by a 1-hr pretreatment with 15 mM NAC followed by a co-treatment of NAC with the As^{III}-treatment.	Griffin et al., 2003
Apoptosis						
K562 cells	As^{III} ATO	2.5	12 hr	2.5	⇑ annexin V, an apoptotic marker	Li and Broome, 1999

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NCI (human myeloma cell line)	As^{III} ATO	1	24 hr	1	Apoptosis was demonstrated by 4,6-diamidino-2-phenylindole staining, by the demonstration of typical DNA ladders corresponding to internucleosomal cleavage, and by annexin-V and PI staining. Various indications of induction of apoptosis were also presented (with less detail) for at least 1 other myeloma cell line and for fresh myeloma cells. In the NCI cells, [3H]thymidine incorporation was also used to assess proliferation: the 50% growth-inhibitory concentration (IC_{50}) in NCI cells was found to be 0.3 μM, based on concentrations tested of 0.05, 0.1, 0.5, 1, 5, 10 over 72 hr. Similar testing of 3 other human myeloma cell lines yielded IC_{50}s of 0.1 for 1 line and ~1 for 2 other lines, with much less detail presented.	Rousselot et al., 1999
MGC-803 cells	As^{III} ATO	0.01–1	24 hr	0.01	Apoptosis detected by flow cytometry and by agarose gel electrophoresis of genomic DNA showing typical DNA ladder; at various doses apoptosis was also induced in 5 other human malignant cell lines.	Zhang et al., 1999
Primary cultures of rat cerebellar neurons	As^{III} SA DMA^{V}	5, 10 5 mM	12 hr 48 hr	5 5 mM	Demonstrated by "DNA ladders" with agarose gel electrophoresis and microscopic examination (nuclear fragmentation and/or condensation)	Namgung and Xia, 2001
MC/CAR (human multiple myeloma cell line)	As^{III} ATO	1, 2, 5, 10	72 hr	2	Apoptosis was demonstrated by an analysis using a FACStar flow cytometer and by detection of cell membrane changes by labeling with annexin V-FITC and annexin PI	Park et al., 2000
V79-C13 Chinese hamster cell line	As^{III} SA	10	24 hr	10	Apoptotic cells appeared by 6 hr after treatment began and included 40% of cells by 24 hr; frequency gradually decreased during 48 hr of observation after treatment ended	Sciandrello et al., 2002
HL-60 cells HaCaT cells	As^{III} SA for both	0.1, 0.5, 1, 10, 20, 40 for both	5 days for both	1 or possibly 0.5 10	By use of Hoechst/PI staining assay: ⇑ in apoptosis for both; for both cell lines, there was the same general response, but to a lesser extent, when same treatments were given over 1 or 3 days.	Zhang et al., 2003

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HL-60 cells HaCaT cells SW13 cells SW480 cells HT1080 cells	As^{III} SA for all	1, 10, 20, 40 for all	5 days for all	1 10 ~20 ~20 1	By use of Hoechst/PI staining assay: ⇑ in apoptosis in all SW13 and SW480 are telomerase negative cell lines, and they showed much less apoptosis at all concentrations than the other 3 cell lines. HT1080 is a telomerase positive cell line, and it was intermediate in the amount of apoptosis at all concentrations to HL-60 (which was higher) and HaCaT. Thus there is a strong positive correlation between telomerase activity and susceptibility to arsenic-induced apoptosis.	Zhang et al., 2003
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM for both	24 hr for both	None 5 mM	Apoptosis demonstrated by TUNEL staining: there was little evidence of induction of apoptosis by MMA^V alone; however, the cells also treated with BSO showed considerable apoptosis.	Sakurai et al., 2005a
TRL 1215 cells	DMA^V	5 mM	24 hr	5 mM	Apoptosis demonstrated by TUNEL staining: huge ⇑, much more extensive that that of the considerable level of apoptosis reported in row above for MMA^V + BSO	Sakurai et al., 2005a
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM for both	12, 24, 36, or 48 hr for both	5 mM 5 mM	Apoptosis demonstrated by FACS analysis after annexin-V and PI staining: 5 mM MMA^V alone caused some apoptosis after 48 hr; however, that response was slight compared to the response of the MMA^V + BSO group after only 24 hr, and the MMA^V + BSO group showed huge ⇑ at 36 hr and even bigger ⇑ at 48 hr. After 48 hr, the percentages of annexin-positive cells were as follows: control, 1.9%, BSO alone, 6.7%; MMA^V alone, 10.6%; MMA^V + BSO, 64%. The PI staining showed that by 48 hr there were also numerous induced necrotic cells in the MMA^V + BSO group.	Sakurai et al., 2005a
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM for both	24 hr for both	None 5 mM	Apoptosis demonstrated by agarose gel electrophoresis showing induced internucleosomal DNA fragmentation: Substantial DNA fragmentation in MMA^V + BSO group; no effect with MMA^V alone.	Sakurai et al., 2005a

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TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	DMA^V for both	5 mM for both	24 hr for both	5 mM 5 mM	Apoptosis demonstrated by agarose gel electrophoresis showing induced internucleosomal DNA fragmentation: Massive ⇑ with DMA^V alone (many times more than with MMA^V + BSO in previous row); slight ⇑ in DMA^V + BSO group (about the same as with MMA^V + BSO in previous row)	Sakurai et al., 2005a
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM for both	12 hr for both	None 5 mM	Cellular caspase-3 activation: ⇑ to ~1.6x in MMA^V + BSO group; no effect without BSO; other experiments showed that co-treatment with 150 μM Z-DEVD-FMK (a caspase 3 inhibitor) during preincubation period and during a 24-hr MMA^V treatment blocked almost all or all of the cytotoxicity detected by AB assay (i.e., ~35% survival without inhibitor, ~92% survival with inhibitor); with a 48-hr MMA^V + BSO treatment, Z-DEVD-FMK caused cytotoxicity to be markedly reduced (i.e., ~7% survival without inhibitor, ~42% survival with inhibitor)	Sakurai et al., 2005a
Primary keratinocytes (in third passage) obtained from foreskins of adults	As^{III} SA	1, 5, 10	48 hr	1	Apoptosis detected by the presence of DNA ladders after agarose gel electrophoresis: much bigger ⇑ at two higher doses, which showed a similar effect	Liao et al., 2004
Primary keratinocytes (in third passage) obtained from foreskins of adults	As^{III} SA	1, 5, 10	48 hr	Various	Protein levels detected by Western blotting: FADD: ⇑ at 1, bigger ⇑ at 5 and 10; caspase-8 (p18, active): ⇑ at 1, huge ⇑ at 5 and 10; caspase-3 (p20, active): huge ⇑ at 5 and 10; cleaved PARP (85 kD): ⇑ at 5 and 10; additional experiments with and without modulators confirmed the involvement of the Fas-associated pathway in iAs-induced apoptosis.	Liao et al., 2004
HeLa cells	As^{III} ATO	2	3 days	2	Induced apoptosis (experimental – control) detected by Annexin V/PI flow cytometry: ~13% for iAs alone; ~3% for 10 μM emodin alone; ~41% for iAs plus 10 μM emodin; ~14% for iAs with both 10 μM emodin and 1.5 mM NAC. Other experiments showed that the effect of emodin in enhancing iAs-induced apoptosis involved a decrease of mitochondrial membrane potential. Emodin was used because it has a semiquinone structure that is likely to increase the generation of intracellular ROS.	Yi et al., 2004

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HeLa cells	As^{III} ATO	2	3 days	2	Induced apoptosis (experimental – control) detected by Annexin V-FITC/PI flow cytometry: 27.0% for iAs alone; 6.9% for 30 μM emodin alone; 44.1% for iAs plus 30 μM emodin; 20.4% for iAs with both 30 μM emodin and 1.5 mM NAC. Emodin and iAs synergistically interacted to greatly ⇑ the ROS level and to cause cytotoxicity. Pretreatment or co-treatment with NAC blocked the synergism for both effects. A 2μM iAs treatment of 90 min caused an ⇑ in ROS to ~2.0x (with wide confidence limits) and, in a treatment lasting 48 hr, about 20% cytotoxicity.	Wang et al., 2005
AR230-s cells, AR230-r cells, KCL22-s cells, KCL22-r cells	As^{III} ATO	1	24 hr	None	Apoptosis detected by Annexin V-FLUOS staining kit and flow cytometry: NSE in any of the 4 cells lines with ATO or 100 μM BSO treatments alone. For the combined treatment, induced rates (experimental – control) were: AR230-s, ~35%; AR230-r, ~35%; KCL22-s, ~10%; KCL22-r, ~13%.	Konig et al., 2007
AR230-r cells, KCL22-r cells	As^{III} ATO	1	24 hr	None	Western blot analyses: iAs alone caused NSE on protein levels of tyrosine phosphorylated Bcr-Abl or total cellular Bcr-Abl in etiher cell line. In both cell lines, combined treatment of iAs with 100 μM BSO yielded huge ⇓ in both proteins. In non-imatinib resistant CML cells, unlike in these 2 imatinib-resistant cell lines, iAs alone had been shown to suppress Bcr-Abl activity.	Konig et al., 2007
U-937 cells NB4 cells HL-60 cells	As^{III} ATO for all	1, 2, 4, 8 0.5, 1, 2, 4 1, 2, 4	24 hr for all	4 1 2	Induced apoptosis (experimental – control) based on chromatin fragmentation: U-937 cells: 1, NSE; 2, ~2%; 4, ~14%; 8, ~85%; NB4 cells: 0.5, NSE; 1, ~5%; 2, ~33%; 4, ~63%; HL-60 cells: 1, NSE; 2, ~5%; 4, ~22%. Induction of apoptosis was potentiated by co-treatment with PI3K inhibitors LY294002 and wortmannin, and by the Akt inhibitor Akt_i5.	Ramos et al., 2005
U-937 cells	As^{III} ATO	4	Various	4	⇓ in Akt phosphorylation after 24 hr (not by 14 hr); ⇑ in caspase 3 activity to ~3x after 24 hr; ⇑ in cytochrome c protein (released from mitochondria) after 14 hr; big ⇑ in activated Bax after 14 hr; big ⇑ in HSP 27 after 14 and 24 hr; big ⇑ in HSP 70 after 14 and 24 hr. The potentiation of apoptosis by inhibitors mentioned in prior row involved more extreme changes in the same direction for p-Akt, caspase 3, cytochrome c, and Bax activation as well as attenuation of HSP27 expression. It also involved increased disruption of the mitochondrial transmembrane potential.	Ramos et al., 2005

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
HK-2 cells	As^{III} SA As^{V}	0.1, 1, 10 for both	6, 24 hr	0.1 at 24 hr Probably 1 at 24 hr	To assess mitochondrial function, depolarization of mitochondrial membrane was detected using MitoTracker Red, a mitochondrion selective dye. Effect of dose of 1 of As^{III} appeared equivalent to that of dose of 10 of As^{V}. Effect increased with dose and time.	Peraza et al., 2006
HK-2 cells	As^{III} SA	0.1, 1, 10, 25	24 hr	0.1	Induced apoptosis (experimental – control) detected by Annexin V-FITC/PI flow cytometry: 0.1, ~36%; 1, ~23%; 10, ~15%; 25, ~15%. Induced necrotic cells (experimental – control) detected by same method: 0.1, ~2.5%; 1, ~3%; 10, ~6%; 25, ~24%. Apoptotic cells detected in this way were said to be in early apoptosis. Examination by transmission electron microscopy showed that most such cells failed to complete apoptosis and ultimately underwent necrosis instead. They suggested that iAs was so toxic to mitochondria that they lost "their ability to keep the cell on course for apoptotic cell death."	Peraza et al., 2006
APL primary cells K562 cells NB4 cells	As^{III} ATO	3	24 hr	3 for all	Apoptosis rates (control rates were not provided), detected by FITC-annexin V and PI double-staining: 52.2% 27.6% 56.6%	Sahu and Jena, 2005
Thymocytes from adult male BALB/cByJ mice	As^{III} ATO As^{V}	5 for both	3, 10, 22 hr for both	None None	NSE at any time point for induction of apoptosis by any of the following types of analysis: (1) "Annexin V-FITC positive" without loss of membrane impermeance (i.e., "7-AAD negative") to identify early apoptotic cells, (2) DNA loss, and (3) both "Annexin V-FITC positive" and "7-AAD positive" for cells in the final stages of cell death	Mondal et al., 2005
Jurkat cells Namalwa cells NB4 cells U937 cells	As^{III} ATO for all	1, 2 for all	24 hr for all	None 2 1 None	Induced apoptosis (experimental – control) detected by fluorescence microscope analysis after staining with AO and EB: Namalwa cells: 1, ~1%; 2, ~16%; NB4 cells: 1, ~12%; 2, ~26%; NSE at dose of 2 in Jurkat and U937 cells. Pretreatment with NAC or Z-VAD-FMK blocked induction of apoptosis in Namalwa and NB4 cells.	Chen et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Jurkat cells Namalwa cells NB4 cells U937 cells	As^{III} ATO for all	2 for all	24 hr for all	None 2 2 None	Western blot analysis: ⇑ in PARP-cleavage and ⇓ in procaspase-3 level in both Namalwa and NB4 cells but not in the other two cell lines; iAs did not induce JNK phosphorylation.	Chen et al., 2006
NB4 cells U937 cells	As^{III} ATO for both	1, 2, 4, 6 for both	24 hr for both	1 4	Induced apoptosis (experimental – control) detected by fluorescence microscope analysis after staining with AO and EB: NB4 cells: 1, ~6%; 2, ~30%; 4, ~70%; 6, 85%; U937 cells: 1, ~0%; 2, ~4%; 4, ~15%; 6, 12%; NB4 cells showed more severe cell growth inhibition at doses of ≥ 2. Also, Western blot analysis showed that iAs induced PARP cleavage in a dose-dependent pattern in NB4 cells. In U937 cells there was only very slight PARP cleavage at the highest dose. JNK phosphorylation did not occur in either cell line.	Chen et al., 2006
MEFs that are wt MEFs that are DKOs for Bax and Bak	As^{III} ATO both	10 for both	8 hr for both	10 None	Various indicators of apoptosis: Induced (experimental – control) DNA fragmentation: wt, ~7%; DKO, NSE; Cytochrome c release: ⇑ in wt, NSE in DKO; Induced caspase-3 activity: wt, ~140 units; DKO, none. Caspase-3 activity was only detected in DKO cells when they were permeabilized and incubated for 1 hr in the presence of 4 μM exogenous cytochrome c. These and other experiments showed that mitochondrial events associated with apoptotic cell death induced at concentrations such as 10 or less required Bax and/or Bak.	Nutt et al., 2005
MEFs that are wt or DKOs for Bax and Bak	As^{III} ATO	10, 125, 500, 1000	Results from several experiments suggested that extramitochondrial thiol oxidation leading to changes in intracellular Ca^{2+} compartmentalization plays a critical role in iAs-induced cytochrome c release. At concentrations of 125 and higher, Bax and Bak became irrelevant to the mechanism of cytotoxicity and cell death resulted form oxidative stress that led to necrosis. ROS seem to be implicated in a concentration-dependent mechanistic switch between apoptosis and necrosis.			Nutt et al., 2005
Namalwa cells NB4 cells	As^{III} ATO for both	1 for both	24 hr for both	1 for both without BSO	Induced apoptosis (experimental – control) detected by fluorescence microscope analysis after staining with AO and EB: Namalwa cells: iAs, ~6%; iAs + 10 μM BSO, ~29%; NB4 cells: iAs, ~8%; iAs + 10 μM BSO, ~47%. BSO treatments markedly reduced GSH levels.	Chen et al., 2006

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer						
Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
Jurkat cells U937 cells	As^{III} ATO for both	1 for both	48 hr for both	None for both without BSO	Induced apoptosis (experimental – control) detected by fluorescence microscope analysis after staining with AO and EB: Jurkat cells: iAs, NSE; iAs + 10 μM BSO, ~25%; U937 cells: iAs, NSE; iAs + 10 μM BSO, ~67%. BSO treatments markedly reduced GSH levels.	Chen et al., 2006
Jurkat cells Namalwa cells NB4 cells U937 cells	As^{III} ATO for all	1 for all	24 hr for Namalwa and NB4 cells, 48 hr for other 2 lines	1 for all with BSO	Results of Western blot analysis in all 4 cells lines following co-treatment of iAs with 10 μM BSO: big ⇑ in PARP-cleavage; big ⇓ in procaspase-3 level; big ⇑ in JNK phosphorylation (the latter effect was not seen in absence of BSO co-treatment).	Chen et al., 2006
Jurkat cells U937 cells	As^{III} ATO for both	1 for both	Various, for 6-72 hr	Time course experiments for co-treatment with 10 μM BSO showed ⇑ in PARP-cleavage; ⇓ in procaspase-3 level; strong ⇑ in JNK phosphorylation. Induced apoptosis increased to ~85% and ~50% by 72 hr in U937 and Jurkat cells, respectively.		Chen et al., 2006
U937 cells	As^{III} ATO	1	48 hr	1, but only with BSO co-treat-ment	Induced apoptosis (experimental – control) detected by fluorescence microscope analysis after staining with AO and EB: ~55% following the co-treatment with BSO; this ⇑ was not significantly decreased by 4-hr treatments with either 10 mM NAC or 200 units of catalase even though those treatments substantially decreased H_2O_2 levels. Moreover, NAC and catalase did not block the JNK activation caused by the iAs + BSO treatment.	Chen et al., 2006
U937 cells	As^{III} ATO	1	48 hr	1, but only with BSO co-treat-ment	Results of Western blot analyses: Huge ⇑ in DR5, huge ⇓ in Bid, and ⇓ in IκBα following co-treatment with 10 μM BSO; NSE on these 3 proteins after iAs or BSO alone. Experiments with inhibitors suggested that (1) both caspase- and JNK-mediated pathways (due to activation of NF-κB) participate in the induction of apoptosis that occurs following co-treatment with iAs and BSO and (2) that JNK increases DR5 protein levels that in turn mediate that apoptosis.	Chen et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NB4 cells, NB4-As^R, and APL primary cells	As^{III} ATO	A series of experiments was conducted involving 24-72 hr treatments with concentrations of iAs of 0.125-10 μM. Tests of MEK1 mRNA knockdown using iAs treatments and MEK1 inhibitors (namely, PD98059 at 40 μM and PD184352 at 1 μM) showed that MEK1 inhibitors and iAs synergize to induce apoptosis. Although iAs induces apoptosis, it also causes ERK1/2 activation, which tends to decrease the extent of apoptosis by causing phosphorylation at Ser112 of the proapoptotic Bad protein. Phophorylated Bad protein does not heterodimerize with the Bcl proteins. The only known function of the Bad protein is to bind (i.e., heterodimerize) with the death antagonist Bcl-2 family proteins, Bcl-2 and Bcl-xL, thereby blocking their antiapoptotic action by preventing them from binding to Bax/Bak. Because MEK1 inhibitors block this ERK1/2 activation and the phosphorylation of BAD, there is more nonphosphorylated Bad protein to heterodimerize with the Bcl-2 proteins and keep them from functioning to block apoptosis. In this way, exposure to iAs in the presence of MEK1 inhibitors greatly increases the extent of apoptosis.				Lunghi et al., 2005
Primary AML blasts from 25 patients with non-APL AML	As^{III} ATO	In experiments involving 48-hr treatments that used concentrations of iAs of 0.125-10 μM in the presence or absence of concentrations of the MEK1 inhibitor PD184352 of 0.1-10 μM, synergistic, additive, or antagonistic interactions in the induction of apoptosis were found in primary cells from 13, 8, and 4 patients, respectively. The p53-related gene p73 was shown to be the molecular target of importance in this interaction, and the synergism had the following basis. iAs induced both the proapoptotic and antiproliferative TAp73 and the antiapoptotic and proproliferative ΔNp73 isoforms, with no net effect on apoptosis because the TAp73/ΔNp73 ratio did not change. The MEK1 inhibitor reduced the level of ΔNp73 and blunted the iAs-induced up-regulation of ΔNp73, with the result that the TAp73/ΔNp73 ratio increased, leading to more apoptosis. At 1 μM, iAs induced only p73, but at doses ≥ 2 μM) it also promoted accumulation of p53 protein levels, which also caused apoptosis.				Lunghi et al., 2006
CHO K1 cells	As^{III} SA	20, 40, 80	4 hr	20	Apoptosis detected by flow cytometry and by the presence of DNA ladders from internucleosomal DNA degradation—ladder effect did not appear until 24 hr after treatment. At dose of 40, it took 8 hours after treatment before apoptosis could be detected by flow cytometry. Reduced levels of apoptosis resulted from treatment with various modulators (antioxidants, a copper ion chelator, a protein kinase inhibitor, and a protein synthesis inhibitor) either simultaneously or, in some instances, immediately following the arsenic treatment.	Wang et al., 1996
Normal human keratinocytes treated with 50 mJ/cm^2 UVB before or after iAs treatment	As^{III} SA for both	1, as pretreatment 1, as post-treatment begun 24 hr after irradiation	24 hr for both	1 None	Apoptosis as detected by PI staining and TUNEL assay: The iAs treatment alone did not induce a significant increase in apoptosis or cytotoxicity; ⇓ the level of UV-induced apoptosis to control levels, with a corresponding ⇓ in cytotoxicity to control levels. A similar amount of apoptosis was seen as with UVB alone, or possibly apoptosis increased slightly; cytotoxicity was similar to that with UVB treatment alone or possibly slightly more extreme.	Chen et al., 2005b
A mouse fibroblast cell line as well as various stable transfectants of JB6 Cl41 cells	As^{III} SA As^V	various	-	-	Various tests indicated that p53 is not involved in arsenic-induced apoptosis. The pathway of JNKs was shown to play an essential role in arsenic-induced apoptosis. For example, such apoptosis was blocked by expression of the dominant-negative mutant of JNK1.	Huang et al., 1999b

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NB4 cells U937 cells HL-60 cells	As^{III} ATO for all	2 for all	2 days for all	2 2 2	Percentages of apoptosis determined by fluorescent microscopy, and units of basal activity of GSTπ, GPx, and CAT, respectively: 67.5%, 94.0, 28.3, 25.8 5.6%, 212.1, 67.6, 170.5 5.8%, 138.6, 55.5, 198.3 These data and others showed that the higher the basal levels of these 3 enzymes, the less the iAs-induced apoptosis. Higher activities of these enzymes decrease the amount of H_2O_2 in cells. Modulators that increase activities of these enzymes were shown to decrease apoptosis and vice-versa.	Jing et al., 1999
Mouse 291.03C keratinocytes	As^{III} SA for both	5 5	48 hr 60 hr	5 5	Apoptosis measured by flow cytometry: ⇑ by 4.20% over control, which was 0.74%. ⇑ by 7.31% over control	Wu et al., 2005
Mouse 291.03C keratinocytes irradiated immediately after the arsenic treatment with a single dose of 0.30k J/m^2 UV	As^{III} SA for all	None (i.e., UV only) 2.5 5.0	- 24 hr 24 hr	- 2.5 5.0	Apoptosis measured by flow cytometry 24 hr after the dose of UV: ⇑ by 26.87% over control, which was 0.74%. ⇑ by 20.62% over control ⇑ by 9.78% over control Thus, both pretreatments with As^{III} SA markedly reduced the amount of UV-induced apoptosis. In parallel with the above, UV-induced caspase 3/7 activity was also decreased by both treatments.	Wu et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HaCaT cells (immortalized, non-tumorigenic human keratinocyte cell line) As-TL cells (arsenic-tolerant cells, which are HaCaT cells that were cultured for 28 weeks in 100 nM As^{III} SA)	As^{III} SA for both	20, 40, 60, 80 for both	24 hr for both	20 40	Apoptosis detected using flow cytometry following staining with Annexin V and PI: ⇑ in apoptosis Much smaller ⇑ in apoptosis; there was a significant decrease in apoptosis compared to HaCaT cells at all 4 dose levels. A similar resistance by As-TL cells was seen to apoptosis induction by 25 J/cm2 of UVA, as well as by cisplatin, etoposide, and doxorubicin. As-TL cells showed greatly increased stability of nuclear P-PKB, and pretreatment with chemicals that inhibit PKB phosphorylation blocked iAs-induced acquired apoptotic resistance.	Pi et al., 2005
MCF-7 cells	As^{III} ATO	3	36 hr	3	Apoptosis detected based on electrophoretic analysis of DNA fragmentation: ~18% of the cells were apoptotic.	Ling et al., 2002
U-2OS cells	As^{III} SA	0.1, 1, 10	24 hr	0.1	TUNEL staining assay was used to detect apoptotic cells after 0, 24, or 48 hr of post-treatment culturing in arsenic-free medium: At dose of 0.1, apoptotic cells were ~0%, ~0.3%, and ~3.6%, respectively. At dose of 1, apoptotic cells were ~0%, ~0.2%, and ~3.4%, respectively. At dose of 10, apoptotic cells were ~0%, ~0%, and ~0%, respectively. Note that a 24-hr treatment with SA affected apoptosis only if there was an additional 24-hr or longer period of culturing in SA-free medium between the end of the SA treatment and when the assay was done.	Komissarova et al., 2005
U-2OS cells	As^{III} SA	0.1, 1, 10	24 hr	0.1	Assay utilizing activation of cellular caspase-3 was used to detect apoptotic cells after 0, 24, or 48 hr of post-treatment culturing in arsenic-free medium: At dose of 0.1, apoptotic cells were ~0%, ~1.3%, and ~6.2%, respectively. At dose of 1, apoptotic cells were ~0%, ~0.3%, and ~5.4%, respectively. At dose of 10, apoptotic cells were ~0%, ~0%, and ~0%, respectively. Note that a 24-hr treatment with SA affected apoptosis only if there was an additional 24-hr or longer period of culturing in SA-free medium between the end of the SA treatment and when the assay was done.	Komissarova et al., 2005
Undifferentiated PC12 cells	As^{III} ATO	8	24 hr	8	Induction of apoptosis detected by annexin V binding and caspase activity: ~55% of cells with apoptotic death, rest with necrotic death; at 6 hrs, ~60% of dead cells were apoptotic	Piga et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	24 hr for both	11.5 11.5	Induction of apoptosis detected by PI and RNase staining and flow cytometry, visualized as sub-G1 population and reported as % of apoptosis (controls were always 0%): ~6% at 11.5, ~9% at 23; ~11% at 11.5, ~21% at 23.	Poonepalli et al., 2005
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	48 hr for both	11.5 11.5	Induction of apoptosis detected by PI and RNase staining and flow cytometry, visualized as sub-G1 population and reported as % of apoptosis (controls were always 0%): ~23% at 11.5, ~32% at 23; ~40% at 11.5, ~62% at 23.	Poonepalli et al., 2005
JB6 C141 cells, transfected with IKKβ-KM to greatly reduce COX-2 induction JB6 C141 cells transfected with vector only	As^{III} SA for both	20, 40 for both	24 hr for both	20 20	Induction of apoptosis detected by PI staining and flow cytometry: ⇑⇑ in apoptosis: medium alone, 0.83%; 20, 12.60%, 40, 41.33% Slight ⇑ in apoptosis: medium alone, 1.03%; 20, 4.58%, 40, 7.23%. Similar conclusion was reached using TUNEL assay and flow cytometry.	Ouyang et al., 2007
JB6 C141 cells, after knockdown of endogenous COX-2 expression to low levels by its specific siRNA JB6 C141 cells transfected with mock vector for the siRNA, with normal COX-2 expression	As^{III} SA for both	10, 20 for both	36 hr for both	10 10	Induction of apoptosis detected by PI staining and flow cytometry: ⇑⇑ in apoptosis: medium alone, 4.14%; 10, 28.45%, 20, 49.22%; Much smaller ⇑ in apoptosis: medium alone, 1.86%; 10, 10.52%, 20, 26.60%. Another experiment showed that pretreatment of normal JB6 C141 cells with NS398, an inhibitor of COX-2, markedly ⇑ amount of apoptosis.	Ouyang et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
MEF cells that were made IKKβ$^{-/-}$ so that they markedly overexpressed COX-2 MEF cells that had the vector only, with normal (low) level of COX-2	As^{III} SA for both	20 for both	36 hr for both	20 20	Induction of apoptosis detected by PI staining and flow cytometry: Slight ⇑ in apoptosis: medium alone, 0.68%; 20, 6.35%; Big ⇑ in apoptosis: medium alone, 0.87%; 20, 49.62%. Thus, COX-2 protects cells from apoptosis.	Ouyang et al., 2007
SY-5Y cells HEK 293 cells	As^{III} ATO for both	1 for both	24 hr 48 hr 72 hr	1 for all	Induction of apoptosis detected by Hoechst staining: response as % of control in SY-5Y and HEK 293 cells, respectively, for each duration of treatment: 266%, 156% 152%, 192% 214%, 200% There was NSE on the mitotic index at any time.	Florea et al., 2007
PMs from CDF_1 mice	As^{III} SA As^{V} MMA^{V} DMA^{V} TMA^{V}	10 1 mM 10 mM 10 mM 10 mM	48 hr for all	10 1 mM 10 mM 10 mM None	Apoptosis detected based on electrophoretic analysis of DNA fragmentation and by TUNEL staining. The particular assay shown in this row used cellular morphological changes to assess apoptosis and the AlamarBlue assay to measure cell death. Approximate resulting percentages of cell death (listed first) and apoptotic cells (listed second) for the 5 compounds follow: For As^{III} SA: 82% and 23% For As^{V}: 65% and 17% For MMA^{V}: 10% and 7% For DMA^{V}: 100% and 100% For TMA^{V}: 12% and none Thus DMA^{V} was unusual in causing almost entirely apoptotic cell death, while the inorganic arsenicals caused mainly necrotic cell death.	Sakurai et al., 1998
TK6 cells	As^{III} SA As^{III} ATO	0.1, 1 for both	24 hr for both	1 1	Apoptosis identified using APO2.7 antibody: ⇑ to 5.0% from 3.6% in control ⇑ to 5.5% from 3.6% in control	Hornhardt et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
TK6 cells irradiated with 1, 2, or 4 Gy of 69 cGy/min gamma radiation at beginning of iAs treatment	As^{III} SA As^{III} ATO	0.1, 1 for both	24 hr for both	None 1	Apoptosis identified using APO2.7 antibody: At dose of 1: 1 Gy, 9.1%; 2 Gy, 10.4%, 4 Gy, 22.6%; SA had no significant effect on any of them. At dose of 1: 1 Gy, 12.5%; 2 Gy, 21.75%, 4 Gy, 38.6%; ATO caused a significant increase over the control (no iAs + radiation) at all 3 radiation doses. This was a synergistic interaction.	Hornhardt et al., 2006
HCT116 cells (securin +/+) HCT116 cells (securin -/-)	As^{III} SA for both	16 for both	24 hr for both	16 16	Induced apoptosis (i.e., experimental – control) detected using fluorescent microscopy after Hoechst staining: securin +/+: ~6%; securin -/-: ~10%; with the amount of apoptosis in the null mutant being significantly higher.	Chao et al., 2006a
NB4 cells NB4-M-AsR2 cells IM9 cells	As^{III} ATO for all	0.5, 1 for all	48 hr for all	0.5 1 0.5	Induced apoptosis (i.e., experimental – control) for ATO alone and for ATO with 100 μM Trolox, detected using PI staining in binding buffer: At 0.5: ~6% alone, ~20% with Trolox; at 1: ~16% alone, ~55% with Trolox; At 0.5: 0% alone, ~11% with Trolox; at 1: ~14% alone, ~45% with Trolox; At 0.5: ~1.5% alone, ~4% with Trolox; at 1: ~6% alone, ~20% with Trolox; Additional support for the conclusion that Trolox enhanced ATO-mediated apoptosis was provided by an annexin V-FITC staining assay and by the observation that Trolox significantly enhanced the percentage of cells with activated caspase-3 and cleaved PARP.	Diaz et al., 2005
$Gclm^{-/-}$ MEF cells, from GCLM knockout mice	As^{III} SA for both durations	25 for both durations	8 hr 24 hr	25 for both dura-tions	Induced apoptosis (i.e., experimental – control) detected by staining with FITC-labeled annexin-V and PI: At 8 hours: ~5% early apoptotic, ~38% late apoptotic, ~8% necrotic; At 24 hours: ~3% early apoptotic, ~79% late apoptotic, ~5% necrotic; Experiments in $Gclm^{+/+}$ cells showed that co-treatment or pretreatment with tBHQ partially or completely blocked iAs-induced apoptosis.	Kann et al., 2005b
MEFs	As^{III} ATO	2, 3, 5	3 days	2	Induced apoptosis (i.e., experimental – control) for ATO alone and for ATO co-treatment with Trolox, detected by PI staining using flow cytometry: ATO alone: 2, ~9%; 3, ~22%; 5, ~62%; ATO & Trolox: 2, ~3%; 3, ~3%; 5, ~20%; thus, in contrast to what happened in malignant cells, Trolox blocked the effects of ATO.	Diaz et al., 2005

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
MEFs that were wt MEFs that were $Bax^{-/-}$ and $Bak^{-/-}$ double knockout (DKO) cells	As^{III} ATO for both	10 for both	12 hr for both	10 10	Induced apoptosis (i.e., experimental – control) detected by PI staining and FACS: ~23% in wt and ~7% in DKO; the results at dose of 500 are ignored here. wt: large ⇑ in release of cytochrome *c*, which was mostly blocked by pretreatment with BAPTA-AM; DKO: trace ⇑ in release of cytochrome *c*. Results showed that cytochrome *c* release and apoptosis occurred largely via a Bax/Bak-dependent mechanism.	Bustamante et al., 2005
Isolated rat liver mitochondria loaded with Ca^{2+}	As^{III} ATO	10, 50, 100	2 min	10	There was a dose-dependent, cyclosporin A-sensitive release of cytochrome *c* via induction of mitochondrial permeability transition and subsequent swelling of mitochondria. Mitochondrial GSH did not seem to be a target for iAs which, however, appeared to cause oxidative modification of thiol groups of pore-forming proteins, notably adenine nucleotide translocase.	Bustamante et al., 2005
SVEC4-10 cells	As^{III} SA	20	24 hr	20	Induced apoptosis (i.e., experimental – control), apoptotic cells were counted by hemocytometer in a fluorescence microscope: ~68%	Hsu et al., 2005
RAW264.7 cells	As^{III} SA	5, 25	24 hr	5	Apoptosis detected by TUNEL assay; results were presented as mean densities of TUNEL staining: there was a positive dose response.	Szymczyk et al., 2006
RAW264.7 cells	As^{III} SA	5, 25	24 hr	5	Apoptosis detected by fluorescence staining of caspase-3 activation: there was a positive dose response. A 30-min pretreatment with DPIC (which inhibits H_2O_2 production) completely blocked caspase-3 activation at both iAs doses, thus showing that it prevented induction of apoptosis by iAs.	Szymczyk et al., 2006
NIH 3T3 cells	As^{III} SA	5, 10, 20, 50, 100, 200	6 hr	10	Induction of caspase 3/7 activity assayed using Caspase-Glo™ assay (an indicator of apoptosis): Units of activity at 0, 10, 50, 100, and 200 were about 2.5, 4, 12, 17, and 36, respectively. Pre-induction of HSP by conditioning heat shock (2 hr at 42°C on prior day) or by constitutive expression of HSP70 markedly reduced the induction, as follows: with heat: NSE at any dose, with constitutive expression: at most a hint of induction at highest 3 doses.	Khalil et al., 2006
HL-60 cells	As^{III} ATO	3	48 hr	3	Induced apoptosis (i.e., experimental – control), based on TUNEL assay: 15%; Effect of intracellular AA (icAA): (Cells were loaded with 4 mM icAA by incubating them with DHA prior to iAs treatments, thus avoiding generation of extracellular ROS in tissue culture media caused by direct addition to it of AA.) Induced apoptosis for iAs + icAA = 1% (NSE) Results using annexin V/FITC assay gave a consistent but milder effect.	Karasavvas et al., 2005

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
H22 cells BAEC cells	As^{III} ATO for both	0.5, 1, 2, 4 for both	24 hr, 48 hr 24 hr, 48 hr	1, 0.5 1, 1	Induced apoptosis index (i.e., experimental – control), based on TUNEL assay: H22, 24 hr: 0.5, NSE; 1, ~8%; 2, ~22%; 4, ~35%; H22, 48 hr: 0.5, ~8%; 1, ~20%; 2, ~36%; 4, ~45%; BAEC, 24 hr: 0.5, NSE; 1, ~6%; 2, ~22%; 4, ~26%; BAEC, 48 hr: 0.5, NSE; 1, ~8%; 2, ~28%; 4, ~40%	Liu et al., 2006e
NB4 cells	As^{III} ATO	3	48 hr	3	% of cells with nuclear fragmentation (NuFr): ~80% Effects of modulators at high doses: Co-treatments with 1000-4000 μM DTT: dose-related marked ⇓ in NuFr reaching ~20%; Co-treatments with 100-400 μM DMSA: dose-related marked ⇓ in NuFr reaching ~20%; Co-treatments with 50-200 μM DMPS: dose-related marked ⇓ in NuFr reaching ~27%	Jan et al., 2006
NB4 cells	As^{III} ATO	1	48 hr	1	% of cells with NuFr: ~20% for experiments with DTT and DMSA; about 12% in experiment with DMPS; Effects of modulators at low doses: Co-treatments with 12.5-50 μM DTT: dose-related marked ⇑ in NuFr reaching ~90%; Co-treatments with 10-40 μM DMSA: dose-related marked ⇑ in NuFr reaching ~75%; Co-treatments with 5-20 μM DMPS: dose-related marked ⇑ in NuFr reaching ~80%	Jan et al., 2006
293 cells	As^{III} ATO	2	48 hr	2	% of cells with sub-G1 DNA content: Untreated = ~5%; dose of 2: big ⇑ to ~53%; Effects of co-treatment (CoTr) with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~26%; CoTr 100 μM DMPS: ⇓ from iAs alone to ~37%. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~83%; CoTr 10 μM DMPS: ⇑ from iAs alone to ~88%.	Jan et al., 2006

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
SV-HUC-1 cells	As^{III} ATO	2	48 hr	2	% of cells with sub-G1 DNA content: Untreated = ~6%; dose of 2: big ⇑ to ~46%; Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~22%; CoTr 100 μM DMPS: ⇓ from iAs alone to ~28%. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~70%; CoTr 10 μM DMPS: ⇑ from iAs alone to ~72%.	Jan et al., 2006
A549 cells	As^{III} ATO	1, 2, 5, 10, 20, 50	48 hr	5	Cell survival determined by MTT assay: LC_{50} = ~27; Cell survival determined by flow cytometry after annexin V and PI staining: iAs at dose of 2: NSE; 200 μM sulindac: NSE; (2 μM iAs + 200 μM sulindac): ~40% cytotoxicity; pretreatment with NAC almost completely blocked this synergistic interaction.	Jin et al., 2006b
A549 cells	As^{III} ATO	2	48 hr	Regarding caspase 3/7 protein levels: 2 μM iAs: NSE; 200 μM sulindac: NSE; (2 μM iAs + 200 μM sulindac): ⇑ to ~1.4x. Regarding caspase 9 protein levels: 2 μM iAs: ⇑ to 1.05x; 200 μM sulindac: NSE; (2 μM iAs + 200 μM sulindac): ⇑ to ~1.5x. There was also a clear synergistic interaction between these treatments in causing big ⇓ of both procaspase-3 and procaspase-9 protein levels. Pretreatment with NAC almost entirely blocked the caspase 3/7 and caspase 9 effects.		Jin et al., 2006b
WM9 cells OM431 cells LU1205 cells	As^{III} SA for all	4	48 hr	4	Induced apoptosis (i.e., experimental – control), based on PI staining and FACS analysis of hypo-diploid content of DNA in the pre-G0/G1 region: WM9, ~32%; OM431, ~17%; LU1205, ~18%. Treatment with soluble recombinant TRAIL was effective in inducing apoptosis; combined treatment with iAs yielded no more than an additive effect.	Ivanov and Hei, 2006
Cancer Promotion						
BALB/c 3T3 A31-1-1 cells (derived from mice)	As^{III} SA As^{V} DA MMA^{V} DMA^{V}	0.2, 0.5, 1, 2, 5 0.5, 1, 2, 5, 10 50, 100, 200, 500, 1000 10, 20, 50, 100, 200	18 days for all	0.5 1 200 None	Caused promotion in a two-stage transformation assay; based on a significant increase in the number of transformed cells after an initiating treatment of 0.2 μg/mL MCA for 3 days followed by post-treatment with an arsenic compound for 18 days. At doses above the LOEC, the responses increased no more than slightly with dose. For As^{III} SA there was a humped dose response with a peak at the dose of 1.	Tsuchiya et al., 2005

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer						
Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
BALB/c 3T3 A31-1-1 cells (derived from mice)	As^{III} SA As^{V} DA MMA^{V} DMA^{V}	1 5 500 50	18 days for all	1 5 500 None	Caused promotion in a two-stage transformation assay; based on a significant increase in the number of transformed cells after an initiating treatment of 10 μM As^{III} SA for 3 days followed by post-treatment with an arsenic compound for 18 days.	Tsuchiya et al., 2005
V79 cells	As^{III} SA As^{V} DA MMA^{V} DMA^{V}	0.15, 0.3, 0.7, 1.5, 2.5 0.5, 1.5, 2.5, 5, 10, 20 0.5, 1.5, 2.5, 5, 10, 20 mM 0.15, 0.3, 0.6, 1.3, 2.7, 5 mM	72 hrs for all	0.7 5 5 mM None	Inhibited gap-junctional intercellular communication, which is a mechanism linked to many tumor promoters; it is based on the metabolic cooperation assay, which detects chemicals that inhibit the transfer of the lethal metabolite of 6-thioguanine from HPRT-proficient to HPRT-deficient cells, thereby allowing recovery of the 6-thioguanine-resistant (HPRT-deficient) cells.	Tsuchiya et al., 2005
Cell Cycle Arrest or Reduced Proliferation						
MGC-803 (human gastric cancer)	As^{III} ATO	0.01–1	24 hr	0.01	Growth inhibition (growth measured by MTT assay): At various doses, growth inhibition was also induced in 5 other human malignant cell lines.	Zhang et al., 1999
MC/CAR (human multiple myeloma cell line)	As^{III} ATO	1, 2, 3, 4, 5	72 hr	1	Growth inhibition (growth measured by MTT assay): About 60% inhibition at 2; cells were arrested in both G1 and G2-M phases. Growth inhibition was also induced in 7 other human multiple myeloma cell lines to various degrees.	Park et al., 2000

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
UROtsa cells	As^{III} SA	0.1, 0.5, 1, 5	24 hr for all	1	Extent of reduction of cell proliferation based on [^{3}H]thymidine incorporation: Cell proliferation reductions at dose of 5 were approximately as follows: $DMA^{III}I$, 15%; As^{III}, 30%; $MMA^{III}O$, 85%.	Drobná et al., 2002
	As^{V}	1, 200		None		
	$MMA^{III}O$	0.1, 0.5, 1, 5		1		
		1, 200		None		
	MMA^{V}	0.1, 0.5, 1, 5		5		
	$DMA^{III}I$	1, 200		None		
	DMA^{V}					
V79 cells	DMA^{V}	1, 2, 5 mM	12 hr	1 mM	Induction of mitotic delay and formation of aberrant mitotic spindles, including tripolar and quadripolar spindles: ~18% aberrant spindles at 1 mM. γ-tubulin was co-localized with the aberrant spindles. The following things were noted to occur after exposure of V79 cells to DMA^{V}: multiple MTOCs, multipolar spindles, amoeboid cells, multinucleated cells and cell death.	Ochi et al., 1999a
HeLa S3 cells	As^{III} SA	1, 3, 5, 10, 20	24 hr	3	Cells arrested at mitotic stage: At dose of 5, 35% of cells were arrested in that stage. Of 7 cell lines tested in this way, two others were almost as sensitive to this effect. Examination of cells arrested in mitosis showed abnormal mitotic figures and spindles, as well as deranged chromosomal congression.	Huang and Lee, 1998
U937 cells	As^{III} SA for both	2.5, 5, 10 for both durations	24 hr 48 hr	2.5 for both dura-tions	Cell numbers counted with a Coulter counter: After 24 hr at the doses of 2.5, 5, and 10, there were approximately 71%, 56%, and 43% as many cells as in the control group, respectively. After 48 hr at the doses of 2.5, 5, and 10, there were approximately 54%, 38%, and 23% as many cells as in the control group, respectively. There was little if any cytotoxicity even at 48 hr at the dose of 5.	McCollum et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
U937 cells	As^{III} SA	5	8 hr	The LOEC was 5. Centrifugal elutriation was used to enrich cells in different phases of the cell cycle so that the effect of iAs could be tested on them. Progression of iAs-treated cells from each cell cycle stage to the next was studied, and it was found that iAs slowed cell growth in every phase of the cycle. For example, in asynchronous populations of untreated cells, DNA synthesis lasted 10 to 12 hr. However, in cells treated with 5 μM iAs, it lasted 16 hr. In the presence of iAs, cells in G1 entered the S phase more slowly, etc. Cell passage from any cell cycle phase to the next was inhibited by 5 μM iAs arsenite. Clearly there was not induction of cell-cycle arrest at one specific checkpoint. The biggest iAs-induced slowdown occurred between M and G1, and the next biggest was between G2 and M. By looking at caspase activity, they showed that iAs induced apoptosis specifically in cell populations delayed in the G2/M phase.		McCollum et al., 2005
$PARP\text{-}1^{+/+}$ MEF cells $PARP\text{-}1^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	24 and 48 hr for both	11.5 for both at both times	iAs caused much disruption of cell cycle as shown by PI and RNase staining and flow cytometry when visualized as proportions of cells that were in G2/M, S, G1, or sub-G1 (i.e., apoptotic) under the different conditions. Disruption was more extreme in $PARP\text{-}1^{-/-}$ MEF cells. Results for apoptosis, which are easier to quantify, are detailed in separate rows. Especially at the highest iAs dose in $PARP\text{-}1^{-/-}$ cells, the proportion of G2/M cells became especially small, at least when the comparison was made to all cells and not just to non-apoptotic ones.	Poonepalli et al., 2005
CGL-2 cells	As^{III} SA	1, 2, 3, 4, 5, 7, 10	24 hr	1	Cell survival was determined using a colony-forming assay: LC_{50} = 1.7. As mitotic cells round-up, they can be separated from the attached interface cells by using the shake-off technique. When that technique was applied to a sample at the dose of 2, 96% of the attached cells were found to be alive, and 96% of the floating (i.e., mitotic) cells were found to be dead, thus indicating that iAs induced mitosis-mediated cell death.	Yih et al., 2005
CGL-2 cells	As^{III} SA	1, 2, 3, 4, 5, 10	24 hr	1	Treatments caused a shift in percentages of cells in G1, S, and G2/M, with a dose-dependent ⇑ in G2/M cells over the range of doses of 0 (~25%) to 4 (~85%), followed by a ⇓ above a dose of 5 that reached ~50% at dose of 10. G2/M cells were predominantly mitotic cells. Mitotic arrest was associated with iAs-induced cell death (see row immediately above). When synchronized cells were treated with dose of 2, all cells, whether treated in the G1, S or G2 stage, progressed into and arrested at mitosis, where they were demonstrated to contain damaged DNA, as demonstrated by the appearance of the DNA double-strand-break marker phosphorylated histone H2A.X (γ-H2AX).	Yih et al., 2005
CGL-2 cells	As^{III} SA	Following on from row above, other experiments showed that iAs appears to inhibit activation of the G2 DNA damage checkpoint and thereby allows cells with damaged DNA to proceed from G2 into mitosis. The subsequent arresting of cells with damaged DNA in mitosis is thought to enhance the induction of apoptosis.				Yih et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HeLa S3 cells	As^{III} SA	5, 10, 20, 50	1 hr	10	Inhibition of mitotic exit after cells were arrested in mitosis by treatment with nocodazole and the nocodazole was removed before arsenic treatment: This shows that such a dose interferes with mitosis.	Huang and Lee, 1998
HLFC cells HLFK cells (Ku70 deficient)	As^{III} SA for both	1, 2.5, 5, 10 for both	4 hr for both	2.5 2.5	Following the 4-hr As^{III} treatment, cells were incubated in arsenic-free medium for 24 hr before determining the proliferation index and the proportions of cells in different parts of cell cycle: Both cell types had ⇓ proliferation index and an ⇑ in G_0/G_1 cells at dose of 2.5. Both effects were more extreme in HLFK than in HLFC cells at the 3 highest doses.	Liu et al., 2007b
Human primary peripheral blood lymphocytes	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	1.25–160 1.25–500 0.1–2.7 10–10000 0.11–12.26 10–10000	24 hr for all	2.5 50 1.5 10000 1.02 3000	Replicative index (RI): All 6 compounds induced significant slowing of the cell cycle. Methylated trivalent arsenicals were 3 orders of magnitude more potent than the methylated pentavalent arsenicals. iAs compounds were substantially more toxic than methylated pentavalent arsenicals.	Kligerman et al., 2003
Human primary peripheral blood lymphocytes	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	1.25–160 1.25–500 0.1–2.7 10–10000 0.11–12.26 10–10000	24 hr for all	20 150 1.8 None 1.02 300	Mitotic index (MI): ⇓ ⇓ ⇓ NSE ⇑ to peak of 3x at 3.07 ⇑ to peak of 6x at 1000 Both decreased abruptly near concentration at which RI showed ⇑ proportion of first division metaphases. This is consistent with disruption of spindle integrity.	Kligerman et al., 2003
Human peripheral lymphocytes	As^{III} SA	5	24 hr	5	There was delayed cell cycle progression. In treated cells, 73 and 32% were still in the first mitotic division at fixation times of 72 and 96 hr, respectively, whereas in untreated cells up to 90% were in second or subsequent divisions at these times.	Jha et al., 1992
TR9-7 cells that were released from being mostly synchronized in G2 (using Hoechst 33342) shortly before iAs treatment began	As^{III} SA	5	1-24 hr	Conclusions based on mitotic indices determined over the 24-hr period in cells made $p53^{(+)}$ or $p53^{(-)}$ by controlling tetracycline levels: iAs delayed entry into mitosis in both $p53^{(+)}$ and $p53^{(-)}$ cells, with peak being delayed by ~3 hr from that of cells unexposed to iAs. Mitotic exit occurred at a normal rate in iAs-treated $p53^{(+)}$ cells but was markedly delayed in $p53^{(-)}$ cells and only reached the baseline level after 24 hr, by which time the iAs-treated $p53^{(+)}$ cells had already reached that level and had begun to cycle again.		McNeely et al, 2006
PCI-1 cells	As^{III} ATO	1, 2, 3, 4	3 days	2	Growth inhibition (growth measured by MTT assay): About 50% inhibition at 2; cells were arrested in the G2-M phases. Growth inhibition was also induced in 3 other human head and neck squamous cell carcinoma cell lines.	Seol et al., 1999

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
CHO cells treated with MMS before or after iAs treatment	As^{III} SA	10, as pretreatment 10, as post-treatment	24 hr 24 hr	10 10	Inhibition of mitosis and cell proliferation: ⇓ in inhibition of both endpoints compared to MMS alone; ⇑ in inhibition of both endpoints compared to MMS alone, synergistic interaction.	Lee et al., 1986
MCF-7 cells	As^{III} ATO	3	24 hr	3	Treatment blocked the cell cycle in mitosis, resulting in a time-dependent accumulation of cells in G_2/M, with about 50% in G_2/M at this time.	Ling et al., 2002
Human lymphocytes	As^{III} SA	10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 0.01, 1	2 hr	10^{-10}	Induction of mitotic arrest: 4 of 5 donors showed statistically significant increase at lowest dose. All showed significant increase from dose of 10^{-8} through 0.01. There was much interindividual variation, but there was a positive dose response within data for each donor. There was a almost no response at dose of 1 because of cytotoxicity.	Vega et al., 1995
Chinese hamster V79 cells	As^{III} SA DMA^{V}	5 2 mM	24 hr for both	5 2 mM	Accumulation of mitotic cells and other abnormal cells as follows (approximate percentages of cells of each type present after 24-hr treatment): Control (assumed same as distribution at starting time): 97% mononucleated, 3% metaphase. As^{III}: 75% mononucleated, 11% metaphase, 10% binucleated, 4% multinucleated. DMA^{V}: 24% mononucleated, 52% metaphase, 1% binucleated, 23% multinucleated. DMA^{V} caused disappearance of microtubule network and abnormalities of mitotic microtubules (i.e., spindles)—there was a big ⇑ increase in frequency of multipolar and aster-like spindles.	Ochi et al., 1999b
SVEC4-10 cells	As^{III} SA	2, 4, 8, 16	24 hr	4	Fraction of cells in G_2/M phases of cell cycle: slight ⇑ at 4, big ⇑ at 8 and 16; also an effect on rate of cell growth (tested at 4, 8, 12, 16): ⇓ at all doses, with a strong dose response	Chao et al., 2006a
HCT116 cells (securin +/+) HCT116 cells (securin -/-)	As^{III} SA for both	4, 8, 12, 16 for both	24 hr for both	12 4	Fraction of cells in G_2/M phases of cell cycle: Similar ⇑ at 12 and 16 to ~39%; ⇑ at 4 to ~38% with a positive dose response, reaching ~49% at highest dose; Consistent with the conclusion, based on the above data, that securin protects against arsenic-induced cell cycle arrest, the -/- cells also showed a much bigger ⇑ in the mitotic index and in the fraction of cells in "anaphase/mitosis". They also showed sister-chromatid separation.	Chao et al, 2006a
SVEC4-10 cells	As^{III} SA	20	24 hr	20	Cell numbers were counted using a hemocytometer: after 6 days of culturing after the iAs treatment, there were ~25% as many cells as in the control.	Hsu et al., 2005

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HT1197 cells	As^{III} SA	1, 5, 10	24 hr	5	Complete inhibition of cell proliferation occurred eventually at the dose of 10, with an accumulation of cells in S-phase. At the dose of 10, after 12 and 24 hr, 1.5x and 2.1x more cells were in S-phase than in control, respectively, with a large deficit of cells in G1.	Hernández-Zavala et al., 2005
CL3 cells, synchronous at G1	As^{III} SA	50	3 hr	50	Cell proliferation, based on cell number: ⇓ to ~35% of control; Survival was cut to 20-25% by co-treatment with PD98059 or U0126.	Li et al., 2006a
Human lymphoblastoid cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	0.2, 0.4, 0.6, 1, 2.5, 5, 10 μM 0.5, 1, 2.5, 5, 7.5, 10 mM 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1 μM 0.5, 1, 2.5, 5, 7.5, 10 mM 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 μM 0.5, 1, 2.5, 5, 7.5, 10 mM	6 hr for all	None 7.5 mM 0.4 μM None None 5 mM	Effect on the mitotic index: NSE effect, but results were confounded by high toxicity; Slight statistically significant ⇑ in slope; Statistically significant ⇑ in slope; Slight statistically significant ⇑ in slope; Equivocal, highly variable, effects probably because of toxicity; Statistically significant ⇑ in slope.	Kligerman et al., 2005
Lyophilized bovine tubulin	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	0.1, 1, 10 mM 0.1, 1, 10 mM 1, 10, 100 μM 0.1, 1, 10 mM 1, 10, 100 μM 0.1, 1, 10 mM	Time course over 1 hr	1 mM None 1 μM 0.1 mM 10 μM 0.1 mM	Effect on GTP-induced polymerization of lyophilized bovine tubulin: ⇓ at 1 mM, ⇓⇓ at 10 mM; NSE; Slight ⇑ at 1 μM, ⇓ at 10 μM, ⇓⇓ at 100 μM; Slight ⇑ at 0.1 and 1 mM, ⇑ at 10 mM; ⇓ at 10 μM, ⇓⇓ at 100 μM; ⇓ at 0.1 mM, NSE at 1 mM, ⇑ at 10 mM.	Kligerman et al., 2005
Cell Proliferation Stimulation						
K562 cells (human erythroleukemia cells)	As^{III} ATO	2.5	12 hr 48 hr	2.5 2.5	~27% of cells are mitotic. (In control, only 4% of cells are mitotic.) ~55% of cells are mitotic.	Li and Broome, 1999

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NHEK cells	As^{III} SA	0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10 for all	24 hr for all	0.005	Stimulation of cell proliferation, but with inhibition of cell proliferation at ≥ 0.05. Stimulation was measured as incorporation of ^{3}[H]thymidine into cellular DNA.	Vega et al., 2001
	As^{V}, MMA^{V}, DMA^{V}			None	No stimulation of cell proliferation; inhibition of cell proliferation at 0.05 or higher.	
NHEK cells	As^{III} SA	0.2, 0.4, 0.8	1 day	0.2	Increase in proliferation based on cell counts: ⇑ of 32%, 58%, and 50%, respectively;	Hwang et al., 2006
			2 days	0.4	⇑ of 20% and 21% at doses of 0.4 and 0.8, respectively;	
			3 days	0.4	⇑ of 27%, only at dose of 0.4; PI staining and FACS analysis after 2 days showed a significant shift from cells in G1 to cells in G2/S at both doses that showed an ⇑ in proliferation.	
HaCaT cells	As^{III} SA	1.25, 2.5, 5	48 hr	1.25	⇑ in fraction of cells in S phase: at doses of 0, 1.25, 2.5, and 5, the percentages of cells in S were 24.9%, 29.8%, 33.8%, and 38.7%, respectively. Since there was a corresponding ⇑ in fraction of cells in G2/M phase, it was concluded that iAs promoted the transition from G1 to S. The 24-hr treatment caused a similar effect at the 2 higher doses.	Ouyang et al., 2005
JB6 C141 cells transfected as described for this assay	As^{III} SA	1.25	72 hr	1.25	Proliferation was measured by using the CellTiter-Glo® Luminescent Cell Viability Assay: ⇑ in proliferation index to ~1.62x	Ouyang et al., 2006
JB6 C141 cyclin D1-Luc mass1 cells	As^{III} SA	5	24 hr	5	Fraction of cells in S phase and cell apoptosis (i.e., cell sub-G1 phase) were measured using PI staining with flow cytometry: ⇑ in fraction of cells in S from ~11.8% to ~14.5%; there was no induction of apoptosis and no evidence of cytotoxicity.	Ouyang et al., 2006
HaCaT cells	As^{III} SA	0.5, 1.0	20 passages	-	Not a significantly increased growth rate, but the trend was in that direction with accumulated population doublings of 58 to 67 in the control and 1.0 groups, respectively, with the value being ~61 in the 0.5 dose group.	Chien et al., 2004

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
C3H 10T1/2 cell line (mouse cells with fibroblast morphology during routine culture but capable of differentiation into adipocytes)	As^{III} SA	6	8 wk	6	Marked increase in FBS-stimulated DNA synthesis (detected using [^{3}H]thymidine incorporation) following dexamethasone/insulin treatment (to induce differentiation), but only after the arsenite exposure has been stopped—the increased mitogenic response is masked while the arsenite treatment continues.	Trouba et al., 2000
C3H 10T1/2 cell line (mouse cells with fibroblast morphology during routine culture but capable of differentiation into adipocytes)	As^{III} SA	6	8 wk	6	Marked increase in cell number compared to control cells following dexamethasone/insulin treatment (to induce differentiation), but increase only occurs after the arsenite exposure has been stopped.	Trouba et al., 2000
Both HL-60 cells and HaCaT cells	As^{III} SA	0.1, 0.5, 1, 10, 20, 40	5 days	0.5 but possibly 0.1	By use of MTT assay: ⇑ in cell number, with peak at 0.5; ⇓ in cell number to below control level at 1, with a continuing decrease at higher concentrations. (Same general response, but to a lesser extent, with same treatments over 1 day or 3 days)	Zhang et al., 2003
UROtsa cells	As^{III} SA	2, 4	72 hr	2	Increase in cell proliferation based on statistically significant increase in [^{3}H]thymidine incorporation; also there was a significantly higher fraction of cells in S-phase of cell cycle.	Simeonova et al., 2000
NHEK cells	As^{III} SA	0.2, 0.4, 0.6, 0.8, 1, 2, 4, 6, 12	24 hr for all; index was then determined immediately	2	Proliferation index based on MTT assay; the statistical comparison was with the untreated control: ⇑ at 3 doses from LOEC through 6;	Mudipalli et al., 2005
	MMA^{III}	0.1, 0.2, 0.4, 0.5, 0.8, 1, 2		0.5	⇑ at 2 doses from LOEC through 0.8;	
	DMA^{III}	0.1, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1, 2, 3		0.6	⇑ at 2 doses from LOEC through 0.7. Significant cytotoxicity occurred at 12 μM and higher for iAs and at 1 μM and higher for the other arsenicals. Cell cycle distributions were changed in many different ways.	

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NHEK cells irradiated with 100 mJ/cm^2 of UVB to arrest 94.5% of cells in G_0/G_1 stages of cell cycle while only killing 2-3% of the cells.	As^{III} SA	0.2, 0.4, 0.6, 0.8, 1, 2, 4, 6, 12	24 hr for all; index was then determined Immediately	0.6	Proliferation index based on MTT assay; the statistical comparison was with the untreated control: ⇑ at 6 doses from LOEC through 6;	Mudipalli et al., 2005
	MMA^{III}	0.1, 0.2, 0.4, 0.5, 0.8, 1, 2		0.4	⇑ at 4 doses from LOEC through 1.0;	
	DMA^{III}	0.1, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1, 2, 3		0.4	⇑ at 5 doses from LOEC through 0.8. Significant cytotoxicity occurred at 12 μM for iAs and at 1 μM and higher for the other arsenicals. At all doses showing a significant effect on the proliferation index after arsenical exposure, the point estimate was always higher in the cells with prior UVB exposure. Cell cycle distributions were changed in many different ways.	
Postconfluent PAEC cells in a monolayer	As^{III} SA for both	1, 2.5, 5, 10, 20	4 hr for both	1	Incorporation of [^{3}H]thymidine into genomic DNA: ⇑ at 1, 2.5, and 5, indicating a mitogenic response. Only the response at 5 is significantly higher, but the 2 lower doses are probably also higher; there was no effect at higher doses.	Barchowsky et al., 1996
PAEC cells in mid-exponential growth in a monolayer		1, 2.5, 5, 10, 20		10	⇓ in rate of DNA synthesis. (In the absence of any treatment, such cells have a higher rate of DNA synthesis than the postconfluent cells in a monolayer.)	
PAEC from freshly harvested vessels	As^{III} probably ATO, but called arsenite	1, 5, 10	24 hr	1	Extent of cell proliferation was estimated using fluorescent Cyquant assay: ⇑ at 1 and 5, but ⇓ at 10	Barchowsky et al., 1999a
U-2OS cells	As^{III} SA	0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5	24 hr	0.01	Cell survival was determined using the clonal survival treat-and-plate method: At doses of 0.01 and 0.05, clonal-forming ability was stimulated to 120-124% of the control, P<0.006. There was no increase at a 72-hr exposure or at higher doses with a 24-hr exposure. Similar results were found with the neutral red and MTT assays, and sometimes with those assays the point estimates still showed an increase at the dose of 0.01 after the 72-hr exposure.	Komissarova et al., 2005
SHE cells	$DMA^{III}I$	0.1, 0.25, 0.5, 1.0	1 day	0.1	Cell growth (No. of viable cells): ⇑ at both 0.1 and 0.25, and also big ⇑ for them after 2 and 3 days. Increase by 1 day at dose of 0.1 was ~8-fold. At dose of 1.0, ~40% cytotoxicity. No clear effect at 0.5 until after 3 days, then ~40% cytotoxicity.	Ochi et al., 2004

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
TM3 cells	As^{III} SA	0.000008, 0.00008, 0.0008, 0.008, 0.08, 0.77	72 hr	0.000008	Increase in cell proliferation: a statistically significant increase at all doses except 0.77; the peak of ~152% of control was at 0.00008.	DuMond and Singh, 2007
UROtsa cells	MMA^{III} for all	0.05 for all	12 weeks 24 weeks 52 weeks	0.05 for all	Shortened cell population doubling times (hr) based on counting cells in trypan blue exclusion assay: (control doubling time = 42 hr) 27 hr 25 hr 21 hr	Bredfeldt et al. 2006
NHEK cells, both with and without irradiation with 100 mJ/cm^2 of UVB to arrest 94.5% of cells in G_0/G_1 stages of cell cycle while only killing 2-3% of the cells	As^{III} SA MMA^{III} DMA^{III}	6 0.8 0.8	24 hr for all	6 0.8 0.8	Examination of expression profiles of more than 10 cell cycle and cell signaling proteins that seem likely to influence cell proliferation showed that many large changes occurred following the UVB and arsenic treatments. As examples, all 3 arsenicals caused a big ⇑ in nuclear cyclin D1 in UVB irradiated cells, and, for nuclear PCNA in UVB irradiated cells, MMA and DMA caused a big ⇑ while iAs had no effect. Activation of JNK phosphorylation and increased EGF expression and phosphorylation of the EGF receptor occurred.	Mudipalli et al., 2005
HELF cells	As^{III} SA	0.1, 0.5, 1, 5, 10	24 hr	0.1 for ⇑ 5 for ⇓	Cell proliferation efficiency based on MTT assay: ⇑ to 150% and 175% of control at 0.1 and 0.5, respectively; ⇓ to 60% of control at 5; significant stimulation of proliferation was also seen at dose of 0.5 after treatments of 12 and 48 hr	Yang et al., 2007
Chromosomal Aberrations and/or Genetic Instability						
HaCaT cells	As^{III} SA	0.5, 1.0	20 passages	0.5	Comparative genomic hybridization showed that all 11 cell lines derived from tumors (see malignant transformation) showed significant loss of chromosome 9q, and 7 lines showed significant gain of chromosome 4q.	Chien et al., 2004
HaCaT cells	As^{III} SA	0.5, 1.0	20 passages	0.5	⇑ MN; detected using the cytokinesis-block micronucleus assay, and scored only in binucleated cells. There was a positive dose response.	Chien et al., 2004
Primary Syrian hamster embryo cells (HEC)	As^{III} SA As^{V}	0.38, 3.8, 7.7 3.2, 8, 16, 32	24 hr for both	0.38 16	SCEs were induced; slight upward trend with dose.	Larramendy et al., 1981
Primary Syrian hamster embryo cells (HEC)	As^{III} SA As^{V}	7.7 32	24 hr for both	7.7 32	CAs were induced: mostly chromatid gaps and breaks, but some chromatid and chromosome exchanges	Larramendy et al., 1981
Human peripheral lymphocytes	As^{III} SA As^{V}	0.77, 1.9 16, 32	48 hr for both	0.77 16	SCEs were induced; dose-independent response	Larramendy et al., 1981

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human peripheral lymphocytes	As^{III} SA As^{V}	7.7 32	48 hr for both	7.7 32	CAs were induced: mostly chromatid and chromosome gaps and breaks, very few exchanges	Larramendy et al., 1981
Human primary peripheral blood lymphocytes	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	1.25–160 1.25–500 0.1–2.7 10–10000 0.11–12.26 10–10000	24 hr for all	None None None 1000 0.34 1000	SCE/metaphase Top 3 in list were negative. Potency of others: $DMA^{III} > DMA^{V} > MMA^{V}$ All were weak inducers of SCE, with the most potent inducing ~1 SCE/metaphase/μM.	Kligerman et al., 2003
Human primary peripheral blood lymphocytes	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	1.25–160 1.25–500 0.1–2.7 10–10000 0.11–12.26 10–10000	24 hr for all	2.5 50 0.6 3000 1.35 3000	Chromosomal aberrations: ⇑ to 42.5% aberrant cells at 10.0 ⇑ to 11.0% aberrant cells at 80.0 ⇑ to 11.0% aberrant cells at 1.2 ⇑ to 6.5% aberrant cells at 3000 ⇑ to 22.0% aberrant cells at 2.70 ⇑ to 57.0% aberrant cells at 10000 All 6 showed a positive dose-response. Chromatid and isochromatid deletions were most prevalent; exchanges were infrequent.	Kligerman et al., 2003
Syrian hamster embryo cells	As^{III} SA As^{V}	0.8, 3.0, 6.2, 10 10, 20, 64, 96	24 hr for both	6.2 64	CAs and endoreduplication (also, with 48 hr treatment, polyploidy) Mainly chromatid gaps, breaks, and exchanges, but a few chromosome-type aberrations (fragments and dicentrics)	Barrett et al., 1989
CHO K1 cells in late G1 of mitotic cycle	As^{III} SA	40	4 hr	40	High frequency of CAs was induced; effect was markedly reduced by prior or simultaneous (but not by subsequent) treatment with 5 mM GSH	Huang et al., 1993
Human peripheral lymphocytes	As^{III} SA	1, 5, 10	48 hr	1	Induction of chromatid aberrations; there was a positive dose response	Jha et al., 1992
Human peripheral lymphocytes	As^{III} SA	0.5, 1.0, 1.5, 2.0	48 hr	2.0	Induction of chromosomal aberrations	Wiencke and Yager, 1992
AS52 cells	As^{III} SA	50, 100	4 hr	100	Induction of gpt mutations at the ypt locus: The mutation frequency was twice that of the spontaneous mutation frequency at a high level of cytotoxicity (15% of the relative survival of the control). Taken as very weak evidence that As^{III} is a gene mutagen; results are grouped here with CAs because most or all of the induced mutations were total deletions of the gene, perhaps caused by the cytotoxicity.	Meng and Hsie, 1996

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
G12 cells	$MMA^{III}O$ $DMA^{III}I$	0.2, 0.4, 0.6, 0.8, 1.0 0.1, 0.2, 0.3, 0.4	3 days for both	0.6 0.3	Induction of mutations at the gpt locus: $DMA^{III}I$: reached 5x control mutant frequency at 7% cell survival; $MMA^{III}O$: reached 5x control mutant frequency at 11% cell survival. Taken as weak evidence that the arsenicals are gene mutagens with sub-linear dose responses; results are grouped here with chromosomal aberrations because ~80% of the induced mutations were deletions of the gene, perhaps caused by the cytotoxicity. ~11% of non-deletion mutants exhibited altered DNA methylation.	Klein et al., 2007
CHO cells	As^{III} SA As^{V}	0.01, 0.1, 1, 10 0.01, 0.1, 1, 10, 100	12 hr for both	1 100	Induction of chromosomal aberrations: A positive dose-response; 36.7% of cells with aberrations at dose of 10. 8.0% of cells with aberrations at dose of 100.	Kochhar et al., 1996
CHO cells	As^{III} SA As^{V}	0.01, 0.1, 1, 10 0.01, 0.1, 1, 10, 100	12 hr for both	1 None	Induction of endoreduplication: A positive dose-response; 22.0% of cells with endoreduplication at dose of 10. -	Kochhar et al., 1996
CHO cells	As^{III} SA As^{V}	0.01, 0.1, 1, 10 0.01, 0.1, 1, 10	12 hr for both	0.01 0.01	Induction of SCEs: 10.94%/cell at lowest dose; 14.08%/cell at highest dose; slight upward trend with dose. 11.38%/cell at lowest dose; 12.84%/cell at highest dose; no dose response.	Kochhar et al., 1996
MRC-5 cells	As^{III} SA	2.5, 5, 10	26 hr	2.5	Induction of SCEs (frequencies): 0, 3.24; 2.5, 5.23; 5, 6.2; 10, no surviving cells could be found to evaluate. There was also much cytotoxicity at dose of 5. High level of cytotoxicity was also reflected in the proliferation index.	Mourón et al., 2006
MRC-5 cells	DMA^{V}	125, 250, 500	26 hr	125	Induction of SCEs (frequencies): 0, 4.25; 125, 5.89; 250, 5.95; 500, 5.91; thus no dose response for SCEs. There was a significant ⇓ in the proliferation index at the highest dose.	Mourón et al., 2005
Human lymphocytes	As^{III} SA	10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 0.01	24 hr	10^{-10}	Induction of hypoploid and hyperploid cells: There was a statistically significant increase in hyperploidy at all dose levels in both 1st and 2nd division cells. There was a positive (but shallow) dose response. For example, in 2nd division cells, the frequency went from 2.3% at dose of 10^{-10} to 11.7% at dose of 0.01. The 4 donors showed variation, with 2 showing no effect at lowest dose. It is unclear at what dose level induction of hypoploidy became significant, but there was a slight positive dose response for it also. Data on CAs, which were reported only briefly, showed that roughly 40% of cells had CAs at the dose of 0.01. A concentration of 1 only μM was highly cytotoxic in these cells with an exposure lasting only 2 hr.	Vega et al., 1995

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human lymphocytes	As^{III} SA	0.001, 0.01, 0.1	24 hr	0.001	Increase in hyperdiploid frequency: (based on FISH analysis, there was a statistically significant dose-related increase for each of the 2 chromosomes tested from both donors). There was also an increase in hypodiploid frequency, but it was only seen (again at all doses) in 1 of the 2 chromosomes tested and in only 1 donor. A related experiment showed that As^{III} can disrupt the microtubule organization of lymphocytes at a dose as low as 0.001.	Ramírez et al., 1997
Primary cultured human umbilical cord fibroblasts	As^{III} SA As^{V} MMA^{V} DMA^{V} TMA^{V}	0.8, 2.3, 3.8, 7.7 16, 32, 64, 160, 321 1.4, 3.6, 7.1 mM 0.7, 1.4, 3.6 mM 3.7, 7.6, 14.7 mM	24 hr for all	0.8 μM 16 μM 1.4 mM 0.7 mM 3.7 mM	Induction of CAs: The percentages of abnormal cells at the LOECs for the 5 chemicals in descending order, as listed to the left, were: 10%, 16%, 19%, 28%, and 26%. Depletion of GSH by pretreatment of cells with BSO increased induction of CAs by As^{III} SA, As^{V}, and MMA^{V} but decreased it for DMA^{V}. In cells pretreated with BSO before treatment with DMA^{V}, the presence of 5 mM or higher GSH in the medium markedly increased induction of CAs. Since GSH does not enter the cells itself, this suggests that some clastogenic chemical is generated in the medium by interaction of DMA^{V} with GSH.	Oya-Ohta et al., 1996
CHO-9 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V} TMA^{V}	10 to 10000 for all	30 min for all	1000 1000 10 None 50 None None	Induction of chromosomal aberrations: Aberrations consisted mainly of chromatid exchanges and breaks; dicentrics and rings occurred rarely. Frequencies of aberrations per 100 cells at the most effective concentration for the 4 positive chemicals ranged form 44-74x that of the control.	Dopp et al., 2004
CHO-9 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V} TMA^{V}	10 to 10000 for all	30 min	1000 1000 10 None 50 None None	Induction of SCEs: For even the most potent inducers of SCE, the number of SCEs/cell was less than double that of the untreated control; thus they were weak inducers.	Dopp et al., 2004
Human primary peripheral blood lymphocytes	As^{III} SA	0.8	48 hr	0.8	SCEs were induced; simultaneous treatment with SOD (an oxygen radical scavenger) blocked induction of SCEs.	Nordenson and Beckman, 1991
CHO K1 cells	As^{III} SA	20	6 hr	20	SCEs were induced; simultaneous treatment with squalene at from 40 to 160 μM significantly and dose-dependently inhibited induction of SCEs	Fan et al., 1996
Human peripheral lymphocytes	As^{III} SA	1, 5, 10	48 hr	1	SCEs were induced; there was a positive dose response	Jha et al., 1992

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human peripheral lymphocytes	As^{III} ATO	0.00036, 0.00072, 0.0014	24 hr	0.00036	SCEs were induced; there was a positive dose response; co-treatment with retinyl palmitate at the highest dose of As^{III} caused a significant ⇓ to a SCE frequency like that seen at the middle dose; the same thing also occurred for PDT and AGT, showing that retinyl palmitate also reversed some of the arsenic-induced decrease in the rate of cell proliferation.	Avani and Rao, 2007
CHO K1 cells	As^{III} SA	5, 10, 20, 40	6 hr	5	Induction of MN in binucleated cells, using cytochalasin B after arsenic treatment to block cytokinesis: simultaneous treatment with 80 μM squalene significantly reduced the effect	Fan et al., 1996
Mouse lymphoma cells (L5178Y/$Tk^{+/-}$ –3.7.2C cells)	MMA^{III} DMA^{III}	0.19, 0.28, 0.38, 0.47, 0.52, 0.57 0.65, 0.83, 1.29, 1.51	4 hr for both	0.28 1.51	Mutations at $Tk^{+/-}$ locus in mouse lymphoma agar assay without exogenous metabolic activation: ⇑ to 2.0x at 0.28, with a positive dose response, reaching 7.2x at 0.57; ⇑ 2.4x control at maximum concentration tested; Both compounds showed large excess of small colonies, which is indicative of chromosomal aberrations; generally similar results were found in a mouse lymphoma microwell assay, which was complicated by higher toxicity.	Kligerman et al., 2003
Human peripheral lymphocytes	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{V} $TMA^{V}O$	0.5, 1, 2, 4 4, 8, 16, 32 0.01, 0.05, 0.1, 0.5, 1, 2 50, 100, 250, 500 50, 100, 250 400, 800, 1000	72 hr for all	2 8 1 100 250 None	Induction of MN in binucleated lymphocytes detected by the cytokinesis-block assay (using cytochalasin B): ⇑ in 2 donors at 2 and in all 3 donors at 4; ⇑ in 1 donor at 8 and in all 3 donors at 2 higher doses; ⇑ in 1 donor at 1 and in all 3 donors at 2; ⇑ in 2 donors at 100 and 250 and in all 3 donors at 500; ⇑ in 1 donor at 250; NSE. Further analysis of MMA^{III} showed ⇑⇑ in centromere-positive micronuclei (~80% of total), which is an indicator of induced anueploidy.	Colognato et al., 2007
SY-5Y cells HEK 293 cells	As^{III} ATO for all	1 for all	24 hr 48 hr 72 hr	1 for all	Induction of MN detected by Hoechst staining; response in comparison to control in SY-5Y and HEK 293 cells, respectively, for each duration of treatment: At 24 hr: 3.70x, 3.35x At 48 hr: 5.14x, 4.81x At 72 hr: 4.00x, 3.16x	Florea et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Mouse lymphoma cells (L5178Y/Tk$^{+/-}$ –3.7.2C cells)	As^{III} SA	2.3. 5.4, 7.7, 8.5, 10.8, 14.6, 16.2	4 hr for all	8.5	Mutations at Tk$^{+/-}$ locus in mouse lymphoma agar assay without exogenous metabolic activation. Very few, if any, large colony mutants were induced by all compounds. Induction of small colony mutants is indicative of induction of CAs.	Moore et al., 1997a
	As^{V}	3.0, 15.2, 30.3, 45.5, 60.6, 75.8, 84.9		45.5		
	MMA^{V}	6.2, 12.3, 15.4, 18.5, 24.7, 30.9 mM		18.5 mM		
	DMA^{V}	12.5, 25.0, 37.5, 50.0, 56.3, 62.5 mM		56.3 mM		
Mouse lymphoma cells (L5178Y/Tk$^{+/-}$ –3.7.2C cells)	As^{III} SA	11.5, 13.1, 15.4	4 hr for all	11.5	Induction of CAs: Aberrations consisted mainly of chromatid exchanges and breaks; all concentrations reported showed induction of CAs except for DMA^{V}, which gave results of borderline significance that were considered negative by the authors. Lower frequencies of induction were seen for MMA^{V} than for the inorganic arsenics in spite of the much higher doses.	Moore et al., 1997a
	As^{V}	60.6, 69.7, 84.9		60.6		
	MMA^{V}	21.6, 24.7, 27.8 mM		24.7 mM		
	DMA^{V}	50.0, 56.3, 62.5 mM		None		
SHE cells	As^{III} SA	4, 6, 8	24 hr	4 for first two effects	Induction of CAs: 0, 1%; 4, 9%; 6, 15%; 8, 32%; Induction of polyploidy and endoreduplication: 0, 0%; 4, 6%; 6, 19%; 8, 27%; Colony-forming efficiency relative to control after 7 days of culturing post-As treatment: 6, 77%; 8, 49%; MI: 0, 9.2; 4, 10.9; 6, 8.7; 8, 1.3	Hagiwara et al., 2006
V79 cells	As^{III} SA	50, 100, 250, 500 for both	1 hr for both	50	Induction of CAs (No. of aberrations in 100 metaphase cells): 0, ~7; 50, ~49; 100, ~99; 250, ~120; 500, ~160.	Sinha et al., 2005a
	As^{V}			50	0, ~6; 50, ~32; 100, ~44; 250, ~62; 500, ~73. Aberrations were mainly chromatid breaks. Co-treatment or pretreatment with tea extracts reduced aberration frequencies by half or more, while post-treatments also reduced the level of effects, which was suggestive of enhanced repair. Tea extracts induced CAT and SOD activity.	

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V79 cells	As^{III} SA As^{V} DMA^{V}	50, 100, 250, 500 for all	1 hr for all	100 or possibly 50 for all	Induction of micronuclei (MN) in cytochalasin B assay: (No. of MN per 1000 binucleated cells): 50, ~105; 100, ~110; 250, ~170; 500, ~300. 50, ~80; 100, ~105; 250, ~125; 500, ~150. 50, 52; 100, 70; 250, 99; 500, 111. Co-treatments with tea extracts reduced MN frequencies by two-thirds or more for As^{III} and by half or more for As^{V} and DMA^{V}. Pretreatments with tea extracts also caused a large ⇓ in MN frequencies for all 3 arsenicals. Post-treatments also reduced MN frequencies, which was suggestive of enhanced repair. The polyphenols EGCG and TF extracted from tea had similar effects in reducing MN frequencies. The LOECs are uncertain because no data were reported for the untreated controls.	Sinha et al., 2005b
Mouse lymphoma cells (L5178Y/$Tk^{+/-}$ –3.7.2C cells)	As^{III} SA As^{V} MMA^{V} DMA^{V}	11.5, 13.1, 15.4 60.6, 69.7, 84.9 21.6, 24.7, 27.8 mM 50.0, 56.3, 62.5 mM	4 hr for all	None 60.6 24.7 mM None	Induction of MN in binucleated cells, using cytochalasin B after arsenic treatment to block cytokinesis: As^{III} SA gave results of borderline significance that were considered negative by the authors.	Moore et al., 1997a
Don Chinese hamster cells	As^{III} SA As^{V} arsenic pent-oxide As^{V} disodium arsenate	7.7 13.9 32.1	28 hr for all	7.7 13.9 32.1	SCEs were induced for all 3 chemicals at 1.56, 1.61, and 1.46 times the control level, respectively. The concentrations tested for SCEs for all 3 chemicals were the “50% inhibition doses” following culturing for 72 hours and using a Giemsa test for viability.	Ohno et al., 1982
CHO cells	As^{III} SA As^{V}	1, 5, 10 50, 80, 100	24 hr for all	1 50	Chromosome aberrations (breaks and exchanges) were induced by both compounds with a dose-response relationship; As^{III} was 5–10 times more effective than As^{V} per unit dose; 80 μM was ~50% growth inhibition dose over 4 days for As^{III}.	Wan et al., 1982
Human lymphocytes	As^{III} SA	0.5, 1.0, 5.0	48 hr	0.5	Chromosome aberrations (breaks and exchanges) were induced.	Wan et al., 1982
CHO cells	As^{III} SA	1, 10	24 hr	1	SCEs were induced with a dose-response relationship.	Wan et al., 1982

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$P388D_1$ macrophage cell line	As^{III} SA	0.01, 0.1, 1	48 hr for all	None	No more than slight hints of induction of SCEs under any of these experimental conditions	Andersen, 1983
	As^{V}	0.1, 1, 10		None		
	DMA^{V}	1,10		None		
Human peripheral lymphocytes	As^{III} SA	1	48 hr	1	Induction of SCEs	Andersen, 1983
	DMA^{V}	1	48 hr	1	Induction of SCEs	
Human peripheral lymphocytes	As^{III} SA	0.5, 1.0, 1.5, 2.0	48 hr	1.0	Induction of SCEs: in 2 of the 3 donors, the LOEC was 1.5. Cells from one donor were more sensitive.	Wiencke and Yager, 1992
BrdU-substituted replicating human lymphocytes	As^{III} SA	0.77, 1.54	24 hr	0.77	Induction of SCEs, but only in 2 of 4 subjects.	Crossen, 1983
	As^{V}	13.5, 26.9	24 hr	13.5	Induction of SCEs, but only in 1 of 4 subjects; in 2 subjects (1 at lower dose and 1 at higher dose) there was a slight but significant decrease in SCEs.	
G_0 human lymphocytes	As^{III} SA	1.54	24 hr	None	No induction of SCEs with either treatment. (4 subjects in each group)	Crossen, 1983
	As^{V}	26.9	24 hr	None		
2BS cells	As^{III} SA	1.0, 3.0, 5.0, 10	5 hr	1.0	DNA-protein crosslinks detected by alkaline elution; peak effect at 3.0; no effect at 10.0; further testing of DNA showed the crosslinks to be protein-associated DNA-strand breaks.	Dong and Luo, 1993
V79-C13 Chinese hamster cell line	As^{III} SA	10	24 hr	10	Cells examined after 6, 12, 18, and 24 hr and after 6, 24, and 48 hr of recovery: by 6 hr of treatment there were multinucleated cells and round cells, by 12 hr there were giant cells. Multinucleated cells persisted at high levels to 48 hr after treatment. Also saw abnormal spindles and persistent (i.e., up to 5 days observed) aneuploidy and hyperdiploidy, but no statistically significant changes in CAs or MI.	Sciandrello et al., 2002
NB4 cells	As^{III} ATO	0.75	3 wk	0.75	Enlarged cells were found that contained chromosomal end-to-end fusions. In 80 karyotypes, there were an average of 2.4 fusion events per cell, and 32 cells had polyploidy. FISH analysis showed that fusions are associated with attrition of telomeres.	Chou et al., 2001
NB4 cells	As^{III} ATO for both	0.25	4, 5, 6 wk	0.25	Southern blot of digested genomic DNA: ⇓ telomere length at all 3 time points.	Chou et al., 2001
HeLa cells		1	3, 4 wk	1	⇓ telomere length at both time points.	

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PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	24 hr for both	None 11.5	Telomere length measured by flow FISH assay: (Point estimate comparisons were made to unexposed cells of the same genotype.) ~98% of control at 11.5, ~91% of control at 23; both are NSEs; ~76% of control at 11.5, ~71% of control at 23.	Poonepalli et al., 2005
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	48 hr for both	23 11.5	Telomere length measured by flow FISH assay: (Point estimate comparisons were made to unexposed cells of the same genotype.) ~99% of control at 11.5, ~79% of control at 23; the one at 11.5 was NSE; ~79% of control at 11.5, ~41% of control at 23. iAs-induced telomere attrition was thus much greater in PARP-1$^{-/-}$ MEFs.	Poonepalli et al., 2005
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	24 hr for both	11.5 11.5	Induced (experimental – control) % of MN in binucleated cells (with cytochalasin B post-treatment to block cytokinesis): ~4% at 11.5, ~5% at 23; ~18% at 11.5, ~13% at 23.	Poonepalli et al., 2005
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	48 hr for both	11.5 11.5	Induced (experimental – control) % of MN in binucleated cells (with cytochalasin B post-treatment to block cytokinesis): ~6% at 11.5, ~6% at 23; ~27% at 11.5, ~15% at 23.	Poonepalli et al., 2005
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	24 hr for both	None 11.5	Induced (experimental – control) frequency of CAs per cell, using FISH with a telomeric PNA probe: ~0.04 at 11.5, ~0.04 at 23; both are NSEs. ~0.09 at 11.5, ~0.05 at 23; only the one at 11.5 was statistically significant. CAs included end-to-end fusions, chromosome breaks, and fragments.	Poonepalli et al., 2005
PARP-1$^{+/+}$ MEF cells PARP-1$^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	48 hr for both	None 11.5	Induced (experimental – control) frequency of CAs per cell, using FISH with a telomeric PNA probe: ~0.04 at 11.5, ~0.04 at 23; both are NSEs; ~0.11 at 11.5, ~0.03 at 23; only the one at 11.5 was statistically significant. CAs included end-to-end fusions, chromosome breaks, and fragments.	Poonepalli et al., 2005

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293 cells	As^{III} ATO	2	24 hr	2	No. MN/1000 binucleated cells (with cytochalasin B during treatments to block cytokinesis): Untreated = ~35; dose of 2: big ⇑ to ~260; Effects of co-treatment (CoTr) with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~155; CoTr 100 μM DMPS: ⇓ from iAs alone to ~170. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~605; CoTr 10 μM DMPS: ⇑ from iAs alone to ~670.	Jan et al., 2006
293 cells	MMA^{III}	2	24 hr	2	No. MN/1000 binucleated cells (with cytochalasin B during treatments to block cytokinesis): Untreated = ~35; dose of 2: big ⇑ to ~230; Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~130; CoTr 100 μM DMPS: ⇓ from iAs alone to ~155. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~465; CoTr 10 μM DMPS: ⇑ from iAs alone to ~470.	Jan et al., 2006
293 cells	DMA^{III}	2	24 hr	2	No. MN/1000 binucleated cells (with cytochalasin B during treatments to block cytokinesis): Untreated = ~35; dose of 2: big ⇑ to ~315; Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~170; CoTr 100 μM DMPS: ⇓ from iAs alone to ~175. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~630; CoTr 10 μM DMPS: ⇑ from iAs alone to ~635.	Jan et al., 2006
SV-HUC-1 cells	As^{III} ATO	2	24 hr	2	No. MN/1000 binucleated cells (with cytochalasin B during treatments to block cytokinesis): Untreated = ~35; dose of 2: big ⇑ to ~330; Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~150; CoTr 100 μM DMPS: ⇓ from iAs alone to ~150. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~680; CoTr 10 μM DMPS: ⇑ from iAs alone to ~645.	Jan et al., 2006

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer						
Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
SV-HUC-1 cells	MMA^{III}	2	24 hr	2	No. MN/1000 binucleated cells (with cytochalasin B during treatments to block cytokinesis): Untreated = ~35; dose of 2: big ⇑ to ~270; Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~145; CoTr 100 μM DMPS: ⇓ from iAs alone to ~150. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~570; CoTr 10 μM DMPS: ⇑ from iAs alone to ~470.	Jan et al., 2006
SV-HUC-1 cells	DMA^{III}	2	24 hr	2	No. MN/1000 binucleated cells (with cytochalasin B during treatments to block cytokinesis): Untreated = ~35; dose of 2: big ⇑ to ~400; Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~160; CoTr 100 μM DMPS: ⇓ from iAs alone to ~145. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~620; CoTr 10 μM DMPS: ⇑ from iAs alone to ~650.	Jan et al., 2006
SHE cells	As^{III} SA $DMA^{III}I$	3, 10 0.5, 1.0	48 hr for both	10 0.5	Aneuploidy detected by flow cytometry: Slight ⇑; Slight ⇑; ⇑⇑⇑ at 1.0. Other experiments showed that $DMA^{III}I$ caused abnormalities of mitotic spindles, centrosomes, and microtubule elongation.	Ochi et al., 2004
Primary rat hepatocytes	As^{III} SA	0.25, 0.5, 1, 2.5, 5, 7.5, 10	27 hr	1	Induction of MN (mean no./1000 cells): 17.4 at dose of 1, increasing with dose to 24.4 at dose of 7.5; control = 13.7; too many cells were dead at dose of 10 to evaluate this endpoint. Co-treatment with 10 or 25 μM $Sb^{III}Cl$: ⇓ in micronucleus frequency below expectation of an additive interaction; that chemical also induced MN.	Hasgekar et al., 2006
CL3 cells, synchronous at G1 CL3 cells, asynchronous (asyn) CL3 cells, synchronous at G2/M	As^{III} SA for all	50 for all	3 hr for all	50 for all	Induction of MN; iAs treatment was followed by culturing with cytochalasin B for 24 hr to block cytokinesis): induced no. of MN (experimental – control)/1000 binucleated cells: G1, ~181; asyn, ~141; G2/M, ~125; when G1 cells were co-treated with iAs and either PD98059 or U0126, this number ⇓ from ~181 to ~75-80. Percentages of binucleated cells: G1, 14%; asyn, 47%; G2/M, 39%.	Li et al., 2006a

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
CL3 cells, synchronous at G1	As^{III} SA	50	3 hr	50	Induction of MN; iAs treatment was followed by culturing with cytochalasin B for 24 hr to block cytokinesis): induced frequency = ~181/1000 binucleated cells (as in row above); percentage of binucleated cells: 14% (as in row above). Culturing of G1 cells with cytochalasin B for 36-48 hr (instead of 24) caused marked ⇑ in percentages of binucleated cells and marked ⇓ in induced numbers of MN (1000 binucleated cells) from 181 to ~40-70. Also, when cultured with cytochalasin B for 40 hr (instead of 24 hr) after the co-treatment of iAs with PD98059 or U0126, these 2 structurally dissimilar inhibitors of MEK1/2 caused no further ⇓ from iAs alone.	Li et al., 2006a
V79-C13 Chinese hamster cells	As^{III} SA	10	24 hr	10	After being expanded through 120 generations in the absence of arsenic and then being cloned, acquired genetic instability persisted and often came to include dicentric chromosomes and telomeric associations. These same clones, which were often aneuploid, micronucleated and/or multinucleated, were affected by the DNA hypomethylation that was seen globally in the cells immediately after the 24-hr treatment.	Sciandrello et al., 2004
CHO cell lines: K1 (parental to the following line) XRS-5 (X-ray and H_2O_2 sensitive)	As^{III} SA	10, 20, 40, 80 for both	4 hr for both	80 10	Induction of MN in binucleated cells, using cytochalasin B after arsenic treatment to block cytokinesis: the much less responsive K1 cells have 6 times as much catalase activity as XRS-5 cells; both lines are similar in arsenic uptake and release, in GSH levels, and in GSH S-transferase activity.	Wang and Huang, 1994
CHO cell lines: K1 (parental to the following lines) XRS-6 (X-ray sensitive) XRS-5 (X-ray and H_2O_2 sensitive)	As^{III} SA	20, 40, 80 20, 40, 60 10, 20, 30, 40, 60	4 hr for all	40 20 10	Frequencies of MN per thousand binucleated cells per μM of arsenic for K1, XRS-6, and XRS-5 cells were 2.1, 4.5, and 10.8, respectively. (Cytochalasin B was used after arsenic treatment to block cytokinesis.) K1 cells have 5.8 times as much catalase activity and 5.4 times as much GPx activity as XRS-5 cells. K1 cells have 3.7 times as much catalase activity and 2.1 times as much GPx activity as XRS-5 cells. The cells with intermediate amounts have an intermediate response. Co-treatment of XRS-5 cells with catalase or GPx eliminates induction of MN by As^{III} SA. Treatment of K1 cells with inhibitors of catalase and GPx makes them much more sensitive to induction of MN by As^{III} SA; when co-treated together, there is a synergistic effect.	Wang et al., 1997

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
CHO-9 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{V} DMA^{III} TMA^{V}	1, 5, 10, 50, 100, 500 for both 1, 5, 10, 30 1, 5, 10, 30, 100, 500, 5000 for both 1, 5, 10 1, 5, 10, 5000	1 hr for all	None None 10 5000 5000 1 5000	Induction of MN in binucleated cells: DMA^{III} was by far the most potent.	Dopp et al., 2004
HFW cells	As^{III} SA for both durations	1.25, 2.5, 5, 10 5, 10, 20, 40, 80	24 hr 4 hr	1.25 10	Induction of MN, with about 70% being kinetochore-positive at maximum induction found at dose of 5. Induction of MN, with about 70% being kinetochore-negative at maximum induction found at dose of 40.	Yih and Lee, 1999
HLFC cells HLFK cells (Ku70 deficient)	As^{III} SA for both	1, 2.5, 5, 10 for both	24 hr for both	2.5 2.5	Induction of micronuclei (% of cells with MN): Control, 5%; 1, 4%; 2.5, 8%; 5, 10%, 10, 15%; Control, 4%; 1, 6%; 2.5, 10%; 5, 21%, 10, 27%; at the 2 higher doses the % is significantly higher in the HLFK cells. Ku70 is 1 of 3 subunits of DNA-dependent protein kinase, and the Ku70 protein plays an important role in repair of DNA double-strand breaks.	Liu et al., 2007b
HLFC cells HLFK cells (Ku70 deficient)	As^{III} SA for both	1, 2.5, 5, 10 for both	24 hr for both	5 2.5	Formation of abnormal nuclei (% of cells with abnormal nuclei): Control, 7%; 1, 9%; 2.5, 10%; 5, 19%, 10, 23%; Control, 10%; 1, 12%; 2.5, 21%; 5, 37%, 10, 42%; at the 3 higher doses the % is significantly higher in the HLFK cells. Ku70 is 1 of 3 subunits of DNA-dependent protein kinase, and the Ku70 protein plays an important role in repair of DNA double-strand breaks.	Liu et al., 2007b
HFF cells	As^{III} SA	5	24 hr	5	cen+ and cen- MN induced per 1000 cells: cen- MN: control, ~10/1000; iAs, ~17/1000 cen+ MN: control, ~2/1000; iAs, ~18/1000 Co-treatment with 170 nM SAM essentially eliminated induction of cen+ MN without having any effect on induction of cen- MN.	Ramírez et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HL-60 cells HaCaT cells	As^{III} SA	0.5, 10, 20	3 days	10 for ⇓ 0.5 for ⇑, 10 ⇓	Analysis of telomere length by TRF analysis using Southern-blot assay: Telomeres were shortened compared to controls at 10 and 20; Telomeres were shortened compared to controls at 10 and 20, but in these cells only, the telomeres were slightly elongated at dose of 0.5.	Zhang et al., 2003
Human-hamster hybrid A_L cells	As^{III} SA	3.8, 7.7, 15.4	1 day or 5 days	Depends on locus	Induction of mutations at both loci, with both showing higher response after 5-day treatment than after 1-day treatment. After only 1 day of treatment, the LOECs were 3.8 at S1 locus and 15.4 at the HPRT locus. This effect is not grouped with gene mutations because most mutations were large deletions; about 28 times as many mutations occurred at the Sl locus, and co-treatment with DMSO eliminated most of the mutation induction.	Hei et al., 1998
Human-hamster hybrid A_L cells	As^{III} SA	11.5, 15.4	24 hr	11.5	Induction of mutations at CD59 locus (formerly known as S1 locus); this effect is not grouped with gene mutations because most mutations were large multilocus deletions; co-treatment with SOD or catalase considerably reduced mutation induction.	Kessel et al., 2002
Human-hamster hybrid A_L cells	As^{III} SA	3.8	24 hr	3.8	Induction of mutations at CD59 locus; pretreatment with BSO (to reduce GSH levels) increased mutation rate about 3-fold.	Kessel et al., 2002
Enucleated A_L hybrid cells treated with As^{III} were fused with untreated nuclei to form reconstituted A_L hybrid cells	As^{III} SA	15.4	3 hr	15.4	Induction of mutations at CD59 locus: Mutant frequency >2x the frequency in control cells reconstituted from untreated enucleated cells and untreated nuclei. Induction of ROS was demonstrated in iAs-treated enucleated cells by using a fluorescent probe. These results suggest that mitochondria may be essential for induction of $CD59^-$ mutations (in nuclear DNA)	Liu et al., 2005
A_L hybrid cells made highly deficient in mitochondrial DNA by long-term ditercalinium treatment; then called ρ^0 cells	As^{III} SA	7.7, 11.5, 13.5, 15.4	18 hr	None	No increase in $CD59^-$ mutations; there was a dose-related increase in cytotoxicity. Analysis of DNA showed that mtDNA was >95% depleted in the ρ^0 cells. Suggests that mitochondrial function may be necessary for induction of $CD59^-$ mutations by iAs.	Liu et al., 2005
Human-hamster hybrid A_L cells	As^{III} SA for both	1.9, 3.8, 7.7 for both	16 days 30 days	1.9 1.9	Induction of mutations at the $CD59^-$ locus: increase in mutation frequency at all doses, with a positive dose response and at least a doubling of the control frequency at the higher dose. These cells showed a dose-related increase in cytotoxicity, with never less than a 60% surviving fraction. After a 60-day exposure, there was an almost 3-fold increase in the number of MN observed over the untreated control, but details were not provided.	Partridge et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human-hamster hybrid A_L cells	As^{III} SA	0.8, 3.8, 7.7, 15.4	24 hr	3.8	Induction of $CD59^-$ mutants: (Addition of BSO, which suppresses GSH, increased mutant frequencies more than 5-fold.)	Liu et al., 2001
Co-carcinogenesis						
Rat lung epithelial cell line Rat lung epithelial cell line exposed to 100 nM B[α]P for 24 hr	As^{III} SA for both	1.5 for both	12 wk without the B[α]P treatment or immediately following that treatment	1.5 for both	Transformation (i.e., anchorage-independent growth in soft agar) occurred with 12-wk iAs treatment alone or with B[α]P treatment alone. There was a synergistic interaction when the B[α]P treatment was followed by the 12-wk iAs treatment, with the transformation rate then exceeding 500 and 200 times that of the iAs or B[α]P treatments alone, respectively.	Lau and Chiu, 2006
Rat lung epithelial cell line Rat lung epithelial cell line exposed to 100 nM B[α]P for 24 hr	As^{III} SA for both	1.5 for both	12 wk without the B[α]P treatment or immediately following that treatment	1.5 for both	Changes in the proteome of the transformed cells detected by MALDI-TOF-MS analysis and other methods: iAs and B[α]P treatments alone caused changes in most of the following proteins alone. The combined treatment often caused a synergistic interaction on the protein levels in the same direction as one or both treatments changed them alone. Affected proteins were as follows: 3 proteins belonging to intermediate filaments were down-regulated; 6 proteins belonging to antioxidative stress-, chaperone-, and glycolytic proteins were up-regulated. Also phosph-ERK1/2 and α-actinin, which are associated with promotion of cell proliferation and de-differentiation, were up-regulated.	Lau and Chiu, 2006
GM04312C cells	As^{III} SA	10, 50	24 hr	10	BPDE-DNA adducts were measured after a 30-min treatment with 0.5 μM BPDE that followed the iAs pretreatment. Compared to no pretreatment, increases in these adducts at the doses of 10 and 50 were 1.4x and 1.6x, respectively. In these NER-deficient cells, which could be used to dissect induction of DNA damage from DNA repair, it was shown that iAs markedly increased the cellular uptake of BPDE in a dose-dependent manner. It was concluded that this effect contributes to the co-carcinogenesis in addition to arsenic's "well demonstrated inhibitory effect on DNA repair."	Shen et al., 2006
Co-mutagenesis						

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
E. coli WP2 irradiated with 5.6 J/m^2 of UV on plates that contained:	As^{III} SA As^{V}	100, 250, 500, 750 100, 300, 500	-	100 None	Plating protocol for Trp^+ revertants: synergistic interaction in inducing Trp^+ revertants at lower 3 dose levels for SA only, with peak effect at 250; synergistic interaction was seen only in a strain of *E. coli* that can carry out excision repair of pyrimidine dimers. Four *E. coli* strains that did not meet that criterion were tested, with no synergism being seen.	Rossman, 1981
CHO K1 cells in late G1 of mitotic cycle exposed to 7 J/m^2 of UV	As^{III} SA	40	2 hr	40	High frequency of chromosome aberrations was induced; effect was markedly reduced by prior or simultaneous (but not by subsequent) treatment with GSH	Huang et al., 1993
CHO cells exposed to 1, 2, 4, or 8 J/m^2 of UV	As^{III} SA	5, 10	24 hr	5	Induction of chromosomal aberrations: synergistic interaction was demonstrated at all dose levels of UV and iAs except for 1 J/m^2 with the 10 μM iAs treatment. At other UV dose levels, the responses at 10 μM arsenic only slightly exceeded those at 5 μM. UV or iAs alone induced mainly chromatid-type aberrations, but in cells treated with both agents there was an apparent increase of chromatid breaks, chromatid exchanges, chromatid gaps, and chromosome breaks.	Lee et al., 1985
CHO cells exposed to 1, 2, 4, or 8 J/m^2 of UV	As^{III} SA	5, 10	24 hr	None	Induction of SCEs: no statistically significant effect of the iAs treatment was observed.	Lee et al., 1985
Human peripheral lymphocytes simultaneously treated with 6 μM DEB	As^{III} SA	0.5, 1.0, 1.5, 2.0	48 hr	1.0	Induction of chromosomal aberrations: There was synergistic interaction between DEB and iAs.	Wiencke and Yager, 1992
Human peripheral lymphocytes simultaneously treated with 6 μM DEB	As^{III} SA	0.5, 1.0, 1.5, 2.0	48 hr	~1.0	Induction of SCEs: Unlike with CAs, there was not a synergistic interaction. Although no statistical comparisons were presented, the trends suggested additivity between the two mutagens.	Wiencke and Yager, 1992
CHO cells exposed to 2 or 4 J/m^2 of UV	As^{III} SA	5, 10	24 hr	5	Induction of gene mutations to 6-thioguanine resistance: synergistic interaction was demonstrated at both dose levels of UV and iAs	Lee et al., 1985
CHO cells exposed to 2 or 4 J/m^2 of UV	As^{III} SA	5, 10	24 hr	5	Induction of gene mutations to ouabain resistance: iAs had no effect	Lee et al., 1985
CHO K1 cells exposed to 1.5 or 2.5 J/m^2 of UV	As^{III} SA	10	24 hr	10	Induction of 6-TG^r gene mutations at the HPRT locus: synergistic interaction was demonstrated at both dose levels of UV; iAs at doses of 10 to 40 had no effect on the mutation frequency by itself.	Yang et al., 1992
CHO cells treated with MMS before or after iAs treatment	As^{III} SA	10, as pretreatment 10, as posttreatment	24 hr 24 hr	10 10	Induction of gene mutations at the HGPRT locus: ⇓ compared to MMS alone; ⇑ compared to MMS alone, synergistic interaction.	Lee et al., 1986

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
CHO cells treated with MMS before or after iAs treatment	As^{III} SA	5, 10, as pretreatments 5, 10, as posttreatments	24 hr 24 hr	None 5	Induction of chromosomal aberrations: No change from MMS alone. ⇑ frequency compared to MMS alone, synergistic interaction with even bigger effect at 10.	Lee et al., 1986
CHO cells treated with MMS before or after iAs treatment	As^{III} SA	5, 10, as pretreatments 5, 10, as posttreatments	24 hr 24 hr	None None	Induction of SCEs: No change from MMS alone. No change from MMS alone.	Lee et al., 1986
Human peripheral lymphocytes	As^{III} SA	5	2 hr before X-rays, 30 min after X-rays	5	Synergistic interaction in causing dicentrics and rings in both donors; synergistic interaction in causing deletions in one of the donors and approximately an additive response in the other; doses of X-rays were 1 Gy or 2 Gy with the dose rate unspecified	Jha et al., 1992
VH16 cell line (human primary fibroblasts) exposed to 7.5 J/m^2 of UV	As^{III} SA	5	24 hr	5	iAs exposure increased the frequencies of MN in binucleated cells and of SCEs over what they would have been with UV alone, but there was not a synergistic effect for MN.	Jha et al., 1992
V79 cells treated with MNU	As^{III} SA	10 5	3 hr 24 hr	10 5	Induction of gene mutations at the HPRT locus: While neither iAs treatment induced mutations by itself, as a post-treatment these iAs treatments both caused an ⇑ in the mutation frequency compared to MNU alone; there was a synergistic interaction.	Li and Rossman, 1989a
V79 cells exposed to 5-15 J/m^2 of UVC	As^{III} SA	10	3 hr	10	Induction of gene mutations at the HPRT locus: While the iAs treatment induced no mutations by itself, as a post-treatment it caused an ⇑ in the mutation frequency compared to UVC irradiation alone; there was a synergistic interaction.	Li and Rossman, 1991
V79 cells exposed to 55-220 KJ/m^2 of UVA	As^{III} SA	10	3 hr	10	Induction of gene mutations at the HPRT locus: While the iAs treatment induced no mutations by itself, as a post-treatment it caused an ⇑ in the mutation frequency compared to UVA irradiation alone; there was a synergistic interaction.	Li and Rossman, 1991

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
V79 cells exposed to: 400-800 J/m^2 of UVB 200 J/m^2 of UVB	As^{III} SA for both	10 5, 10, 15	3 hr 24 hr	None 10	Induction of gene mutations at the HPRT locus: While the iAs treatments induced no mutations by themselves, the 24-hr post-treatment caused an ⇑ in the mutation frequency compared to UVB irradiation alone; there was a synergistic interaction.	Li and Rossman, 1991
Mouse 291.03C keratinocytes irradiated immediately after the arsenic treatment with a single dose of 0.30 kJ/m^2 UV	As^{III} SA	2.5, 5.0	24 hr	5.0	Effect on repair rate of UV-induced photodamage to genomic DNA measured at 2 and 6 hr after the UV exposure ended: ⇓ in repair rate of 6-4PPs by 48%, but no effect on the repair of CPDs	Wu et al., 2005
TK6 cells	As^{III} SA As^{III} ATO	0.1, 1, 10 for both	24 hr for both	10 10	Induction of MN using flow cytometry assay: ⇑ to 24.7% from 3.4% in control. ⇑ to 17.4% from 3.4% in control; the text noted that it was sometimes difficult to distinguish between the MN and necrotic cell fragments due to toxicity at the dose of 10 for SA and ATO.	Hornhardt et al., 2006
TK6 cells irradiated with 1 or 3 Gy of 69 cGy/min gamma radiation at beginning of iAs treatment	As^{III} SA As^{III} ATO	0.1, 1, 10 for both	24 hr for both	1 1	Induction of MN using flow cytometry assay: At dose of 1: 1 Gy, 10.2%; 3 Gy, 12.2%; 12.2% was significantly higher than 9.8% in control. There was a statistically significant (additive) effect. At dose of 1: 1 Gy, 10.0%; 3 Gy, 16.3%; 16.3% was significantly higher than 9.8% in control. There was a statistically significant (possibly slightly synergistic) effect. Interpretation of results at dose of 10 was complicated by difficulty of distinguishing micronuclei and necrotic cell fragments. Responses were extremely different for the 2 arsenicals at dose of 3 Gy: 30.2% for SA and only 15.9% for ATO.	Hornhardt et al., 2006
Cytotoxicity						

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
NHEK cells	As^{III} SA As^{V}, MMA^{V}, DMA^{V}	0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10for all	24 hr 24hr	0.005 0.5	Extent of viability determined by neutral red assay; viability was significantly reduced	Vega et al., 2001
HOS cells AG06 cells W138 cells	As^{III} SA, As^{V}	IC_{50} determinations	100 hr for all	-	Extent of viability determined by neutral red assay IC_{50}s: 3.5 for As^{III}, 11 for As^{V}. IC_{50}s: 1.1 for As^{III}, 16 for As^{V}. IC_{50}s: 8.8 for As^{III}, 30 for As^{V}.	Hu et al., 1998
WI38 cells	As^{III} SA	0.25, 0.5, 1, 2	7 days	0.25	Clonal survival determined by crystal violet assay LD_{50}: ~1.85	Vogt and Rossman, 2001
HaCaT cells	As^{III} SA	0.5, 1.0	20 passages	0.5	⇑ resistance to cytotoxicity caused by exposure to concentrations of As^{III} of 1–16 μM for 72 hr	Chien et al., 2004
HepG2 cells	Dimethyl-arsinate, the usual form of DMA^{V} in this table Thio-DMA^{V} (i.e., Thio-dimethyl-arsinate)	0.01, 0.1, 0.5, 1, 5, 10, 50 mM for both	48 hr for both	0.5 mM 0.1 mM	Cell survival was determined by WST-8 assay: LC_{50}s: regular DMA, ~0.2 mM; Thio-DMA, ~0.02 mM; At 0.1 mM, regular DMA showed no cytotoxicity, but thio-DMA resulted in only 22% cell survival.	Raml et al., 2007
17 human cancer cell lines: 4 bladder cell lines: 2 lung cell lines: 2 liver cell lines: 1 leukemia cell line: and various others	As^{III} ATO	IC_{50} determinations	96 hr	-	Viability determined by sulphorhodamine B method: Bladder: IC_{50}s: 0.34, 0.47, 0.93, 1.38 Lung: : IC_{50}s: 3.27, 4.17 Liver: IC_{50}s: 5.17, 7.17 Leukemia: IC_{50}s: 0.64 All 17 lines: LC_{50} range was 0.34–7.17. There was a strong positive correlation between GSH content of cells and magnitude of IC_{50}:	Yang et al., 1999

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
4 of above 17 human cancer cell lines with high levels of GSH	As^{III} ATO	IC_{50} determinations	96 hr	-	Viability determined by sulphorhodamine B method: 10 μM BSO, which depletes cellular GSH, was incubated with cells for 4 days, causing them all to become very sensitive to arsenic, as follows: IC_{50}s without BSO: 0.47, 2.59, 2.08, 9.89 IC_{50}s with BSO: 0.19, 0.14, 0.40, 0.20, respectively	Yang et al., 1999
Hepa-1 cells (mouse hepatoma)	As^{III} SA	2, 5, 10, 25, 50	12 hr 24 hr	None 10	Viability determined by LDH release method	Maier et al., 2000
NHEK cells	As^{III} SA	IC_{50} determinations	72 hr	-	Extent of viability determined by neutral red assay: IC_{50}: 10.8	Snow et al., 1999
AG06 cells	As^{III} SA	0.1, 0.3, 1, 3	48 hr	3 3 0.3 0.1	Extent of viability determined by neutral red assay: Values below at 3: ~90% of cells viable if no pretreatment (pt) to change GSH level; ~85% of cells viable if NAC pt to ⇑ GSH level; ~20% of cells viable if BSO pt to ⇓ GSH level; ~20% of cells viable if CHE pt to ⇓ GSH level.	Snow et al., 1999
Human-hamster hybrid A_L cells	As^{III} SA	0.8, 3.8, 7.7, 15.4	24 hr	3.8	No. of colonies counted to determine surviving fraction: LC_{50} = about 7.7 (Addition of BSO, which suppresses GSH markedly, increased cytotoxicity.)	Liu et al., 2001
Primary cultures of rat cerebellar neurons	As^{III} SA DMA^{V}	5, 10, 15 1, 5, 30 mM	12 hr 48 hr	5 5 mM	Viability determined using MTT metabolism assay	Namgung and Xia, 2001
Chang human hepatocytes	As^{III} SA, As^{V}, MMA^{III}, MMA^{V}, DMA^{V}	LC_{50} determinations	24 hr	-	LC_{50}s using LDH leakage assay in phosphate media As^{III}: 68.0 As^{V}: 1,628 MMA^{III}: 6.0 MMA^{V}: 8,235 DMA^{V}: 9,125	Petrick et al., 2000
Chang human hepatocytes	As^{III} SA, As^{V}, MMA^{III}, MMA^{V}, DMA^{V}	LC_{50} determinations	24 hr	-	LC_{50}s using K^{+} leakage assay in phosphate media: As^{III}: 19.8 As^{V}: 1,006 MMA^{III}: 6.3 MMA^{V}: 9,283 DMA^{V}: 4,109	Petrick et al., 2000

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Chang human hepatocytes	As^{III} SA, As^{V}, MMA^{III}, MMA^{V}, DMA^{V}	LC_{50} determinations	24 hr	-	LC_{50}s using the XTT assay in phosphate media: As^{III}: 164 As^{V}: 3,050 MMA^{III}: 13.6 MMA^{V}: 42,000 DMA^{V}: 91,440	Petrick et al., 2000
Raji cells (human B-lymphocytes)	As^{III} SA MMA^{III} DMA^{III}	0.2, 1, 10, 20, 40, 100 for all	4 hr 4hr 2 hr	10 40 10	Extent of viability determined by trypan blue assay: Viabilities at maximum dose for each: As^{III}: ~85% MMA^{III}: ~85% DMA^{III}: 60%	Gómez et al., 2005
Jurkat cells	As^{III} SA MMA^{III} DMA^{III}	0.2, 1, 10, 20, 40, 100 for all	4 hr 4hr 2 hr	40 0.2 10	Extent of viability determined by trypan blue assay: Viabilities at maximum dose for each: As^{III}: ~95% MMA^{III}: ~52% DMA^{III}: ~58%	Gómez et al., 2005
A549 cells	As^{III} SA As^{V} DMA^{V}	0.016, 0.08, 0.4, 2.0, 10 30, 100, 300 2, 20, 200, 2000	7 days for all	0.016 30 None	Colony-forming efficiency assay with Giemsa staining: LC_{50}s: As^{III}, ~0.08; As^{V}, ~100	Mass and Wang, 1997
CHO K1 cells	As^{III} SA	10	4 hr	None	Clonogenic survival assay for cytotoxicity: 12-hr pretreatment with BSO depletes GSH; with BSO at 50 and 400 μM, survival was 9% and 1%, respectively; other experiments showed that an increase in GSH markedly reduced the cytotoxicity of an As^{III} treatment following UV irradiation.	Huang et al., 1993
CHO cells: Wild-type V 850 R 120	As^{III} SA for all	5, 10, 15, 20, 30, 50, 75, 100 for most	48 hr for all	5 20 10 (lowest for it)	Comparative inhibition of cell growth was based on numbers of cells present compared to control: V 850 cells were adapted to 850 μM H_2O_2 over about 4 months of exposures to increasing concentrations; R 120 cells had then been cultured 4 months without exposure to H_2O_2. IC_{50} values: Wild-type, 17.2; V 850, 62.45; R 120, 26.6. Results after pretreatment with BSO suggest that intracellular thiol levels (GSH mainly) may account for the arsenic resistance seen in V 850 cells.	Cantoni et al., 1994
CHO K1 cells	As^{III} SA	20, 40, 80	4 hr	20	Colony formation assay	Wang et al., 1996

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
CHO cell lines: K1 (parental to the following line) XRS-5 (X-ray and H_2O_2 sensitive)	As^{III} SA	10, 20, 40 for both	4 hr	40 20	Clonogenic survival with crystal violet staining: ID_{50}s: line K1, 37.8; line XRS-5, 17.0; the much less responsive K1 cells have 6 times as much catalase activity as XRS-5 cells; both lines are similar in arsenic uptake and release, in GSH levels, and in GST activity.	Wang and Huang, 1994
CHO cell lines: K1 (parental to the following lines) XRS-6 (X-ray sensitive) XRS-5 (X-ray and H_2O_2 sensitive)	As^{III} SA	20, 40, 80, 160 20, 40, 80, 160 20, 40, 80, 160	4 hr	160 80 20	Inhibition of cell growth: ID_{50} values were: K1, 235; XRS-6, 108; XRS-5, 33. K1 cells have 5.8 times as much catalase activity and 5.4 times as much GPx activity as XRS-5 cells. K1 cells have 3.7 times as much catalase activity and 2.1 times as much GPx activity as XRS-5 cells. The cells with intermediate amounts have an intermediate response.	Wang et al., 1997
CHO-9 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V} $TMA^{V}O$	0.1, 1, 10, 100, 500 for all	1 hr for all	1 1 500 100 0.1 500 None	Extent of viability determined by trypan blue assay: DMA^{III} was by far the most cytotoxic at all concentrations tested, with the percentages of living cells at 1, 10, and 100 being approximately 45, 41, and 0%, respectively.	Dopp et al., 2004
BFTC905 cells and NTUB1 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	IC_{50} determinations	7 days	-	Clonogenic survival in a colony-forming assay, IC_{50} values in BFTC905 and NTUB1 cells, respectively: 0.13, 0.16 9.25, 9.00 0.13, 0.15 3.04, 2.64 0.52, 0.58 0.38, 0.63	Wang et al., 2007
CHO K1 cells	As^{III} SA	20	6 hr	20	Colony-forming assay: this concentration caused ~32 % survival; squalene at up to 160 μM had no effect on cytotoxicity	Fan et al., 1996

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
CHO cells treated with MMS before or after iAs treatment	As^{III} SA	5, 10, as pretreatments 5, 10, as post-treatments	24 hr 24 hr	None 5	Colony-forming assay: No change from MMS alone ⇑ in cytotoxicity compared to MMS alone, synergistic interaction with even less survival at 10	Lee et al., 1986
CHO K1 cells exposed to 1.5 or 2.5 J/m^2 of UV	As^{III} SA	10	24 hr	10	Colony-forming assay: Synergistic ⇑ in cytotoxicity because of the iAs post-treatment	Yang et al., 1992
C-33A cells HeLa cells Jurkat cells LCL-EBV cells	As^{III} SA for all	0.1, 1, 10, 25, 50 for all	24 hr	10 50 0.1 10	Cell viability determined by Trypan blue exclusion: ~35% viability at 50; ~75% viability at 50; ~55% viability at 50; ~60% viability at 50.	Salazar et al., 1997
Jurkat cells and human lymphocytes	As^{III} SA	0.1, 1, 10, 25, 50 for both	24 hr for both	0.1 for both	Cell viability determined by Trypan blue exclusion: When both of these cell types were transfected with mutant p53 genes (by electroporation) there was substantially increased cytotoxicity. This ⇑ was already apparent at a dose of 0.1 (i.e., the LOEC) in the 1 p53 mutation tested in Jurkat cells and in 1 of 2 p53 mutations tested in PHA-stimulated lymphocytes.	Salazar et al., 1997
Mouse 291.03C keratinocytes	As^{III} SA	0.05, 0.1, 0.5, 1, 5	7 days	0.5	Cytotoxicity based on colony survival, using crystal violet staining: LC_{50} = 0.9; almost all dead at dose of 5	Wu et al., 2005
Chinese hamster V79 cells	As^{III} SA DMA^{V}	1, 2, 5, 10 ~0.8, 1, 2, 5, 10 mM	24 hrs for both	1 2 mM	Cytotoxicity based on number of viable cells compared to control: LC_{50}s: As^{III}, ~5.5; DMA^{V}: ~3.5 mM	Ochi et al., 1999b
A2780 cells H460 cells MCF-7 cells	As^{III} ATO for all	IC_{50} determinations	72 hr	-	Cell survival was determined using the MTT assay: IC_{50} values: A2780, 2.80; H460, 14.60; MCF-7, 3.00	Ling et al., 2002

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
BALB/c 3T3 cells (derived from mice)	As^{III} SA As^{V} MMA^{V} DMA^{V} TMA^{V}	IC_{50} determinations	18 hr	-	Cell survival was determined using the MTT assay: IC_{50} values: As^{III} SA, 16.9; As^{V}, 64; MMA^{V}, 14.7 mM; DMA^{V}, 4.35 mM; TMA^{V}, >74 mM. Depletion of GSH in cells by co-treatment with 0.2 mM BSO markedly reduced the cytotoxicity of DMA^{V} even though it markedly increased the cytotoxicity of the other 4 compounds.	Ochi et al, 1994
G12 cells	As^{III} SA $MMA^{III}O$ $DMA^{III}I$	0.05, 0.1, 0.5, 1, 2.5, 5, 10 0.2, 0.4, 0.6, 0.8, 1 0.1, 0.2, 0.3, 0.4	72 hr	1 0.2 0.1	Cell survival was determined using the clonal survival assay: LC_{50} values: As^{III} SA, ~8; $MMA^{III}O$, 0.51; $DMA^{III}I$, 0.15 The 2 methylated forms were also tested at 4 and 24 hr and showed cytotoxicity at both; for $MMA^{III}O$, cytotoxicity was > 50% at both times at highest dose.	Klein et al., 2007
U-2OS cells	As^{III} SA	0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5	10 days	0.05	Cell survival was determined using the clonal survival assay: LC_{50} = 0.68; 100% cell killing at 2.5	Komissarova et al., 2005
U-2OS cells	As^{III} SA for all	LC_{50} Determinations	24 hr 48 hr 72 hr	- - -	Cell survival was determined using the clonal survival treat-and-plate (CSTP), neutral red (NR), and MTT assays, for the different durations: LC_{50}: CSTP, 1.1; MTT, 3.8; NR, 4.8; LC_{50}: CSTP, 0.9; MTT, 0.99; NR, 1.05; LC_{50}: CSTP, 0.8; MTT, 0.8; NR, 0.84.	Komissarova et al., 2005
U118MG cells	As^{III} ATO	1, 5, 10, 25, 50	24 hr	5	Cell survival was determined using the MTT assay: Slightly > 50% survival at dose of 5; co-treatment with lipoic acid blocked cytotoxicity. Other tests showed no ⇑ in either apoptotic cell death or intracellular peroxide levels; cell death was shown to be autophagic.	Cheng et al., 2007
Undifferentiated PC12 cells	As^{III} ATO	1, 10, 100, 1000	24 hr	1	Cell survival was determined using the MTT assay: LC_{50} = 8. (At dose of 8, about 75% cell survival at 12 hr.) Effects of pretreatment or co-treatment with antioxidants on cytotoxicity: NAC: big ⇓, but α-Toc, GSH, 17β-estradiol, or BO653: NSE	Piga et al., 2007
FGC4 cells HepG2 cells Rat hepatocytes	As^{III} SA for all	50, 75, 100, 125 25, 50, 75, 100, 125 2, 10, 25, 35, 45, 55	24 hr for all	75 50 25	Cell survival was determined by the NR uptake assay: LC_{50}s: FGC4, ~90; HepG2, ~70; hepatocytes, ~30	Gottschalg et al., 2006
SVEC4-10 cells	As^{III} SA	2, 4, 8, 12, 16	24 hr	4	Cytotoxicity determined by the MTT assay: LC_{50} = ~13	Chao et al., 2006a

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HCT116 cells (securin +/+) HCT116 cells (securin -/-)	As^{III} SA for both	4, 8, 12, 16 for both	24 hr for both	4 4	Cytotoxicity determined by the MTT assay: LC_{50}s = securin +/+, ~17; securin -/-, ~11. There was significantly more cytotoxicity in null mutant at doses of 8, 12 and 16.	Chao et al., 2006a
RKO cells (p53 wt) SW480 cells (p53 mutant)	As^{III} SA for both	8, 16, 24, 32 for both	24 hr for both	8 8	Cytotoxicity determined by the MTT assay: LC_{50}s = RKO, ~20; SW480, ~27. There was significantly more cytotoxicity in wt p53 cell line at doses of 16, 24 and 32.	Chao et al., 2006a
U-2OS cells	As^{III} SA for all	0.1, 1, 10	24 hr	1 or 10; see explana-tion	Trypan blue exclusion assay to identify necrotic cells (which take up stain) after additional periods of post-treatment culturing of 0, 24, or 48 hr in arsenic-free medium: At dose of 0.1, no increase in necrotic cells at any time. At dose of 1, necrotic cells were ~0%, ~20%, and ~40% of total cells, respectively. At dose of 10, necrotic cells were ~70%, ~95%, and ~95% of total cells, respectively. Note that a 24-hr treatment with SA affected the amount of necrosis at a dose of 1 only if there was an additional 24-hr or longer period of culturing in SA-free medium between the end of the SA treatment and when the assay was done.	Komissarova et al., 2005
HaCaT cells CRL1675 cells THP-1 + A23187 cells	As^{III} ATO for all	LD_{10} and LD_{25} determinations for each cell line	72 hr	-	Cytotoxicity assessed using fluorescein diacetate assay: LD_{10} = 1.9 ; LD_{25} = 15.2 LD_{10} = 1.0; LD_{25} = 1.9 LD_{10} = 1.9; LD_{25} = 3.8	Graham-Evans et al., 2004
HaCaT cells CRL1675 cells THP-1 + A23187 cells	As^{III} ATO for all	LD_{10} and LD_{25} determinations for each cell line	72 hr under chronic exposure conditions	-	Testing for cytotoxicity was preceded by exposure to 1.0 μM As^{III} ATO for at least 8 passages to establish chronic-exposure conditions. Then, following exposures to various doses for 72 hr, cytotoxicity was assessed using fluorescein diacetate assay: LD_{10} = 2.0 ; LD_{25} = 4.0 LD_{10} = 0.5; LD_{25} = 1.3 LD_{10} = 0.5; LD_{25} = 5.1	Graham-Evans et al., 2004

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Alveolar macrophages (AMs) from CDF_1 mice Peritoneal macrophages (PMs) from CDF_1 mice	As^{III} SA As^{V} MMA^{V} DMA^{V} TMA^{V}	IC_{50} determinations	48 hr	-	Cell survival was determined using the AlamarBlue assay (said to be similar to the MTT assay): IC_{50} values of AM cells: As^{III} SA, 4; As^{V}, 400; MMA^{V}, >10 mM; DMA^{V}, 5 mM; TMA^{V}, >>10 mM IC_{50} values of PM cells: As^{III} SA, 5; As^{V}, 650; MMA^{V}, >10 mM; DMA^{V}, 5 mM; TMA^{V}, >>10 mM. DMA^{V} caused almost entirely apoptotic cell death, while the inorganic arsenicals caused mainly necrotic cell death. SOD, CAT and a peptide inhibitor ICE inhibited the cytotoxicity of As^{III} but not of DMA^{V}.	Sakurai et al., 1998
RHMVE cells	MMA^{V} DMA^{V} $TMA^{V}O$	0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100 mM for all	24 hr	25 mM 1 mM None	Cell survival was determined using a modified MTT assay: LC_{50}s: MMA^{V}, 33.6 mM; DMA^{V}, 2.54 mM; $TMA^{V}O$, cell number increased by dose of 1 mM, reaching 135% of control at dose of 25 mM. Another study showed LC_{50}s: As^{III}, 36; As^{V}, 220 (both μM); Co-treatment with NAC caused ⇓ in cellular arsenic content and cytotoxicity by DMA^{V} but not by MMA^{V}. Co-treatment with BSO caused big ⇑ in cytotoxicity of MMA^{V} but slight ⇓ in cytotoxicity of DMA^{V}.	Hirano et al., 2004
HLFC cells HLFK cells (Ku70 deficient)	As^{III} SA for both	5, 10, 20, 40, 80 for both	24 hr for both	10 for both	Viability was determined by trypsin blue exclusion assay: LC_{50}s: HLFC, 27.38; HLFK, 21.80; cytotoxicity was significantly greater for HLFK than HLFC at doses of 20, 40 and 80	Liu et al., 2007b
NB4 cells NB4-M-AsR2 cells IM9 cells	As^{III} ATO for all	0.5, 1 2, 4 0.5, 1	6 days for all	0.5 4 0.5	Cell viability (% of control) for ATO alone and for ATO with 100 μM Trolox, determined using trypan blue exclusion: At 0.5: 75% alone, 43% with Trolox; at 1: 30% alone, 3% with Trolox; at 2: 100% alone, ~80% with Trolox; at 4: ~63% alone, ~30% with Trolox; at 0.5: ~80% alone, ~70% with Trolox; at 1: ~50% alone, ~25% with Trolox; Thus, Trolox enhanced ATO-induced cytotoxicity (or growth inhibition) in all 3 cell lines.	Diaz et al., 2005
MCF-7 cells, T47D cells, and MDA-MB-231 cells	As^{III} ATO for all	IC_{50} determinations	3 days	-	Cell viability for ATO without and with 100 μM Trolox co-treatment, respectively, determined using trypan blue exclusion assay: MCF-7: 2.07 and 1.02; T47D: 3.22 and 1.56; MDA-MB-231: 2.27 and 0.98. Thus, co-treatment with Trolox enhanced ATO growth inhibition similarly to what was seen in the row above.	Diaz et al., 2005
Human PBMCs cultured in various ways	As^{III} ATO	1	15 days	1	Colony-forming ability was assessed for ATO alone and for co-treatment with Trolox by counting CFU-erythrocytes, CFU-granulocytes-macrophages, and BFU-erythrocytes. Biggest effect of ATO alone: 62% ⇓ for CFU-erythrocytes. In all 3 cases, co-treatment with Trolox had little or no effect.	Diaz et al., 2005

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MDAH 2774 cells	As^{III} ATO	1, 2, 5, 8	72 hr	1 or 2	Cytotoxicity assessed using trypan blue exclusion assay: uncertainty about LOEC exists because control value was not reported: LC_{50}: 5	Terek et al., 2006
SVEC4-10 cells	As^{III} SA	5, 10, 20, 40	24 hr	10	Cell survival was determined using the MTT assay: LC_{50} = ~13	Hsu et al., 2005
$1RB_3AN_{27}$ cells	As^{III} SA	0.1, 0.5, 1, 5, 10	72 hr	1	Cell survival was determined using the MTT assay: there probably was cytotoxicity at dose of 1; statistically significant cytotoxicity at dose of 5; LC_{50} = ~8; all experiments on ROS or induction of transcription factors were at doses of ≤ 10 for ≤ 4 hr, and under those conditions, there was no cytotoxicity	Felix et al., 2005
BEAS-2B cells	As^{III} ATO	10, 20, 50	24 hr	10	Cell survival was determined using the MTT assay: LC_{50} = ~15	Han et al., 2005
HT1197 cells	As^{III} SA	1, 5, 10, 25, 50	24 hr	10	Cell survival was determined using the trypan blue exclusion assay: LC_{50} = ~35	Hernández-Zavala et al., 2005
HL-60 cells U266 cells	As^{III} ATO for both	1, 2, 3, 5, 10 for both	24 hr for both	2 1	Cell survival was determined using the trypan blue exclusion assay: LC_{50}s = HL-60, ~7; U266, ~2. Effects of modulators in both cell lines: (Cells were loaded with high concentrations of intracellular AA (icAA) by incubating them with DHA prior to iAs treatments, thus avoiding generation of extracellular ROS in tissue culture media caused by direct addition to it of AA.) icAA caused big ⇓ in cytotoxicity of iAs. GSH depletion by BSO treatment caused big ⇑ in iAs-induced cytotoxicity. icAA caused big ⇓ in cytotoxicity caused by iAs in GSH-depleted cells. Extracellular AA caused big ⇑ in iAs-induced cytotoxicity, including after GSH depletion. Relatively limited data from a methylcellulose colony-forming assay in both cell lines (with 48-hr iAs treatment and 10-14 days to form colonies) and from cytotoxicity testing of RPMI-8226 cells supported some of the above conclusions. Effect of NAC was tested in HL60 cells; it caused big ⇓ in iAs-induced cytotoxicity.	Karasavvas et al., 2005
Embryonic mesenchymal cells prepared from CF-1 mouse conceptuses at gestation day 11	As^{III} SA	5.8, 11.6, 15.4, 30.8	2 hr	5.8	Cell survival was determined using the MTT assay: LC_{50} = ~27; 15-min pretreatment with 0.5% (v/v) DMSO completely blocked the iAs effect at dose of 15.4, whereas 15-min pretreatment with 0.1% or 0.2% (v/v) DMSO partially blocked it.	Pérez-Pastén et al., 2006
HCT15 cells HeLa cells PLC/PR/5 cells Chang cells	As^{III} SA for all	LC_{50} determinations	24 hr	-	Cell survival determined by MTT cell proliferation assay: LC_{50}s: HCT15, 278.33; HeLa, 200.33; PLC/PR/5, 376.66; Chang, 328.33	Othumpangat et al., 2005

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K562 cells, AR230-s cells, AR230-r cells, KCL22-s cells, KCL22-r cells, NB4 cells	As^{III} ATO	IC_{50} determinations for all	3 days	-	Antiproliferative activity as determined by MTS assay—some would interpret such results as cytotoxicity and present results as LC_{50}s: IC_{50}s: K562, 0.9; AR230-s, 2.6; AR230-r, 6.9; KCL22-s, 2.6; KCL22-r, 2.8; NB4, 0.4. A dose of 2 represents the upper margin of the clinically useful range for ATO. There was a positive correlation between GSH content of cells and resistance to the antiproliferative (i.e., cytotoxic) effect.	Konig et al., 2007
AR230-s cells, AR230-r cells, KCL22-s cells, KCL22-r cells	As^{III} ATO	1	2 days	None	Cell survival was determined by trypan blue assay: 100 μM BSO treatment was shown to greatly ⇓ GSH levels in all 4 cells types both with and without iAs exposure. In all 4 cell types, the iAs + BSO treatment caused big to huge ⇓ in number of viable cells, whereas untreated cells or cells treated with iAs or BSO showed ~2-fold ⇑. A similar assay in primary cultures of mononuclear cells from 4 patients in blast crisis with imatinib-resistant CML also showed maximum cytotoxicity for the combined iAs + BSO treatment.	Konig et al., 2007
H1355 cells	As^{III} ATO	3.125, 6.25, 12.5, 25, 50, 100, 200	24 hr	6.25	Cell survival was determined using the MTT assay: Cytotoxicity increased with dose, with ~57% cell survival at dose of 200	Cheng et al., 2006
TRL 1215 cells = X in this row TRL 1215 cells that had been treated with 1.3 mM MMA^V for 20 weeks prior to acute arsenic treatments = Y in this row	As^{III} SA, As^V, DMA^V for both	LC_{50} determinations for both	48 hr for both	-	Cell survival based on AB assay: LC_{50}s for As^{III}: X, 16.3; Y, 74.1 LC_{50}s for As^V: X, 157.1; Y, 2743.8 LC_{50}s for DMA^V: X, 2090; Y, 6950. Thus the MMA^V pretreatment caused marked resistance to cytotoxicity for all 3 arsenicals. Much of this resistance was lost if Y cells were cultured for 8 more weeks with no arsenic in media. The 20-week pretreatment caused no cytotoxicity, gave the Y cells an arsenic content of 135.4 ± 12.0 ng/mg cellular protein, and did not induce malignant transformation. Arsenicals were not methylated or demethylated in these cells.	Kojima et al., 2006
TRL 1215 cells = X in this row TRL 1215 cells that had been treated with 0.7 mM DMA^V for 20 weeks prior to acute arsenic treatments = Y in this row	As^{III} SA, As^V, DMA^V for both	LC_{50} determinations for both	48 hr for both	-	Cell survival based on AB assay: LC_{50}s for As^{III}: X, 16.3; Y, 19.2 LC_{50}s for As^V: X, 157.1; Y, 182.2 LC_{50}s for DMA^V: X, 2090; Y, 4730. Thus the DMA^V pretreatment caused marked resistance to cytotoxicity for only the DMA^V treatment, and the slight differences for the other 2 arsenicals were not statistically significant. When Y cells were cultured for 8 more weeks with no arsenic in media, there was no change regarding the lack of resistance to As^{III}, but the resistance to the other 2 arsenicals increased substantially. The 20-week pretreatment caused no cytotoxicity, gave the Y cells an arsenic content of 41.8 ± 2.5 ng/mg cellular protein, and did not induce malignant transformation. Arsenicals were not methylated or demethylated in these cells.	Kojima et al., 2006

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TRL 1215 cells = X in this row TRL 1215 cells that had been treated with 10.0 mM TMA^VO for 20 weeks prior to acute arsenic treatments = Y in this row	As^{III} SA, As^V, DMA^V for both	LC_{50} determinations for both	48 hr for both	-	Cell survival based on AB assay: LC_{50}s for As^{III}: X, 16.3; Y, 54.8 LC_{50}s for As^V: X, 157.1; Y, 684.1 LC_{50}s for DMA^V: X, 2090; Y, 4500. Thus the TMA^VO pretreatment caused marked resistance to cytotoxicity for all 3 arsenicals. Much of this resistance was lost regarding DMA^V, and all of it was lost regarding the other 2 arsenicals, if Y cells were cultured for 8 more weeks with no arsenic in media. The 20-week pretreatment caused no cytotoxicity, gave the Y cells an arsenic content of 543.8 ± 12.0 ng/mg cellular protein, and did not induce malignant transformation. Arsenicals were not methylated or demethylated in these cells.	Kojima et al., 2006
NB4 cells	As^{III} SA As^{III} ATO MMA^{III} DMA^{III}	1, 2, 3, 4 1, 2, 3, 4 0.25, 0.5, 1, 2 2, 4, 6, 8	72 hr	1 1 0.25 4	Cell survival was determined using the MTT assay: LC_{50}s: As^{III} SA, ~3.4; As^{III} ATO, ~2.2; MMA^{III}, ~1.2; DMA^{III}, ~5.8. Co-treatment (CoTr) with 3000 μM DTT markedly decreased cytotoxicity of all arsenicals: Maximum cytotoxicities with 3000 μM DTT CoTr: As^{III} SA, ~17%; As^{III} ATO, ~12%; MMA^{III}, ~25%; DMA^{III}, ~12%. CoTr with 100 μM DTT markedly increased cytotoxicity of all arsenicals: LC_{50}s with 100 μM DTT CoTr: As^{III} SA, ~2.2; As^{III} ATO, ~1.0; MMA^{III}, ~0.28; DMA^{III}, ~4.0.	Jan et al., 2006
293 cells	As^{III} ATO	0.5, 1, 2, 3, 4	12 days	1	Cell survival was determined by colony-forming assay (% of cells forming colonies): ~73% at dose of 4; LC_{25} = ~3.6; Co-treatment with 200 μM DMSA increased survival: ~87% at dose of 4. Co-treatment with 20 μM DMSA decreased survival: ~61% at dose of 4; LC_{25} = ~1.6. Co-treatment with 100 μM DMPS increased survival: ~86% at dose of 4. Co-treatment with 10 μM DMPS decreased survival: ~50% at dose of 4; LC_{25} = ~1.2.	Jan et al., 2006
SV-HUC-1 cells	As^{III} ATO	0.5, 1, 2, 3, 4	12 days	0.5	Cell survival was determined by colony-forming assay (% of cells forming colonies): ~62% at dose of 4; LC_{25} = ~2.2. Co-treatment with 200 μM DMSA increased survival: ~73% at dose of 4; LC_{25} = ~3.5. Co-treatment with 20 μM DMSA decreased survival: ~43% at dose of 4; LC_{25} = ~1.4. Co-treatment with 100 μM DMPS increased survival: ~79% at dose of 4. Co-treatment with 10 μM DMPS decreased survival: ~47% at dose of 4; LC_{25} = ~1.2	Jan et al., 2006
HeLa cells	As^{III} SA	10, 100	24 hr	10	Cell survival determined using a LIVE/DEAD viability/cytotoxicity kit: LC_{50}: ~95	Hansen et al., 2006
Primary rat hepatocytes	As^{III} SA	2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50	24 hr	7.5	Cell survival was determined using the MTT assay: LC_{50} = ~18	Hasgekar et al., 2006

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
A431 cells	As^{III} ATO	1.25, 2.5, 5, 10, 20 for both	24 hr 48 hr	2.5 1.25	Cell survival was determined using the MTT assay: At 24 hr: LC_{50} = ~20; At 48 hr: LC_{50} = ~3	Huang et al., 2006
RAW264.7 cells	As^{III} SA	2.5, 5, 10, 25	24 hr	2.5	Cell survival based on neutral red uptake assay: LC_{50} = ~13	Szymczyk et al., 2006
NIH 3T3 cells	As^{III} SA	5, 10, 20, 50, 100, 200	6 hr	20 for ⇓	Cell viability assayed using CellTiter-Glo assay: possibly slight ⇑ at 5 and 10; ⇓ at 20, LC_{50} = ~90. Pre-induction of HSP by conditioning heat shock (2 hr at 42°C on prior day) or by constitutive expression of HSP70 markedly reduced the cytotoxicity, as follows: with heat: LOEC = 100 and ~80% viability at dose of 200, with constitutive expression: LOEC = 50 and ~70% viability at dose of 200	Khalil et al., 2006
NHEK cells	As^{III} SA	0.2, 0.4, 0.8	1, 2, 3, 4 days	0.2 for ⇑ on all days	Cell survival was determined using the NR uptake assay: ⇑ to ~1.1-1.4x at doses of 0.2 and 0.4 on all days; point estimates at dose of 0.8 were always higher than control, but the ⇑ was always a NSE.	Hwang et al., 2006
BAEC cells	As^{III} SA	1, 5, 10	24 hr 48 hr	5 1	Cell survival was determined using a variation of the MTT assay: LC_{50}s: ~7.5 at 24 hr, ~5.0 at 48 hr. Unlike co-treatment with Zn^{II}, Fe^{II}, or Cu^{II}, only co-treatment with Mn^{II} increased iAs toxicity at concentrations at which it (the metal) did not cause cytotoxicity alone.	Bunderson et al., 2006
H22 cells BAEC cells	As^{III} ATO for both	0.5, 1, 2, 4 for both	24 hr, 48 hr 24 hr, 48 hr	1, 0.5 2, 1	Cell survival (also called the proliferation index) was determined using the MTT assay: LC_{50}s for H22: ~2.0 at 24 hr, ~1.2 at 48 hr; LC_{50}s for BAEC: ~4.5 at 24 hr, ~2 at 48 hr	Liu et al., 2006e
HEK 293 cells HEK 293 cells transfected with OATP-C	As^{III} SA As^{V} MMA^{V} DMA^{V}	LC_{50} determinations	72 hr	Cell survival was determined using the MTT assay: LC_{50}s without and with OATP-C, respectively: As^{III}: 10.9, 5.6; As^{V}: 98.1, 53; MMA^{V}: 4319.3, 4211.6 (this comparison: NSE); DMA^{V}: 994.1, 899.3 (this comparison: NSE); The OATP-C transfected cells accumulated 43% more As^{III} and 34% more As^{V} than the non-transfected cells while they did not accumulate more of the methylated arsenicals. Co-treatment of the As^{III}- or As^{V}-treated cells with rifampin or taurocholic acid eliminated the difference between the two cell types. OATP-C can transport iAs in a (GSH)-dependent manner but this may not be the major pathway for arsenic transport.		Lu et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
U937 cells	As^{III} SA for all	0.5, 1, 2.5, 5, 10, 20 for all	24 hr 48 hr 72 hr	20 10 10	Cell survival was determined using the PI-exclusion assay: At 24 hr, ~74% survival at dose of 20; At 48 hr, ~62% survival at dose of 20; At 72 hr, ~40% survival at dose of 20, LC_{50}: ~17.5.	McCollum et al., 2005
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^{V} for both	1.25, 2.5, 5, 10 mM for both	48 hr for both	10 mM 2.5 mM	Cell survival was determined using the AB assay: Without BSO: ~80% cell survival at dose of 10 mM; at 5 mM, survival may have been higher than that of control; LC_{50} with BSO: 3.2 mM. Similar results were obtained using CV assay.	Sakurai et al., 2005a
$Gclm^{+/+}$ MEF cells $Gclm^{+/-}$ MEF cells $Gclm^{-/-}$ MEF cells, from GCLM knockout mice	As^{III} SA for all	4, 8, 16, 32, 64 for all	8 hr for all	16 or 64 16 or 64 4	Cell survival was determined using the MTT assay: It was unclear which of the first two genotypes had the LOEC of 16; one had an LOEC of 64, and the LOEC for the other one was 16; LC_{50}s: +/+, 86; +/-, 86; -/-, 11; pretreatment with tBHQ protected $Gclm^{-/-}$ and $Gclm^{+/-}$ MEF cells from iAs-induced cytotoxicity in a dose- and time-dependent manner.	Kann et al., 2005b
HeLa cells U937 cells Primary human skin fibroblasts	As^{III} ATO for all	2 for all	3 days	2 2 None	Cell survival was determined using the MTS assay: ~77% survival in HeLa and ~85% survival in U937; no hint of cytotoxicity in fibroblasts. Co-treatment with 10 μM emodin apparently sensitized HeLa and U937 cells (but not fibroblasts) to cytotoxicity. The addition of 1.5 mM NAC to the co-treatment of HeLa cells with 10 μM emodin and 2 μM iAs eliminated all cytotoxicity; effect of NAC was not tested in U937 cells. Emodin was used because it has a semiquinone structure that is likely to increase the generation of intracellular ROS.	Yi et al., 2004
MCF-7 cells	As^{III} ATO	0.5, 1, 2, 4, 8, 16	24 hr, 48 hr, or 96 hr	2 at 24 hr; 1 at 48 and 96 hr	Cell survival was determined using the MTT assay: LC_{50}s at 24, 48, and 96 hr were 8.6, 3.3, and 1.86, respectively. Apoptosis was shown to be the mechanism of cell death after treatment with a dose of 5 for 3 days.	Ye et al., 2005

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MYP3 cells	As^{III} SA As^{V} MMA^{III} DMA^{III} DMA^{V} $TMA^{V}O$	2, 3 35, 40 1, 1.5 0.6, 1 0.6 mM, 1 mM 15 mM, 20 mM	7 days for all	2 35 1 0.6 0.6 mM 15 mM	Cell survival was determined using the MTT assay: ~33% at 2, ~9% at 3; ~37% at 35, ~28% at 40; ~60% at 1, ~7% at 1.5; ~28% at 0.6, ~10% at 1; ~45% at 0.6 mM, ~28% at 1 mM; ~28% at 15 mM, ~18% at 20 mM; Co-treatments with antioxidants that work by different mechanisms yielded the following results: melatonin slightly inhibited cytotoxicity of As^{III}. NAC inhibited cytotoxicity of MMA^{III}, DMA^{III}, DMA^{V} and $TMA^{V}O$. Vitamin C inhibited cytotoxicity of As^{III}, As^{V}, MMA^{III} and DMA^{III}. Tiron and Trolox did not affect cytotoxicity of any arsenical.	Wei et al., 2005
Primary keratinocytes (in third passage) obtained from foreskins of adults	As^{III} SA	0.001, 0.01, 0.1, 1, 5, 10, 100, 1000	24 hr 48 hr 72 hr	5 for ⇓ 1 ⇑; 5 ⇓ 0.1 ⇑; 5 ⇓	Cell survival was determined using the XTT assay: ⇑ in viability (proliferation) to 1.18x and 1.32x at dose of 1 at 48 and 72 hr, respectively. LC_{50}s at 24, 48, and 72 hr were ~160, ~10, and ~4.2, respectively.	Liao et al., 2004
Huh7 cells	As^{III} SA	0.5, 1, 3, 5, 10, 20	24 hr	1 for ⇑ 20 for ⇓	Cell survival was determined using the MTT assay: ⇑ to ~1.1x at doses of 1 and 3; ⇓ to 58% at dose of 20. In co-treatments with 10nM TCDD, iAs doses of 5 and 10 caused 0% and 10% cytotoxicity, respectively.	Chao et al., 2006b
CL3 cells, synchronous at G1 CL3 cells, asynchronous (asyn) CL3 cells, synchronous at S CL3 cells, synchronous at G2/M	As^{III} SA for all	50, 100 for all	3 hr for all	-	Cell survival was determined using a colony-forming assay: % survival at dose of 50: G1, 45%; asyn, 35%; S, 29%; G2/M, 17%; Survival at dose of 50 in G1 cells was cut from 45% to 25-30% by co-treatment with PD98059 or U0126, which are these 2 structurally dissimilar inhibitors of MEK1/2.	Li et al., 2006a

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human neuroblastoma cell lines: IMR-32 SK-N-DZ SK- N-BE(2) SK-N-AS SH-SY5Y All 4 lines ± co-treatment with 25 μM DCHA	As^{III} ATO	1	48 hr	- or + DCHA None, 1 None, 1 1, 1 1,1 1,1	Cell survival (% of control) was determined using the MTT assay: iAs alone, DCHA alone, (iAs + DCHA) NSE, NSE, 35%; NSE, NSE, 45%; 73%, NSE, 41%; 56%, NSE, 39%; 61%, NSE, 40%; co-treatment of (iAs +DCHA) with vitamin E blocked much of the cytotoxicity in line IMR-32.	Lindskog et al., 2006
HaCaT cells	As^{III} SA	0.5, 1, 1.5, 2.5, 4, 6, 7, 8, 10, 12, 13, 14, 16, 18, 20, 22	24 hr for all	0.5 for ⇑ 12 for ⇓	Cell survival was determined using the MTT assay, with a proliferative effect being seen at lower doses: Peak of 141% at dose of 1; first point estimate below 100% at dose of 8; about 50% cytotoxicity at 22.	Ganyc et al., 2007
	As^{V}	10, 20, 30, 40, 50, 60, 80, 100, 120, 160, 200, 240, 280, 320, 360		10 for ⇑ 100 for ⇓	Peak of 145% at dose of 10; first point estimate below 100% at dose of 80; about 50% cytotoxicity at 320.	
	MMA^{III}	0.1, 0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 8, 10		0.1 for ⇑ 2.5 for ⇓	Peak of 160% at dose of 1; first point estimate below 100% at dose of 2.5; about 50% cytotoxicity at 4.5.	
	DMA^{III}	0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11		None for ⇑ 3 for ⇓	About 60% cytotoxicity at 11.	

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Mouse lymphoma cells (L5178Y/Tk$^{+/-}$ –3.7.2C cells)	As^{III} SA	2.3, 3.1, 4.6, 6.2, 7.7, 9.2, 10.8, 11.5, 13.1, 13.9, 14.6, 15.4	4 hr for all	4.6	Cell survival, as percent of relative total growth compared to the vehicle control: Estimates of LC_{50}s: As^{III} SA, ~7.3 μM; As^{V}, ~50.3 μM; MMA^{V}, ~16.1 mM; DMA^{V}, ~38.8 mM	Moore et al., 1997a
	As^{V}	6.1, 15.2, 30.3, 45.5, 48.5, 54.6, 60.6, 66.7, 69.7, 72.8, 78.8, 84.9		15.2		
	MMA^{V}	12.3, 15.4, 18.5, 21.6, 24.7, 27.8 mM		12.3 mM		
	DMA^{V}	12.5, 18.8, 25.0, 31.3, 37.5, 43.8, 50.0, 56.3, 62.5 mM		18.8 mM		
V79 cells treated with MNU	As^{III} SA	10	3 hr	10	Cell survival, percent of control: Both iAs treatments caused 4% or less cytotoxicity; however, as post-treatments they both considerably increased the cytotoxicity caused by the MNU treatments.	Li and Rossman, 1989a
		5	24 hr	5		
V79 cells exposed to UVA, UVB, or UVC over a wide range of doses	As^{III} SA	10	3 hr	10	Cell survival, percent of control: The iAs treatments caused 8% or less cytotoxicity; however, as post-treatments they increased the cytotoxicity caused by the UV treatments.	Li and Rossman, 1991
Human-hamster hybrid A_L cells	As^{III} SA	3.8, 7.7, 15.4	1 day or 5 days	3.8	Colony-forming assay; ~55% survival with 1-day treatment at 7.7	Hei et al., 1998
1T1 cells MYP3 cells	As^{III} SA, As^{V}, $MMA^{III}I_2$, MMA^{V}, $DMA^{III}I$ DMA^{V}, $TMA^{V}O$	LC_{50} determinations for all	7 days	-	LC_{50}s based on trypan blue assay for viability: As^{III} SA: 4.8 in 1T1; 0.4 in MYP3 As^{V}: 31.3 in 1T1; 5.3 in MYP3 $MMA^{III}I_2$: 1.0 in 1T1; 0.8 in MYP3 MMA^{V}: 1.7 mM in both lines $DMA^{III}I$: 0.8 in 1T1; 0.5 in MYP3 DMA^{V}: 0.50 mM in 1T1; 1.1 mM in MYP3 $TMA^{V}O$: 1.7 mM in 1T1; 4.5 mM in MYP3	Cohen et al., 2002

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
AG06 cells	As^{III} SA	0.2, 4, 20	24 hr pretreatment	0.2 for ⇑ 4 for ⇓	Extent of viability determined by NR assay: ⇑ in viability over that seen for MNNG alone at ~1–15 μM MNNG. ⇓ in viability below that seen for MNNG alone at ~15–40 μM MNNG (synergistic interaction)	Snow et al., 1999
Human cells: AG06 (keratinocytes) AG06 (keratinocytes) HaCaT (keratinocytes) NHEK (keratinocytes) GM847 (fibroblasts) WI38 (fibroblasts)	 As^{III} SA MMA^{III} As^{III} SA As^{III} SA As^{III} SA As^{III} SA	IC_{50} determinations	48 hr	-	Extent of viability determined by NR assay: IC_{50}: 7.2 IC_{50}: ~7.5 IC_{50}: 11.6 IC_{50}: 12.3 IC_{50}: 10.7 IC_{50}: 11.2	Snow et al., 2001
AG06 cells	As^{III} SA MMA^{III}	1, 5, 10, 20, 30	5 hr	-	Extent of viability determined by NR assay: ~20 kills 20% of cells ~20 kills 50% of cells	Snow et al., 2001
K562 cells	As^{III} ATO	2.5	12 hr	2.5	~50% of cells die	Li and Broome, 1999
Human kidney carcinoma cell lines: UOK123 UOK109 UOK121 Human lung carcinoma cell line: A549	As^{III} SA	IC_{50} determinations	7 days	-	Extent of viability determined by colony-formation efficiency assay: 0.020 0.021 0.020 0.4	Zhong and Mass, 2001
HFW cells (diploid human fibroblasts)	As^{III} SA	2.5, 5, 10, 20	6 hr	2.5	Cytotoxicity determined by a colony-forming assay; co-treatment with catalase (but not heat-inactivated catalase) at 100 μg/mL markedly reduced cytotoxicity; increasing GSH levels with ß-mercaptoethanol reduced cytotoxicity; decreasing GSH levels with BSO increased cytotoxicity	Lee and Ho, 1995
HFW cells (diploid human fibroblasts)	As^{III} SA	1.25, 2.5, 5, 10 5, 10, 20, 40, 80	24 hr 4 hr	1.25 ~10	Cytotoxicity determined by a colony-forming assay	Yih and Lee, 1999

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V79-C13 Chinese hamster cell line	As^{III} SA	5, 10, 20, 30, 40, 50, 60	24 hr	10	Cytotoxicity determined by a colony-forming assay: Survival at 10 was 76.3 ± 2.61% of control; IC_{50}: ~20	Sciandrello et al., 2002
Syrian hamster embryo cells	As^{III} SA As^{V}	~0.7, 1.4, 2, 3, 4, 5, 6 ~5, 10, 20, 50, 75, 100, 130, 160, 200	7 days for all	0.7⇑, 5⇓ 10⇑, 100⇓	Cytotoxicity determined by measuring CFE: Small but reproducible ⇑ from 0.7 to about 1.5 followed by a logarithmic decrease in CFE with a linear increase in dose Small but reproducible ⇑ from 10–50 followed by a logarithmic decrease in CFE with a linear increase in dose	Barrett et al., 1989
UROtsa cells	As^{III} SA	0.1, 10, 25, 50, 100, 200	24 hr	50	Viability determined using MTT assay: IC_{50} = ~100; doses ≤ 10 were said to stimulate mitochondrial activity (i.e., the curve went up; the assay tests mitochondrial function), but the stimulation was not statistically significant. Co-treatment with BSO: big ⇑ in cytotoxicity, with IC_{50} = ~15	Bredfeldt et al. 2004
UROtsa cells	MMA^{III} for all	0.5, 1, 2, 5, 10 for all	24 hr 48 or 72 hr	5 5	Viability determined using MTT assay: IC_{50} = ~5 All cells (or almost all cells) were dead at LOEC.	Bredfeldt et al. 2006
UROtsa cells	As^{III} SA As^{V} $MMA^{III}O$ MMA^{V} $DMA^{III}I$ DMA^{V}	0.1, 0.5, 1, 5 1, 200 0.1, 0.5, 1, 5 1, 200 0.1, 0.5, 1, 5 1, 200	24 hr for all	None None 5 None None None	Viability determined using MTT assay: With $MMA^{III}O$: 50% cytotoxicity was estimated to result from dose of about 2.5, with about 90% cytotoxicity at dose of 5	Drobná et al., 2002
BALB/c 3T3 A31-1-1 cells (derived from mice)	As^{III} SA As^{V} DA MMA^{V} DMA^{V}	2, 5, 10, 15, 20 10, 15, 20, 25, 30 1, 2, 5, 10 mM 0.5, 1, 2, 5 mM	72 hr for all	5 10 5 mM 1 mM	Cytotoxicity based on percent cell growth compared to treatment with distilled water: IC_{50} values: As^{III} SA, 4.8; As^{V} DA, 17; MMA^{V}, 9.8 mM; DMA^{V}, 3.2 mM	Tsuchiya et al., 2005

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TK6 cells	As^{III} SA As^{III} ATO	0.1, 0.5, 1, 10, 100, 1000 0.1, 1, 10, 100	24 hr for both	10 10	Cytotoxicity based on trypan blue exclusion assay: For both: LC_{50} between 3 and 4	Hornhardt et al., 2006
HL-60 cells	As^{III} ATO	0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 13, 25, 50, 100	24 hr	0.8	Viability determined using MTT assay: LC_{50} = 32	Yedjou and Tchounwou, 2007
IEC cells (primary culture) IEC-6 cells	As^{III} SA for both	7.7, 15, 38, 77, 116, 154 for both	24 hr for both	15 15	Viability determined using MTT assay: At dose of 77: IEC, ~45% dead; IEC-6, ~55% dead; the cytotoxicity of the 2 cells types was almost identical at most doses; based on this and their rather similar concentration-dependent declines in membrane enzymes and constituents (e.g., alkaline phosphatase, hexose, sialic acid, cholesterol, and phospholipid), the primary and established cultures gave approximately similar toxic responses.	Upreti et al., 2007
MDAH 2774 cells	As^{III} ATO	1, 2, 5, 8	72 hr	1 or 2 (uncertain since control not shown)	Cytotoxicity estimated by XXT proliferation assay and alternatively by trypan blue dye-exclusion assay (for which treatment time was either 72 or 96 hr—it was unclear from methods): IC_{50} by both methods: 5	Askar et al., 2006
HPBMs exposed to M-CSF for 7 days and considered M-macrophages	As^{III} SA As^{V} MMA^{V} DMA^{V}	LC_{50} determinations	48 hr	-	Viability based on AB assay: LC_{50} values: As^{III}, 7.0; As^{V}, 1900; MMA^{V}, 2500; DMA^{V}, 800.	Sakurai et al., 2006
HPBMs exposed to GM-CSF for 7 days and considered GM-macrophages	As^{III} SA As^{V} MMA^{V} DMA^{V}	LC_{50} determinations	48 hr	-	Viability based on AB assay: LC_{50} values: As^{III}, 5.8; As^{V}, 2800; MMA^{V}, 2000; DMA^{V}, 2000.	Sakurai et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
The following cell lines: HL-60, U-937, TIG-112, CRL-1609, RAW264.7, mouse normal embryo cells, mouse embryo cells that were MT +/+ and MT -/-, and the following 3 types of human immune cells: peripheral T-lymphocytes, immature dendritic cells and multi-nucleated giant cells	As^{III} SA	LC_{50} determinations	48 hr	-	Viability based on AB assay: LC_{50} values: HL-60, 13; U-937, 12; TIG-112, 25; CRL-1609, 17; RAW264.7, 25; MT +/+ cells, 4.8; MT -/- cells , 5.8; T-lymphocytes, 3.3; dendritic, 8.2; giant, 2.3	Sakurai et al., 2006
GM04312C cells	As^{III} SA	2.5, 10, 50	24 hr	2.5	Viability based on neutral red assay: LC_{50} = ~20. However, when viability was based on colony-forming assay: LC_{50} = ~6 with LOEC of 2.5	Shen et al., 2006
Primary mouse hepatocytes	As^{III} SA	60, 100, 200	24 hr	60	Viability determined using MTT assay: LC_{50} = ~200 (LC_{50} = 30 for 48-hr treatment). Pretreatment with SFN caused big ⇓ in cytotoxicity. SFN activates transcription factor Nrf2 and causes significant ⇑ of protein expressions responsible for excretion of As into extracellular space. SFN caused big ⇑ in intracellular GSH levels and big ⇓ in intracellular arsenic levels. Also, pretreatments with BSO, EA, or MK-571, which ⇑ As accumulation in hepatocytes, caused big ⇑ in cytotoxicity.	Shinkai et al., 2006
SV-HUC-1 cells	As^{III} SA MMA^{III} DMA^{III}	0.5, 1, 2, 5, 10 0.1, 0.25, 0.5, 1 0.25, 0.5, 1, 2, 5	3 days for all	1 0.25 0.5	Viability determined by SRB assay: LC_{50} values: As^{III}, 2.91; MMA^{III}, 0.46; DMA^{III}, 1.59	Su et al., 2006
JB6 C141 cells JB6 C141 cells exposed to 0.1, 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, or 8 kJ/m^2 of UVB at end of pretreatment with iAs	As^{III} SA for both	0.1, 1, 5, 10, 20, 50, 100, 500, 1000 10	24 hr for both	5 10	Viability determined by MTS assay: LC_{50} = ~15, decreased with dose until reached ~12% of control at top 3 doses. Probably some cytotoxicity at UVB dose of 5, and there was significant cytotoxicity at UVB dose of 6. Viability was ~70% of control at highest UVB dose.	Tang et al., 2006

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DNA Damage						
WRL-68 (human hepatic cell line)	As^{III} SA	0.001, 0.01, 0.1, 10	16 hr	0.001	Induction of DNA-protein crosslinks (Methylated forms of arsenic could not be detected in the cells.)	Ramírez et al., 2000
Human aorta VSMCs (vascular smooth muscle cells)	As^{III} SA	~1.2, 2.5, 5, 10	4 hr	~1.2	DNA strand breaks (double and single strand breaks and alkali-labile sites) detected by comet assay: The effect was similar in nonproliferating VSMCs.	Lynn et al., 2000
PHA-stimulated and unstimulated human lymphocytes	As^{III} ATO	10	2 hr	10	Oxidative damage to DNA measured by the comet assay, including SSBs—after digestion with FPG, arsenic-induced base damage was converted to a large increase in SSBs and some FPG-created DSBs. (FPG cleaves purines including 7,8-dihydro-8-oxoguanine (8-oxoG), formamidopyrimidines, and AP sites.) Like the damage induced by H_2O_2, arsenic-induced DNA damage was repaired more slowly in unstimulated lymphocytes.	Li et al., 2001
L-132 cells (human diploid alveolar epithelial type II cells)	As^{III} SA MMA^{V} DMA^{V}	100 100 5, 10, 100	6 hr for all	None None 5	Induction of DNA SSB resulting from inhibition of repair polymerization by polymerization inhibitors aphidicolin and hydroxyurea. DMA^{V} induced them in a dose-dependent manner (measured by alkaline elution)	Yamanaka et al., 1997
L-132 cells (human diploid alveolar epithelial type II cells)	As^{III} SA MMA^{V} DMA^{V}	100 for all	3 hr for all	None None 100	Induction of DNA repair synthesis using the BrdU photolysis assay (single-strand DNA breaks induced by UV-irradiation were measured by alkaline elution). Follow-up experiment with same DMA^{V} treatment for 1, 3, or 6 hr showed increases with longer durations of treatment.	Yamanaka et al., 1997
L-132 cells (human diploid alveolar epithelial type II cells)	MMA^{V}	100 with 10 mM SAM present	6 hr	100	Induction of DNA repair synthesis using the BrdU photolysis assay (single-strand DNA breaks induced by UV-irradiation are measured by alkaline elution). This and other evidence strongly suggests that the DNA damage was not directly induced by MMA^{V} but by dimethylated arsenic that was produced metabolically by reaction of MMA^{V} with SAM.	Yamanaka et al., 1997
ϕX174 RF I DNA Naked double-stranded circular DNA	As^{III} SA MMA^{III} DMA^{III} DMA^{V}	0.1, 1, 10, 100, 300 mM 10, 15, 20, 25, 30, 60 mM 40, 80, 150, 250 μM 0.1, 1, 10, 100, 300 mM	2 hr for all	None 30 mM 150 μM None	Nicked DNA in DNA nicking assay	Mass et al., 2001

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human primary peripheral blood lymphocytes	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	1–1000 1–1000 1.25–80 Not reported–875 1.4–91 Not reported–1000	2 hr for all	Not reported for any of them	Breaks and/or alkali-labile lesions in DNA detected in the single-cell gel comet assay— the relative potencies based on slopes are shown below (the larger the number, the bigger the effect) As^{III} 1 As^{V} 1.4 MMA^{III} 77 MMA^{V} <1 DMA^{III} 386 DMA^{V} <1 As^{III} and As^{V} caused a significant effect, and they were not significantly different from each other. MMA^{III} and DMA^{III} were thus 77 and 386 times more potent in causing DNA damage than SA.	Mass et al., 2001
E. coli WP2s(λ) (lon_{11}, $sulA_1$, $trpE_{65}$, $uvrA_{155}$, $lamB^+$)	MMA^{III} DMA^{III}	0.01, 0.10, 1.0, 10 for all	Overnight for all	None None	Assay to test for induction of prophage with and without exogenous metabolic activation: No statistically significant induction of prophage by either compound.	Kligerman et al., 2003
Raji cells (human B-lymphocytes)	As^{III} SA MMA^{III} DMA^{III}	0.2, 1, 10, 20, 40, 100 for all	4 hr 4 hr 2 hr	10 0.2 10	Extent of DNA damage detected by single-cell gel electrophoresis (comet) assay: At 0.2 & 1: MMA^{III} >> DMA^{III} = As^{III}. At 100: all 3 chemicals had roughly the same level of DNA damage as MMA^{III} had at 0.2, but MMA^{III} still has significantly more DNA damage than the other two chemicals.	Gómez et al., 2005
Jurkat cells	As^{III} SA MMA^{III} DMA^{III}	0.2, 1, 10, 20, 40, 100 for all	4 hr 4hr 2 hr	10 0.2 10	Extent of DNA damage detected by single-cell gel electrophoresis (comet) assay: at 0.2 & 1: MMA^{III} >> DMA^{III} = As^{III}; at 40 & 100: DMA^{III} > MMA^{III} > As^{III}.	Gómez et al. , 2005
NB4 cells HL-60 cells CHO-K1 cells	As^{III} SA for all	0.25, 0.5, 1 0.25, 0.5, 1 0.25, 0.5, 1, 2	4 hr for all	0.25 0.25 None	The LOECS shown are for DNA strand breaks (termed ADSB by the authors) detected by the comet assay without any additional treatments of DNA to digest and reveal ODA or DPC. They also treated the damaged DNA with FPG or PK to yield estimates of ODA or DPC, respectively. The LOEC was 0.25 for all 3 cell types for ODA, DPC, or ODA+DPC. Clearly much more DNA damage is revealed by treatments with FPG, PK, or both. DNA damage was induced at levels causing no cytotoxicity.	Wang et al., 2001

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human peripheral blood lymphocytes from 2 donors, with results reported separately	As^{III} SA	5, 10	4 hr for all	None	The LOECs apply to the extent of DNA damage detected by SCGE (comet) assay at pH > 13. There was no cytotoxicity at doses up to 20. Much lower responses for all arsenicals were seen in comet assay at pH of 12.1, with the difference between this and pH 13 being defined as alkaline labile sites. DNA damage by both methylated arsenicals was markedly reduced by co-exposures to the antioxidants Se-Met or Vitamin C. DNA-double strand breaks were not induced.	Soto-Reyes et al., 2005
	MMA^{III}	2.5, 5, 10, 20, 40, 80, 100		2.5		
	DMA^{III}	2.5, 5, 10, 20, 40, 80		5		
MRC-5 cells	As^{III} SA	2.5, 5, 10	2 hr	2.5	DNA SSBs detected by the standard alkaline (pH > 13) comet assay: ⇑ with dose of both tail length and tail moment at doses of 2.5 and 5, but a ⇓ for both effects at dose of 10 to less than effect seen at dose of 2.5. NSE on cytotoxicity at any of the tested doses.	Mourón et al., 2006
MRC-5 cells	As^{III} SA	2.5, 5, 10	2 hr	2.5 for SSBs 10 for protein-DNA adducts	Protein-DNA adducts and DNA SSBs detected by alkaline (pH > 13) comet assay done with and without posttreatment with proteinase K, respectively: Experiment without proteinase K: ⇑ of both tail length and tail moment at doses of 2.5 and 5, but a ⇓ of both effects at dose of 10 to less than effect seen at other doses. Experiment with proteinase K: ⇑ of both tail length and tail moment at doses of 2.5 and 5, and a further large ⇑ in both parameters at dose of 10. NSE on cytotoxicity at any of the tested doses in either experiment. Evidence for protein-DNA adducts (or crosslinks) came from ⇑ observed at dose of 10, which is thus the LOEC for that effect. Proteinase K breaks the crosslinks that hinder the DNA fragmentation caused by the DNA SSBs.	Mourón et al., 2006
MRC-5 cells	DMA^{V}	125, 250, 500	2 hr	500 for ⇓ in SSBs (see row below)	DNA SSBs detected by the standard alkaline (pH > 13) comet assay: slight ⇑ in tail moment (TM) at dose of 125 (a NSE); point estimates of TM were below control at 2 higher doses, with that at 500 being significantly below it; actual data: TMs: 0, 13.4; 125, 14.6; 250, 13.1; 500, 9.7. NSE on cytotoxicity at any of the tested doses.	Mourón et al., 2005
MRC-5 cells	DMA^{V}	125, 250, 500	2 hr	125 for both protein-DNA adducts and SSBs	Protein-DNA adducts and DNA SSBs detected by alkaline (pH > 13) comet assay done with and without posttreatment with proteinase K, respectively: Experiment without proteinase K (buffer only): progressive ⇓ in tail moment (TM) with increasing dose; actual data: TMs: 0, 7.7; 125, 6.7; 250, 5.3; 500, 4.9. Experiment with proteinase K: ⇑ in TM, with a positive dose response; actual data: TMs: 0, 8.3; 125, 11.9; 250, 22.2; 500, 23.3. NSE on cytotoxicity at any of the tested doses in either experiment. Proteinase K breaks the crosslinks that hinder the DNA fragmentation caused by the DNA SSBs.	Mourón et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
L-132 cells (human alveolar type II cells)	DMA^V	5, 7.5, 10 mM	12 hr	5 mM	DNA SSB detected by alkaline elusion: There was a dose response. Early in the exposure period, there was marked suppression of replicative DNA synthesis, and the chain length of the nascent DNA was shorter than that of the control, which suggests that the template DNA was modified by more than just strand breaks.	Tezuka et al., 1993
HepG2 cells	As^{III} SA	7.5	24 hr	7.5	Induction of DNA DSBs by immunodetection of γH2A.X foci: ⇑ to ~6x control level; co-treatment with 170 nM SAM did not change the induced DSB frequency.	Ramírez et al., 2007
NB4 cells	As^{III} SA As^{III} ATO MMA^{III} DMA^{III}	0.5	30 min	0.5	Experiments with ENIII, FPG and NE (from NB4 cells) as well as experiments using immunodepletion of NE with antibodies directed against proteins known to be involved in excision repair suggest that these trivalent arsenicals induce only oxidative DNA adducts and that OGG1, MYH and APE are involved in the excision of the oxidative DNA adducts.	Pu et al., 2007
HL-60 cells	As^{III} ATO	12.5, 25, 50	24 hr	12.5	DNA damage detected by alkaline SCGE (comet) assay: while the response was barely statistically significant at the lowest dose, it was strong at the other 2 doses, with a positive dose response.	Yedjou and Tchounwou, 2007
$PARP\text{-}1^{+/+}$ MEF cells $PARP\text{-}1^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	30 min for both	11.5 11.5	Extent of DNA damage detected by SCGE (comet) assay at pH >13, reported as induced damage (experimental – control) in units of TM length: ~0.4 at 11.5, ~0.7 at 23; ~2.9 at 11.5, ~3.4 at 23; All 4 estimates were statistically significant.	Poonepalli et al., 2005
$PARP\text{-}1^{+/+}$ MEF cells $PARP\text{-}1^{-/-}$ MEF cells	As^{III} SA for both	11.5, 23 for both	24 hr for both	11.5 11.5	Extent of DNA damage detected by single-cell gel electrophoresis (comet) assay at pH >13, reported as induced damage (experimental – control) in units of tail moment length: ~2.0 at 11.5, ~3.6 at 23; ~4.8 at 11.5, ~5.5 at 23; All 4 estimates were statistically significant.	Poonepalli et al., 2005
HaCaT cells CRL1675 cells THP-1 + A23187 cells	As^{III} ATO for all	72-hr LD_{10} and LD_{25} for each cell line: 1.9, 15.2 1.0, 1.9 1.9, 3.8	72 hr for all	15.2 None 1.9	DNA single-strand breaks detected by SCGE (comet assay) following alkaline treatment: NSE at LD_{10}; ⇓ at LD_{25} (perhaps stimulates repair) NSE at LD_{10}; NSE at LD_{25} ⇑ at LD_{10}; ⇑ at LD_{25}	Graham-Evans et al., 2004

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HaCaT cells CRL1675 cells THP-1 + A23187 cells	As^{III} ATO for all	72-hr LD_{10} and LD_{25} for each cell line under chronic-exposure conditions, as follows: 2.0, 4.0 0.5, 1.3 0.5, 5.1	Under chronic exposure conditions: 72 hr for all	2.0 1.3 0.5	Testing for DNA single-strand breaks was preceded by exposure to 1.0 μM As^{III} ATO for at least 8 passages to establish chronic-exposure conditions. Then, following exposures to various doses for 72 hr, DNA single-strand breaks were detected by single-cell gel electrophoresis (comet assay) following alkaline treatment: ⇑ at LD_{10}; ⇑⇑ at LD_{25} NSE at LD_{10}; ⇑ at LD_{25} ⇑⇑ at LD_{10}; ⇑⇑ at LD_{25}	Graham-Evans et al., 2004
293 cells	As^{III} ATO	1	6 hr	1	DNA damage reported in units of tail moment in a comet assay that used nuclear extraction incubation: Untreated = ~11 units; dose of 1: big ⇑ to ~58 units. Effects of co-treatment (CoTr) with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~38 units; CoTr 100 μM DMPS: ⇓ from iAs alone to ~39 units. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~104 units; CoTr 10 μM DMPS: ⇑ from iAs alone to ~84 units.	Jan et al., 2006
SV-HUC-1 cells	As^{III} ATO	1	6 hr	1	DNA damage reported in units of tail moment in a comet assay that used nuclear extraction incubation: Untreated = ~10 units; dose of 1: big ⇑ to ~49 units. Effects of CoTr with modulators at high doses: CoTr 200 μM DMSA: ⇓ from iAs alone to ~34 units; CoTr 100 μM DMPS: ⇓ from iAs alone to ~35 units. Effects of CoTr with modulators at low doses: CoTr 20 μM DMSA: ⇑ from iAs alone to ~99 units; CoTr 10 μM DMPS: ⇑ from iAs alone to ~89 units.	Jan et al., 2006
UROtsa cells	As^{III} SA MMA^{III}	1, 10 0.05, 0.5, 5	30 min for both	1 0.05	Detection of 8-oxo-dG (measure of oxidative DNA damage): ⇑ to 3x control at 1, ⇑ to 2x control at 10; ⇑ to 5x control at 0.05, ⇑ to 4x control at 0.5, NSE at 5	Eblin et al., 2006
UROtsa cells	As^{III} SA MMA^{III}	1, 10 0.05, 0.5, 5	60 min for both	10 0.05	Detection of 8-OHdG formation (measure of oxidative DNA damage): NSE at 1, big ⇓ from control at 10; ⇑ to 3x control at 0.05, ⇑ to 3.3x control at 0.5, ⇑ to 4.3x control at 5 Thus MMA^{III} showed a time delay just as it did for ROS production.	Eblin et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
E. coli strain $WP2_S(\lambda)$	As^{III} SA	Up to 3.2 mM	20 hr	None	No induction of λ phage (part of "SOS" system) using 8 serial twofold dilutions from a concentration that inhibits growth	Rossman et al., 1984
Human-hamster hybrid A_L cells	As^{III} SA	30.8	24 hr	30.8	Induction of 8-OHdG; co-treatment with SOD or catalase considerably reduced induction of this oxidative DNA damage.	Kessel et al., 2002
HaCaT cells	As^{III} SA As^{V}	5, 10, 20, 30 10, 20, 30, 50, 100	24 hr	10 20	Induction of 8-OHdG; pre-incubation with SOD, CAT or DMSO almost completely blocked this; oxidative DNA damage by 20 μM As^{III} SA: pre-incubation with MnTMPyP, *L*-NAME or FeTMPyP substantially blocked such damage.	Ding et al., 2005
TK6 cells	As^{III} SA As^{III} ATO	0.1, 1, 10 for both	22 hr for both	1 10	Induction of DPCs detected by a decrease in DNA damage detected in the comet assay when an As treatment was followed by exposure to 1 or 2 Gy of 69 cGy/min gamma radiation. The DPCs kept the damaged DNA from moving during electrophoresis. While both SA and ATO caused a significant effect, the effect was more pronounced for SA.	Hornhardt et al., 2006
ϕX174 RF I DNA Naked double-stranded circular DNA	As^{III} un-specified MMA^{III} DMA^{III}	10μM–30 mM in log increments 10, 20, 30, 40, 50 37.5, 75, 150, 300, 1000	24 hr for all	None 10 37.5	Nicked DNA in DNA nicking assay	Nesnow et al., 2002
Supercoiled DNA (plasmid pBR 322) Similar results were found for plasmid φX174, but details were not reported.	As^{III} SA As^{V} MMA^{III} MMA^{V} Mono-methyl-arsine DMA^{III} DMA^{V} Dimethyl-arsine Tri-methyl-arsine	≥ 5 mM ≥ 5 mM ≥ 5 mM ≥ 5 mM ≥ 5 mM <5 mM ≥ 5 mM <0.5 mM <0.5 mM	2 hr for all	None None ≥ 5 mM None ≥ 5 mM <5 mM None <0.5 mM <0.5 mM	Damage to DNA detected by agarose gel electrophoresis: The arsines were produced in aqueous reaction mixtures of sodium borohydride and the appropriate arsenical. Trimethylarsine and dimethylarsine were about 100 times more potent than DMA^{III}. When NADH or NADPH, which are biological hydride donors, were incubated with DMA^{III} for 2 hr, DNA damage was increased by at least 10-fold, possibly because of the generation of dimethylarsine.	Andrewes et al., 2003

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DNA Repair Inhibition or Stimulation						
CHO K1 cells	As^{III} SA	5, 10, 20, 40, 80	6 hrs	5	DNA single-strand breaks detected by alkaline elution: those induced by MMS were repaired after incubation in a drug-free medium for 6 hr; however, posttreatment with sodium arsenite accumulated MMS-induced breaks with a dose-response for the arsenite exposure. Both alkali-labile sites and frank breaks were enhanced, with the latter occurring at higher doses of MMS and arsenite.	Lee-Chen et al., 1993
V79 cells, strain 743-3-6	As^{III} SA for both	10 μM	3 hr	10 μM	Similar decreases in inducible total nuclear DNA ligase activity and in inducible nuclear DNA ligase II activity were demonstrated after arsenic treatments given before or after MNU treatments, thereby demonstrating that most of the inhibited activity was DNA ligase II.	Li and Rossman, 1989b
HeLa S3 cells	MMA^{III} MMA^{V} DMA^{III} DMA^{V}	0.0001, 0.001, 0.01, 0.1, 1 0.01, 0.1, 1, 10, 100, 500 0.0001, 0.001, 0.01, 0.1 0.01, 0.1, 1, 10, 100, 250	For all: 18 hr + 5 min more while also being treated with 100 μM H_2O_2	0.001 None 0.001 None	Effect on H_2O_2-induced poly(ADP-ribosyl)ation: ⇓ with dose, 59% of control at dose of 1; NSE; ⇓ with dose, 49% of control at dose of 0.1; NSE. — Other experiments showed that the above effects were real decreases (not merely delayed responses). All above measurements were at dose levels with little to no cytotoxicity. After 18 hr incubation, these arsenicals had NSE on the extent of gene expression of PARP-1 at doses up to 0.1 and 100 for methylated and pentavalent arsenicals, respectively.	Walter et al., 2007
Isolated recombinant PARP-1	As^{III} SA MMA^{III} DMA^{III}	10, 50, 100, 200, 500 for all	For all: 10 min preincubation before PARP-1 reaction with a nicked plasmid as substrate	10 10 10	Effect on activity of PARP-1: ⇓ with dose, 58% of control at dose of 500; ⇓ with dose, 24% of control at dose of 500; ⇓ with dose, 15% of control at dose of 500. These data suggest that trivalent arsenicals inhibit cellular poly(ADP-ribosyl)ation by reducing PARP-1 activity.	Walter et al., 2007

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Jurkat cells	As^{III} SA	0.01, 0.1, 1, 5, 10	24 hr	0.01	⇓ ERCC1 mRNA level; not said to be statistically significant until dose of 1, but means ± SDs suggest 45% ⇓ at 0.01 and 60% ⇓ at 0.1. Decreases of 60%, 95%, and 85% at doses of 1, 5, and 10, respectively	Andrew et al., 2006
Jurkat cells	As^{III} SA	1	24 hr	1	⇓ in repair following a 2-hr *in vitro* treatment with 4 μM 2-AAAF immediately after the iAs treatment. DNA damage measured by SCGE (comet) assay: iAs group had ⇑ DNA damage after 2-hr 2-AAAF treatment and following a 4-hr repair period. No difference in DNA damage before 2-AAAF.	Andrew et al., 2006
HLFC cells HLFK cells (Ku70 deficient)	As^{III} SA for both	1, 2.5, 5, 10 for both	2 hr for both	2.5 1	DNA DSB damage as measured with neutral SCGE assay: This type of damage was significantly greater for HLFK than HLFC at all 4 doses.	Liu et al., 2007b
HLFC cells HLFK cells (Ku70 deficient)	As^{III} SA for both	5 for both	2 hr for both	5 5	The LOECs are for induction of DNA DSBs. After the 2-hr As^{III} treatment, cells were incubated in arsenic-free medium to measure repair of DNA DSBs using the neutral SCGE assay at 0.5, 1, 1.5, and 2 hr. At all time points there was significantly and substantially less repair in HLFK, showing that the Ku70 deficiency decreases the efficacy of repair of arsenic-induced DSBs.	Liu et al., 2007b
CHO-K1 cells	As^{III} SA	0.1, 0.5, 1, 5, 10	24 hr	0.1	DNA polymerase β promoter activity: big ⇑ at 0.1; slight ⇑ at 0.5; no effect at 1; big ⇓ at 5 and 10	Snow et al., 2005
GM847 cells HaCaT cells	As^{III} SA for both	0.1, 0.5, 1, 5, 10 for both	24 hr for both	0.1 0.1	DNA polymerase β protein levels: Big ⇑ at 0.1 and 0.5, slight ⇑ at 1, no effect at 5 and big ⇓ at10; Big ⇑ at 0.1 and 0.5, no effect at 1, big ⇓ at 5 and 10	Snow et al., 2005
W138 cells for both	As^{III} SA for both	0.1, 0.5, 1, 5, 10 for both	24 hr 48 hr	0.1 0.1	DNA ligase activity: ⇑ at 0.1, big ⇑ at 0.5, huge ⇑ at 1, ⇓ at 5, big ⇓ at 10; No effect at 0.1, big ⇑ at 0.5 and 1, no effect at 5, big ⇓ at 10. Two other experiments of 72 and 96 hr duration showed generally even more subdued increases and decreases than the 48-hr experiment.	Snow et al., 2005
Effects Related to Oxidative Stress (ROS)						
Hepa-1 cells (mouse hepatoma) stably transformed with pEpREβgeo	As^{III} SA	0.1, 1, 5, 25, 50	6 hr	5	Activated a β-galactosidase gene reporter system: suggests there was induced oxidative stress—5.6 fold response; progressively and markedly decreasing responses at 2 higher doses	Maier et al., 2000

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WI38 (human fibroblasts)	As^{III} SA	0.05, 0.5, 5 (24 hr pretreatment) followed by 60 min exposure to H_2O_2 at 1, 10 or 50 mM for 1 hr and then 24 hr to recover	24 hr pretreatment	0.05	Extent of viability determined by NR assay: Compared to control cells exposed to H_2O_2, with no pretreatment: ⇑ viability at 1 mM H_2O_2 only. At dose of 5, there was an ⇑ in viability at 10 mM H_2O_2 but a ⇓ in viability at 50 mm H_2O_2	Snow et al., 2001
Purified thioredoxin enzyme from mouse liver; to test the NADPH-dependent reduction of DTNB	As^{III} SA MMA^{III} DMA^{III} As^{V} MMA^{V} DMA^{V}	- ~0.2–800 ~0.2–800 ~0.2–800 ~10–6000 ~10–6000 ~10–6000	-	- ~100 ~0.2 ~3 ~300 - -	Approximate IC_{50}s (inhibition of enzyme activity): ~200 ~0.4 ~30 ~3000 Never more than ~80% inactivation Never more than ~80% inactivation	Lin et al., 1999
Primary culture of rat hepatocytes	As^{III}SA MMA^{III}	1–50 0.1–10	30 min for both	-	Decreased thioredoxin enzyme activity (the NADPH-dependent reduction of DTNB) IC_{50}: >> 100 IC_{50}: ~3	Lin et al., 2001
Human-hamster hybrid A_L cells	As^{III} SA	30.8	within 5 min	30.8	Production of ROS, measured by ESR and with about a 3-fold increase in amplitude of signals; concurrent treatment with the radical scavenger DMSO eliminates the effect	Liu et al., 2001
Human aorta VSMCs (vascular smooth muscle cells)	As^{III} SA	~1.2, 2.5, 5, 10	4 hr	~1.2	Numerous experiments in this study led to the conclusion that arsenite activates NADH oxidase to produce superoxide, which then causes oxidative DNA damage.	Lynn et al., 2000
HFW cells (diploid human fibroblasts)	As^{III} SA	1.25, 2.5, 5, 10 5, 10, 20, 40, 80	24 hr 4 hr	1.25 20	Micronuclei were induced in both protocols; the yield of micronuclei was greatly reduced by the presence of the antioxidants catalase or NAC (the precursor of GSH), which suggests that oxidative stress was involved in the induction of micronuclei.	Yih and Lee, 1999
Jurkat cells	As^{III} SA MMA^{III} DMA^{III}	0.2, 10, 20, 100 for all	2 hr 2hr 2 hr	None 10 10	Level of intracellular peroxides determined by flow cytometry using cell permeable fluorogenic marker DHR123: At 10 and 20: DMA^{III} >> MMA^{III} >> As^{III}. At 100: MMA^{III} > DMA^{III} about equal to As^{III} (Cell lysis may explain the reduction of DMA^{III} at dose of 100 to 1/3 level seen at 20.) Control value was not reported. If control value was actually 0 (and thus the baseline in the figure), then the LOEC for all 3 arsenicals would have been 0.2, with a rather similar slight response for all of them.	Gómez et al., 2005

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Whole blood lymphocytes from 2 human donors, with results reported separately	MMA^{III} DMA^{III}	2.5, 5, 10, 20 for both	4 hr for all	2.5 10	Levels of MDA as lipid peroxidation marker in human plasma: For MMA^{III} both donors showed significant increase over control at all doses except 10, for which only 1 was significant. For DMA^{III} both donors showed significant increase over control at 20, but only 1 did at 10. There was no cytotoxicity at the dose levels tested.	Soto-Reyes et al., 2005
HaCaT cells	As^{III} SA As^{V}	5, 10, 15, 20 for both	24 hr	5 10	Induction of 3-NT, which is a diagnostic marker for RNS *in vivo*; pre-incubation with SOD, MnTMPyP, *L*-NAME or FeTMPyP almost completely blocked this protein damage by 20 μM As^{III} SA; pre-incubation with CAT or DMSO had no effect, in sharp contrast to what happened for ROS-damage to DNA.	Ding et al., 2005
L-132 cells	DMA^{V}	10 mM alone 10 mM + 0.5 mM PQ	2 hr 1 hr	None 10 mM	DNA single-strand breaks detected by alkaline elusion: co-exposure with PQ or sequential exposures of 1 hr (with either one first) yielded a strong response.	Kawaguchi et al., 1996
PAEC cells harvested from freshly isolated vessels	As^{III} SA	5, 10	1 hr	5	Various experiments showed that iAs activates a NADPH-dependent oxidase located in the plasma membrane that results in superoxide accumulation. Both the $p67^{phox}$ and Rac1 subunits of the oxidase were shown to be essential for the response, and the oxidase is dependent on exogenous NAD(P)H for activity. The peak effect occurred within 1 hr and was higher at a dose of 5 than 10.	Smith et al., 2001
NB4 cells	As^{III} ATO	1	4 hr	1	Generation of ROS led to decrease (and eventual loss, with continued treatment) of mitochondrial membrane potential, with subsequent outer mitochondrial membrane permeability changes.	Jing et al., 1999
PAEC from freshly harvested vessels	As^{III} probably ATO, but called arsenite	5	5-15 min	5	⇑ in superoxide and H_2O_2 accumulation	Barchowsky et al., 1999b
HFW cells	As^{III} SA	5, 10, 20	24 hr	5	DCF fluorescence to indicate formation of cellular oxidants; co-treatment with BHT (a radical scavenger) completely blocked this effect.	Lee and Ho, 1995
Cell free buffer	$DMA^{III}I$	-	-	-	Oxidative damage was induced in thymine to form cis-thymine glycol. SOD and CAT did not alter this reaction. Other tests suggest that the reaction requires the formation of a reactive arsenic peroxide, probably dimethylated arsenic peroxide.	Yamanaka et al., 2003
Postconfluent PAEC cells in a monolayer	As^{III} SA	1, 2.5, 5, 10, 20	30 min	1	DCF fluorescence as a direct measure of intracellular oxidant concentrations (i.e., accumulation of ROS): Likely ⇑ at all doses, with a peak at 5 that is ~45% higher than control, a difference that is statistically significant.	Barchowsky et al., 1996

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Human-hamster hybrid A_L cells	As^{III} SA	11.5, 15.4	24 hr	11.5	Induction of $CD59^-$ mutations: dose-related increase in mutation frequency; pretreatment + co-treatment with L-NMMA (a nitric oxide synthase inhibitor) substantially reduced the mutation frequencies at both doses. Similar treatment with D-NMMA (the inactive enantiomer) had no effect. These finding were taken as evidence that peroxynitrites have an important role in iAs-induced genotoxicity. That conclusion was supported by a Western-blot analysis of nitrotyrosine-modified proteins induced by iAs treatments and mostly blocked by L-NMMA.	Liu et al., 2005
HepG2 cells	As^{III} ATO	20	6 hr	20	Analysis of 481 selected genes in a DNA microarray experiment: hierarchical clustering analysis showed that iAs exposure closely resembled DMNQ exposure (and was extremely different from DMN or phenol exposure) regarding patterns of genes that were up-regulated and down-regulated. In phase 1 of this experiment, DMNQ was selected as a model chemical that generates ROS and is known to induce genes associated with cell proliferative responses. Exposure to iAs caused significant up-regulation of 38 genes and down-regulation of 20 genes; dose used had > 80% cell viability.	Kawata et al., 2007
NB4 cells NB4-M-AsR2 cells IM9 cells	As^{III} ATO for all	0.5, 1 2, 4 0.5, 1	24 hrs for all	0.5 2 0.5	HMOX-1 protein (a stress-responsive protein) levels after treatment with ATO alone and co-treatment with 100 μM Trolox: At 0.5: slight ⇑ alone, big ⇑ with Trolox; at 1: ⇑ alone, huge ⇑ with Trolox; At 2: slight ⇑ alone, big ⇑ with Trolox; at 4: ⇑ alone, huge ⇑ with Trolox; At 0.5: slight ⇑ alone, big ⇑ with Trolox; at 1: ⇑ alone, huge ⇑ with Trolox	Diaz et al., 2005
NB4 cells	As^{III} ATO for all	Regarding row above, other indications that Trolox potentiates ATO-mediated oxidative stress: bigger ⇑ in protein carbonyls (indicator of oxidative damage to proteins) and 8-iso-$PGF_{2\alpha}$ (indicator of lipid peroxidation) by combined ATO and Trolox treatment(s) than by ATO treatment(s) alone. Other experiments showed that the synergistic effect of Trolox on ATO-mediated apoptosis was not related to extracellular H_2O_2 production. ATO was shown to induce the formation of Trolox phenoxyl radicals by electronic spin resonance spectroscopy.				Diaz et al., 2005
BFTC905 cells and NTUB1 cells	As^{III} SA As^V MMA^{III} MMA^V DMA^{III} DMA^V	0.2 for all	24 hr for all	0.2 0.2 0.2 None 0.2 None	Relative extent of oxidative damage (peroxidation) in lipids, measured as malonaldehyde formation; ranking of those with statistically significant ⇑ over control (i.e., unranked arsenicals had NSE): in BFTC905 cells: As^{III}>DMA^{III}>MMA^{III}>> As^V; in NTUB1 cells: DMA^{III}>>MMA^{III}>As^{III}	Wang et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
BFTC905 cells and NTUB1 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	0.2 for all	24 hr for all	0.2 0.2 0.2 0.2 0.2 None	Relative extent of oxidative damage (carbonylation) in proteins; ranking of those with statistically significant ⇑ over control (i.e., unranked arsenicals had NSE): in BFTC905 cells: MMA^{III}>As^{III}>DMA^{III}>> As^{V}; in NTUB1 cells: As^{III}>MMA^{III}>DMA^{III}>>As^{V}>MMA^{V}; consistent with these effects, increased levels of nitric oxide, superoxide ions, hydrogen peroxide, and the cellular free iron pool were consistently detected in both cell lines after treatments by the 3 trivalent arsenicals.	Wang et al., 2007
BFTC905 cells and NTUB1 cells	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	0.2 for all	24 hr for all	0.2 0.2 0.2 0.2 0.2 0.2	Relative extent of oxidative damage (comet assay) in DNA; ranking of those with statistically significant ⇑ over control (i.e., unranked arsenicals had NSE): without enzyme digestion: in BFTC905 cells: As^{III} = MMA^{III}>MMA^{V}> DMA^{V}; in NTUB1 cells: As^{III} = MMA^{III}>DMA^{III} = MMA^{III} = DMA^{V}; with EnIII + FPG digestion: in BFTC905 cells: As^{III}>MMA^{III}>DMA^{III}> MMA^{V}; in NTUB1 cells: As^{III}>MMA^{III}>DMA^{III}>MMA^{V}> DMA^{V} = As^{V}	Wang et al., 2007
$Gclm^{-/-}$ MEF cells, from GCLM knockout mice	As^{III} SA for all	See rows under Apoptosis and Cytotoxicity for this citation for experimental conditions. The high level of As-induced oxidative stress from some treatments was not significantly decreased by tBHQ. Yet, tBHQ pretreatment or co-treatment greatly decreased iAs induced apoptosis and cytotoxicity.				Kann et al., 2005b
NB4 cells	As^{III} ATO	0.75	Results were obtained from various experiments, including Affymetrix oligonucleotide microarray analysis using a chip that contained 22,000 open reading frames from the human genome. Treatment for 10 days increased the expression of a set of genes responsible for ROS production. Genes were identified that responded to iAs and H_2O_2 but whose response to iAs was reversed by NAC. It was found that 26% of the genes significantly responsive to iAs might have been directly altered by ROS. iAs treatment induced ROS, which in turn oxidized the Sp1 transcription factor, with a corresponding decrease in its *in situ* binding to the promoters of the 3 genes hTERT, C17, and c-Myc, with the result that their expressions were significantly suppressed (e.g., hTERT: ⇓ expression to < 1% normal)			Chou et al., 2005
$1RB_3AN_{27}$ cells	As^{III} SA	0.1, 0.5, 1, 5, 10	2 hr	0.5	ROS production using DCFH-DA assay: ⇑ with a positive dose response; the increase at dose of 1 was blocked by co-treatment with either NAC or α-Toc	Felix et al., 2005
BEAS-2B cells	As^{III} ATO	5, 10, 20	24 hr	5	Production of 8-isoprostane, a by-product of lipid peroxidation: ⇑ with a positive dose response; 2x control at 5, 6x control at 20. In addition, electron spin resonance studies (involving co-treatments with CAT, SF, NAC, or NADPH) and confocal microscope studies showed that iAs can produce ROS, such as H_2O_2 and $^{\bullet}OH$, as a result of reduction reactions within cells.	Han et al., 2005

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Embryonic mesenchymal cells prepared from CF-1 mouse conceptuses at gestation day 11	As^{III} SA	5.8, 11.6, 15.4	2 hr	5.8	Production of ROS detected by a variant of the DCF assay using CM-H_2DCFDA: Induced RFUs: (i.e., experimental – control): 5.8, ~950; 11.6, ~2050; 15.4, ~2700; 15-min pretreatment with 0.2 or 0.5% (v/v) DMSO blocked all or almost all iAs-induced production of ROS at dose of 15.4, whereas 15-min pretreatment with 0.1% (v/v) DMSO partially blocked it	Pérez-Pastén et al., 2006
RAW264.7 cells	As^{III} SA	2.5, 5, 10, 25	3 hr	5	Extracellular H_2O_2 production quantified using the Amplex Red Hydrogen Peroxide Assay: there was a positive dose-response, reaching ~1.4x control	Szymczyk et al., 2006
HELF cells	As^{III} SA	0.1, 0.5, 1, 5, 10	4 hr	0.5	Production of ROS detected by the DCFH-DA assay in the 15 min after iAs treatment: ⇑ with dose to >2x control at dose of 10.	Yang et al., 2007
HELF cells	As^{III} SA	0.1, 0.5, 1, 5, 10	3, 6, 12, 24, or 48 hr	Various	SOD activity after 24 hr: ⇑ at 0.5, ⇓ at 5 and 10; hint of similar change of direction in response also in treatments of some other durations; GPx activity after 24 or 48 hr: ⇓ at 5 and 10; but hints of ⇑ at lower doses and ⇓ at higher doses in treatments of some durations; MDA content (measure of LPO) after 24 or 48 hr: ⇑ at 5 and 10; tended to increase with time and dose in treatments of all durations	Yang et al., 2007
NB4 cells	As^{III} ATO	1, 3	16 hr	1	Effect on cellular total antioxidant capacity determined using the ABTS assay (Troiloc-equivalent antioxidant capacity in units of nmol/mg protein): Control = ~420; iAs at dose of 3: ~150; iAs at dose of 1: ~240; Effects of co-treatment (CoTr): iAs at 3 + CoTr with 1000 μM DTT: ~275; iAs at 3 + CoTr with 2000 μM DTT: ~340 iAs at 1 + CoTr with 25 μM DTT: ~150; iAs at 1 + CoTr with 50 μM DTT: ~125	Jan et al., 2006
NB4 cells	As^{III} ATO	0.5	2 hr	0.5	Intracellular H_2O_2 level (units of Amplex red assay): Control = ~20; iAs ~45; Effects of co-treatment (CoTr): CoTr with 80 μM DTT: ~72; CoTr with 100 μM DMSA: ~67; CoTr with 20 μM DMPS: ~72	Jan et al., 2006
BFTC905 cells and NTUB1 cells	DMA^{V}	1, 2	24 hr for all	1 in at least one cell line for all 3 effects	⇑ oxidative damage (peroxidation) in lipids, measured as malonaldehyde formation: at both doses in BFTC905 cells, at dose of 2 in NTUB1 cells; ⇑ oxidative damage (carbonylation) in proteins: at higher dose in BFTC905 cells, at lower dose in NTUB1 cells; ⇑ oxidative damage (comet assay) in DNA, without enzyme digestion: at both doses in both cell lines	Wang et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
A549 cells	As^{III} ATO	2	48 hr	Loss of MMP determined by flow cytometry using JC-1: 2 μM iAs: ⇑ to ~1.25x; 200 μM sulindac: ⇑ to ~1.15x; (2 μM iAs + 200 μM sulindac): ⇑ to ~1.9x. There was also a synergistic interaction between these treatments in causing big ⇑ in cytochrome C protein level in the cytosol, which is thought to result from damage to mitochondrial membranes that permits cytochrome C release to the cytosol. Pretreatment with NAC almost entirely blocked the MMP and cytochrome C effects. (Sulindac is a NSAID that inhibits COX-2.)		Jin et al., 2006b
A549 cells	As^{III} ATO	2	48 hr	2	Production of ROS using carboxy-H_2DCFDA assay: control, ~0.8 unit; 2 μM iAs, ~4.2 units; 200 μM sulindac: ~4.5x; (2 μM iAs + 200 μM sulindac): ~7.5x. Thus there was only additivity. Pretreatment with NAC before combined treatment: ⇓ to ~3.9 units.	Jin et al., 2006b
HeLa cells	As^{III} SA	10, 100	4 hr	10 for Trx1 and Trx2; none for GSH/ GSSG	Effects on Trx1 and Trx2 redox states determined using Redox Western blot methods: Trx1: ⇑ in oxidation at 10, slightly bigger ⇑ at 100; Trx2: huge ⇑ in oxidation at 10, slightly bigger ⇑ at 100; In contrast, iAs had little effect on the GSH/GSSG redox state, as determined by HPLC.	Hansen et al., 2006
BAEC cells	As^{III} SA	5, 10	1 hr	5	⇑ in peroxynitrite to ~1.4x and ~1.6x at 5 and 10, respectively.	Bunderson et al., 2006
BAEC cells	As^{III} SA	10	24 hr	10	⇑ in nitrotyrosine formation to ~1.15x	Bunderson et al., 2006
HEK 293 cells and SV-HUC-1 cells	As^{III} SA MMA^{III} DMA^{III}	0.2 for all	24 hr for all	0.2 0.2 0.2	Relative extent of oxidative damage (peroxidation) in lipids, measured as malonaldehyde formation; ranking of those with statistically significant ⇑ over control (all were significant): in HEK 293 cells: As^{III}>>MMA^{III}>DMA^{III}; in SV-HUC-1 cells: As^{III}>DMA^{III}>MMA^{III}	Wang et al., 2007
HEK 293 cells and SV-HUC-1 cells	As^{III} SA MMA^{III} DMA^{III}	0.2 for all	24 hr for all	0.2 0.2 0.2	Relative extent of oxidative damage (comet assay) in DNA; ranking of those with statistically significant ⇑ over control (all were significant): without enzyme digestion: in HEK 293 cells: As^{III}>MMA^{III} = DMA^{III}; in SV-HUC-1 cells: MMA^{III} = As^{III} = DMA^{III} with EnIII + FPG digestion: in HEK 293 cells: As^{III} = MMA^{III} = DMA^{III}; in SV-HUC-1: As^{III}>DMA^{III} = MMA^{III}	Wang et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM	24 hr	None 5 mM	Cellular ROS levels based on DCFH-DA assay: MMA^V: NSE MMA^V + BSO: ⇑ to ~2.22x	Sakurai et al., 2005a
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM	48 hr	None 5 mM	Cell survival determined by AB assay: MMA^V: 100% survival; MMA^V + BSO: ~3% survival; co-treatment with 10 mM DMPO during the MMA^V + BSO treatment blocked most of the cytotoxicity, resulting in ~72% survival. DMPO effectively scavenged cellular radical molecules.	Sakurai et al., 2005a
TRL 1215 cells TRL 1215 cells pretreated with 50 μM BSO for 24 hr to deplete GSH levels and then co-treated with 50 μM BSO	MMA^V for both	5 mM	48 hr	None None with DMPO	Caspase 3 activity (related to apoptosis): MMA^V: NSE; MMA^V + BSO: ⇑ to ~1.66x; co-treatment with 10 mM DMPO during the MMA^V + BSO treatment completely blocked the ⇑ of caspase 3 activity. DMPO effectively scavenged cellular radical molecules.	Sakurai et al., 2005a
HeLa cells	As^{III} ATO	2	Various up to 24 hr	2	ROS levels were shown by DCFH-DA assay to be significantly elevated by iAs and to ⇑ roughly 3x higher than for iAs alone following a combined iAs plus 10 μM emodin treatment; the addition of 1.5 mM NAC as a co-treatment attenuated (but did not completely block) that ⇑ in ROS levels.	Yi et al., 2004
HeLa cells	As^{III} ATO	2	1 hr	2	Analysis of GSH/GSSG ratios showed that co-treatment of iAs with emodin had a major oxidative impact on the cellular redox state, as shown by following ratios: control, ~62; iAs, ~52; 10 μM emodin, ~34; iAs plus 10 μM emodin, ~13; pretreatment with 1.5 mM NAC attenuated (but did not completely block) this effect.	Yi et al., 2004

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Jurkat cells Namalwa cells NB4 cells U937 cells	As^{III} ATO for all	2	24 hr for all	None 2 2 None	⇑ in H_2O_2 levels as detected by FACS after staining with DCFH-DA: large effect seen in Namalwa and NB4 cells only; NSE in other cell lines	Chen et al., 2006
U937 cells	As^{III} ATO	1	24 hr	None	⇑ in H_2O_2 levels as detected by FACS after staining with DCFH-DA: large effect was seen only following a co-treatment with BSO for 24 hr; the ⇑ was substantially decreased by a 4-hr treatment with either 10 mM NAC or 200 units of catalase	Chen et al., 2006
HEK293 cells	As^{III} ATO	2	48 hr	2	Cell survival was determined by the WST-1 cell proliferation assay: iAs treatment resulted in ~22% cell survival; co-treatment with 1 mM Tiron or 400 U/mL CAT significantly ⇑ cell survival although more than 60% of the cells still died; co-treatment with 200 U/mL SOD markedly ⇓ cell survival. These and other data suggested that iAs induced both superoxide anion and H_2O_2 through the activation of NAD(P)H oxidase. Presence of superoxide anion in cells that resulted from iAs treatment was confirmed.	Sasaki et al., 2007
PRCCs HEK293 cells	As^{III} ATO for both	0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20 for both	48 hr for both	0.5 0.1	Cell survival was determined by the WST-1 cell proliferation assay: LC_{50}s in PRCC: iAs, ~10; co-treatment of iAs with 10 μM α-lipoic acid, ~25; LC_{50}s in HEK293: iAs, ~1; co-treatment of iAs with 10 μM α-lipoic acid, ~7. In both cell types, this antioxidant markedly attenuated iAs's cytotoxicity, and in HEK293 cells it was shown to suppress superoxide anion generation.	Sasaki et al., 2007
NB4 cells HL60 cells KMS12BM cells U266 cells	As^{III} ATO for all	~0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 50 for first three ~0.05, 0.1, 0.6, 1.2, 6	48 hr for all	~0.1 ~5 ~0.2 ~0.6	Cell survival was determined by the WST-1 cell proliferation assay: LC_{50}s: NB4, ~0.2; HL60, ~8; KMS12BM, ~0.3; U266, ~0.3. In all 4 cells lines, co-treatment of iAs with 10 μM α-lipoic acid resulted in a remarkably similar dose-related pattern of cell survival to that seen with iAs alone, this being in sharp contrast to the attenuation of cytotoxicity caused by it that was seen in PRCCs and HEK293 cells. Note that the LOEC is higher than the estimated LC_{50} of 0.3 for U266 cells because the next lower dose of 0.1 had no effect, and the LC_{50} was estimated from the dose-response curve that was presented.	Sasaki et al., 2007
JAR cells	As^{III} ATO	5	2, 4, 6, 16, 24 hr	5	⇑ in HMOX-1 protein level in cytoplasm by 2 hr, with time-related response becoming huge by 16 hr.	Massrieh et al., 2006

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JAR cells	As^{III} ATO	5	6 hr	5	Intracellular H_2O_2 level detected by DCFH-DA and flow cytometry assay: ⇑ to 2x	Massrieh et al., 2006
BEAS-2B cells	As^{III} SA	1, 2.5, 5 for mRNA 2.5, 5 for protein	8 hr for both	1 for mRNA 2.5 for protein	Big ⇑ in HMOX-1 mRNA level at 1, bigger ⇑ of the same at 2.5, huge ⇑ of the same at 5. Big ⇑ in HMOX-1 protein level at 2.5, huge ⇑ of the same at 5.	O'Hara et al., 2006
Undifferentiated PC12 cells	As^{III} ATO	8	Various up to 6 hr	8	Detection of ROS shown by increase of DCF-fluorescence in DCFH-DA assay: ⇑ to ~2x control for several time points during first hr; no hint of effect at 3-6 hr; fluorescence was observed before the onset of cell death	Piga et al., 2007
UROtsa cells	As^{III} SA MMA^{III}	1, 10, 100 0.05, 0.5, 5	10 min 50 min	1 0.05	Detection of ROS using CM-H_2DCFDA assay: Slight ⇑ at 1, big ⇑ at 10, huge ⇑ at 100; when quantified at dose of 10 over 10 min: 20 RFU by 4.5 min, 110 RFU by 10 min; pretreatment with PEG-SOD or PEG-CAT blocked most ROS production. ⇑ at 0.05, huge ⇑ at 0.5, slightly weaker response at dose of 5 than at dose of 0.05; when quantified at dose of 0.5 over 10 min: 0 RFU; when quantified at dose of 0.5 over 50 min: 10 RFU by 42 min, 65 RFU by 50 min; pretreatment with PEG-CAT blocked most ROS production, and co-treatment with PEG-SOD blocked some ROS production; less effect for both than for iAs^{III}, suggesting a difference in the ROS they produce.	Eblin et al., 2006
Human-hamster hybrid A_L cells	As^{III} SA For both	7.7	60 days	7.7	Effects related to mitochondria: Fluorescence microscopy showed that arsenic treatment led to considerable variation in the distribution of mitochondria between cells and caused the fraction of them with elongated morphology to increase from 6% to 66%; ~50% ⇓ in COX activity; ~40% ⇓ in oxygen consumption; ~40% ⇑ in citrate synthase activity.	Partridge et al., 2007
Human-hamster hybrid A_L cells	As^{III} SA for both	1.9, 3.8, 7.7 1.9, 3.8, 7.7	60 days 1 day	3.8 for copy # 1.9 for deletions	mtDNA copy number: ⇓ to ~0.84x at 3.8; ⇓ to ~0.65x at 7.7. SA induced large heteroplasmic deletions in mitochondrial DNA, and the frequencies of induction increased with dose and time of exposure.	Partridge et al., 2007
Splenic lymphocytes from $Sod1^{tm1Leb}$ knockout mice	As^{III} SA	50, 100, 200	2 hr	50	Breaks and/or alkali-labile lesions in DNA detected in the single-cell gel (comet) assay: Big ⇑ in effect in the SOD -/- mice, which were also shown to have big ⇓ in levels of SOD in spleens (and also in livers and kidneys). SOD +/- mice were intermediate in SOD levels and DNA damage. Results suggest ROS may be involved in As^{III}- induced DNA damage.	Kligerman and Tennant, 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Lyophilized bovine tubulin	DMA^{III}	50	Time course over 1 hr	50	Big ⇓ in GTP-induced polymerization of lyophilized bovine tubulin Effects of modulators: NAC blocked the inhibition by DMA^{III}, while AA, CAT, DMSO, Tiron, or Trolox® had NSE on it, which suggests that ROS is not involved in the inhibition. Premixing of iAs^{V}, MMA^{V}, or DMA^{V} for 2 hr with a 5-fold molar excess of GSH greatly decreased the polymerization of tubulin (i.e., increased the inhibition)	Kligerman and Tennant, 2007
W138 cells and HaCaT cells	As^{III} SA	0.5	24 hr	0.5	ROS (peroxide) levels based on DCF assay:⇓ in both cell lines compared to control, and less in W138 than in HaCaT. The average activities of 3 important intracellular redox agents, GSH, GR, and GST are ~3X higher in WI38 cells than in HaCaT cells. After the iAs treatment, there was a 60-min menadione treatment followed by a 60-min recovery period. During this 120 min, ROS levels in W138 cells never reached control levels, while the control level was substantially exceeded in HaCaT cells after 60 min of the menadione treatment and later.	Snow et al., 2005
NB4 cells HL-60 cells	As^{III} SA for all	2 for all assays, which tested effects of various co-treatments described in Results column	4 hr for all	- -	This row relates only to the effects seen after co-treatments in an attempt to learn how SA causes DNA damage. They assayed DNA strand breaks (ADSB) detected using the comet assay. In the absence of a co-treatment, a significant increase would be expected with a dose of only 0.25. Conclusions always were supported by data on ODA and DPC individually. Chemicals used individually in co-treatments were: catalase, calcium chelators, and inhibitors of nitric oxide synthase, SOD, and myeloperoxidase. On the basis of the large reduction in DNA strand breaks seen following the co-treatments, they concluded that arsenite induces DNA adducts through calcium-mediated production of peroxynitrite, hypochlorous acid, and hydroxyl radicals.	Wang et al., 2001
PAEC cells isolated from freshly harvested vessels	As^{III} SA	5	Up to 20 min	5	⇑ oxygen consumption associated with ⇑ superoxide (O_2^-) formation; ⇑ extracellular accumulation of H_2O_2, with same time and dose dependence as superoxide formation. Pretreatment of the cells with DPI, apocynin, or SOD abolished arsenite-stimulated superoxide (O_2^-) formation.	Barchowsky et al., 1999b
CHO K1 cells	As^{III} SA	20, 40, 80, 160	4 hr	40	⇑ intracellular peroxide level (strong hint of same effect at dose of 20)	Wang et al., 1996
ϕX174 RF I DNA Naked double-stranded circular DNA in presence of ROS inhibitors	MMA^{III} DMA^{III}	10, 20, 30, 40, 50 37.5, 75, 150, 300, 1000	24 hr for all	- -	This row relates only to the effects seen after co-treatments in an attempt to learn how SA causes DNA damage. Significant (and usually complete) reduction in nicked DNA (in DNA nicking assay) was found when ROS inhibitors Trolox, melatonin, or Tiron were present individually during the arsenic treatment. Spin trap agent DMPO was also effective in preventing DNA nicking by these compounds. Thus, production of ROS by these chemicals is associated with their DNA-cutting activity. Genotoxicity is an indirect effect via the generation of ROS.	Nesnow et al., 2002

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Both HL-60 cells and HaCaT cells	As^{III} SA	0.1, 0.5, 1, 10, 20, 40	5 days	0.5 but possibly 0.1	By use of MTT assay, in presence of 2.5 mM DMPO: ⇑ in cell number, with peak at 0.5 (DMPO has no effect); ⇓ in cell number to below control level at 1 for HL-60 and at 10 for HaCaT, and DMPO significantly lessens reduction in cell number at ≥ 10 (possibly 1) for HL-60 and at ≥ 20 (possibly 10) for HaCaT	Zhang et al., 2003
HL-60 cells	As^{III} SA	10	3 days	-	Analysis of TRF using Southern-blot assay in presence of 2.5 mM DMPO: With DMPO present, telomere length was longer than it was with arsenic alone; interpreted to mean that DMPO provided some protection against arsenic-induced telomere shortening	Zhang et al., 2003
HL-60 cells HaCaT cells	As^{III} SA for both	0.1, 0.5, 1, 10, 20, 40 for both	5 days for both	1 but possibly 0.5 10	By use of Hoechst/PI staining assay, in presence of 2.5 mM DMPO: ⇑ in apoptosis for both; however, DMPO significantly reduced the amount of apoptosis at ≥ 1 for HL-60 and at ≥ 10 for HaCaT	Zhang et al., 2003
Enzyme Activity Inhibition						
AG06 cells were pretreated for 24 hr with unspecified low dose of As, and then extracts of the cells were tested for activity of: GSH peroxidase and Ligase	As^{III} SA	IC_{50} determinations	Rate over 6 min	-	IC_{50}s: 2.0 (was 0.13 mM for purified enzyme with no As pretreatment) 14.5 (was 6.5 mM with no As pretreatment). The same paper presented the IC_{50}s for a similar treatment with As^{V} for GSH peroxidase, and it was 173 μM. The paper also presented IC_{50}s for numerous purified enzymes with both SA and As^{V}, but they were almost all far above a physiologically interesting range and are thus not presented here. Most were in the range of 6.3 to 381 mM for SA and usually even higher for As^{V}.	Snow et al., 1999
Cell-free system using purified human enzymes	As^{III} SA As^{V}	IC_{50} determinations	Rate of reaction over 6 min	-	Inhibition of PDH: IC_{50}s: 5.6 μM for iAsIII, 206 mM for As^{V}; 7 other enzymes involved in aspects of DNA repair and/or cellular stress response had IC_{50}s for As^{III} of 6.3–381mM. Only PDH, with its lipoic acid cofactor, was inhibited by physiologically relevant, micromolar concentrations of As^{III}.	Hu et al., 1998
Cell-free system using purified human enzymes	As^{III} SA As^{V}	~0.0007, 0.001, 0.007, 0.01, 0.07, 0.1 ~0.01, 0.07, 0.1, 1, 10, 25, 75, 100, 125	Rate of reaction over 1 min	~0.001 ~25	Inhibition of PDH	Hu et al., 1998

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Cell-free system using purified porcine heart PDH	As^{III} SA MMA^{III}	25, 75, 100, 200 (all approximate) 8, 16, 30, 50, 100	30 min for both	~25 ~8	Inhibition of PDH (IC_{50}s): 106.1 17.6	Petrick et al., 2001
Cell-free system using hamster kidney PDH	As^{III} SA MMA^{III}	~20 to ~400 ~20 to ~400	30 min for both	-	Inhibition of PDH (IC_{50}s): 115.7 61.0	Petrick et al., 2001
Gene Amplification						
Mouse 3T6 cells	As^{III} SA As^{V}	0.2, 0.4, 0.8, 1.6, 3.2, 6.4 1, 2, 4, 8, 16	Not reported	0.4 2	Gene amplification of dhfr gene detected by MTX-selection assay: Both compounds showed positive dose response extending to highest concentrations tested.	Barrett et al., 1989
AG06 cells	As^{III} SA	7, 10, 17, 20	3.5 hr	None	Amplification of SV40: none observed at concentrations causing from 40–98% cytotoxicity.	Rossman and Wolosin, 1992
AG06 cells	As^{III} SA	6	Assay's maximal response time	6	Amplification of endogenous dhfr genes (determined by MTX-selection assay): highly effective at this concentration, which caused 50% survival. "Amplification factor" was ~3 even though it was 1 (i.e., no induction) for same concentration for amplification of SV40.	Rossman and Wolosin, 1992
Human osteosarcoma TE85 (HOS) cells	As^{III} SA	0.0125, 0.025, 0.05, 0.1 for both durations	6 wk 8 wk	0.025 0.0125	Amplification of endogenous dhfr genes (determined by MTX-selection assay): dose response was the same for both durations beginning with 0.025; it increased with dose to 0.05 and then plateaued.	Mure et al., 2003
SHE cells	As^{III} SA As^{V}	6, 8 50, 100, 150	48 hr for both	6 50	From among these treatment groups, 5 neoplastic transformed cell lines were produced that were shown to be tumorigenic, of these: 3 had c-Ha-ras (oncogene) gene amplification; 2 had c-myc (oncogene) gene amplification; a few other arsenic-treated cell lines also showed this same gene amplification.	Takahashi et al., 2002
Gene Mutations						
E. coli (several strains)	As^{III} SA	Up to 25 mM	Various	None	Several assays (spot tests, treat and plate protocols, and fluctuation tests) for Trp^{+} revertants yielded no evidence of induction of gene mutations. Also, there was no induction of λ prophage.	Rossman et al., 1980

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
V79 cells	As^{III} SA	0.5 5, 20, 100	2 days Up to 1.5 hr	None None	In several assays, ouabain resistance and thioguanine resistance were used as genetic markers. No evidence was found of induction of gene mutations. Only the dose of 100 caused cytotoxicity (33.1% the survival of the control).	Rossman et al., 1980
G12 cells	As^{III} SA	5, 10, 15 10, 25, 50	24 hr 3 hr	None None	No statistically-significant induction of mutations at the *gpt* locus in an assay that can detect multilocus deletions, point mutations, and small deletions (tested up to cytotoxicity of 61.9% of cells killed)	Li and Rossman, 1989a
Salmonella typhimurium strains TA98, TA100, TA104	As^{III} SA As^{V} MMA^{III} MMA^{V} DMA^{III} DMA^{V}	Tested up to concentrations limited by cytotoxicity or to the limit concentration for the assay	3 days for all	None	*Salmonella* mutagenicity plate incorporation assay with and without exogenous metabolic activation: There was no indication of any induction of gene mutations over background levels by any of the compounds.	Kligerman et al., 2003
Syrian hamster embryo cells	As^{III} SA As^{V}	~0.8, 1.6, 3, 3.5, 5 ~8, 16, 32, 64, 128	Not reported	None None	Gene mutation assays for the Na^{+}/K^{+} ATPase and HPRT loci	Barrett et al., 1989
Human osteosarcoma TE85 (HOS) cells	As^{III} SA MMA^{III}	0.0125, 0.025, 0.05, 0.1 0.00625, 0.0125, 0.025, 0.05	8 wk for all	0.0125 None	Mutations in the HPRT gene: positive dose response to highest concentration for As^{III}; no increase until almost 15 generations of continuous exposure	Mure et al., 2003
TM3 cells	As^{III} SA for both	0. 008, 0.77. 7.7 for both	~25 days ~75 days	0. 008 0. 008	Detection of DNA changes by RAPD-PCR: gain or loss of loci and changes in the intensity of loci were detected at the DNA sequence level; although the nature of the "mutations" and whether they were actual gene mutations is unknown.	Singh and DuMond, 2007
Hypermethylation of DNA						
Human kidney carcinoma cell lines: UOK123 UOK109 Human lung carcinoma cell line: A549	As^{III} SA for all	 0.010, 0.020, 0.050 0.007, 0.021, 0.093 0.08, 0.4, 2.0	 4 wk 4 wk 2 wk	 ≤ 0.050 ≤ 0.093 ≤ 2.0	The number of specific DNA sequences shown to undergo hypermethylation changes by methylation sensitive AP-PCR following exposure to SA: 1 from line UOK123; 4 from line UOK 109, and 1 from line A549. The concentrations used to treat these lines were known to be the IC_{30}, IC_{50}, and IC_{80} concentrations for UOK cells and the IC_{20}, IC_{50}, and IC_{80} concentrations for A549 cells. It was not reported which concentrations yielded the hypermethylation changes, but the LOECs could not be higher than the maximum concentration used for each cell line.	Zhong and Mass, 2001

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
A549 cells (human adenocarcinoma)	As^{III} SA As^{V} DMA^{V}	0.08, 0.4, 2.0 3, 10, 30, 100, 300 2, 20, 200, 2000	7 days for all	0.08 30 None	Hypermethylation within a 341-base-pair fragment of the promoter of p53 For the two inorganic forms, there was a positive dose response throughout the range of concentrations tested.	Mass and Wang, 1997
Hypomethylation of DNA						
TRL 1215 cells (normal rat liver)	As^{III} SA	0.125, 0.250, 0.500	19 wk	0.125	Global DNA hypomethylation, thought to be caused by continuous methyl depletion.	Zhao et al., 1997
Human kidney carcinoma cell line: UOK121 Human lung carcinoma cell line: A549	As^{III} SA for all	0.009, 0.020, 0.074 0.08, 0.4, 2.0	4 wk 2 wk	≤ 0.074 ≤ 2.0	The number of specific DNA sequences shown to undergo hypomethylation changes by methylation sensitive AP-PCR following exposure to SA: 1 from line UOK121 and 1 from line A549. The concentrations used to treat these lines were known to be the IC_{30}, IC_{50}, and IC_{80} concentrations for UOK121 cells and the IC_{20}, IC_{50}, and IC_{80} concentrations for A549 cells. It was not reported which concentrations yielded the hypermethylation changes, but the LOECs could not be higher than the maximum concentration used for each cell line.	Zhong and Mass, 2001
Untransformed and immortalized RWPE-1 cells (human prostate epithelial cell line)	As^{III} SA	5	30 wk	5	Global hypomethylation of DNA (up to 131% increase in unmethylated DNA compared to the control); hypomethylation still present 6 weeks after end of exposure. The cells became tumorigenic after 29 weeks of treatment and were then called the CAsE-PE cell line.	Benbrahim-Tallaa et al., 2005
SHE cells	As^{III} SA As^{V}	6, 8 50, 100, 150	48 hr for both	-	From among these treatment groups, 5 neoplastic transformed cell lines were produced that were shown to be tumorigenic. Testing of them using the methylation-sensitive restriction endonuclease isoschizomers HpaII and MspI revealed hypomethylation of c-myc and c-Ha-ras in the 5'-CCGG sequence. Both of these oncogenes were often shown to exhibit gene amplification and ⇑ mRNA expression.	Takahashi et al., 2002
TM3 cells	As^{III} SA for both	0. 008, 0.77. 7.7 for both	~25 days ~75 days	0.008 0.008	Detection of methylation changes in DNA by RAPD-PCR using methylation-sensitive restriction endonuclease isoschizomers HpaII and MspI: methylation changes were detected at 18 loci, with some showing hypomethylation and others hypermethylation. Some loci were only affected by the shorter-term exposure, and vice-versa.	Singh and DuMond, 2007
HaCaT cells	As^{III} SA	0.2	For 10 serial passages in folic-acid-depleted media	0.2	Genomic hypomethylation as demonstrated by a 27% ⇓ in the level of 5-methyl-dCMP compared with cells cultured for the same number of passages in medium without As^{III}. This dose was too low to have much, if any, effect on the proliferation rate.	Reichard et al., 2007

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HaCaT cells	As^{III} SA	0.5, 1.5, 5	72 hr	Various	⇓ DNMT1 mRNA at 0.5, and progressively larger decreases at 2 higher doses; ⇓ DNMT3A mRNA at 1.5, and larger ⇓ at dose of 5. These cells did not show any detectable quantities of the other 2 mammalian DNA methyltransferases. Big ⇑ HMOX-1 RNA at 1.5 with very big ⇑ at 5.	Reichard et al., 2007
Immune System Response						
(Human myeloma-like cell lines) RPMI 8226 Karpas 707 U266	As^{III} ATO	0.5, 1, 2	72 hr	0.5	Induction of cell lysis by LAK effector cells was apparent by 36 hours and maximal at 72 hours. The extent of lysis was determined by the ^{51}Cr release assay. At these concentrations, arsenic trioxide had no effect on viability (using trypan-blue assay) or apoptosis.	Deaglio et al., 2001
HPBMs co-exposed to M-CSF	As^{III} SA As^{V} MMA^{V} DMA^{V}	IC_{50} determinations	7 days	-	Viability of M-type macrophages based on AB assay was used to estimate the arsenic concentration at which maturation into M-type macrophages was inhibited by 50%: IC_{50} values: As^{III}, 0.06; As^{V}, 200; MMA^{V}, 750; DMA^{V}, 300.	Sakurai et al., 2006
HPBMs co-exposed to GM-CSF	As^{III} SA As^{V} MMA^{V} DMA^{V}	IC_{50} determinations	7 days	-	Viability of GM-type macrophages based on AB assay was used to estimate the arsenic concentration at which maturation into GM-type macrophages was inhibited by 50%: IC_{50} values: As^{III}, 0.38; As^{V}, 300; MMA^{V}, 700; DMA^{V}, 550.	Sakurai et al., 2006
HPBMs co-exposed to GM-CSF and IL-4	As^{III} SA	IC_{50} determination	7 days	-	Viability of immature dendritic cells based on AB assay was used to estimate the arsenic concentration at which maturation into immature dendritic cells was inhibited by 50%: IC_{50} value: 0.70	Sakurai et al., 2006
HPBMs co-exposed to GM-CSF and IL-4	As^{III} SA	IC_{50} determination	14 days	-	Viability of multinucleated giant cells based on AB assay was used to estimate the arsenic concentration at which maturation into multinucleated giant cells was inhibited by 50%: IC_{50} value: 0.33	Sakurai et al., 2006
HPBMs co-exposed to GM-CSF	As^{III} SA	With regard to 4 rows immediately above this one, SA at doses of 0.05 to 0.5 induced abnormal morphological changes in the HPBMs to form small nonadhesive and CD14-positive cells called arsenite-induced cells that displayed a dendritic morphology with delicate membrane projections. This response was not produced by treatments with many other metallic compounds (e.g., chromium, mercury, and zinc) including iAs^{V}, MMA^{V} and DMA^{V}. This effect was not seen at doses exceeding 1.				Sakurai et al., 2006
HPBMs co-exposed to GM-CSF	As^{III} SA	0.5	7 days	0.5	In comparison to the cells not treated with iAs, there was 43.3% less metabolic activity, 0.6% as much adherent ability, a 76% higher cellular GSH concentration, 256% as much NO_2^- production, 185% as much IL-1α production in the supernatant, 412% as much IL-1α production in the lysate, and 576 ng/g cellular protein of IL-12 in the lysate even though none was detected in arsenic-untreated cells.	Sakurai et al., 2006

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HUVECs	As^{III} SA	0.5	3 hr	0.5	Both HUVECs and PMNs were pretreated for 24 hr with GLN (glutamine) at 0, 300, 600, or 1000 μM. Those HUVECs were then exposed to the same concentration of GLN with or without the iAs treatment for 3 hr. The pretreated PMNs were added to wells and allowed to migrate across the pretreated HUVECs for 2 hr, after which surface expressions on HUVECs of ICAM-1 and VCAM-1 were measured, with the following results: ICAM-1: ⇑ in iAs only group and huge ⇑ at all 3 dose levels of GLN; VCAM-1: NSE in iAs only group and ⇑ at all 3 dose levels of GLN, with largest ⇑ at 300 μM. Clearly HUVECs were activated by iAs. Also at this time, PMN expressions of CD11b and IL-8 receptor were measured, with the following results: CD11-b: ⇑ in iAs only group and bigger ⇑ at all 3 dose levels of GLN; IL-8 receptor: ⇑ in iAs only group and at all 3 dose levels of GLN. Clearly PMNs were activated by the iAs treatment of the HUVECs. Effects on PMN migration: In absence of GLN pretreatment, iAs caused slight ⇓ from 36% to 30% migrated. In the iAs + 300 μM GLN group: ⇑ from ~40% (for GLN alone) to ~50% migrated (for iAs + GLN), which was the most migration observed.	Hou et al., 2005
PBMCs co-treated with GM-CSF	As^{III} ATO	0.125, 0.25, 0.5, 1, 2	6 days	0.125	Induced apoptosis (i.e., experimental – control) detected using A5/SG assays: approximate induced frequencies at the 5 doses: 6%, 20%, 29%, 48%, and 62%, respectively, with all being statistically significant except first one. Induced frequency of necrotic cells was ~20% at the highest dose, and there were smaller numbers of necrotic cells induced at the lower doses. After dose of 1 for 3 days: ⇑ caspase-3 activity, ⇑ caspase-8 activity, big ⇑ in active caspase-3 subunit p17. ATO was shown to reduce DNA binding of the transcriptionally active p65 NF-κB subunit to the κB consensus sites in GM-CSF treated PBMCs, which was thought to be important in development of apoptosis. Other experiments showed that ATO inhibited macrophagic differentiation of PBMCs.	Lemarie et al., 2006a
PBMCs co-treated with M-CSF	As^{III} ATO	1	6 days	1	Induced apoptosis (i.e., experimental – control) detected using A5/SG assays: ~44%. The induced frequency of necrotic cells was ~23%.	Lemarie et al., 2006a
U937 cells co-treated with PMA	As^{III} ATO	1, 4	4 days	4	Induced apoptosis (i.e., experimental – control) detected using A5/SG assays: approximate induced frequencies at the 2 doses: 3% and 35%, respectively, with the higher one being statistically significant. Induced frequency of necrotic cells was ~9% at the highest dose. Other experiments showed (1) that ATO induced apoptosis through inhibition of NF-κB signals and (2) that ATO inhibited macrophagic differentiation of U937 cells.	Lemarie et al., 2006a
U937 cells co-treated with PMA	As^{III} ATO	4	4 days	4	⇓ $FLIP_L$ protein level, ⇓ XIAP protein level	Lemarie et al., 2006a

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PBMCs co-treated with GM-CSF	As^{III} ATO	1	3 days	1	⇓ $FLIP_L$ protein level and ⇓ $FLIP_L$ mRNA level; ⇓ XIAP protein level and ⇓ XIAP mRNA level	Lemarie et al., 2006a
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	1, 4 1, 4	3 days 6 days	4 4	Induced apoptosis (i.e., experimental – control) detected using A5/SG assays: No induced apoptosis at dose of 1 at either time. At dose of 4: ~22% and ~50% after 3 and 6 days, respectively; thus these cells are resistant to induction of apoptosis by ATO at low doses.	Lemarie et al., 2006a
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	1	6 days	None for ⇓	NSE regarding $FLIP_L$ protein level, big ⇑ XIAP protein level	Lemarie et al., 2006a
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	4	3	4	big ⇓ $FLIP_L$ protein level; big ⇓ XIAP protein level	Lemarie et al., 2006a
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	0.25, 0.5, 1	6 days	0.25	Major alterations in the morphology, adhesion, and actin organization with the impression that iAs "de-differentiated' macrophages back into monocytic cells. The effect was time-dependent with rounded and contracted morphology first observed at dose of 1 after only 8 hr. By 6 days at dose of 1 only 31% as many cells were adherent as in control. iAs induced a reorganization of the F-actin cytoskeleton. The series of experiments suggested that the effects occurred because of the activation of a RhoA/ROCK pathway.	Lemarie et al., 2006b
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	0.5, 1, 2, 4	6 days	2	Induced apoptosis (i.e., experimental – control) detected using A5/SG assays: approximate induced frequencies at the 4 doses: 0%, 0%, 20%, and 50%, respectively. Induced frequency of necrotic cells was ~4% at the highest dose. 18 days of treatment at dose of 1 caused no cytotoxicity.	Lemarie et al., 2006b

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Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	1	6 days	1	Changes in surface markers: CD14: ⇑ 5.1x; CD71: ⇓ to 45% of control; CD29: ⇓ to 49% of control; CD11b: ⇓ to 42% of control. Changes in major functions: marked ⇓ in endocytosis and phagocytosis. Changes in surface markers and morphology were shown to be reversible when iAs was removed and cells were cultured with GM-CSF for 6 days.	Lemarie et al., 2006b
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	1	6 days	1	Ability to secrete inflammatory cytokines in response to co-treatment of iAs (dose of 1) and 200 ng/mL LPS for 8 or 24 hr: (control = macrophages treated with LPS only) TNF-α secretion: ⇑ ~3.0x and ~3.0x at 8 and 24 hr, respectively; IL-8 secretion: ⇑ ~3x and ~4.5x at 8 and 24 hr, respectively. Much more extreme potentiation was demonstrated for both cytokines at the mRNA level at 8 hr. The text implies that the potentiation of both secretion and mRNA production does not occur without the 6-day iAs treatment.	Lemarie et al., 2006b
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	1	6 days	1	The iAs-treated macrophages differentiated into dendritic-like cells when exposed to GM-CSF and IL-4 in the absence of iAs for 6 days. This conclusion was based on the ~9x increase in the expression of the typical dendritic marker CD1a. The increase was similar to that seen in PBMCs treated with GM-CSF and IL-4 for 6 days, and in both cases the dendritic-like cells were nonadherent. In contrast, fully differentiated macrophages (i.e., PBMCs treated with GM-CSF for 6 days without iAs) did not show this response.	Lemarie et al., 2006b
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days before iAs treatment	As^{III} ATO	1	8 hr	1	⇑ GTP-binding fraction of RhoA; ⇑ phospho-Moesin protein level (Phosphorylated-Moesin is a major cytoskeleton adaptor protein involved in RhoA regulation. RhoA is a small GTPase protein known to regulate the actin cytoskeleton in the formation of stress fibers.)	Lemarie et al., 2006b

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
Differentiated macrophages developed from PBMCs treated with GM-CSF for 6 days and then pretreated with ROCK inhibitor Y-27632 for 2 hr before iAs treatment	As^{III} ATO	1	72 hr	1	Pretreatment with the ROCK inhibitor prevented both the F-actin reorganization and cellular rounding of macrophages treated with iAs. It also considerably blunted damage to the phagocytosis function caused by the iAs treatment.	Lemarie et al., 2006b
HepG2 cells	As^{III} SA	0.04, 0.4, 4, 40	48-hr pretreatment	4	After the iAs pretreatment, there was a 30-min treatment with IL-6, which induced STAT3 activity unless inhibited by the pretreatment. Level of STAT3 activity: huge ⇓ at 4; no activity at 40.	Cheng et al., 2004
HepG2 cells	As^{III} SA	0.04, 0.4, 4, 40, 400	48-hr pretreatment	40	After the iAs pretreatment, there was a 30-min treatment with IL-6, which induced both STAT3 tyrosine phosphorylation and STAT3 serine phosphorylation. Only the tyrosine phosphorylation was inhibited by the iAs pretreatment, with slight ⇓ at 40 and huge ⇓ at 400. iAs is thought to inactivate the JAK-STAT signaling pathway by means of inhibition of STAT3 tyrosine phosphorylation. Other inflammatory stimulants, stress agents, and cadmium failed to induce similar effects on the tyrosine phosphorylation of STAT3.	Cheng et al., 2004
HepG2 cells	As^{III} SA	4, 40, 400	30-min pretreatment and 1-hr co-treatment with IL-6	4	Huge ⇓ in Cis mRNA and in SOCS mRNAs for 5 of 6 forms tested (⇓ for the other form); the ⇓ at higher doses was usually the same or more severe; ⇓ in STAT mRNAs for 4 of 6 forms tested, the ⇓ at higher doses was usually the same or more severe. The decreases for STAT mRNAs were very slight compared to those for SOCS. The inhibition of induction of SOCS mRNA confirmed that JAK-STAT signaling had been turned off. Other experiments showed that the effect of iAs on JAK-STAT inactivation is independent of ligand-receptor action and is a result of the direct action of arsenic on the JAK1 protein.	Cheng et al., 2004
HepG2 cells	As^{III} SA	0.04, 0.4, 4, 40, 400	iAs (in co-treatment with IL-6 for unknown duration) activated all 3 subfamilies of MAP kinases (i.e., there was phosphorylation of ERK 1/2, p38, and JNKs) with LOECs of 40, 0.04, and 0.04 respectively. Such activation was independent of IL-6 stimulation at least at higher doses. Experiments with specific inhibitors of the 3 MAP kinases showed that iAs selectively targeted JAK tyrosine kinase and that the inhibition of JAK-STAT activity by iAs did not require the participation of any MAP kinases.			Cheng et al., 2004
PBMCs treated with 1000 U/mL of M-CSF at the same time as with iAs	As^{III} SA	0.005, 0.010, 0.050, 0.10, 0.50	7 days	0.050	Cell survival demonstrated by trypan blue exclusion assay: LC_{50}: 0.22; about 25% survival at dose of 0.5. The cells differentiated into adhesive M-type macrophages that were elongated and had a spindle-like morphology.	Sakurai et al., 2005b

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
PBMCs treated with 5000 U/mL of GM-CSF at the same time as with iAs	As^{III} SA	0.005, 0.010, 0.050, 0.10, 0.50	7 days	0.10	Cell survival demonstrated by trypan blue exclusion assay: ~85% survival at 2 highest doses; up to dose of 0.050, all cells differentiated into GM-Mp, which had a round saucer-like appearance; at dose of 0.10, ~80% of living cells were GM-Mp and the rest were abnormal "arsenite-induced cells"; at dose of 0.50, ~10% of living cells were GM-Mp and the rest were "arsenite-induced cells".	Sakurai et al., 2005b
PBMCs treated with 5000 U/mL of GM-CSF at the same time as with iAs	As^{III} SA	0.50	7 days	0.50	In comparison to controls (i.e., PBMCs treated with 5000 U/mL of GM-CSF and no iAs), the resulting morphologically, phenotypically, and functionally altered "arsenite-induced cells" had: ⇑ HLA-DR to 5.0x; ⇓ CD11b to 0.71x; ⇑ CD14 to 1.4x; ⇓ CD54 to 42% of control; big ⇓ in phagocytic ability; ⇑ in effectiveness in inducing allogeneic or autologous T-cell responses; and huge ⇑ in response to bacterial LPS by inflammatory cytokine release.	Sakurai et al., 2005b
PBMCs treated with 5000 U/mL of GM-CSF at the same time as with iAs	As^{III} SA	0.50	7 days	0.50	The resulting high numbers of "arsenite-induced cells" were markedly reduced by co-treatment with DMPO, DMSO, or BHT, all of which are membrane-permeable radical trapping reagents. Further indication that ROS might impact development of the "arsenite-induced cells" was that by using DCFH-DA it was shown that ROS levels were much higher throughout the 7 days of culturing and ≥ 2x higher on days 1-4 of that period.	Sakurai et al., 2005b
PBMCs treated with 1000 U/mL of M-CSF or 5000 U/mL of GM-CSF at the same time as with iAs	As^{V}	LC_{50} determinations	7 days	> 1	Cell survival demonstrated by trypan blue exclusion assay: LC_{50}: 300 for simple cytotoxicity for both treatments and with no toxic effect on differentiation into macrophages up to dose of 1.	Sakurai et al., 2005b
PBMCs stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment	As^{III} SA	1, 2, 3, 4, 5	120 hr	1	Number of rounds of cell division estimated using CFSE dilution assay with FACS: (Control had 6 rounds.) 5, 4, 3, 2, and 1 rounds of cell division were observed after doses of 1, 2, 3, 4, and 5, respectively; there was a marked dose-related ⇓ in both proliferation and the percentage of divided cells. Additional staining with 7-AAD revealed that, at even the higher doses, most cells were viable but unable to divide. The reduced proliferation resulted from an ⇑ in the fraction of non-dividing cells and a delay in the cell cycle, with only a comparative small ⇑ in the number of dead cells. At the highest dose, 63% of the cells were non-dividing, and 2/3 of them were alive.	Tenorio and Saavedra, 2005

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
PBMCs stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment	As^{III} SA	1, 2, 3, 4, 5	120 hr	1	Expression of CD4 and CD8 molecules was determined using CFSE staining during the iAs treatment and then, after the 96 hr incubation, by indirect immunofluorescence using OKT4 or OKT8 hybridoma supernatants and goat anti-mouse IgG-PE, followed by 7-AAD staining and FACS analysis. The dose of 1 slightly modified the expression of both CD4 and CD8. At doses ≥ 3: a marked ⇓ in number of cells expressing CD4; at doses ≥ 4: a marked ⇓ in number of cells expressing CD8.	Tenorio and Saavedra, 2005
PBMCs stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment	As^{III} SA	1, 2, 3, 4, 5	120 hr	1	Evaluation of blast transformation of both $CD4^+$ and $CD8^+$ T cells suggested that they have different sensitivities to iAs. There was an accumulation of resting $CD8^+$ cells with a positive dose response; that accumulation was not seen for $CD4^+$ cells.	Tenorio and Saavedra, 2005
Human $CD4^+$ cells stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment Human $CD8^+$ cells stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment	As^{III} SA for both	1, 2, 3, 5 for both	120 hr for both	1 for both: a slight but signifi-cant effect	Number of rounds of cell division estimated using CFSE dilution assay with FACS: (Control $CD4^+$ and $CD8^+$ cells had 6 and 5 rounds, respectively.) At a dose of 1: only 5 rounds in $CD4^+$ but no ⇓ in rounds in $CD8^+$; however, $CD8^+$ cells had ⇓ in cell number in the last 3 rounds. As doses increased in both cell types: decreasing numbers of cell divisions and of numbers of cells in each round. Effects were generally more extreme in $CD8^+$ cells.	Tenorio and Saavedra, 2005
Human $CD4^+$ cells stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment Human $CD8^+$ cells stimulated with PHA for 96 hr starting 24 hr after the beginning of the iAs treatment	As^{III} SA for both	1, 2, 3, 4, 5 for both	120 hr for both	1 for both: a slight but signifi-cant effect	CFSE dilution assay with 7-AAD staining and FACS: In both cell types there were apparent differences from the control at the dose of 1, and there was a progressive ⇓ in viable proliferating cells with increasing dose. As doses increased from 0 to 3, there was a much faster ⇑ in the fraction of resting cells that was alive among $CD8^+$ cells than among $CD4^+$ cells, and that fraction remained higher.	Tenorio and Saavedra, 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
PBMCs stimulated with PHA during the iAs treatment, $CD4^+$ and $CD8^+$ T cells were analyzed separately	As^{III} SA	1, 2, 3, 4, 5	24 hr 48 hr 72 hr	2 1 1	LOECs were based on FACS patterns that seemed substantially different as to kinetics of expression of CD25 and CD69 in $CD4^+$ T cells. iAs delayed both the expression of CD25 and the down-regulation of CD69, suggesting that iAs delays the activation kinetics of $CD4^+$ T cells. $CD4^+$ T cells exposed to the highest dose for 72 hr showed a very similar pattern to that seen in non-iAs-exposed cells stimulated for only 24 hr. A similar analysis of $CD8^+$ T cells showed similar results; however, with them there were somewhat more $CD25^-$ $CD69^-$ cells (i.e., cells unable to activate) as dose increased.	Tenorio and Saavedra, 2005
SV-HUC-1 cells	As^{III} SA	2, 4, 8, 10, 40	48 hr	2 for all effects	Labeling indices (LIs) for immunochemistry of cells: Bcl-6: ⇑ at 2, increases with dose as follows: LIs of 0, 1.04, 3.05, 6.01, 8.24, and 23.94 for control and doses listed, respectively. JAK2: ⇓ at 2, decreases with dose as follows: LIs of 100, 58.1, 48.9, 13.0, 5.1, and 0.8 for control and doses listed, respectively. p-STAT3 (Tyrosine 705): ⇑ at 2 with peak at dose of 4 before decreasing, as follows: LIs of 100, 111.7, 151.0, 125.2, 119.0, and 50.8 for control and doses listed, respectively. All experimental LIs above differed from control, $p<0.05$.	Huang et al., 2007b
SV-HUC-1 cells	As^{III} SA	2, 4, 8, 10, 40	48 hr	2 for all effects	Effects on protein levels determined by Western blotting: Bcl-6: ⇑ at 2 and increases with dose; JAK2: ⇓ at 2 and decreases with dose; p-STAT3 (Tyrosine 705): ⇑ at 2, peak at 4, less than control at 40; results at different doses were highly consistent with results obtained using immunochemistry, as shown in row above.	Huang et al., 2007b
SV-HUC-1 cells	As^{III} SA	2, 4, 8, 10, 40	48 hr	2 for all effects	Microscopy and immunochemistry showed Bcl-6 and p-STAT3 (Tyrosine 705) to be localized in the nucleus and JAK-2 to be localized in the cytoplasm. Morphological changes began to appear at dose of 2. At dose of 4, cells became round and exhibited nuclear condensation. At highest two doses, there was cellular shrinkage and cytoplasmic vacuolization.	Huang et al., 2007b
BAEC cells	As^{III} SA	10	48 hr	10	⇑ in LTE_4 to ~5x. Co-treatment with 50 μM Mn^{II}, which caused ~9x ⇑ by itself, caused an approximately additive ⇑ to ~12x. Addition of *L*-NAME to the iAs/Mn co-treatment boosted LTE_4 level to ~24x. Addition of ETU to iAs/Mn co-treatment boosted LTE_4 level to slightly above that of iAs/Mn combination. Addition of AA-861 to iAs/Mn co-treatment reduced LTE_4 level by ~80%.	Bunderson et al., 2006
Thymocytes (freshly isolated)	As^V	67, 150, 315, 680, 1000, 2000	24 hr	315	Cell survival determined using XTT assay: LC_{50}: 442	Stepnik et al., 2005

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer						
Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
Splenocytes (freshly isolated)	As^{V}	67, 150, 315, 680, 1000, 2000	24 hr	150	Cell survival determined using XTT assay: LC_{50}: 427	Stepnik et al., 2005
Thymocytes (freshly isolated)	As^{V}	67, 315, 680	24 hr	315	Point estimates of induced apoptosis (experimental minus control) determined by TUNEL staining: 5% at 67 (NSE); 16% at 315 (NSE); and 24% at 680. 27% of control cells were apoptotic. Agarose gel electrophoresis of DNA showed high (and indistinguishable) levels of apoptosis in control group and at the 3 experimental dose levels.	Stepnik et al., 2005
Splenocytes (freshly isolated)	As^{V}	67, 315, 680	24 hr	315	Point estimates of induced apoptosis (experimental minus control) determined by TUNEL staining: 1% at 67 (NSE); 16% at 315; and 33% at 680. 29% of control cells were apoptotic. Agarose gel electrophoresis of DNA showed high (and indistinguishable) levels of apoptosis in control group and at the 3 experimental dose levels.	Stepnik et al., 2005
Inhibition of Differentiation						
C3H 10T1/2 cell line (mouse cells with fibroblast morphology during routine culture but capable of differentiation into adipocytes)	As^{III} SA	6	8 wk	6	Complete inhibition of differentiation into adipocytes induced by dexamethasone/insulin (dexI) treatment. The effect is the same if arsenic is removed just before the dexI treatment.	Trouba et al., 2000
C3H 10T1/2 cell line (mouse cells with fibroblast morphology during routine culture but capable of differentiation into adipocytes)	As^{III} SA	0.1, 1, 3, 6, 10	48 hr	3	Dose-related inhibition of differentiation into adiopocytes induced by dexamethasone/insulin (dexI) treatment. These concentrations do not cause cytotoxicity.	Trouba et al., 2000
SIK cells treated in surface cultures beginning when they reached confluence, which is when their rate of division decreases as differentiation increases	As^{III} SA	2	Various	2	CFE based on assay using Rhodanile blue staining: on 1 day post-confluence both experimental and control groups had CFEs of ~11%, by 4 days their CFEs were ~9.2% and ~5.2%, and by 14 days they were ~4.7% and ~0.6%, respectively. Thus, iAs decreased the exit of cells from the germinative compartment under conditions that promote differentiation.	Patterson et al., 2005

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
hEp cells treated in surface cultures beginning when they reached confluence, which is when their rate of division decreases as differentiation increases	As^{III} SA	2	Various	2	CFE based on assay using Rhodanile blue staining: at 4 days post-confluence experimental and control groups had CFEs of ~1.1% and 0.25%, by 11 days their CFEs were ~1.0% and ~0.05%, and by 14 days they were ~1.0% and ~0%, respectively. Thus, iAs decreased the exit of cells from the germinative compartment under conditions that promote differentiation.	Patterson et al., 2005
SIK cells, with iAs treatment beginning 1 day before suspension and continuing while cells were in suspension, which drives such cells prematurely into the differentiation pathway	As^{III} SA	2	1, 2, 3, 4 or 5 days, when including the 1 day of treatment before being put into suspension	2	CFE based on assay using Rhodanile blue staining comparison with control (C): at 1 day: C, ~11.0%, iAs, ~10.8%; at 2 days: C, ~0.5%; iAs, ~2.3%; at 3 days: C, ~0.1%; iAs, ~2.0%; at 4 days: C, ~0%; iAs, ~1.3%; at 5 days: C, ~0%; iAs, ~0.8%	Patterson et al., 2005
hEp cells, with iAs treatment beginning 1 day before suspension and continuing while cells were in suspension, which drives such cells prematurely into the differentiation pathway	As^{III} SA	2	1 or 2 days, when including the 1 day of treatment before being put into suspension	2	CFE based on assay using Rhodanile blue staining comparison with control (C): at 1 day: C, ~1.15%, iAs, ~1.37%; at 2 days: C, ~0.08%; iAs, ~0.68%	Patterson et al., 2005
SIK cells, with iAs treatment beginning when they were put into suspension, which drives such cells prematurely into the differentiation pathway	As^{III} SA	0.1, 0.2, 0.5, 2	4 days	0.1	CFE based on assay using Rhodanile blue staining: (control CFE = ~ 0.03%) Experimental CFEs: 0.1, ~ 0.10%; 0.2, ~ 0.23%; 0.5, ~ 0.40%; 2, ~ 0.80%.	Patterson et al., 2005

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
RACs from either the SIK or hEp cell line (did not specify which) treated in surface culture	As^{III} SA	2	4, 7, 11, or 14 days	2	Mean no. of colonies present, comparison with control (C): at 4 days: C, ~3.0; iAs, ~18.2; at 7 days: C, ~0.8; iAs, ~10.5; at 11 days: C, ~0.7; iAs, ~7.8; at 14 days: C, ~0.4; iAs, ~5.0;	Patterson et al., 2005
SACs from either the SIK or hEp cell line (did not specify which) treated in surface culture	As^{III} SA	2	4, 7, 11, or 14 days	2	Mean no. of colonies present, comparison with control (C): at 4 days: C, ~1.3; iAs, ~5.5; at 7 days: C, ~0.5; iAs, ~1.8; at 11 days: C, ~0.6; iAs, ~1.5; at 14 days: C, ~0.3; iAs, ~1.3;	Patterson et al., 2005
SIK cells, with iAs treatment beginning when cultures reached 90% confluence	As^{III} SA	2	3 days	2	Relative CFEs based on Rhodanile blue assay, with values relative to the CFE of untreated cells in medium normally contained insulin (was set at 1): ⇑ to ~2.6; if iAs + EGF in medium: ⇑ to ~4.1; if EGF alone: ~1.9; if no insulin in medium (± EGF): ~3.5. Thus, iAs delays differentiation and preserves the proliferative potential of keratinocytes.	Patterson and Rice, 2007
hEp cells, with iAs treatment beginning when cultures reached 90% confluence	As^{III} SA	2	3 days	2	Relative CFEs based on Rhodanile blue assay, with values relative to the CFE of untreated cells in medium normally contained insulin (was set at 1): ⇑ to ~2.6; if iAs + EGF in medium: ⇑ to ~4.1; if EGF alone: ~2.1; if neither EGF nor insulin: ~2.1; if EGF but no insulin: ~5.3. Thus, iAs delays differentiation and preserves the proliferative potential of keratinocytes.	Patterson and Rice, 2007
SIK cells, with iAs treatment beginning when cultures reached 90% confluence	As^{III} SA	2	9 days	2	Relative CFEs based on Rhodanile blue assay, with values relative to the CFE of untreated cells in medium normally contained insulin (was set at 1): ⇑ to ~3.8; if iAs + EGF in medium: ⇑ to ~5.1; if EGF alone: ~1.3; if no insulin in medium: ~5.5. In the absence of insulin, EGF substantially augmented CFE while iAs had no effect. Thus, iAs delays differentiation and preserves the proliferative potential of keratinocytes.	Patterson and Rice, 2007
SIK and hEPcells, with iAs treatment beginning when cultures reached 90% confluence	As^{III} SA	2	With regard to effects in the previous 3 rows, EGFR inhibitors AG1478 and PD 158780 kept iAs from increasing the CFE regardless of the addition of EGF. They did not affect CFE in the presence of insulin but largely prevented the ⇑ in CFE resulting from insulin removal. iAs treatment caused big ⇑ in active Ras protein, a downstream effector of EGFR; co-treatment with AG1478 blocked that effect. Other experiments showed that the iAs treatment blocked the ⇓ in active EGFR protein and the ⇓ in active β-catenin that normally occur after confluence as cells exit the proliferative pool and differentiate. Also, expression of a dominant negative β-catenin suppressed the ⇑ in colony-forming ability and yield of putative stem cells induced by iAs and EGF.			Patterson and Rice, 2007
Interference with Hormone Function						

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
EDR3 cells transfected as described in paper	As^{III} SA	0.045, 0.09, 0.18, 0.27, 0.36, 0.45, 0.54, 0.675, 0.9, 1.8, 2.7	~18 hr	~0.09	⇑ of hormone-activated GCR-mediated gene transcription of reporter genes containing TAT glucocorticoid response elements in the presence of activated GCR; peak response was at ~0.5; however, iAs was inhibitory at doses of 1.8 and 2.7. Other experiments showed a similar effect on the endogenous TAT gene and also that the central DNA binding domain of the GCR is the minimal region required for the arsenic effect.	Bodwell et al., 2004
COS-7 cells transfected as described in paper	As^{III} SA	0.1, 0.5, 1.0, 2.0, 3.0	~18 hr	None	iAs had no effect on transcriptional repression by GCR. That is, arsenic had no effect on the ability of hormone-activated GCR to inhibit AP1 expression or NF-κB-mediated gene expression.	Bodwell et al., 2004
EDR3 cells transfected as described in paper	As^{III} SA	0.045, 0.09, 0.18, 0.27, 0.36, 0.45, 0.54, 0.675, 0.9, 1.8, 2.7	~18 hr	~0.09	⇑ of hormone-activated GCR-mediated gene transcription of reporter genes containing TAT response elements in the presence of activated PR; peak response was at ~0.5; however, iAs was inhibitory at doses of 0.9, 1.8 and 2.7.	Bodwell et al., 2006
EDR3 cells transfected as described in paper	As^{III} SA	0.045, 0.09, 0.18, 0.27, 0.36, 0.45, 0.54, 0.675, 0.9, 1.8, 2.7	~18 hr	~0.09	⇑ of hormone-activated GCR-mediated gene transcription of reporter genes containing TAT response elements in the presence of activated MCR; peak response was at ~0.5; however, iAs was inhibitory at doses of 1.8 and 2.7.	Bodwell et al., 2006
EDR3 cells transfected as described in paper	As^{III} SA	For all 3 steroid receptors tested (GCR, PR and MCR—see 3 rows immediately above this one), the degree of stimulation at lower iAs concentrations or repression at higher iAs concentrations was highly dependent on, and inversely related to, the amount of activated steroid receptor within cells. The relative increases in transcription noted above, which were up to ~2x or more above control levels, were at the lowest levels of activated steroid receptor within cells that were tested. Other studies showed that iA (1) had no significant effect on cellular steroid levels or on binding of steroid to the receptor, (2) did not activate or act as an agonist for GCR, (3) did not act as a competitive antagonist, (4) did not appear to affect the ability of the hormone to bind to or activate GCR, (5) did not appear to affect hormone-stimulated nuclear translocation of GCR, and (6) did not significantly alter the level of GCR for either cells expressing endogenous GCR or those expressing stably integrated GCR. Dimerization is not critical for the response to iAs. In summary, it is clear that iAs can simultaneously disrupt multiple hormone receptor systems.				Bodwell et al., 2006
NHEK cells	As^{III} SA As^{V}, MMA^{V}, DMA^{V}	0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 for all	24 hr 24 hr	0.001 0.001-0.5	For cytokines GM-CSF, TNF-α, and IL-6: substantial ⇑ at 0.001–0.01, but no change or ⇓ (sometimes markedly) at 0.05–5. No change or ⇓ (sometimes markedly).	Vega et al., 2001
Hormone-responsive H4IIE (rat hepatoma cell line)	As^{III} SA	0.3, 1.0, 2.0, 3.3	2 hr	0.3	⇓ in hormone-inducible expression of GRE2-Luc with a 2-hr As^{III} pretreatment before an 18-hr Dex treatment. The pretreatment did not block the normal Dex-induced nuclear translocation of glucocorticoid receptor. As^{III} selectively inhibited glucocorticoid-receptor-mediated transcription.	Kaltreider et al., 2001
Malignant Transformation or Morphological Transformation						

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
HaCaT cells	As^{III} SA	0.5, 1.0	20 passages	0.5	Cells became tumorigenic; tumors were produced by 2 months after injection of cells into Balb/c nude mice; cells from tumors were much more malignant.	Chien et al., 2004
TRL 1215 cells (normal rat liver)	As^{III} SA	0.125, 0.250, 0.500	18 wk	0.250	Transformed cells produced aggressive tumors capable of metastasis after inoculation into nude mice.	Zhao et al., 1997
JB6 Cl41 cells simultaneously treated with 10 ng/mL EGF	As^{III} SA As^{V}	25, 50, 200 12.5, 50, 200	14 days for both	50 12.5	Inhibition of EGF-induced cell transformation: The effect was much stronger for As^{V} (sodium arsenate) with complete blockage of transformation at 50 and 200.	Huang et al., 1999b
JB6 Cl41 cells	As^{III} SA	25, 50, 100	4 wk followed by 4 wk at lower concentration	25	Transformed cells, as shown by growth of colonies in soft agar; transformation did not occur at the 2 higher doses; SA-induced transformation was blocked by introduction of dominant negative Erk2.	Huang et al., 1999a
Primary Syrian hamster embryo cells (HEC)	As^{V}	13, 27	7–8 days	13	Morphologically transformed cells	DiPaolo and Casto, 1979
Syrian hamster embryo cells	As^{III} SA As^{V}	~0.8, 1.6, 3, 3.5, 5 ~8, 16, 32, 64, 128	7 days for all	0.8 8	Morphological transformation: For both chemicals: a positive dose response throughout the dose range tested	Barrett et al., 1989
Human osteosarcoma TE85 (HOS) cells	As^{III} SA MMA^{III}	0.0125, 0.025, 0.05, 0.1 0.00625, 0.0125, 0.025, 0.05	6 and 8 wk for both	0.025 at 8 wk; 0.05 at 6 wk None	Transformation to anchorage-independence in soft agar As^{III}: positive dose response to highest concentration; 8 weeks was ~40 generations; MMA^{III} was more toxic than iAs^{III}.	Mure et al., 2003
Untransformed and immortalized RWPE-1 cells (human prostate epithelial cell line)	As^{III} SA	5	29 wk	5	Aggressive tumors were produced after cells showing ⇑ secretion of MMP-9 were inoculated into nude mice	Achanzar et al., 2002
SHE cells	As^{III} SA $DMA^{III}I$	1, 3, 10 0.1, 0.2, 0.4, 1.0	48 hr for both	1 0.1	Morphological transformation (% of surviving colonies transformed at each concentration): 1, 0.11%; 3, 0.23%; 10, 0.48%; 0.1, 0.28%; 0.2, 0.51%; 0.4, 3.41%, 1.0, 3.35%	Ochi et al., 2004
SHE cells	As^{III} SA As^{V}	3, 6, 8, 10 50, 100, 150	48 hr for both	6 50	Neoplastic transformation based on anchorage-independent growth and/or tumorigenicity in newborn hamsters. All 5 anchorage-independent cultures tested were tumorigenic.	Takahashi et al., 2002

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
UROtsa cells	MMA^{III}	0.05	52 weeks	0.05	Anchorage-independent growth as detected by colony formation in soft agar; cells from those colonies showed enhanced tumorigenicity in SCID mouse xenografts. After only 24 weeks there was also much anchorage-independent growth, but those cells did not show the enhanced tumorigenicity.	Bredfeldt et al. 2006
NIH 3T3 cells	As^{III} SA	2, 5, 10, 20, 50, 100, 200	7 days	2	Anchorage-independent growth in soft agar assayed using AlamarBlue dye assay and microscopic examination: ⇑ to ~1.4x control at 2 and 5; NSE at 10, marked dose-related ⇓ at higher doses. A daily 2-hr 42°C heat shock (which would induce HSPs) boosted induction of anchorage-independent growth for up to 3 repetitions, but 5 heat-shock repetitions markedly reduced such growth. When the same experiment was repeated in R-3T3 (transformed) cells, there was NSE by iAs or heat shock on the already high level of anchorage-independent growth; iAs caused ⇓ at dose of 20, and at higher doses the ⇓ became marked, as it did also at all doses following 5 daily repetitions of the heat-shock treatment.	Khalil et al., 2006
BALB/c 3T3 A31-1-1 cells (derived from mice)	As^{III} SA As^{V} DA MMA^{V} DMA^{V}	2, 5, 10, 15, 20 10, 15, 20, 25, 30 1, 2, 5, 10 mM 0.5, 1, 2, 5 mM	72 hr for all	5 15 10 mM 1 mM	Caused initiation in a two-stage transformation assay; based on a significant increase in the number of transformed cells after an initiating treatment with an arsenic compound for 72 hr followed by post-treatment with 0.1 μg/mL TPA for 18 days. Except for As^{III} SA, responses were stronger at higher doses; with it, the peak response was at 10, with a steep decline by 20. Slight but significant transformation occurred even without TPA at the 2 highest doses of As^{III} SA and for 2 mM DMA^{V}. The ranges of positive effects in foci/dish in the two-stage transformation assay (from the LOEC to the peak) for each arsenical were as follows: As^{III} SA, 1.80-3.90; As^{V} DA, 1.20-2.90; MMA^{V}, 1.10 (only 1 positive value); DMA^{V}, 1.0-3.10. The control value was 0.30.	Tsuchiya et al., 2005
Signal Transduction						
MGC-803 (human gastric cancer)	As^{III} ATO	0.01–1	48 hr	0.01	Increase in intracellular Ca^{2+} as measured by a Ca^{2+} sensitive fluorescent probe Indo-1/AM in flow cytometric assays, which parallels the effect on apoptosis.	Zhang et al., 1999
Primary cultures of rat cerebellar neurons	As^{III} SA DMA^{V}	10 5 mM	4 hr 8hr	10 5mM	For both: ⇑ in activated p38 MAP kinase.	Namgung and Xia, 2001
Primary cultures of rat cerebellar neurons	As^{III} SA	10	1 hr	10	⇑ JNK3 MAP kinase. No change in JNK1 and JNK2 MAP kinases. (Blocking the p38 and JNK signaling pathways inhibited arsenite-induced apoptosis.)	Namgung and Xia, 2001

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
SY-5Y cells HEK 293 cells	As^{III} ATO for both	0.1, 1 for both	~1 hr for both	0.1 0.1	The Ca^{2+} concentration in cells was substantially increased (and by rather similar amounts) by both doses; iAs triggered 3 different kinds of Ca^{2+} signals: slow (sustained), transient elevations, and calcium spikes. The irreversible increases were independent of extracellular Ca^{2+} and dependent on internal Ca stores, which could become depleted. Little or no cytotoxicity resulted from these doses during the time of measuring Ca^{2+} concentrations.	Florea et al., 2007
UROtsa cells	As^{III} SA As^{V} $MMA^{III}O$ MMA^{V} $DMA^{III}I$ DMA^{V}	0.1, 0.5, 1, 5 1, 10, 100 0.1, 0.5, 1, 5 1, 10, 100 0.1, 0.5, 1, 5 1, 10, 100	Up to 2 hr for all	Various None Various None Various None	Phosphorylation of ERK2: ⇑ with potencies: $MMA^{III}O$ >> $DMA^{III}I$ >> As^{III} AP-1 binding activity: for As^{III}: ⇓ at 0.1 and 0.5; for $MMA^{III}O$, big ⇑ at 0.1, 0.5, 1.0 but no increase at 5; for $DMA^{III}I$: ⇓ at 0.1, 0.5, and 1, and big ⇑ at 5. Phosphorylation of c-Jun: for As^{III}: ⇓ at 0.1 and 0.5 and ⇑ at 1 and 5; for $MMA^{III}O$, big ⇑ at 1 and 5; for $DMA^{III}I$: ⇑ at 0.1, big ⇑ at 5. Also trivalent arsenicals caused changes in Fra-1 and induced AP-1 dependent gene transcription. There was no effect on c-Jun N-terminal kinases or p38 kinases.	Drobná et al., 2002
Postconfluent PAEC cells in a monolayer	As^{III} SA	0.5, 2, 5	1 hr	2	EMSA analysis: ⇑ nuclear retention of NF-κB binding proteins; ⇑ nuclear translocation of NF-κB binding proteins. Supershift analysis showed that p65/p50 heterodimers accounted for the majority of proteins binding consensus κB sequences in cells treated with As^{III} or oxidants. These and other experiments suggest that As^{III} initiates vascular dysfunction by activating oxidant-sensitive endothelial cell signaling. Increased binding of proteins to genomic κB sites could induce a mitogenic or inflammatory response.	Barchowsky et al., 1996
$Gclm^{+/+}$ MEF cells	As^{III} SA for all	See rows under Apoptosis and Cytotoxicity for this citation for experimental conditions. iAs inhibits NFκB activation and nuclear translocation. Co-treatment or pretreatment with tBHQ appears to reverse the iAs-mediated inhibition of NF-κB translocation, and it triggers the nuclear accumulation of the transcription factor Nrf2. tBHQ may cause its cytoprotective effects by inducing gene expression changes though activation of at least NF-κB and Nrf2.				Kann et al., 2005b
Hepa-1c1c7 cells	As^{III} SA	6, 12, 25, 50	1 hr	6	⇑ AhR nuclear translocation, with a positive dose response; other experiments showed that the translocation occurs by different mechanisms from those followed by ligands and that AhR-dependent gene expression is only weakly up-regulated by iAs.	Kann et al., 2005a

Table C-3. *In Vitro* Studies Related to Possible Modes of Action of Arsenic in the Development of Cancer

Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
$1RB_3AN_{27}$ cells	As^{III} SA	0.1, 0.5, 1, 5, 10	240 min	Various	Activation of nuclear transcription factors detected by EMSA: NF-κB: slight ⇑ at 0.5, ⇑ at 1, huge ⇑ at 5 and 10; effect at dose of 1 was considerably suppressed by co-treatment with NAC. iAs-induced degradation of IκBα was demonstrated in the cytosolic fraction. AP-1: ⇑ at 0.1, slight ⇑ at 0.5, huge ⇑ at 1, 5 & 10; effect at dose of 1 was completely blocked by co-treatment with NAC.	Felix et al., 2005
$1RB_3AN_{27}$ cells	As^{III} SA	0.1, 0.5, 1	120 min	0.1	Phosphorylation (activation) of ERK detected by EMSA: huge ⇑ at all 3 doses.	Felix et al., 2005
JB6 C141 cells	As^{III} SA for both	20, 40 40	12 hr for both	20 40	⇑ COX-2 protein level. ⇑ COX-2 transcription. However, deletion of NF-κB binding sites from the COX-2 promoter blocked this effect. Other experiments, including some in MEF cells, confirmed the requirement of the IKKβ/NF-κB pathway for the induction of COX-2 by As^{III} (shown at protein and transcription levels).	Ouyang et al., 2007
Hepa-1c1c7 cells	As^{III} SA	2, 5, 10 0.1, 1, 2, 5, 10 2, 5, 10 2, 5, 10	5 hr 48 hr 5 hr 5 hr	2 0.1 None 2	⇑ Nqo1 mRNA expression, with a positive dose response. ⇑ Nqo1 enzyme activity, with a slightly higher and rather similar response at doses 1-10. NSE on Nrf2 mRNA levels. ⇑ Nrf2 protein level, with a positive dose response. These and other experiments showed that Nqo1 induction occurred through the Nrf2/ARE pathway with the following important steps: (1) iAs markedly stabilizes Nrf2; (2) iAs disrupts the Nrf2-Keap1-Cul3 complex in the nucleus, and (3) iAs recruits Nrf2 and Maf to the ARE of Nqo1. iAs does not recruit Keap1, Cul3, ubiquitin, c-Jun, or c-Fos to the ARE of Nqo1.	He et al., 2006
WM9 cells OM431 cells K1735-SW1 cells and other melanoma cell lines	As^{III} SA	2, 4, 6	A series of experiments (usually with durations of treatment between 30 min and 16 hr) demonstrated that iAs up-regulated TRAIL-mediated apoptosis. iAs up-regulated surface levels of death receptors, TRAIL-R1 and TRAIL-R2, through increased translocation of these proteins from cytoplasm to cell surface. Furthermore, activation of cJun and suppression of NF-κB by iAs caused up-regulation of the endogenous TRAIL and down-regulation of cFLIP gene expression, which was followed by cFLIP protein degradation and, finally, by acceleration of TRAIL-induced apoptosis. cFLIP is one of the main anti-apoptotic proteins in melanomas.				Ivanov and Hei, 2006
HeLa cells	As^{III} SA	100	4 hr	100	Big ⇑ in autophosphorylation (activation) of ASK1 determined by autoradiography	Hansen et al., 2006

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
A431 cells	As^{III} ATO	20	Many experiments, usually at dose of 20, and over various durations, and often involving inhibitors or other modulators, yielded the following information and conclusions: iAs had following effects: ⇑ p21 promoter activity, ⇑ p21 mRNA level, ⇑ p21 protein level. Transfection with a p21 siRNA reduced iAs-induced p21 expression and reduced the iAs-induced cytotoxicity after 24 hr by half. Conclusions: iAs induced p21 activation via the EGFR-Ras-Raf-ERK1/2 pathway. ERK1/2 and JNK may differentially contribute to iAs-induced p21 expression via the EGFR-Ras-Raf-ERK1/2 pathway. The ERK 1/2 and JNK pathways play opposing roles in iAs-induced cytotoxicity.			Huang et al., 2006
NHEK cells	As^{III} SA	0.4	1, 3, 5, 7	0.4 on days 3 and 5 only	cyclin D mRNA level: ⇑ to ~3.2x on day 3; ⇑ to ~1.5x on day 5; NSE on other days	Hwang et al., 2006
NHEK cells	As^{III} SA	0.4	1, 3, 5, 7	0.4 on day 3 only	Binding of transcription factors to their respective binding motifs within the cyclin D1 promoter by demonstrated by EMSA: ⇑ for AP1 to 1.9x; NSE on other days; ⇑ for CREBP to 1.6x; NSE on other days. Note the correspondence with ⇑ in mRNA level in row above; there was a hint of an ⇑ for both on day 7.	Hwang et al., 2006
NHEK cells	As^{III} SA	0.1, 0.2, 0.4	1, 2, 3, 4, 7	0.4 on days 2-7	cyclin D protein level: ⇑ to ~1.35x on days 2-4; ⇑ to 2x on day 7	Hwang et al., 2006
DU145 cells	As^{III} SA	50, 100, 200	1 hr	50	AMPK activation: ⇑ at 50, big ⇑ at 100, ⇑ at 200; activation was blocked by preincubation with CAT, GSH, or NAC; tests with a dominant negative form of AMPK showed that AMPK activity is necessary for iAs-induced VEGF expression. Other experiments showed that the arsenic-induced AMPK signaling pathway is independent of the p38 MAP kinase and PI-3 kinase pathways and that the blocking of AMPK activation markedly increased cytotoxicity from iAs exposures of 50 or 100.	Lee et al., 2006c
HaCaT cells, trans-fected for use in a luciferase reporter assay	As^{III} SA	1.25, 2.5, 5	12 hr	1.25	⇑ cyclin D1 transcription to 1.9x and then ⇑ with dose to 2.4x at highest dose	Ouyang et al., 2005
HaCaT cells	As^{III} SA	0.31, 1.25, 5	12 hr	0.31	Protein levels determined by Western-blot assay: ⇑ cyclin D1 and then ⇑ with dose to highest dose; other experiments showed that induction of cyclin D1 required activation of NF-κB and also required IKKβ. It was suggested that the iAs-induced stimulation of the transition from G1 to S phase that was reported in this paper occurred through a IKKβ/NF-κB/cyclin D1-dependent pathway.	Ouyang et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
Primary keratinocytes (in third passage) obtained from foreskins of adults	As^{III} SA	0.1, 1, 5, 10	48 hr	Various	Enzyme activities detected by luciferase assays: NF-κB: ⇑ to ~1.8x at 0.1, ⇑ to ~5x at 1, ⇑ to ~3.8x at 5, NSE at 10. AP-1: NSE at 0.1, ⇑ to ~1.7x at 1, ⇑ to ~2.7x at 5, ⇑ to ~4.5x at 10. All results were confirmed at the protein level.	Liao et al., 2004
H9c2 cells	As^{III} SA	1, 2.5, 5 for 1 or 2 days	There was a large dose-related ⇓ in cell migration rates at the 2 higher doses at both durations. NSE on viability (based on MTT assay) at these 3 doses, but at the dose of 10, which was not tested for other effects, there was a ⇓ in viability. There was a dose-related ⇓ in focal adhesions per cell at all doses and a ⇓ in F-actin content of cells at the dose of 5. At doses of 2.5 and 5, there was a ⇓ in tyrosine phosphorylation of FAK, a ⇓ in phosphorylation of FAK at phosphotyrosine 397, and a ⇓ in tyrosine phosphorylation of FAK's substrate paxillin. iAs affected focal adhesion structure or formation and not the turnover or amounts of focal adhesion proteins. Focal adhesions are involved in integrin signaling, and the iAs-induced changes may disrupt cell contraction and signaling. It was concluded that iAs decreases cell migration through an effect on focal adhesions and by disruption of cell interactions with the extracellular matrix.			Yancy et al., 2005
MEFs from wild type or Ikkβ gene knockout ($IKK\beta^{-/-}$) mouse embryos	As^{III} ($AsCl_3$)	Various between 1.25 and 50	In a series of experiments lasting for 2-32 hr, the main findings were as follows. In knockout MEFs, which exhibit NF-κB inhibition due to IKKβ deficiency, (a) there was a big ⇑ in basal levels of mRNAs of the following genes: gadd45α, gadd45β, gadd45γ and gadd153, (b) there was a big ⇑ in iAs-induced (usually at 20 μM for 4 hr) levels of mRNAs for gadd45α and gadd153, and (c) there was no induction by As (same conditions) of mRNAs for gadd45β and gadd45γ. It appears that NF-κB activation is an inhibitory signal for the expression of gadd45α and gadd153. C-myc expression was reduced in knockout cells, and IKKβ deficiency did not affect p53 or Akt signaling and the expression of FoxO3a.			Zhang et al., 2005
JAR cells	As^{III} ATO	0.5, 1, 2.5, 5, 10	6 hr	0.5	Big ⇑ in nuclear Nrf2 protein level, with dose-related ⇑ becoming huge by dose of 10; also similar ⇑ in cytoplasmic Nrf2 protein level.	Massrieh et al., 2006
JAR cells	As^{III} ATO	5	2, 4, 6, 16, 24 hr	Various	Big ⇑ in nuclear Nrf2 protein level at first 4 time points, but small ⇑ at 24 hr. Slight ⇑ in MafF protein level at many time points, but NSE on 2 other dimerization partners of Nrf2, namely MafG and MafK. Experiments done in part in HEK293T cells suggested that in JAR cells there is an ⇑ in binding of endogenous Nrf2/small Maf DNA-binding complexes to a StRE site.	Massrieh et al., 2006
BEAS-2B cells	As^{III} SA	5	4 hr	5	Huge ⇑ in nuclear Nrf2 protein level. Other experiments showed iAs caused ⇑ in Nrf2 transcriptional complex binding to the HMOX-1 ARE *cis* element.	O'Hara et al., 2006
SVEC4-10 cells	As^{III} SA	10	3 min to 4 hr	10	iAs induced actin filament reorganization to form lamellipodia and filopodia structures at the leading edge of the cells and rosette-like structures in the cell bodies. Effects were noted after only 3 min; longer treatments did more damage. Reorganization of actin filament occurred through the activation of Cdc42.	Qian et al., 2005
SVEC4-10 cells	As^{III} SA	10	3 min to 4 hr	10	Huge ⇑ in activation of Cdc42 already after 3 min and level of activation stayed almost as high for at least 1 hr; by 4 hr the level of activation was similar to that of control. See comment in row above.	Qian et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
SVEC4-10 cells	As^{III} SA	10	Various	As more information about series of experiments described in 2 rows immediately above, its was shown that iAs-stimulated Cdc42-induced actin filament reorganization regulated the activation of NADPH oxidase. Authors suggested that the formation of superoxide anion radicals observed after iAs treatment occurred through the activation of NADPH oxidase. Rac activities were required for Cdc42-mediated superoxide anion radical production, and NADPH oxidase activity was involved in iAs-stimulated cell migration via Cdc42-mediated actin filament reorganization.		Qian et al., 2005
JB6 C141 cyclin D1-Luc mass1 cells	As^{III} SA	5	24 hr	5	Protein level determined by Western blot assay: huge ⇑ in cyclin D1; separate treatments with vanadate, cadmium, or $NiCl_2$: NSE	Ouyang et al., 2006
JB6 C141 cyclin D1-Luc mass1 cells	As^{III} SA	2	12 hr	2	mRNA level determined by luciferase reporter assay: ⇑ in cyclin D1 to ~3.2x	Ouyang et al., 2006
JB6 C141 cells	As^{III} SA	0.1, 0.5, 1, 5, 10	1 hr	Various	Protein levels determined by Western blot assay: phosphorylation of Akt Ser473: ⇑ at 0.1-5, big ⇑ at 10; phosphorylation of Akt Thr308: slight ⇓ at 0.1, ⇓ at 0.5, ⇑ at 1 and 5, big ⇑ at 10; phosphorylation of $p70^{S6K}$ Thr389: big ⇑ at 0.1-10; phosphorylation of $p70^{S6K}$ Thr421/Ser424: ⇑ at 0.1-10	Ouyang et al., 2006
JB6 C141 cells	As^{III} SA	5	20 min	5	Protein level determined by Western blot assay: big ⇑ in PI-3K activation as shown by big ⇑ in PIP3; inhibition experiments showed that iAs triggered a PI-3K/Akt/IKKβ/NF-κB signal cascade that played essential roles in inducing cyclin D1 expression.	Ouyang et al., 2006
HEL cells	As^{III} ATO	0.25, 0.5, 1, 2, 5, 8, 10 for P-STAT3 0.5, 1, 2, 4, 6, 8, 10 for HSP70	6 hr for both	0.5 1	Western blot analysis: ⇓ P-STAT3 protein level (IC_{50}s = 1.334); 3 HSP90 inhibitors all markedly potentiated the effect with iC_{50}s of 0.0468, 0.395, and 0.745. ⇑ HSP70 protein level. Dose of ~2.9 doubled the control level. 3 HSP90 inhibitors all markedly potentiated the effect. HSP70 inhibits apoptosis. Much more iAs was needed to kill half the cells in 6 hr ($LC_{50,\ 6\ hr}$ = 80) than to down-regulate P-STAT3 by 50% in 6 hr (1.334, as above). The trypan blue assay was used to determine cell survival.	Wetzler et al., 2007
A549 cells	As^{III} SA	1, 5, 10, 20	24 hr	10	iAs activated the binding of IRP-1 to IRE: ⇑ to 1.35x, with smaller ⇑ to 1.25x at dose of 20; 10 and 20 μM iAs caused a slight ⇑ in HIF-1α protein level (only ~2% above control). iAs at dose of 20 had NSE on ferritin protein level.	Li et al., 2006b

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Type of cell/tissue	**Arsenic species**	**Concentration(s) tested (μM)**	**Duration of treatment**	**LOEC[1] (μM)**	**Results (compared with controls, with all concentrations being in μM unless noted)**	**Reference**
CL3 cells, synchronous at G1	As^{III} SA	50, 100	3 hr	50	At 50, ⇑ in phosphorylation (activation) of ERK1/2 to 1.7x right after treatment and to 2.0x after a 24-hr recovery period. At 100: ⇑ to 2.3x immediately; no activation of ERK1/2 occurred following co-treatment with PD98059 or U0126.	Li et al., 2006a
CL3 cells, synchronous at G1	As^{III} SA	5, 10, 25, 50	3 hr	Varied	Dose-related ⇑ in phosphorylation (activation) of ERK1/2 to ~1.45x at 25 and ~1.8x at 50, LOEC = 10. Dose-related ⇓ in efficiency of synthesis of NER to ~50% and ~41% of control at doses of 25 and 50, respectively. LOEC = 25; for both ERK1/2 and NER, the changes at 5 and 10 were NSE. NER synthesis efficiency was determined based on whole cell extracts of treated cells in an assay with UV-irradiated pUC19 plasmids. Co-treatment of iAs with either PD98059 or U0126 blocked much of the phophorylation of ERK1/2 and restored 50-80% of the NER synthesis efficiency. In summary, co-treatments of iAs with inhibitors that blocked activation of ERK1/2 did the following: (1) ⇑ NER synthesis efficiency, (2) ⇓ induction of micronuclei, (3) ⇓ survival, and (4) ⇓ rate of proliferation.	Li et al., 2006a
A431 cells	As^{III} ATO	20	Effects detected by Western blot assay following 30-min treatment: increases in p-EGFR, p-ERK, p-JNK, pp-38, and $p21^{WAF1/CIPI}$ (an immunoblot assay was used for p21). NSE for JNK. A series of experiments involving modulators and reporter genes showed that: (1) EGFR activation can mediate iAs-induced p21 expression, (2) activation of EGFR by iAs occurred later than by EGF, (3) c-Src was involved in iAs-induced ERK activation and p21 expression, (4) the EGFR/Ras/Raf/ERK pathway is involved in iAs-induced p21 gene expression, (5) Sp1 binding sites in the promoter are essential for iAs-induced p21 activation, and (6) a post-transcriptional or post-translational stabilization mechanism is essential for iAs-induced p21 expression.			Liu and Huang, 2006
MDA-MB-435 cells	As^{III} SA	1, a non-cytotoxic dose	0.5 hr, 1 hr 2 hr 4 hr 6 hr	-	Effects on the nuclear binding of the following 4 transcription factors, relative to control and in sequential order of the 5 time periods: AP-1: NSE, NSE, ⇑ 2.5x, ⇑ 2.5x, NSE; NF-κB: NSE, ⇓ 0.5x, ⇑ 3.5x, ⇑ 3.5x, ⇑ 1.5x; Sp1: ⇓ 0.5x, ⇓ 0.5x, ⇑ 3x, NSE, NSE; YB-1: NSE, NSE, ⇑ 9x, ⇑ 3x, ⇑ 3x Another experiment using a highly cytotoxic dose of 100 resulted in markedly different patterns over time within approximately the same ranges of effect.	Kaltreider et al., 1999

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
H411E cells	As^{III} SA	0.33, a non-cytotoxic dose	0.5 hr, 1 hr 2 hr 4 hr 6 hr	-	Effects on the nuclear binding of the following 4 transcription factors, relative to control and in sequential order of the 5 time periods: AP-1: NSE, ⇑ 5.5x, ⇑ 8.5x, NSE, ⇓ 0.5x; NF-κB: ⇑ 1.3x, NSE, ⇑ 1.5x, NSE, NSE; Sp1: NSE at any time; YB-1: ⇑ 3x, ⇑ 3x, ⇑ 3.2x, NSE, ⇓ 0.5x Another experiment using a highly cytotoxic dose of 333 resulted in markedly different patterns over time within approximately the same ranges of effect.	Kaltreider et al., 1999
SIK cells, with iAs treatment beginning 1 day before suspension and continuing while cells were in suspension	As^{III} SA	2	2 or 5 days, when including the 1 day of treatment before being put into suspension	2	Protein levels determined by immunoblotting assay: β-catenin: ⇑ to 3.2x on day 2 and to 3.6x on day 5; β1-integrin: ⇑ to 2.7x on day 2 and to 4.0x on day 5; p-GSK3β (the inactive form): ⇑ to 2.5x on day 2 and to 2.2x on day 5. On day 1, in cells harvested before suspension, there was ⇑ in p-GSK3β to 1.5x and NSE for other two proteins. Consistent with iAs maintaining the cell's proliferative potential, levels of these 3 proteins decreased much less rapidly during the 4 days in suspension if treated with iAs.	Patterson et al., 2005
SIK cells treated while being maintained in surface cultures	As^{III} SA	2	Various	2	Protein levels determined by immunoblotting assay: nuclear β-catenin: ⇑ to 3.4x on day 9; cytoplasmic β-catenin: ⇑ to 2.5x on day 11; p-β-catenin: ⇓ to 0.45x on day 1; β1-integrin: ⇑ to 1.6x on day 7 and to 4.5x on day 11; p-GSK3β (the inactive form): ⇑ to 1.8x on day 7 and to 3.1x on day 11. Consistent with iAs maintaining the cell's proliferative potential, iAs decreased the rate of post-confluent loss of all of these proteins except P-β-catenin.	Patterson et al., 2005
B16-F10 cells	As^{III} SA for all	0.01, 0.1, 1, 10 0.01, 0.1, 1, 10 0.01, 0.033, 0.1	4 hr 72 hr 7 days	None 0.01 0.01	HIF-1α protein levels: No ⇑ seen; no mention if there were decreases. Small ⇑, but decreased to no change from control at 0.1, and at higher doses a ⇓ from control. Big ⇑, but decreased to almost 2 times control at 0.033, and there was no change from control at 0.1.	Kamat et al., 2005

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Type of cell/tissue	Arsenic species	Concentration(s) tested (μM)	Duration of treatment	LOEC[1] (μM)	Results (compared with controls, with all concentrations being in μM unless noted)	Reference
B16-F10 cells	As^{III} SA for all	0.01, 0.1, 1, 10	4 hr	0.1	VEGF secretion: Small ⇑ seen at two middle doses.	Kamat et al., 2005
		0.01, 0.1, 1, 10	72 hr	0.01	Big ⇑; much smaller increase over control at 0.1;no change from control at 1.0, and a ⇓ from control at 10.	
		0.01, 0.1	7 days	0.01	Big ⇑, but ⇓ from control at 0.1. Also, both 7-day treatments showed ⇑ HRE transactivation that was mostly or completely blocked by co-treatment with YC-1.	
J82 cells	As^{III} SA for all	0.01, 0.1	7 days for all	0.01	HIF-1α protein levels: ⇑, but ⇓ from control at 0.1;	Kamat et al., 2005
HMEC-1 cells		0.01, 0.1		0.01	⇑, but ⇓ from control at 0.1;	
SMC cells		0.01, 0.1		0.01	Big ⇑ at both dose levels.	
J82 cells	As^{III} SA for all	0.01, 0.1	7 days for all	0.01	VEGF secretion: ⇑, but no change from control at 0.1	Kamat et al., 2005
HMEC-1 cells		0.01, 0.1		0.01	⇑, but no change from control at 0.1	
SMC cells		0.01, 0.1		None	-	
H22 cells	As^{III} ATO	0.5, 1, 2, 4	36 hr	0.5	Huge ⇑ in VEGF protein level in cell lysates, with similar response at all doses (Westen blot assay)	Liu et al., 2006e
MCF-7 cells	As^{III} ATO	0.5, 2, 5, 10	60 min	Various	Results of Western blot analysis: ⇑ phosphorylation (i.e., activation) of ERK1/2 at 2 with a dose-related increase to 10; ⇑ phosphorylation (i.e., activation) of p38 at 2 with a dose-related increase to 10; NSE on JNK1/2. Thus, iAs activates the prosurvival mitogen-activated MEK/ERK pathway.	Ye et al., 2005
MCF-7 cells	As^{III} ATO	2, 5	The findings in the row above prompted investigation whether a combination treatment with either of the MEK inhibitors U0126 (at 10 μM) and PD98059 (at 20 μM) could lead to enhanced growth inhibition and apoptosis. They did, augmenting apoptosis approximately 2x compared to the effects of iAs and either inhibitor alone, based by Hoechst 33258 or annexin V/PI staining and flow cytometry. Treatment with a p38 inhibitor did not prevent iAs-induced apoptosis; there was a slight but nonsignificant ⇑ in apoptosis.			Ye et al., 2005
HeLa cells	As^{III} ATO	2	Various	None	Experiments showed that NF-κB and AP-1 activation served as prosurvival or antiapoptotic forces and that their activation by PMA was suppressed by co-treatment with 10 μM emodin plus iAs, whereas emodin or iAs alone had rather little or no suppressive effect. The synergistic suppression was thought to be caused, at least in part, by cellular ROS because pretreatment or co-treatment with NAC reduced the effect.	Yi et al., 2004

[1]Lowest Observed Effect Concentration

Appendix D. Immunotoxicity

Arsenic has been observed to affect the immune system. While changes to the immune system are not directly related to any specific disease or cancer endpoint, disruptions to the immune function can impact the individual and likely increase their risk for developing a disease or cancer outcome. This may, in part, be why there are a vast array of cancers and diseases associated with arsenic exposure. In addition, arsenic's effects on the immune response may play some role in acting as a co-carcinogen with other compounds. Therefore, immunotoxicity is listed as a key event in this review, and many studies are detailed in the MOA section (4.4.1). The effects, however, on the immune system are important to note in and of themselves, and a few are detailed here.

Gonsebatt et al. (1994) selected two populations from Comarca Lagunera (Mexico) to study the labeling, mitotic, and replication indexes (LI, MI, and RI, respectively) of peripheral blood lymphocytes. The exposed population consisted of 33 individuals from Santa Ana, Coahuila, who had arsenic levels in the drinking water ranging from 375 to 392 ppb (92% in the form of As^{V} and 8% in the form of As^{III}) for several years. Approximately 50% of the exposed individuals had cutaneous signs of arsenic poisoning. The control population consisted of 30 individuals (selected based on similar proportions of age and sex as the exposed population) from Nazareno, Durango, who had arsenic levels from the preceding 2 years ranging from19–26 ppb (>95% as As^{V}). Urine and blood samples were obtained from all subjects. Average total arsenic in the urine and blood of the control group was 36.7 and 37.2 µg/L, respectively, and 758.4 and 412.0 µg/L, respectively, in exposed subjects. Peripheral blood lymphocyte counts were significantly greater in the exposed population ($3.1x10^{6}$ cells) compared to the control population ($2.6x10^{6}$ cells), with a greater increase noted in females. In females, the average 36-hour LI was lower in the exposed population compared to the control population, regardless of the presence or absence of skin lesions (Table D-1 below). Exposed males, however, only exhibited a lower average 36-hour LI in those with skin lesions; males without skin lesions had an increase in LI. MI were significantly increased in both sexes after a 72-hour incubation period (Table D-1), but were not after 48 or 60 hours of incubation. Exposed females had a significantly lower RI after 48, 60, and 72 hours of incubation, while males were unaffected.

Table D-1. Lymphocyte Counts and Labeling, Mitotic, and Replication Indexes (Mean ± SE) in the Peripheral Blood Lymphocytes in Populations Exposed to Low (Control) and High (Exposed) Levels of Arsenic (Gonsebatt et al., 1994)

	Control			Exposed		
	Males	**Females**	**Total**	**Males**	**Females**	**Total**
Lymphocyte count ($x10^6$ cells/ml)	2.7±1.2	2.4±1.1	2.6±1.1	3.0±1.2	3.1±0.8*	3.1±1.0*
Labeling Index (36 hours)						
with skin lesions				2.14±0.86	2.74±0.32	2.42±0.49*
without skin lesions	3.32±1.06	4.77±1.06	3.37±0.61	4.05±1.31	3.90±0.49	3.95±0.56
Mitotic Index						
48 hours	1.15±0.23	2.52±0.48	1.89±0.30	1.59±0.29	1.59±0.26	1.59±0.20
60 hours	2.53±0.30	4.90±0.79	3.85±0.50	3.35±0.39	3.65±0.48	3.50±0.32
72 hours	3.52±0.37	3.96±0.52	3.78±0.34	6.00±0.55**	6.60±0.69**	6.34±0.45**
Replication Index						
48 hours	1.07±0.01	1.16±0.02	1.12±0.01	1.05±0.01	1.10±0.02*	1.08±0.01*
60 hours	1.43±0.03	1.54±0.04	1.49±0.03	1.39±0.04	1.37±0.04*	1.38±0.02*
72 hours	1.93±0.09	2.08±0.04	2.01±0.04	1.89±0.09	1.84±0.05*	1.86±0.05*

* Statistically different ($p < 0.05$) from the control (two-tailed Mann-Whitney U-test)
** Statistically different ($p < 0.001$) from the control (two-tailed Mann-Whitney U-test)

A previous study by Ostrosky-Wegman et al. (1991), in which cell-cycle kinetics of peripheral blood lymphocytes showed a significantly longer average generation time (AGT) for the highly exposed group as compared to the control group. The AGT was 19.90 hours in the low-exposure group compared to 28.70 hours in the high-exposure group. The AGT in the control group was 19.02 hours. The exposed group consisted of 11 individuals (9 females and 2 males) from Santa Ana, Coahuila with drinking water containing an average of 390 ppb (98% as As^V). The control group consisted of 13 individuals (11 females and 2 males) from Nuevo Leon, Coahuila (drinking water concentrations not reported). Average urine arsenic/creatinine levels were 0.121 µg/mL in the control group and 1.565 µg/ml in the exposed group. There were no incidence of skin lesions in the control subjects, but 4 of the 11 exposed subjects had skin lesions (i.e., hyperkeratosis, hypo- and hyperpigmentation, and skin horns).

IgG and IgE levels were significantly elevated in arsenic-exposed individuals who presented clinical symptoms of arsenic exposure (i.e., skin lesions) (Islam et al., 2007). As exposure duration increased, so did the severity of the skin lesions. The level of IgE also was greater with longer durations of arsenic exposure. IgG levels were highest during the initial stages of skin lesions. There was a smaller, but significant, increase in IgA in individuals with arsenicosis compared to the control group, but no change was observed in IgM levels. Arsenic exposed individuals also had a greater incidence (63% of subjects) of respiratory complications, such as chest sounds, shortness of breath and breathing complications, irritation of the upper and lower respiratory tract, couch, bronchitis and asthma as compared to the control group (7%). The IgE level in individuals with respiratory complications was greater than in arsenic-exposed individuals without complications. Because the difference in IgE levels could not be explained by differences in eosinophil levels, it was suggested that the reason may be due to inflammatory reactions due to arsenic exposure.

Yu et al. (1998) found that patients with Bowden's disease (skin carcinoma *in situ*) from an arsenic endemic area in the southwest coast of Taiwan had significantly decreased T-cells, increased B-cells, decreased T-helper cells, decreased IFN-γ release, TNF-α release, increased IL-2 release, decreased soluble IL-2 receptor release, changes in soluble CD4 and soluble CD8 release (increases in spontaneous release, but decreases in phytohaemagglutinin-induced release) in comparison to normal controls, as well as non-Bowden's patients in the endemic region. Results indicate a depressed cell mediated immunity in patients with Bowden's disease. The deficient immune response appears to be related to an impairment of the membrane IL-2R expression in lymphocytes after stimulation. This study, however, could not associate arsenic with these changes because individuals without Bowden's disease who lived in the endemic region did not demonstrate the same effects. In addition, a cause and effect relationship could not be determined. Since arsenic has been demonstrated to affect the immune response in other studies, it is possible that individuals developing Bowden's disease were more susceptible to the effects of arsenic on the immune system.

The development of skin lesions is a typical symptom of arsenic-exposed individuals. However, not all individuals exposed, even those within the same family, develop skin lesions. Mahata et al. (2004) examined some effects on peripheral lymphocytes in arsenic-exposed individuals with or without skin lesions and compared those results to a group of subjects that were unexposed. Six individuals (3 males and 3 females) were selected from each group:

symptomatic (with lesions and exposure), asymptomatic (without lesions and exposure), and unexposed. Where possible, symptomatic and asymptomatic were selected from the same family. The 6 controls (3 males and 3 females) were selected for similar socio-economic status, age, and gender. Levels of arsenic in urine, nail, and hair demonstrated that the control group had little exposure to arsenic. Individuals with skin lesions were noted to have less arsenic in their urine and more in their hair and nails. This indicated individual differences in distribution and excretion (for more information on this see Section 4.7.3.1 on genetic polymorphism) that may be related to the individual's susceptibility to developing skin lesions. When the blood of the individuals from all three groups was exposed to further arsenite *in vitro*, all groups demonstrated a dose-dependent increase in chromosomal aberrations in the lymphocytes with a significant increase observed even at the lowest concentration (1 µM). Untreated lymphocytes, however, had a greater level of chromosome aberrations in arsenic exposed individuals. In addition, individuals with skin lesions had a 1.7-fold increase in "background" chromosomal aberrations compared to asymptomatic individuals. Although the arsenic exposed individuals had more chromosomal aberrations in the absence of additional arsenic exposure *in vitro*, the *in vitro* exposure to arsenite caused a smaller increase in chromosome aberrations in lymphocytes of exposed individuals compared to unexposed individuals, indicating a greater sensitivity in the control lymphocytes to the *in vitro* effects of As^{III}.

The JAK-STAT pathway is essential in mediating the normal functions of different cytokines in the hematopoietic and immune systems. *In vitro* studies by Cheng et al. (2004) suggest that arsenic exposure in the range of 0.4 to 400 µM caused inactivation of the JAK-STAT signaling pathway in HepG2 cells (a human hepatocarcinoma cell line). This inactivation was caused by the direct interaction of arsenic with JAK tyrosine kinase and was independent of arsenic activation of mitogen-activated protein (MAP) kinase. Exposure to sodium arsenite abolished the STAT activity-dependent expression of cytokine signaling suppressors by inhibiting IL-6-inducible STAT3 tyrosine phosphorylation. This effect on the STAT3 tyrosine phosphorylation induced by arsenic was not observed with other inflammatory stimulants, stress agents, or cadmium (metal).

Harrison and McCoy (2001) performed an *in vitro* study to examine the role of apoptosis and enzyme inhibition on arsenic suppression of the immune response. Cysteine cathepsins are lysosomal enzymes that are critical in antigen processing. Because of As^{III} interactions with sulfhydryl groups, cathepsin L (a member of the papain superfamily of cysteine proteases and a major lysosomal protease involved in cleaving exogenous protein antigens into peptide fragments) was examined as a potential target for arsenic inhibition. As^{III} caused a dose-dependent inhibition of cathepsin L, both as a purified enzyme and in active murine B cells. Inhibition occurred in TA3 cells even at concentrations that did not affect cell viability; greater inhibition was obtained with the purified enzyme. Addition of DTT caused a complete reversal of the inhibition. As^{V} was not able to inhibit cathepsin L, therefore, indicating the involvement of the sulfhydryl groups. Although cathepsin L was inhibited by 4 hours, exposure for 18 hours lead to increases in apoptosis even at the lowest concentration (0.8 µM). Apoptosis was observed at concentrations about 100-fold lower than those inhibiting cathepsin L, suggesting

that apoptosis is likely a more important role in immunosuppression than inhibition of lysosomal cathepsins.

De La Fuente et al. (2002) found a significant increase in apoptosis in PMBCs from healthy donors at concentrations of 15 µM As^{III} after 48 hours of exposure; an increase also was noted at 5 µM, but did not reach statistical significance. Results did not show a dose response; instead apoptosis levels were similar between 15 and 75 µM of arsenite. Lower concentrations of As^{III} (i.e., 1 µM and 2.5 µM) also were able to increase apoptosis levels, but required at least 96 hours of exposure compared to only 16 hours of exposure needed with 15 µM of As^{III}. Measuring the levels of apoptosis in the PMBCs of children chronically exposed to arsenic (urinary levels of arsenic between 94 and 240 µg/g of creatine) also demonstrated an increase in apoptosis when compared to the control group. However, exposing the cells of chronically exposed children to arsenic *in vitro*, demonstrated a decrease in apoptosis when compared to controls. Therefore, this data supports the data by Mahata et al. (2004), which suggested that control PMBCs are more sensitive to *in vitro* arsenic exposure.

In contrast, González-Rangel et al. (2005), found the opposite response. Although their data also show an increase in basal apoptosis in PMBCs lymphocytes and monocytes (but not natural killer [NK] cells) in an exposed individual compared to six non-exposed individuals, the data also show an increased sensitivity to *in vitro* arsenite-mediated apoptosis in lymphocytes and NK cells in the exposed individual. This study, however, used a higher concentration of arsenite (30 µM) compared to the other studies (which used at most 15 µM) and only used one exposed individual compared to 6 unexposed individuals. Therefore, results could be different due to dose or because of inter-individual variation.

Although Harrison and McCoy (2001) and De La Fuente et al. (2002) observed an increase in the apoptosis in PMBCs, Chen et al. (2005b) did not observe any effect on the apoptosis of human keratinocytes (obtained from the adult foreskin through routine circumcision) with 1 µM of arsenite. When cells were exposed to As^{III} for 24 hours prior to exposure to UVB, however, the As^{III} protected against UVB-induced apoptosis, thus, indicating a possible mechanism for arsenic's observed co-carcinogenic nature. Exposing the cells to As^{III} after UVB exposure did not cause a reduction in apoptosis and possibly increased the level of apoptosis.

Galicia et al. (2003) isolated PBMC from healthy, non-smoking, males (22-40 years old) who were not exposed to arsenic to examine the affects of As^{III} on cell proliferation. Although there was individual variability, a dose-dependent decreased in cell proliferation in PHA-induced cells was observed. In all cases, the highest concentration used (1 µM) decreased the percent of dividing cells, with a reduction of 12 to 54%. Although cell proliferation was affected, there was relatively little affect on cell viability. After further examination, it was suggested that proliferation of T lymphocytes was affected and there was a reduction in CD3+ cells producing IL-2. Although arsenic prevents cells from entering the cell cycle and slows down the progression through the cell cycle, no alteration in the expression of CD69 or CD25 activation molecules was observed. Thus, it was concluded that the reduction in T cell proliferation was caused by a decrease in the production and secretion of IL-2, thereby blocking the T cells in G_1.

Di Gioacchino et al. (2007) examined the effects of arsenic compounds (i.e., As^{III}, As^{V}, MMA^{V}, and DMA^{V}) on PBMC proliferation and cytokine release. As^{III} had the greatest effect on the cells. At 10^{-4} M, As^{III} caused the greatest decrease in PHA-induced cell proliferation and the greatest reduction in IFN-γ and TNF-α release. At 10^{-4} M, the affect on cell proliferation by compound was As^{III}>As^{V}>DMA^{V}>MMA^{V}. At 10^{-7} M, however, As^{III} caused a significant increase in cell proliferation. DMA^{V} also caused a significant increase in cell proliferation at10^{-7} M, but had no affect on cell proliferation at 10^{-4} M. DMA^{V} and As^{V} caused a significant decrease in IFN-γ release at 10^{-4} M, but did not affect TNF-α release. Although the text indicates that dose responses analyses were performed, there is no data provided in the article. It was concluded that the immunotoxicity of arsenic was dependent on the chemical speciation of arsenic.

As^{V} (0.5, 5, or 50 mg As/L) administered for 12 weeks via drinking water to female C57BL/6J/Han mice (8-12 weeks old) was determined to decrease NO and superoxide production (Arkusz et al., 2005). While there was a concentration-dependent decrease in ROS production, the decrease observed in NO was similar across the three doses. The As^{V} did not appear to affect TNF-α production. It should be noted, that in testing for the NO and superoxide production, $2x10^{5}$ cells/well were plated. Therefore, a cell to cell comparison was made between the isolated macrophages from the control group and arsenate-treated mice. The As^{V} treatment, however, was noted to cause a concentration-dependent increase in the number of peritoneal macrophages isolated. The percent increase compared to control (55, 77, and 101%, respectively) was such that it may have compensated for the changes noted in NO and superoxide production. This, however, was not tested.

Nayak et al. (2007), however, did test the immune response in zebra fish to virus or bacterial infection. Zebra fish embryos (one-cell stage) were exposed to 2 or 10 ppb As^{III} in water until 4 days post fertilization. Seven days later fish were infected or left uninfected. Viral and bacterial loads were then examined. Viral load was significantly increased in both As^{III} treatment groups compared to the infected control group, with a concentration-dependent increase in the viral load. There also was a significant increase in the bacterial load in treated fish; however, the increase was similar across both treatments. As^{III} was also found to decrease ROS burst, interferon, Mx mRNA expression, IL-1β, and TNF-α mRNA levels. The maximum response for these cytokines was also found to be delayed compared to the controls.

Appendix E. Quantitative Issues in the Cancer Risk Assessment for Inorganic Arsenic

As discussed in Section 5.3, the arsenic cancer risk assessment involved two distinct phases. The first phase involved the derivation and fitting of dose-response models using the Taiwanese epidemiological data of Chen et al. (1988a, 1992) and Wu et al. (1989). The outputs of this phase of the analysis were arsenic dose-response coefficients that described the relationship between estimated arsenic intake in the Taiwanese population and proportional increases in age-specific lung and bladder cancer mortality risk. A key assumption underlying this relative risk model is that the risk of arsenic-related cancer is a constant multiplicative function of arsenic dose and the ”background” age profile of risks.

The second phase of the risk assessment involved the estimation of arsenic-related cancer risks in a (hypothetical) U.S. population exposed to arsenic at varying levels in drinking water. This phase of the analysis involved the application of the dose-response coefficients for arsenic derived from the Taiwanese data to the age-specific background population risks for the U.S. population. In addition, the risk estimates were converted from mortality-based values to incidence-based estimates. The following sections describe each of these phases.

E.1 Cancer Risk Assessment for the Taiwanese Population

The calculation of cancer risks from the Taiwanese epidemiological data was performed using Excel workbook files. The files contained the input data for the dose-response models and spreadsheets to accept user-specified inputs, perform calculations, and summarize outputs from the assessment. Input data included male and female lung and bladder cancer mortality and person-years at risk (PYR) data for arsenic exposed populations from 42 villages obtained from Morales et al. (2000), village water arsenic concentrations (minimum, median, and maximum data sets), and southwest Taiwan and all Taiwan reference population mortality and PYR data.

The user first specifies drinking water consumption and body weights for the Taiwanese population in the “Poisson Model” page of the risk calculation files. Solver® is then invoked to estimate that age coefficients (a1, a2, and a3) and the arsenic dose-response coefficient (b) in equation E-1 by maximizing the likelihood function that is coded into the spreadsheets. Solver is then reconfigured to calculate the upper confidence limit (UCL) on “b” using the profile likelihood method (see below). The resulting UCL value is then input to the “BEIR Model” sheet and the LED_{01} for cancer incidence is calculated, again using Solver®. The LED_{01} value is transferred to the “Summary” sheet, where other risk metrics (Unit Risk, cancer risks at different drinking water concentrations, and the drinking water concentration corresponding to 10^{-4} lifetime risk) are calculated. Risk metrics are calculated based on user-specified drinking water intake and body weight for the U.S. population. Likelihood calculations for most of the endpoints were replicated using a different optimizer in the Non-linear Estimation module of Statistica® software package.

E.1.1 MLE Estimation of Dose-Response Parameters

The Taiwan risk model spreadsheets calculate the dose-response parameters for the Poisson model, fitting separate models for each endpoint:

$$h(x,t) = \exp(a_1 + a_2*age + a_3*age^2) * (1 + b* dose) \qquad (E\text{-}1)$$

In this model, the midpoints of the age group strata are normalized (placed on a "z-scale") prior to estimating risk; age is thus treated as a "nuisance parameter" in the model. Dose, as noted above, is calculated from dietary arsenic and village water concentrations and is expressed in terms of mg/kg-day. Each age-dose group is represented by a row on the spreadsheet. There are 42 villages with arsenic well water data and the reference population, each divided into 13 age strata, for a total of 559 population groups. The model begins with randomly selected values for the four parameters and then calculates the Poisson log likelihood values for each group:

$$\text{Log Likelihood} = \text{Observed} * \ln[h_{CURRENT}(x,t)] - \text{Predicted} \qquad (E\text{-}2)$$

Where:

Observed =	The number of cancer deaths in groups age t, exposed at dose x)
$h_{CURRENT}(x,t)$ =	The estimated total cancer risk in age group t at dose x, based on the current parameter estimates
Predicted =	The predicted number of cancer deaths in age group t at dose x, = $h_{CURRENT}(x,t)$ * PYR, where PYR = person years at risk

The sum of the log likelihood across all the age groups is then maximized using standard optimization methods (Excel Solver®) to provide the MLE estimates of the age and dose parameters.

E.1.2 Estimation of Upper Confidence Limits (UCLs) on the Arsenic Dose-Response Parameters

ED_{01} values are derived based on the MLE dose-response parameter estimates. The LED_{01} estimates are derived from the 95% UCLs on the dose-response parameters. The UCLs on the dose response "b" parameters were estimated using the "profile likelihood" method (Venson and Moolgavkar, 1988). In this approach, the value of the dose parameter, b, was varied from its estimated mean value, and the changes in log-likelihood were calculated. The ratio of the log likelihood for the best-fit model to the log likelihood for other values of "b" is known to follow an approximate chi-squared distribution with one degree of freedom. Thus, the 5^{th} and 95^{th} confidence limits on the dose coefficient "b" correspond to the values where the likelihood ratio is equal to 1.92. Upper and lower confidence limits were calculated using Solver®. The fact that the profile likelihood method ignores the likelihood impact of the age "nuisance parameters" implies that the calculated confidence limits are only approximate. Confidence limit calculations using other methods (empirical Bayesian simulation and "bootstrap-t") gave similar values (within a few percent).

E.2 Estimation of Risk for U.S. Populations Exposed to Arsenic in Drinking Water

LED_{01} values were calculated using a life-table method that is a variation on the "BEIR IV" model recommended by NRC (2001). Specifically, the approach includes a modification suggested by Gail et al. (1999) for obtaining more accurate estimates of incidence within multi-year age strata. The BEIR IV relative risk models, takes as its inputs the arsenic dose-response "b" coefficient from the Poisson model, background cancer incidence data, along with age-specific mortality data to directly estimate lifetime bladder and lung cancer incidence for the target (U.S. adult) population. Lung and bladder cancer incidence reference data for the years 2000-2003 were obtained from the National Cancer Institute's SEER program (NCI, 2006). U.S. gender and age-specific population data and all-causes mortality data came from the National Center for Health Statistics (NCHS, 2000).

Formulas for calculating LED_{01} values were implemented on separate Excel spreadsheets for each endpoint. The following calculations were implemented in separate lines on each spreadsheet. In all of the equations, the subscript "i" refers to age group:

$$L(x) = \text{lifetime risk of cancer incidence at dose x}$$
$$= \sum_i \frac{c_i(x)}{s_i(x)} T_i (1 - r_i)$$

Numerator Terms:

$c_i(x)$ = cancer incidence hazard at dose (x), age interval (i)

$c_i(x) = c_i(0) * (1 + \text{beta} * \text{dose})$ (beta comes from the linear Poisson model) (1)

$c_i(0) =$ <u>background</u> cancer incidence$_i$ / cancer free population$_i$ (2)

Background cancer incidence c_i comes from SEER, cancer-free population$_i$, see (7)

$b_i =$ <u>background</u> cancer incidence$_i$ / alive population$_i$ (SEER data) (3)

$Y_i = \exp(-5b_i)$ (4)

F_i = Initial estimated background probability of survival without cancer to the end of interval (i - 1)

$$Fi = \prod_{j=1}^{j=i-1} Yi \quad (5)$$

G_i = Initial estimated background probability of survival without cancer to the middle of interval (i)

$$Gi = Fi^{(-2.5*bi)} \quad (6)$$

Cancer-free population$_i$ = alive population$_i$ * G_i (7)

Denominator Terms:

$s_i(x)$ = total noncancer mortality and cancer incidence hazard, at dose (x) in age interval (i)

$s_i(x)$ = background noncancer mortality (x, i) + cancer incidence hazard (x, i)

$s_i(x) = (d_i - h_i) + c_i(0) * (1 + \text{beta} * \text{dose})$ (8)

d_i = Total mortality (background) in age interval (i)

d_i = total deaths$_i$ / population$_i$ (Census, Vital Stat. U.S.) (9)

h_i = cancer deaths$_i$ / population$_i$ (Census, Vital Stat. U.S.) (10)

Survival (T_i and r_i) Estimation:

T_i = Probability of survival without cancer to end of interval (i - 1)

$$Ti = \prod_{j=1}^{j=i-1} ri = \prod_{j=1}^{j=i-1} Wi * \prod_{j=1}^{j=i-1} Wib \quad (11)$$

r_i = Probability of survival cancer free through interval (i), given survival to beginning of interval (i)

$r_i = W_i * W_{ib}$ (12)

$W_i = \exp(-5d_i + 5h_i - 5c_i)$ (13)

$W_{ib} = \exp(-5c_i * \text{Beta} * x)$ (14)

To calculate ED_{01} values, the value of the daily arsenic dose used to calculate $h_i(x)$, and hence L(x), was varied until L(x) = 0.01 (one percent). For the MLE estimation, Solver was used to estimate LED_{01} values in the model spreadsheets.

Appendix F. Risk Assessment for Townships and Low-Exposure Taiwanese Populations

F.1 Recent Studies of the Taiwanese Populations that Do Not Find Consistent Exposure-Response Relationships

As discussed in Section 5.3.8.5, several recently published studies have called into question the strength and significance of the exposure-response relationship for arsenic in the Taiwanese populations studies (Chen et al., 1988a, 1992 and Wu et al., 1989). This appendix provides a brief analysis of some of these concerns.

Based on "graphical and regression analysis," Lamm et al. (2003) found no significant dose-response relationship for arsenic-related bladder cancer in the subset of the Taiwanese population with median drinking water well concentrations less than 400 ppb (µg/L). Significant, positive dose-response slopes were found for villages with median well concentrations above 400 ppb. They also observation that all of the villages "solely dependent" on artesian wells had median arsenic concentrations above 350 ppb, and that the median well concentrations in villages not solely dependent on artesian wells were generally below this values. Based on these observations, Lamm et al. (2003) suggested that the nature of the villages water sources (artesian vs. non-artesian), rather than arsenic concentration, explained the observed variations in bladder cancer risk in the Taiwanese population.

Kayajanian et al. (2003) have also argued that the data from the Taiwanese population are being misinterpreted by EPA. In their analysis, they stratify median well arsenic concentration into ten ranges from 10 to 934 ppb. They then calculated combined mortality rates for lung, bladder, and liver cancer for each stratum of the population. They calculated that crude (age-unadjusted) cancer mortality for both males and females were significantly elevated in the lowest exposure groups, decreased to minimums for villages with water arsenic concentrations in the range between 42 and 60 ppb and then again increased with increasing arsenic exposure. They argued on this basis (and based on the analysis of cancer mortality data from another epidemiological study), that health standards for arsenic should be set in the vicinity of 50 µg/L (ppb) in order to minimize the risk of arsenic-associated cancer, and that lower exposures would actually result in increased risk in the U.S. population.

In a more recent study, Lamm et al. (2006) reported additional analyses of the relationship between cancer risks and drinking water arsenic in the same Taiwanese population. In this analysis, they divided the epidemiological data according to six "township" designations provided by the original Chinese investigators (townships 0, 2, 3, 4, 5, and 6).[8] They stratified the data into two groups; townships that (by their characterization) "showed a significant dose-response relationship" with arsenic (2, 4, 6) and townships "that did not" (0, 3, and 5). They then applied linear regression to characterize the relationship between combined bladder and lung cancer Standardized Mortality Ratios (SMRs) and arsenic exposures in the Taiwanese villages. They found that (1) dummy variables related to township were significant (along with arsenic well concentration) when all the townships were included in the analysis, and (2) the dose-response parameter for arsenic exposure became insignificant for arsenic well

[8] Each township included subsets of the 42 "villages" used as the basic units of analysis in the current assessment.

concentrations less than 151 ppb when only townships 2, 4, and 6 were included in the regression. Based on these results, they concluded that location (township), was an important explanatory variable for cancer risks and that 151 ppb represented a "threshold" well arsenic concentration below which no exposure-response relationship for arsenic could be detected.

F.2 Limitations of the Recent Studies

The studies discussed above all have significant limitations, relating both to the methods used to select or stratify data for the risk assessment, and to the methods used in analyzing exposure-response data. In the first place, it is important to recognize the complexity and limitations of the data. Cancer mortality and person-years at risk observations are provided for a large number (559) of relatively small age- and village-stratified populations (median person-years at risk ~ 340 for both males and females). Most population groups have zero cancer deaths, and the data are very "noisy." Cancer mortality is strongly age-dependent, and simultaneously evaluating the age-and dose-dependence of cancer mortality based on a data set in which cancer deaths are "rare events" requires appropriately structured models. All of these features of the data drove the selection of the Poisson regression methods described in Section 5, and the use of simpler models (linear regression, for example) can (and did) lead to misleading results.

With regard to the Lamm et al. (2003) paper, it is likely that the use of linear regression and the failure to account for the age-dependency of bladder cancer risks combined to make it impossible to detect a significant exposure-response relationship in villages with water arsenic levels less than 400 ppb. In addition, it should be noted that Lamm et al. (2003) did not have data regarding the actual sources of drinking water in the various villages; instead they relied on the arsenic concentration to assess the degree of dependency of specific villages on artesian (generally high arsenic) versus shallow (low arsenic) wells. When defined in this circular fashion, it is inevitable that including the degree of "artesian well dependence" in a multiple regression along with arsenic concentration would deprive the latter variable of much of its explanatory power and statistical significance. Finally, the rationale for excluding valid data on southwestern or all-Taiwan reference populations from the analysis is highly questionable, and again lowers the likelihood of detecting significant exposure-response relationships.

The major limitation of Kayajanian's (2003) analysis of the Taiwanese data is that it breaks the data into strata that are too small to be used to calculate reliable mortality risks, and that it is very sensitive to the specific way that the data are stratified. The relatively high cancer mortality risks seen in the low-dose strata are associated with a small number of villages that happen to have a (relatively) large number of deaths. The observed trend in cancer mortality versus arsenic dose would be very different if only few cancer deaths were misclassified, or if the pattern of cancer deaths had been slightly different by chance. Again, failure to use a model that adequately addresses the distribution of cancer deaths as rare events (and that does not incorporate age dependence) resulted in results that are misleading.

Lamm et al.'s (2006) failure to find a significant exposure-response relationship in villages with arsenic water concentrations below 151 ppb can also be explained by (1) the use of linear regression without age-adjustment and (2) the omission of data from three of the six

townships from some of the regressions. Lamm et al. (2006) did not explain the specific criteria for determining if a township "showed a dose-response relationship," but based on the description of their methods provided in the article, it may be assumed that the used linear regression to characterize the relationship between SMRs and arsenic exposure in each village in the various townships. Given the small number of villages in each township, this approach, and the rationale for leaving townships 0, 3, and 5 out of the analysis appear arbitrary and unjustified.

In the following sections, we present alternative analyses that further investigate the nature of arsenic exposure-response relationships in the various townships and in villages with low arsenic drinking water concentrations.

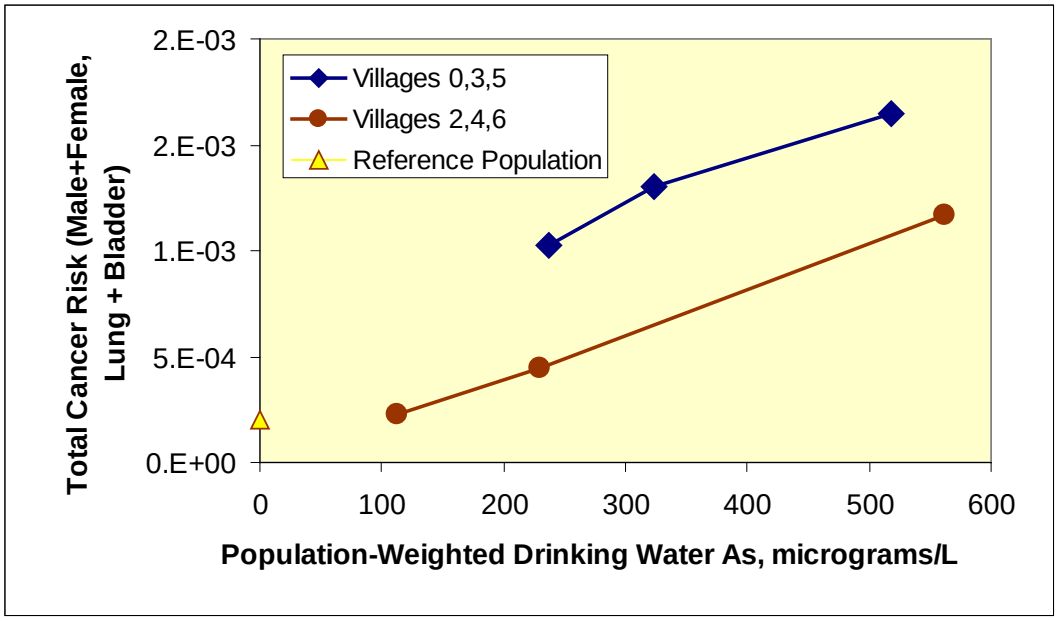
F.3 Calculations of Risks for Township Groups

To address the issues raised by Lamm et al. (2003, 2006), the patterns of cancer risks for subjects living in two townships (0, 3, and 5 vs. 2, 4, and 6) villages were evaluated to see whether there were any differences. As noted above, it is not believed that Lamm et al.'s approach to omitting townships because they lack an internal dose-response relationship is valid, so EPA did not do so.

First, to get a rough idea of the patterns in cancer incidence versus exposure, the crude cancer risks (population-weighted deaths per person-year for all age groups) and population-weighted average arsenic exposure concentrations were calculated for each of the six villages. The results are shown in Figure F-1. This figure simply illustrates that, even without age-adjustments, arsenic dose-response relationships across the villages are quite robust.

For both sets of villages (low and high exposure), crude cancer risks increase with average arsenic drinking water concentration. Age distributions were very similar in all cohorts, so the lack of age-adjustment did not seriously bias the results. While total cancer risks are dominated by male lung cancer, the other endpoints also showed generally the same pattern. This finding suggests that the positive exposure-response relationship for arsenic is not being seriously confounded by a "village effect." Given the small populations, populations at risk, and numbers of cancer deaths in the individual villages, it is not clear that analyzing exposure-response relationships within these villages (as defined by Lamm et al.) is justified.

Figure F-1. Lifetime Crude Total Cancer Risk (Male + Female) for the Low- and High-Exposure Villages



Exposure-response relationships in the various townships were also investigated using a variant of the multiple regression method applied by Lamm et al. (2006). In this analysis, however, the non-linear relationship between cancer risk and age was explicitly recognized, and the analysis was conducted for township both "with" and "without" significant exposure-response relationships by Lamm et al.'s definition. First, male and female combined cancer mortality risks (bladder + lung) were regressed against the same non-linear age dependency incorporated into the Poisson model shown in Equation 5-2. That is, the following equation was fit to both the male and female cancer data from the various age groups in the low- and high-exposure villages:

$$\text{Risk (age)} = \exp(a_1 + a_2*\text{age} + a_3*\text{age}^2)$$

Then, the residuals from these regressions (the cancer risks with the effect of age removed) were regressed against estimated arsenic dose levels. The dose levels were calculated assuming a non-water arsenic intake of 10 μg/day for exposed and reference populations, which is consistent with the assumptions outlined in Section 5.3.5. The regressions were population- (person-year) weighted, in effect giving a linear regression of age-adjusted cancer risks versus arsenic dose. The results are shown in Table F-1.

The estimated dose coefficients for age-adjusted women's cancer risk (the linear "slope" of the relationship between cancer mortality, with the effect of age removed, and arsenic dose[9]) are positive and statistically significant for all combinations of townships. Coefficients for male age-adjusted cancer risk are positive and significant when all townships are included (although marginally significant when the reference population is excluded). Similarly, age-adjusted male cancer risk coefficients are positive and highly significant for townships 2, 4, and 6, with or without the reference population. In contrast, the arsenic dose-response coefficients for male age-adjusted cancer risks are negative, but very small and not significant, for townships 0, 3, and 5.

Table F-1. Coefficients from Linear Regressions of Age-Adjusted Cancer Risk versus Arsenic Doses for Townships Identified by Lamm et al. (2006)

Township Numbers	All Townships		Townships 2, 4, and 6		Townships 0, 3, and 5	
Reference Population[a]	Included	Excluded	Included	Excluded	Included	Excluded
Male Arsenic Dose coefficient (p-value)	0.035 (0.043)	0.032 (0.068)	0.092 (0.0002)	0.091 (0.001)	-0.0093 (0.787)	-0.002 (0.487)
Female Arsenic Dose (p-value)	0.12 (0.0002)	0.12 (0.0004)	0.11 (0.0001)	0.12 (0.0001)	0.14 (0.015)	0.13 (0.026)

[a]Southwest Taiwan

This analysis illustrates that, even using the less-desirable linear regression approach, when the cancer risk for the genders separated, and with proper age adjustment, female arsenic dose-response relationships are robust and significant for both village groups. For males, the arsenic dose-response relationships are significant when a reference population is included, except for townships 0, 3, and 5. As noted above, the rationale for analyzing groups of townships separately is questionable, as is the omission of a reference population. The results showing apparently insignificant associations between male cancer risks and arsenic exposure more than anything reflect the limitations of this less-than-optimal approach to risk modeling for these data.

F.4 Calculation of Arsenic-Related Cancer Risks for Low-Exposure Villages

Rather than stratify the Taiwanese population by township, a better way to test the significance of exposure-response relationships at low doses is to simply restrict the analysis to the villages with low arsenic water concentrations, but use the appropriate Poisson regression methodology. In the analysis summarized in Table F-2, the Poisson model shown in Equation 5-

[9] This approach is not particularly desirable from the standpoint of finding the best fit to the data because it restricts the effect of arsenic on cancer risk to being linear, and assumes that regression residuals are normally distributed, which is unlikely to be true. This approach has been used to illustrate that even using simple models, positive dose-response relationships can be detected in the data. Due to the different form of this model, the slope coefficients derived in this section are also not comparable to those shown in Tables 5-3 and 5-4.

2 was fit to data from the approximately one-half of subject groups with median arsenic drinking water concentrations less than 150 ppb. Lamm et al. (2006) considered this concentration to be a natural breakpoint because the median arsenic concentrations in the Wu et al. (1989) and Chen et al. (1992) population cluster into two groups, one group with 10-126 ppb and the other with 256-934 ppb. Arsenic "b" coefficients (the dose coefficients in the Poisson model) were estimated separately for lung and bladder cancer and for both endpoints combined, for men and women.

Table F-2. Arsenic Dose Coefficients for Study Populations with Median Well Water Arsenic Concentrations Less than 127 ppb

End Point	Arsenic "b" Coefficient (95% UCL, LCL)
Male Lung	85.7 (13.1, 172.1)
Male Bladder	586 (335, 877)
Male Combined	160 (83.4, 247)
Female Lung	615 (412, 836)
Female Bladder	2639 (2021, 3307)
Female Combined	924 (721, 1139)

For all of the endpoints, the arsenic dose coefficients are positive with lower confidence limits that are also positive.[10] This finding indicates that for population groups with water arsenic concentrations less than or equal to 126 ppb, the dose-response relationships are positive and statistically significant.

On the whole, the analyses presented in this section provide support for statistically significant dose-response relationships for arsenic-related cancer even in the population groups with relatively low exposures. When the data are artificially stratified, when no reference population is included, and when inappropriate statistical models are employed, it is possible to find insignificant or negative dose-response relationships for arsenic for some portions of the data. When appropriate models are used, however, the Taiwanese data show robust and significant positive associations between arsenic exposures and cancer risks for all of the endpoints analyzed, even in low-exposure groups. No evidence was found that either 400 ppb or 150 ppb represent "threshold" arsenic concentrations in drinking water below which cancer risks are not increased. Likewise, the analyses do not support the existence of a "village effect" related to the degree of dependence on artesian versus shallow wells.

[10] As in Section 5.3.8, the upper and lower confidence limits were calculated using profile likelihood; similar results are obtained using Bootstrap methods.