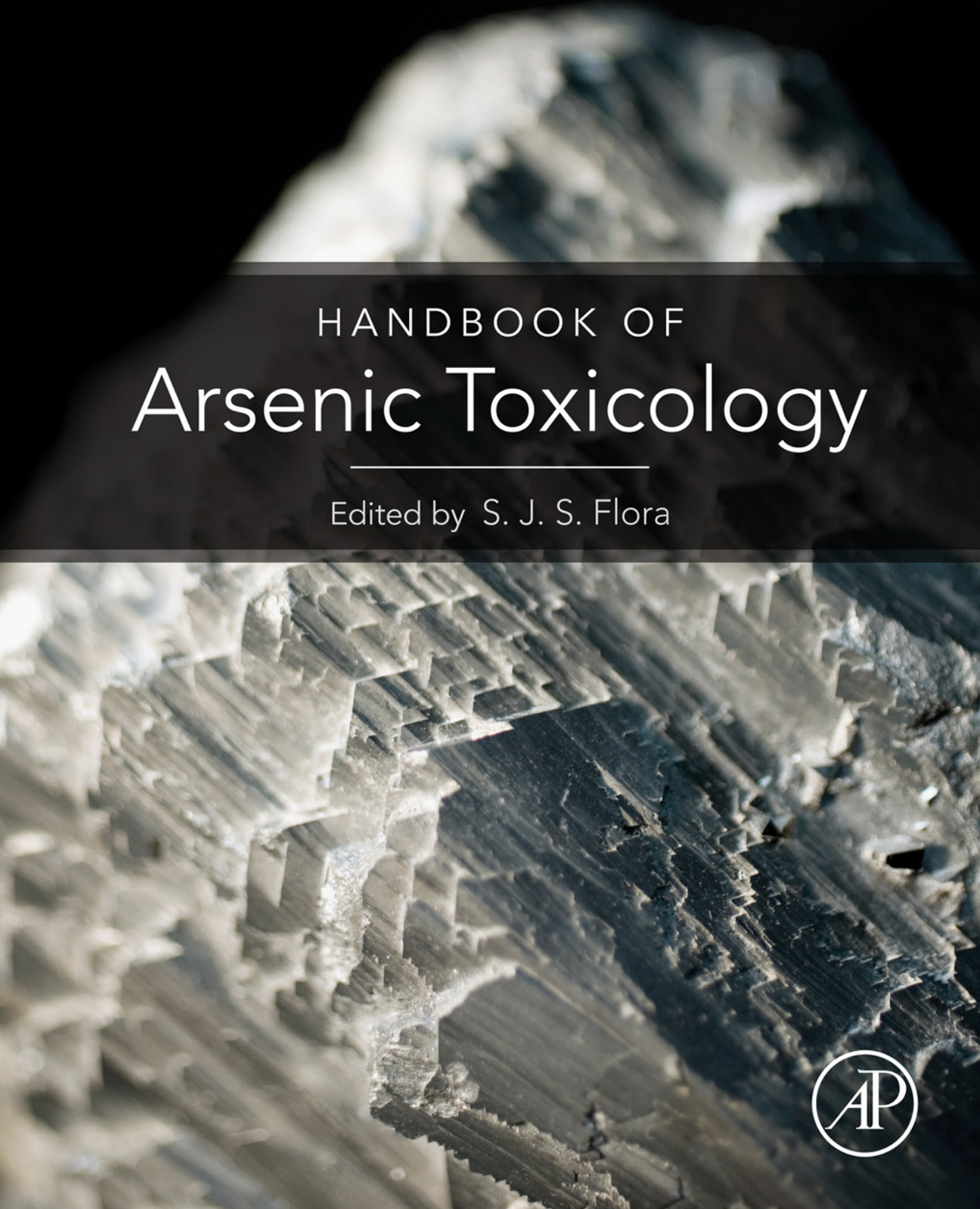
HANDBOOK OF
Arsenic Toxicology
Edited by S. J. S. Flora

Handbook of Arsenic Toxicology

In the memory of my mother
Paramjit
who always told me,
'do not be successful but be valuable'

Handbook of Arsenic Toxicology

Edited by

S. J. S. Flora

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Contents

Foreword

Handbook of Arsenic Toxicology is a significant addition to the literature dealing with the toxicology of this very important metalloid. The chapters in this book are relevant to arsenic exposure via drinking water, the usual route of exposure of an estimated 200 million people in the world – many in developing countries. In Bangladesh, arsenic exposure is also more common through ingestion of food. Populations with high intake of rice and vegetables may also be at risk.

Arsenic is a recognized human carcinogen and it can also cause cardiovascular and metabolic disease. It is a unique toxicant to the respiratory system – the only one following ingestion that is associated with malignant and non-malignant respiratory diseases.

Arsenic for many years was known as the King of Poisons, and the Poison of Kings. It was the poison of choice for homicides because no chemical test was available to identify and measure it. Arsenic trioxide, As_2O_3, was the highly favored poison form, because it is odorless, easily incorporated into food and drink, and before the development of the Marsh test, it was untraceable in the body. This chemical test was developed in about the 1830s after which time the number of homicides using arsenic compounds was greatly reduced.

The metabolism of inorganic arsenic has been carefully investigated during recent years. It was discovered surprisingly that inorganic arsenite was metabolized to the more toxic +3-methylated metabolites, methylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}). The latter compound is so toxic that it is wise to use a fume hood when working with it.

In his chapter on arsenical kidney toxicity, Bruce Fowler aptly points out that "the mitochondria are a major target organelle for arsenical toxicity with resultant inhibition of respiratory function, loss of ATP production, and generation of reactive oxygen species leading to altered cell signaling pathways, gene expression patterns, and induction of apoptosis."

Many, but not all, chapters of this book are written by the new generation of arsenic investigators. They bring to the forefront many newer thoughts and information about the toxicity of compounds of this toxic metalloid. One cannot ignore, however, the contributions by some of the well-known established authors. The first chapter by Dr. Flora on the chemistry, occurrence and exposure to arsenic compounds sets the tone as to what is to come. Dr. D.N. Guha Mazumder is another highly respected investigator with a lengthy list of significant publications dealing with the human clinical effects of exposure to arsenic compounds.

One also needs to remember other highly respected investigators like Dr. Mariano Cebrian. Although he is not an author of a chapter in this book, he was among the earliest investigators of such research. His first arsenic paper was published in 1983, entitled *Chronic Arsenic poisoning in the North of Mexico.* This early paper is very relevant because it dealt with a human population and answered some questions dealing with arsenic toxicity. The paper also presented

new questions concerning arsenic exposure and human health and stimulated the entrance of other investigators into this previously neglected area of scientific investigation. The chapters of the present book expand these questions and answers.

With the number of people at risk today, it is surprising that no useful treatment of chronic arsenic toxicity for large populations has become available. Prevention and/or limiting exposure remain the only approaches, and the information supplied in this book is a great step forward in this area.

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Preface

I have been a chemical toxicologist for nearly 35 years. In this time, I have studied and evaluated the toxicities of toxic metals and the health effects produced by human exposure to metals.

Arsenic, a naturally occurring metalloid, is ubiquitously present in the environment. Arsenic is ranked first among toxicants posing a significant potential threat to human health based on known or suspected toxicity. This naturally occurring metalloid is a known poison, a co-carcinogen, and in lower concentrations has been shown to cause damage to almost all major organs including liver, lungs, bladder and brain. Currently, the permitted concentration of arsenic in water is 10 μg/L (10 ppb). Yet, an estimated 100 million people worldwide are exposed to excessive amounts of arsenic via drinking water (in the ppm, not ppb, range). Many of these individuals obtain drinking water from unregulated sources (wells) or live in regions where arsenic levels are high, such as Bangladesh. Arsenic leaches from rock formations into water sources as the water table recedes, and hence exposure to high amounts of arsenic will continue to persist whilst the demand for clean water increases. This phenomenon particularly affects the Western region of the United States, where it is estimated that certain areas contain up to 3100 μg/L arsenic (31 ppm) in drinking water, on par with levels reported in Taiwan, China, Bangladesh and India.

Although the largest number of people affected worldwide by the arsenic contamination of drinking water are in Bangladesh, the problem is not unique to that area. As early as 1960, scientists reported the link between various forms of cancer and arsenic in drinking water in Taiwan. Communities in North and South America, Europe, Asia and Australia also face the problem of arsenic-contaminated drinking water. The problem of arsenic-contaminated groundwater is found in communities throughout Canada and western USA that use groundwater as their source of drinking water. It is now almost certain that arsenic contamination is a worldwide problem; however, some of the most affected regions lie in the flood plains of the great rivers of Bangladesh, Nepal, and West Bengal, India. In Bangladesh alone, seventy million people are impacted. Problems associated with drinking groundwater were first noticed in Bangladesh by healthcare workers in the early 1990s. While the World Health Organization (WHO) and the Environmental Protection Agency (EPA) regulate water sources of arsenic, lack of strict regulations on food, beverages, and air quality can lead to increased arsenic exposure. Ingestion of arsenic activates metabolic pathways for excretion, resulting in a number of metabolites, some of which are more potent and toxic than the originally ingested inorganic form of arsenic.

Inorganic arsenic exposure of humans, by the inhalation route, has been shown to be strongly associated with lung cancer, while ingestion of inorganic arsenic by humans has been

linked to a form of skin cancer and also to bladder, liver, and lung cancer. The EPA has classified inorganic arsenic as a human carcinogen. This explosion of information in the recent years reflects the vast increase in number of researchers studying about the mechanisms of action of arsenic. The specific knowledge of the chemistry, biochemistry, toxicology, and epidemiology of arsenic is far greater than that for any other environmentally-occurring chemical carcinogen.

This book really began over 20 years ago, when I was confronted with the first of scores of instances in Uttar Pradesh, Maharashtra, West Bengal and Bihar in India, where individuals exposed to arsenic subsequently developed symptoms and effects that could notably be explained by the known toxicological effects. In some instances the exposures led to effects far in excess of what would be expected. In others, effects were noted following exposures to extremely low levels of arsenic; in even more instances the body organs targeted were not those known to be impacted by arsenic. I carried out number of studies during these years; a few of them with my colleagues in West Bengal. These studies led to one serious concern: that we do not have a safe, specific and effective chelating drugs in this part of the world, and those drugs available in the developed countries are largely ineffective against arsenic toxicity.

As time progressed, I began to think that the task was too big and a solution remained elusive. The breakthrough came when I got a reprint from Prof. M.M. Jones, Vanderbilt University in which his group synthesized and evaluated the efficacy of number of di- and monoesters of meso 2,3-dimercaptosuccinic acid (DMSA) with limited success against cadmium intoxication. There was a small but an interesting note on the top of the reprints written in red ink, where he asked me to try these esters against arsenic. This led me to my interest in arsenic poisoning and in particular searching for a new chelating agent. A review of the literature and our research group's own studies highlighted the shortcomings with DMSA, DMPS and BAL, and it was then hypothesized that monoesters of DMSA might be a better option to treat cases of arsenicosis.

I have attempted to bring together as comprehensive a group of scientists as possible in assembling this book. Whilst at first glance, the literature on arsenic toxicology seems exhaustive and systematic, this is not the case. There is no comprehensive and in-depth analysis of its effects on major organs, preventive and therapeutic measures; additionally, there are a few new topics where not much work has been undertaken but could be of potential future interest. This book thus promises to provide a comprehensive coverage of arsenic and its toxic effects, including its toxicokinetics, mode(s) of action, effects on all major organs and medical countermeasures. To my knowledge, this book perhaps is the first in-depth analysis of data on toxicology, risk assessment, and management. Included in these 28 chapters are detailed reviews of the many important mechanistic aspects of arsenic.

Chapters1 and 2 provide an orientation and introduction to the subject of arsenic. The focus of these chapters is to provide an overview of various critical factors affecting arsenic chemistry, the natural and anthropogenic sources of exposure. The focus of Chapters 3 and 4 are risk assessment following arsenic exposure while Chapter 5 provides data for the removal of arsenic using activated alumina (AA) and modified AA adsorbents. Chapters 5 and 6 provide information on the general health effects of arsenic and the role of arsenic metabolites in

the toxic manifestation, respectively. Chapters 7–9 focus on various proposed modes of actions for arsenic, exposure pathways and toxicokinetics, various alterations in mediating genotoxic effects such as altered DNA repair, signal transduction, cellular proliferation, and altered DNA methylation. One of the major mechanisms of arsenic-induced toxic manifestation is oxidative stress. These chapters provide in-depth information regarding alterations at the biochemical level, detailed mechanisms of toxicity and oxidative injury, and the links between arsenic, oxidative stress and cancer. Chapter 10 discusses the gastrointestinal tract as one of the target organs of arsenic and a factor affecting its toxicity and the resultant risk assessments required. The authors suggest that arsenic species with higher toxicity degree than those ingested may appear in the intestinal lumen as a result of interactions with food components and from metabolism by enterocytes and micro biota. Also, the biotransformation may modulate arsenic intestinal absorption and therefore adverse effects.

Although arsenic impacts on the physiological cellular processes in numerous organ systems, the outcomes of its toxicity are usually first seen in the skin. The major focus of Chapter 11 is on skin manifestations from acute toxicity such as flushing, erythema, facial edema, acrodynia, urticarial, alopecia, loss of nails, and Mees lines visible on nails. The liver is the target organ of arsenic and many important various metabolizing reactions take place in liver, rendering it the most susceptible organ to any xenobiotic. Exposure to arsenic leads to various hepatic disorders, which has been discussed in Chapter 12. Arsenic, the only environmental toxicant has been linked to both malignant and non-malignant respiratory disease following ingestion, rather than inhalation, making arsenic a unique toxicant to the respiratory system. Chapter 13 suggests that chronic exposure to arsenic has been associated with the development of respiratory symptoms, impaired lung function and chronic lung disease. Chapter 14 provides an overview of information that arsenic disturbs various vital renal functions such as the excretion of nitrogenous waste products and maintenance of electrolyte balance, which leads to immediate effects on circulating blood and hence whole body. Chapter 15, 16, 17, and 19 make a strong argument for the potential role of arsenic in disrupting the normal functions of the central nervous system, thereby causing impairment of learning, concentration and short term memory. It also alters the release of various neurotransmitters. Since the brain is the most vital organ, it's important to fully understand the effect of arsenic intoxication and associated neuropathologies, which are discussed here.

Arsenic accumulates in the urinary bladder epithelium, causing activation of specific signaling pathways, leading to increased cell proliferation and increased incidence of urinary bladder tumors. Such manifestations are discussed in Chapter 18. Chronic arsenic exposure induces high oxidative stress which affect the structure and function of the cardiovascular system, resulting in cardiac anomalies such as arrhythmia and atherosclerosis. Moreover, newer technologies like the use of pluripotent stem cells could provide a better opportunity to study the effects of arsenic in a cell type-specific manner. Chapter 18 also briefly discusses emerging technologies that provide a new dimension to evaluate diseases.

Immunosuppression caused by arsenic is another major cause of cancer development in humans. Chronic exposure to arsenic has been shown to significantly compromise the host response to infection, thereby leading to development of fatal conditions. Chapter 21 provides

comprehensive information about such immunosuppressive effects. There have been many reports exhibiting significant relationship between arsenic exposure and adverse reproductive health outcomes such as stillbirths, low birth-weight, childhood stunting, neural tube defects and under-weight children. Chapter 22 specifically deals with various adverse effects of arsenic on reproductive system and potential mechanisms. Arsenic is classified as a human carcinogen and has been linked with various malignancies such as skin, lungs and liver. Chapter 23 specifically explores the association between arsenic exposure and cancer development and current mechanisms that have been proposed to contribute to such neoplastic processes. An association between chronic arsenic exposure and Type 2 diabetes is still inconclusive. Chapter 24 deals with the analysis of 27 studies including 15 cross-sectional, 4 case-control and 3 cohort studies. Authors conclude that their meta-analysis suggests that chronic arsenic exposure is likely to increase the risk of Type 2 diabetes. Chapter 25 attempts to highlight the advancement made so far in the development of arsenic biosensors – analytical devices that have high sensitivity, portability, small sample requirements and are easily used for quantitative and qualitative monitoring of various analytes.

Both chemical and synthetic medical countermeasures have been used against chronic arsenic poisoning. Chelating agents such as Dimercaprol and DMSA were found to be quite effective; however they are compromised with a few shortcomings. Extensive research in future is required to establish an effective drug against chronic arsenicosis. Also development of specific biomarkers is an important strategy. Chapter 26 focuses on available arsenic antidotes, their clinical uses, their drawbacks, side effects and the new developments in the area. Chapter 27 deals with recent progress on the mechanisms of arsenic uptake, toxicity, and detoxification in microbes and in plants. Chapter 28 provides information about arsenic concentration and biogeochemical cycle in marine environment (marine animals, bacteria, phytoplankton, etc).

I intend that this book be useful for health scientists, including nutritionists and dietitians, pharmacologists, public health scientists, and colleges, epidemiologists, health workers and practitioners, agriculturists, botanists, healthcare professionals of various disciplines, policymakers, and marketing and economic strategists. It is also designed for teachers and lecturers, undergraduates and graduates.

Finally, I am deeply indebted to all authors for their sincere and dedicated contributions to this book. I have tried to get all contributions written by specialists in the respective area; however, we have missed a few well-known names who expressed their inability due to various professional and personal commitments. The editor views these contributions as excellent summaries by some of the world's outstanding scientists/ researchers practitioners of arsenic toxicology and hope the readers will find them highly enlightening and useful. A special thanks to Molly McLaughlin, the project manager at Elsevier/Academic Press, who was a big support throughout the process and provided vital input in the preparation of this book.

Swaran J.S. Flora

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I am grateful to all of the contributors for taking the time and effort to complete these chapters in a thorough yet timely manner. In addition to the individual contributions of each of our chapter authors, who are recognized leaders in their respective areas of specialization, many other scientists from around the world have contributed extensively to our understanding of arsenic biochemistry and toxicology. I would like to acknowledge the work of all these contributors. Their efforts made it much easier to do my job of compiling and editing this book.

Many of us who are involved in research about arsenic find that our work is not just fascinating and compelling, but are drawn to this metalloid for its public health significance in the developing world. The daily exposure to arsenic in different parts of the world are manifold. In some countries, human exposure can exceed 3000 ppb and in parts of the developed world, the exposure is much less than 10 ng per day. Given this situation it is hoped that the understanding of basic mechanisms of the action of arsenic will lead to the design of effective prevention strategies for both the developed and developing world.

In addition to the contributors, several other scientists have also provided scientific substance for this book and they should be congratulated for their efforts, I also thank Molly McLaughlin, Editorial Project Manager at Elsevier/Academic Press for her extensive and excellent administrative support and unfailing help in the development of this book from its inception. This book would not have been physically possible without her tireless work and extraordinary skills. I am also grateful to the many people at Elsevier/Academic Press for their assistance, patience, and understanding in working with me to complete this book in a timely manner. A heartfelt thanks is also extended to Melissa Read at Elsevier/Academic Press for the fine production work.

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I dedicate this book to Gurpyari, my life-long companion, for her unconditional support in many projects. I also dedicate this book to my daughter Preeti, my son Ujjwal, and brother Govinder for providing me with reasons to fight for the protection of the environment and especially to my parents for their support during my studies and thereafter. I also dedicate this book to my mentor Dr S.K. Tandon (in memoriam) for his guidance, foresights and for opening my eyes to the science of toxicology.

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1

Arsenic: Chemistry, Occurrence, and Exposure

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CHAPTER OUTLINE

1.1 Introduction

Arsenic, the king of poisons, has influenced the human population more than any other element or toxic compound for thousands of years. Today, millions of people are being chronically exposed to elevated doses of arsenic from air, food, water, and soil. Throughout the history of human progress, arsenic has been seen as a bizarre and frightful element. Toxic effects of arsenic are highly prevalent in both developed and developing countries. Arsenic toxicity has become a principal concern owing to the escalating contamination of air, water, and soil. It has the ability to readily change its oxidation state and bonding configuration, thus showing diverse chemical behavior in the environment and forming large numbers of organic and inorganic compounds. Specific electronic configurations of valence shells with filled s orbitals and half-filled p orbitals enable arsenic to easily donate electrons and overlap in covalent bonds. Naturally, arsenic forms bonds with oxygen and sulfur and generates large

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numbers of oxides and sulfides. It is also capable of forming large numbers of bio-molecules as it forms stable bonding with the methyl group. The peculiar chemistry of arsenic is the basis for its dual action as a toxin and as a curative.

Drinking water and contaminated soils are the major means by which arsenic gains its entry into the food chain. Most of the arsenic compounds are readily soluble in water and so can easily enter water bodies such as rivers, lakes, and ponds, and by surface runoff. The main pathways of human exposure to arsenic are ingestion of drinking water, consumption of food, and inhalation of air. Among all sources, drinking water has been reported to be the main route of arsenic exposure around the globe [1,2]. Occupational exposure to arsenic is also very common in individuals working in wood preservation, desiccant, chemical warfare agent, pigment, drug and arsenic-based pesticide industries, and those involved in smelting and mining operations and residing in the vicinity of mining areas.

Prolonged exposure to arsenic leads to various dermatological, respiratory, neurological, and reproductive disorders and it is therefore referred to as a carcinogen and mutagenic agent [3,4]. The US Department of Health and Human Services in its 9th Report on Carcinogens listed arsenic compounds as human carcinogens. Arsenic exposure may cause severe health manifestations including cancers, melanosis (hyperpigmentation or hypopigmentation), hyperkeratosis (hardened skin), blackfoot disease (peripheral vascular disorder), gangrene, diabetes mellitus, hypertension, ischemic heart disease, etc. [5,6].

Due to increasing health concerns and adverse effects of arsenic on humans, US EPA in 2002 reduced the maximum permissible limit of arsenic in drinking water from 50 to 10 μg/L [7]. Despite a number of corrective and preventive measures, the increase of arsenic contamination in ground water continued to develop with the addition of new areas to the list of arsenic-contaminated regions. In view of the global health issues associated with arsenic exposure, it has become essential to understand arsenic sources, geochemistry, interaction with water, and various mechanisms associated with arsenic release into the environment. This chapter deals with the occurrence of arsenic in air, water, and soil, and its chemistry in the entire medium along with its global distribution. The chapter also discusses a number of treatment methodologies for the effective removal of arsenic from ground water and to reduce the global arsenic burden.

1.2 Chemistry of Arsenic

1.2.1 Origin and History

Arsenic has been known since ancient times in its sulfide form. The Greek philosopher Theophrastus knew about two arsenic minerals: bright yellow orpiment (As_2S_3) and red colored realgar (As_4S_4). Greek historian Olympiodorus of Thebes (5th century AD) was the first to obtain white arsenic (As_2O_3) by heating arsenic sulfide. The discovery of the element arsenic is attributed to Albertus Magnus, a German philosopher in the 1200s, who was the first to report the metallic behavior of arsenic. *De Mineralibus* described pure arsenic being obtained by the heating of orpiment with soap. Arsenic trioxide (As_2O_3), a by-product of copper refining, when mixed with olive oil and heated gives arsenic metal. Chinese scientist Tsao Kan-Mu studied

toxicity of arsenic compounds in the 1500s during the Ming dynasty and mentioned their use as pesticides in rice fields. The elemental name is believed to come from the Greek word *arsenikos* meaning potent. Arsenic ranks as the 20th most common element in Earth's crust, 14th in the sea, and 12th in the human body.

Humans have been using arsenic since ancient times both as a poison and a curative. It has also been used in pyrotechnics, metallurgy, warfare, and pigmentation, and for decoration. One of the most popular oxides of arsenic (arsenic trioxide) is a tasteless, odorless, white powder used in the past as a chemical warfare agent; however, green colored copper acetoarsenate was traditionally used in wallpapers as a pigment [8].

1.2.2 Atomic Structure and Bonding

Arsenic ranks 33rd in the periodic table, as part of the elements in Group 15, being a member of the nitrogen family. Its atomic number is 33 and its atomic weight is 74.921, placing it as heavier than iron, nickel, and manganese but lighter than silver, lead, or gold. The most stable and non-radioactive isotope of arsenic is arsenic-75 (^{75}As) with 33 protons and 42 neutrons inside the nucleus surrounded by 33 electrons in different energy shells. Other unstable and radioactive isotopes of arsenic are listed in Table 1–1. The isotopes are converted to stable elements by electron capture, electron emission, positron emission, neutron emission, and internal transition [9]. The electronic configuration of the stable form of arsenic, As(0), can be written as shown below to illustrate how the presence of five valence electrons allows arsenic to participate in chemical bonding, with an empty p orbital for electron occupation; this can also be illustrated by the electron dot model of arsenic (Figure 1–1).

$$1s^2\,2s^2\,2p^6\,3s^2\,3p^6\,3d^{10}\,4s^2\,4p^3$$

The electronic configuration suggests that the first shell has two electrons, the second has eight electrons, the third has 18 electrons, while the fourth with five electrons is considered as

Table 1–1 Various Isotopes of Arsenic with their Decay Properties

Isotope	Mass	Mode of Decay	Half-life
^{68}As	67.9368	EC to ^{68}Ge	2.53 min
^{69}As	68.93228	EC to ^{69}Ge	15.2 min
^{70}As	69.93093	EC to ^{70}Ge	52.6 min
^{71}As	70.927114	EC to ^{71}Ge	2.72 d
^{72}As	71.926753	EC to ^{72}Ge	26.0 h
^{73}As	72.923825	EC to ^{73}Ge	80.3 d
^{74}As	73.923829	β^- to ^{74}Se; EC to ^{74}Ge	17.78 d
^{76}As	75.922394	β^- to ^{76}Se	26.3 h
^{77}As	76.920648	β^- to ^{77}Se	38.8 h
^{78}As	77.92183	β^- to ^{78}Se	1.512 h
^{79}As	78.92095	β^- to ^{79}Se	9.0 min

Abbreviations used: min = minutes; d = days; h = hours; EC = electron capture; β^- = electron emission; As = arsenic; Ge = germanium; Se = selenium.

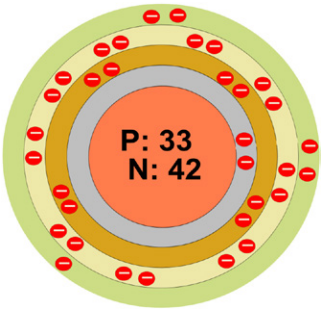

FIGURE 1–1 Electron dot model of arsenic (^{75}As) depicting number of protons (P) and neutrons (N) inside the nucleus and electrons (−) in various energy shells.

incomplete. In the fourth shell, the s orbital is completely filled and the p orbital is half filled, and therefore arsenic shows four common redox states: −3, 0, +3, and +5. Oxidation state −3 arises by the addition of three more electrons to the p orbital in order to complete the total of six electrons in this orbital. Elemental arsenic (^{0}As) equally shares its three electrons present in the 4p orbital with the other three surrounding arsenic atoms in a trigonal pyramidal structure and appears as a brittle gray metal. The +3 oxidation state forms when three electrons of the 4p orbital become more attracted towards a non-metal, usually oxygen or sulfur, whereas arsenic exhibits the +5 oxidation state when all the five electrons of the 4s- and 4p orbitals become more associated with the non-metal.

Table 1–2 lists selected physical properties of elemental arsenic, which suggest that the electronegativity of arsenic is greater than that of nitrogen and similar to that of phosphorus. Arsenic has a greater ability to lose electrons (oxidation potential) as compared to nitrogen and phosphorus, which increases the cationic character of arsenic and it thus can easily exhibit +3 and +5 oxidation states. Variable oxidation states of arsenic suggest that it can combine with many elements to form covalent compounds; however, in nature it most commonly bonds to oxygen and sulfur. Arsenic can occupy electrons in its bonding and anti-bonding orbitals and can exhibit the properties of ligands by sharing its valence electrons. Thus, it shows the ability to shift from an electropositive state (oxo-anions) to an electronegative state (metal arsenides). Arsenic in its pure form is a brittle, gray metal but in nature it is found with other metals, such as iron, copper, silver, and nickel as oxides and sulfides.

Arsenite is known to be more toxic and 25–60 times more mobile than arsenate [10]. In both oxidation states, it can combine with methyl groups to form organic species. Common organic species of arsenic are monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). However, their natural occurrence is low compared to inorganic forms. Inorganic arsenicals are more toxic than organic arsenicals and the trivalent oxidation state is more toxic than the pentavalent oxidation state. Arsenic toxicity is also related to the rate at which it is metabolized and the degree to which it accumulates in the tissues. Generally, the As toxicity pattern is $AsH_3 > As^{3+} > As^{5+} >$ RAs-X.

Table 1–2 Physical and Electronic Properties of Elemental Arsenic

Property	Value
Atomic number	33
Atomic mass	74.9216 g/mol
Appearance	Gray, brittle, non-metal flakes
Electronegativity according to Pauling	2.0
Density	5.7 g/cm^3 at 14°C
Molar volume	13.08 cm^3/mole
Melting point	814°C (36 atm)
Boiling point	615°C (sublimation)
Specific heat	0.33 J/g K
van der Waals radius	0.139 nm
Ionic radius	0.222 nm (−2) 0.047 nm (+5) 0.058 (+3)
Isotopes	8
Electronic shell	[Ar] 3d^{10} 4s^2 4p^3
Energy of first ionization	947 kJ/mol
Energy of second ionization	1798 kJ/mol
Energy of third ionization	2736 kJ/mol
Standard potential	−0.3 V (As^{3+}/As)
Enthalpy of atomization	301.3 kJ/mole @ 25°C
Enthalpy of fusion	24.44 kJ/mole
Enthalpy of vaporization	34.76 kJ/mole
Electrical conductivity	0.0345 10^6/cmΩ
Thermal conductivity	0.502 W/cmK

1.2.3 Arsenic Oxidation and Reduction

Oxidation and reduction of arsenic mainly depend upon redox potential and pH conditions. In natural water bodies, arsenic predominantly exists as oxyanions of trivalent arsenite (As^{III}) or pentavalent arsenate (As^{V}). Under oxidizing conditions, the pentavalent form of arsenic predominates and the oxyanions' existence depends upon pH—at a pH less than 6.9, $H_2AsO_4^-$ predominates, while at a higher pH, $HAsO_4^{-2}$ becomes dominating. Under reducing conditions at a pH less than 9.2, the neutral trivalent arsenic species H_3AsO_3 exists, which dissociates to form anions under high pH conditions only. Most often, more trivalent arsenic than pentavalent arsenic is found in reducing groundwater conditions, whereas the pentavalent arsenic prevails under oxidizing groundwater conditions. The stabilities of various arsenic species under different pH conditions are shown in Figure 1-2, suggesting the significance of pH in the formation and occurrence of various forms of arsenic in natural water bodies. The dissociation reactions and corresponding equilibrium constants of H_3AsO_4 and H_3AsO_3 are as shown below:

Arsenite (As^{III})

$$H_3AsO_3 \leftrightarrow H_2AsO_3^- + H^+ \quad \text{pKa: 2.24} \tag{1.1}$$

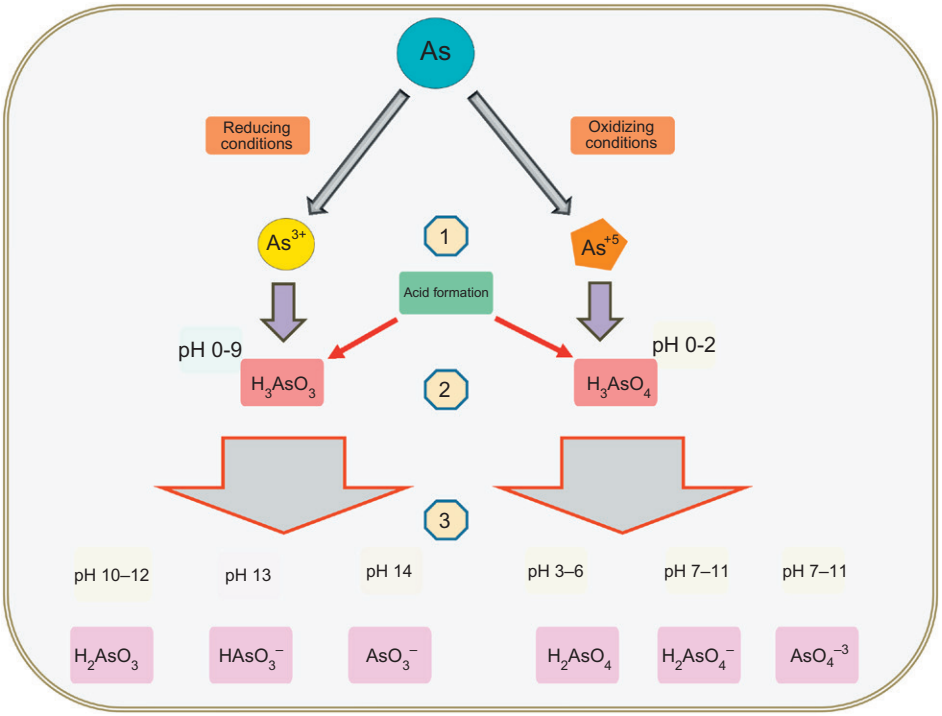

FIGURE 1–2 Pictorial depiction of (1) oxidation of arsenic under oxidizing and reducing conditions, (2) formation of acids by $As(^{+3})/As(^{III})$ and $As(^{+5})/As(^{V})$ under different pH conditions, and (3) dissociation of acids to oxyanions under various sets of pH conditions.

$$H_2AsO_3^- \leftrightarrow HAsO_3^{-2} + H^+ \quad \text{pKa: 6.69} \tag{1.2}$$

$$HAsO_3^{-2} \leftrightarrow AsO_3^{-3} + H^+ \quad \text{pKa: 11.5} \tag{1.3}$$

Arsenate (As^V)

$$H_3AsO_4 \leftrightarrow H_2AsO_4^- + H^+ \quad \text{pKa: 9.2} \tag{1.4}$$

$$H_2AsO_4^- \leftrightarrow HAsO_4^{-2} + H^+ \quad \text{pKa: 12.1} \tag{1.5}$$

$$HAsO_4^{-2} \leftrightarrow AsO_4^{-3} + H^+ \quad \text{pKa: 13.4} \tag{1.6}$$

1.2.4 Arsenic Methylation

Methylation is a process of adding one or more methyl (-CH_3) groups to the chemical species. Bio-methylation plays an important role in the metabolism of inorganic arsenic and the mechanism involves alternate steps of two-electron reduction followed by oxidative addition

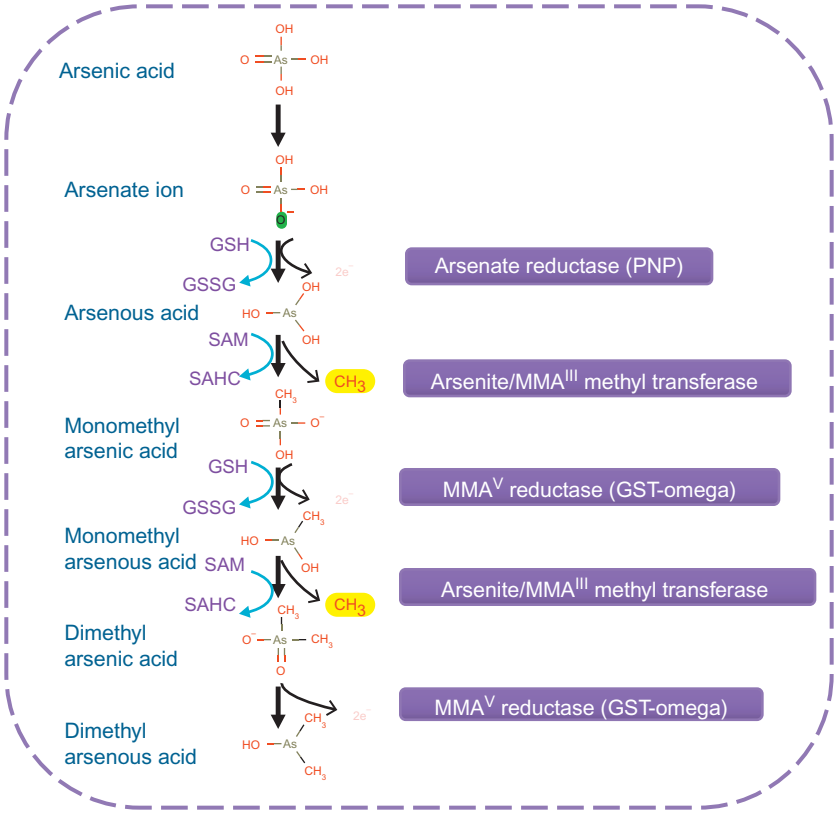

FIGURE 1–3 Biotransformation of inorganic arsenic in the mammalian system. Abbreviations: SAM—S-adenosylmethionine; SAHC—S-adenosylhomocysteine; GSH—reduced glutathione; GSSG—glutathione disulfide; PNP—purine nucleoside phosphorylase.

of a methyl group known as oxidative methylation [11]. Arsenate reduction can also occur non-enzymatically under low oxygen concentration or at pH 2 or lower. S-Adenosylmethionine, a methyl donor, methylates inorganic arsenic into monomethylarsonic acid (MMA) and then to dimethylarsinic acid (DMA). This reaction is catalyzed by methyltransferases in the presence of glutathione, which acts as a co-factor. It has been reported that arsenic methylation does not depend upon arsenic concentration in water [12]; rather it is assumed to be a detoxification mechanism of arsenic as, in organic form, arsenic cannot ionize readily to interact with bio-molecules and becomes less available for chemical and bio-chemical reactions [13]. The methylation process of arsenic via alternate reduction and oxidative methylation is depicted in Figure 1–3.

1.2.5 Historical and Modern Applications of Arsenic

Burning of coal in thermal power plants and disposal of fly ash, long-term mining and smelting of the sulfide ores, runoff from mine tailings, and application of pesticides and herbicides release

huge amounts of arsenic in to the biosphere. Additionally, arsenic is also used in the production of semiconductors, lead-acid batteries, and pesticides and herbicides, in the glass industry and copper refining industry, and in the hardening of metal alloys. Use of arsenic in wood preservation is very common and has increased significantly in the last few decades [14]. Wood may deteriorate by the attack of insects, fungi, bacteria, and animals, but can be protected by impregnating with CCA with the composition CuO (18.5%), Cr_2O_3 (47.5%), and As_2O_3 (18.5%). At one time, arsenic compounds such as lead arsenate, calcium arsenate, and sodium arsenate were used as pesticides for debarking trees, to control ticks, fleas, and lice, and in aquatic weed control. However, these applications have been banned due to the toxic effects of arsenic and later public awareness about food safety and environmental contamination [15].

1.3 Arsenic Minerals and Compounds

A mineral is a naturally occurring crystalline and inorganic solid. Arsenic minerals primarily include elemental arsenic, arsenolamprite, para-arsenolamprite, and nearly 320 other inorganic compounds. Arsenic minerals can be classified under five categories: elemental arsenic, arsenides, arsenosulfides, arsenites, and arsenates. Arsenides and arsenosulfides are commonly found associated with anoxic hydrothermal ore deposits, and with metamorphic and igneous rocks. When these minerals come in contact with water and oxygen, they are rapidly converted into arsenites and arsenates. Examples of arsenic minerals falling under the five categories are shown in Table 1–3.

1.3.1 Arsenosulfides

Arsenopyrite (FeAsS), orpiment (As_2S_3), and realgar (AsS/As_4S_4) are the most common arsenic sulfide minerals, occurring primarily in hydrothermal and magmatic ore deposits. Arsenic is commonly found in sulfide-bearing mineral deposits; especially with gold mineralization. Orpiment, due to its golden color, was used in ancient times as a pigment and dye, while realgar was a common red pigment for paints and dyes. Realgar decomposes in air to a yellow-orange compound para-realgar; consequently, old unrestored paintings have a yellow-orange tinge over a red color. Arsenic can exist in sulfide minerals either as a dominant mineral-forming element or as an impurity. Arsenic release into nature is a slow process as a result of mineral weathering; however, physical forces such as grinding, crushing, and pulverization from mining activities greatly increase the release rate. Arsenosulfides also combine with transition metals such as Co, Ni, and Cu to form a variety of other sulfides and sulfosalts [16].

In arsenic sulfides, elemental arsenic and sulfur are covalently bonded with different arrangements of As-S and As-As units. In arsenopyrite, each Fe atom is octahedrally coordinated by three As and three S atoms through edges and corners. In dimeric form ($[As\text{-}S]^{-2}$) each As or S atom is tetrahedrally coordinated to three Fe atoms and one S-As atom. In the presence of water and oxygen, the arsenic present in arsenopyrite rapidly oxidizes to As^{+2}, As^{+3}, As^{+5}, and a precipitate in the form of scorodite ($FeAsO_4{\cdot}2H_2O$) or amorphous Fe(III) arsenate [17].

Table 1–3 Examples of Arsenic Minerals Classified under the Five Categories

Group	Mineral	Formula
Elemental arsenic		
	Native arsenic	As
	Arsenolamprite	As
	Paraarsenolamprite	As
Arsenic sulfides		
	Arsenopyrite	FeAsS
	Cobaltite	CoAsS
	Orpiment	As_2S_3
	Realgar	AsS/As_4S_4
	Gersdorffite	NiAsS
	Enargite	Cu_3AsS_4
Metal arsenides		
	Domeykite	Cu_3As
	Lollingite	$FeAs_2$
	Nickeline or niccolite	NiAs
	Rammelsbergite	$NiAs_2$
	Sperrylite	$PtAs_2$
	Safflorite	$CoAs_2$
Arsenite		
	Arsenolite	As_2O_3
	Claudetite	As_2O_3
	Sodium arsenite	$NaAsO_2$
	Leiteite	$ZnAs_2O_4$
	Reinerite	$Zn_3(AsO_3)_2$
	Gebhardite	$Pb_8(As_2O_5)_2OCl_6$
Arsenate		
	Johnbaumite	$Ca_5(AsO_4)_3(OH)$
	Mimetite	$Pb_5(AsO_4)_3(Cl)$
	Austinite	$CaZn(AsO_4)(OH)$
	Scorodite	$FeAsO_4 \cdot 2H_2O$
	Erythrite	$Co_3(AsO_4)_2 \cdot 8H_2O$

In realgar and its polymorphs As-As and S-S, dimers form a discrete molecular cage-like structure in which units are connected by van der Waals forces. Polymorphs of realgar, alacrinite (As_8S_9), and amorphous arsenic sulfide can also occur in hydrothermal deposits, volcanic emissions, intrusive igneous rocks, and hot springs. Realgar exists as high and low temperature polymorphs and at about 240°C α-realgar converts to β-realgar. α-Realgar in exposure to oxygen and sunlight converts to another polymorph, para-realgar.

In orpiment (As_2S_3), molecular units are present in chain form and connected by bridging S atoms and cross-linked by van der Waals attraction forces. Both the minerals realgar and

orpiment are stable over a wide range of temperature; however, orpiment possesses a greater stability range due to reduced sulfur fugacity [18].

Oxidation of arsenopyrite is the widespread mechanism for the distribution of arsenic into the environment. Arsenopyrite is formed under high temperature and a reductive environment, such as areas around buried plant roots or other nuclei of decomposing organic matter. Pyrite readily oxidizes in aerobic conditions with the formation of iron oxides and traces of arsenic. Arsenic is also found associated with phosphate minerals; however, the concentrations are less than those of oxide and sulfide minerals. By substituting Si^{4+}, Al^{3+}, Fe^{3+}, and Ti^{4+} arsenic can also be found associated with many other minerals; however, the concentrations are comparatively less.

Other arsenosulfides of transition metals are also known and the most common are enargite (Cu_3AsS_4), cobaltite (CoAsS), and gersdoeffite (NiAsS). Enargite is orthorhombic thioarsenate where each arsenic atom is coordinated with four sulfur atoms as $(AsS_4)^{-3}$ [19]. Below 300°C enargite is converted to its polymorph luzonite with tetragonal geometry.

1.3.2 Metal Arsenides

A large number of metal arsenides are known and generally can be considered as metal alloys with the formula MAs_n, where M is a metal and $n = 1$, 2, or 3. In these compounds arsenic exists in a negative oxidation state; however, bonding is strongly covalent and the compounds are semiconducting in nature. The well-known semiconductor is GaAs while other arsenides can be formed with Fe, Co, Ni, and Cu. Naturally occurring common metal arsenides are domeykite (Cu_3As), lollingite ($FeAs_2$), nickeline or niccolite (NiAs), nickel-skutterudite (Ni,Co)As_3, rammelsbergite ($NiAs_2$), safflorite ($CoAs_2$), and sperrylite ($PtAs_2$).

In skutterudite, each Co atom or any other divalent metal atom is surrounded by six arsenic atoms in octahedral mode, while in lollingite each Fe atom is coordinated with six arsenic atoms in orthorhombic geometry [20]. Another important metal arsenide is GaAs, which is synthetic in nature and each arsenic atom is coordinated by four Ga atoms [21]. It has various applications as a semiconductor in solar cells, light emitting diodes (LEDs), laser windows, etc. Other synthetic arsenides are indium (III) arsenide (InAs) and mixed indium gallium arsenide (InGaAs) with similar applications.

1.3.3 Arsenites

The most common arsenites are polymorphs of As(III) oxides, arsenolite (As_2O_3, isometric), and claudetite (As_2O_3, monoclinic), with similar thermodynamic stability, while claudetite is thought to be slightly more stable at standard conditions [18]. These oxides occur naturally as secondary weathering products of arsenic sulfides and industrially during combustion of arsenic-bearing minerals and coal in gaseous form, which upon condensation gives the powdered form of white As_2O_3. The structure of arsenolite consists of $<As_4O_6>$ cages, while in claudetite each arsenic atom is surrounded by three oxygen atoms in a trigonal pyramidal structure. Arsenic trioxides are moderately soluble in water and have been used industrially as pigments

and pesticides for a long time. The solubility of arsenite salts is largely due to the type of cation in the compound. The most soluble are the alkali arsenites, whereas metal arsenites are the least soluble.

1.3.4 Arsenates

Arsenates are usually considered a subclass of the phosphate mineral group owing to the similarity in size and charge of the phosphate and arsenate anionic unit. In arsenate, the unit $(AsO_4)^{-3}$ is either tetrahedrally or octahedrally coordinated to transition metal or alkaline earth metal with anions (OH^-, Cl^-, and F^-) for charge balancing. Similarly to phosphate minerals arsenate minerals also occur in a variety of soil and oxidized environments with a range of waters of hydration. During the period of the 1870s – 1970s arsenates of copper, lead, and calcium were extensively used as herbicides and insecticides and significantly increased the environmental burden of arsenic. As_2O_5 is rarely found in nature; however, it was synthesized and widely used at one time in the production of copper arsenate, a wood preservative. As_2O_5 decomposes at 315°C to As_4O_6 and O_2. In water it can dissolve to form various species, H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{-2}$, and AsO_4^{-3}, depending upon the pH of the solution.

1.4 Organoarsenicals

In organoarsenic compounds arsenic combines easily with carbon to form various organic compounds with one or more As-C bonds and is widely used in agriculture and plant protection agents. A wide variety of organoarsenic compounds are found naturally in the environment as a consequence of bio-methylation and other biosynthetic pathways. They can be grouped into aliphatic and aromatic organoarsenic compounds. The most common classes of natural organoarsenic compounds are methylated forms of arsenite and arsenate. These can be generated by replacing a hydroxyl (-OH) ligand by a methyl group ($-CH_3$). The structures of major organoarsenic compounds are depicted in Figure 1–4. By the replacement of one or more methyl or hydroxyl groups around arsenic by other organic moieties such as sugars, lipids, or cyclic groups, a wide variety of arsenic-bearing organic compounds are generated. Major aliphatic organoarsenic compounds methane-arsenic acid and dimethylarsenic acid were at one time used as fungicides, herbicides, and desiccants. On the other hand, aromatic organic arsenicals such as arsanilic acid have been used as corrosion inhibitors for iron and steel, and as additives for motor fuel, agricultural bactericides, herbicides, and fungicides. In organoarsenicals arsenic can exist in three oxidation states: As(I), As(III), and As(V). Compounds of oxidation state As(I) feature three bonds to As but only an As-As single bond and are used in antisyphylitic drugs such as Salvarsin and Neosalvarsan. Arsenic (V) compounds show the general formula $RAsO(OH)_2$ or $R_2AsO(OH)$ (R = alkyl or aryl). Monomethylarsonic acid ($CH_3AsO(OH)_2$) and dimethylarsinic acid, commonly known as cacodylic acid ($(CH_3)_2AsO_2H$), figure prominently throughout the chemistry of organoarsenic compounds. Arsenic (III) compounds are prepared by alkylation of $AsCl_3$ and its derivatives

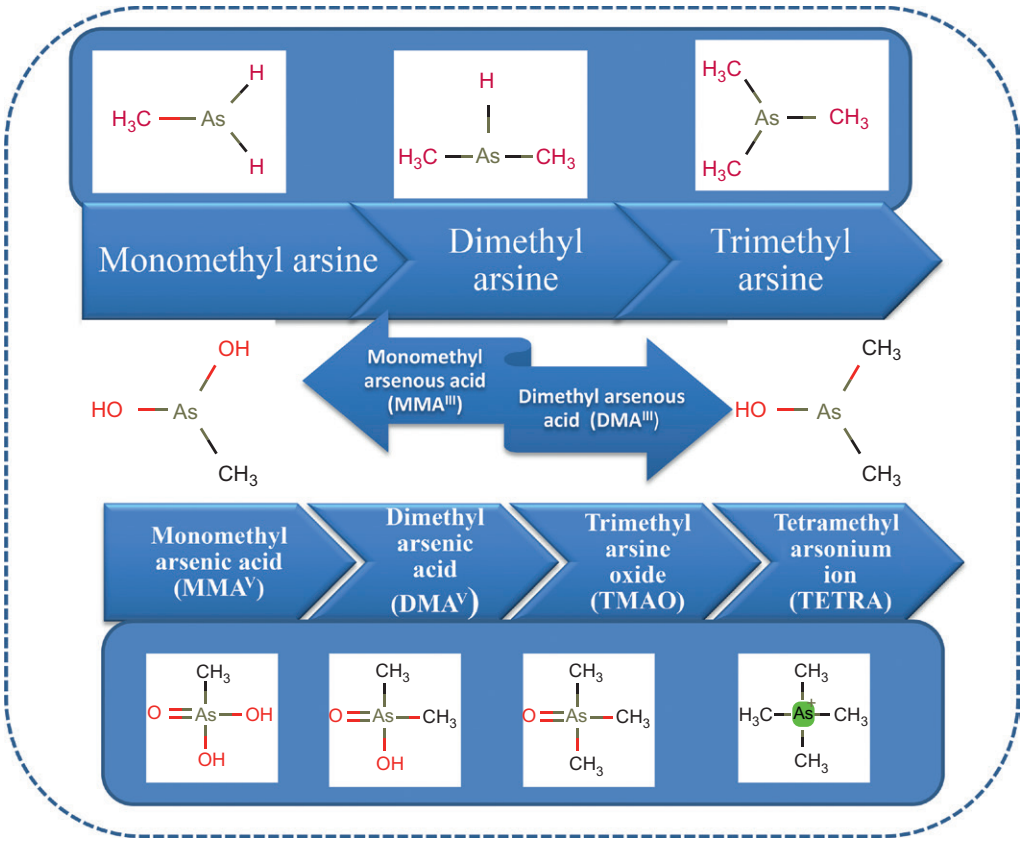

FIGURE 1–4 Structure of major methylated organoarsenic compounds.

using organolithium and Grignard reagents [22], e.g., methylarsenic dichloride (CH_3AsCl_2), dimethylarsenic chloride ($(CH_3)_2AsCl$), and trimethylarsine ($(CH_3)_3As$). Reduction of these chloride derivatives produces hydrides, such as dimethylarsine ($(CH_3)_2AsH$) and methylarsine (CH_3AsH_2). Trimethylarsine and triphenylarsine are symmetrical organoarsenic (III) compounds, commonly used as ligands in coordination chemistry.

1.4.1 Organoarsenicals in the Food Chain

Inorganic arsenic and its compounds enter the food chain and metabolize through a methylation process known as bio-methylation in the presence of specific enzymes related to vitamin B_{12}. The organic compound arsenobetaine is found in some marine foods such as fish and algae, and is considered a non-toxic form of organoarsenic compound. Carbohydrates bound to arsenic known as arsenosugars are found especially in seaweeds, and if lipid is bound to arsenic an arsenolipid is formed [23].

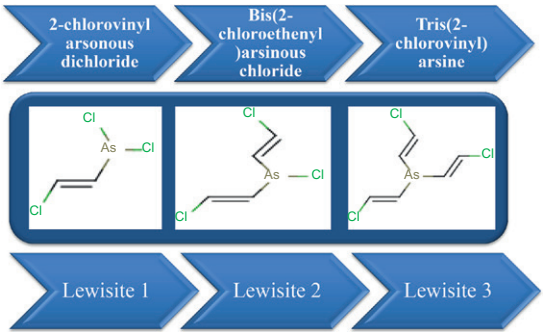

FIGURE 1–5 Structure of three different lewisites illustrating the number of chlorine atoms in each that are responsible for its reaction with bio-molecules.

1.4.2 Organoarsenicals in Chemical Weapons

Lewisite (2-chloroethenylarsinous dichloride), an organoarsenic compound initially manufactured in the USA and Japan as a chemical weapon, acts as a blister agent and lung irritant. It is synthesized by the addition of arsenic trichloride to acetylene in the presence of a suitable catalyst. It is usually found as a mixture of 2-chlorovinylarsonous dichloride (lewisite 1) as well as *bis*(2-chloroethenyl) arsinous chloride (lewisite 2) and *tris*(2-chlorovinyl)arsine (lewisite 3) (Figure 1–5). It diminishes energy metabolism of the human body by inhibiting activity of pyruvate dehydrogenase necessary for the generation of ATP via converting pyruvate to acetyl-CoA. Sequentially, nervous pathology arises as the nervous system essentially depends on glucose as a catabolic fuel. It can easily penetrate ordinary clothing and rubber, and causes immediate pain and itching on skin with a rash and swelling, which convert to large, fluid-filled blisters after approximately 12 hours. Eye exposure causes stinging and strong irritation to blistering and scarring of the cornea. Sufficient absorption causes systemic poisoning leading to liver necrosis or death.

1.4.3 Methylation in Mammals

In mammals, methylation of inorganic arsenic compounds occurs in the liver, via the activity of methyltransferases, to $(CH_3)_2As^{III}OH$ (dimethylarsinous acid) and $(CH_3)_2As^{V}(O)OH$ (dimethylarsinic acid). The source of the methyl groups is methionine, in the form of S-adenosyl methionine in the process of detoxification of inorganic arsenicals [24]. When the liver's methylation capacity is inhibited by the excess exposure to inorganic arsenic compounds, an imbalance occurs, which has deleterious effects on various organs and leads to carcinogenesis.

1.5 Arsenic Mobilization in the Environment

Arsenic is mobilized in the environment via various natural processes such as weathering of rocks, hydrothermal and geothermal activities, biological activities, and a range of

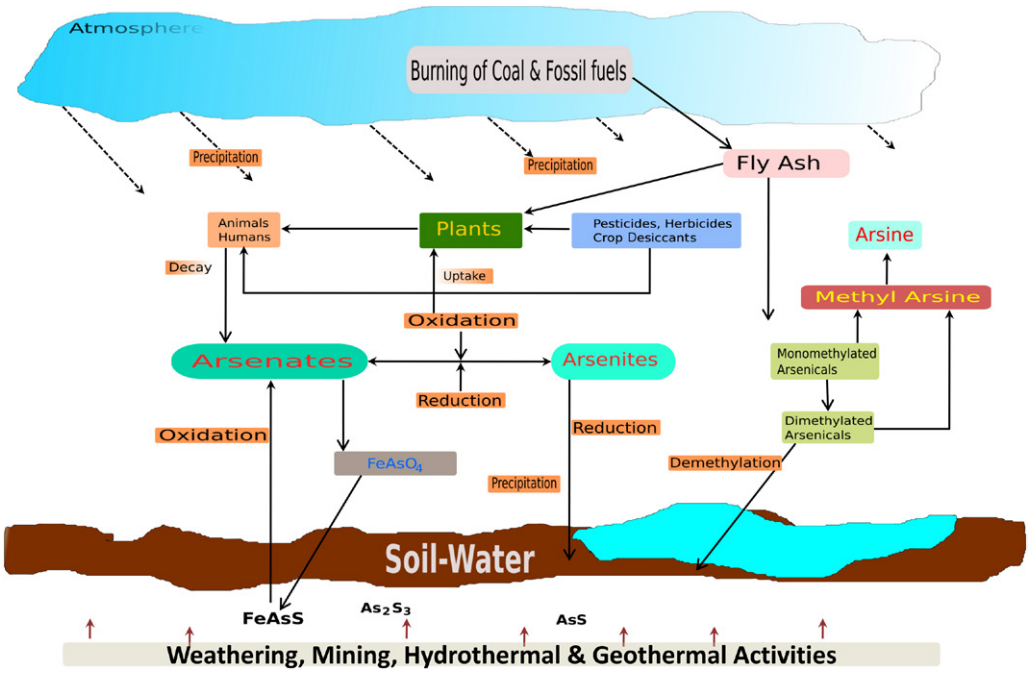

FIGURE 1–6 Arsenic mobilization in air, water, and soil. Arsenic accumulation via weathering and mining, and oxidative dissolution of arsenic-bearing mineral releases to the soil. Arsenic undergoes reduction, methylation, demethylation, and precipitation reaction and finally returns to soil and forms arsenic-rich sediments. Industrial processes, burning of coal, and use of pesticides release arsenic into the atmosphere. None of the various species of arsenic escapes from the environment; rather they continue to cycle from one species to another and from one medium to another.

anthropogenic activities. Widespread anthropogenic activities include mining, combustion of fossil fuels, use of arsenical pesticides, herbicides and crop desiccants, and the use of arsenic as an additive to livestock feed, particularly for poultry (Figure 1–6). Arsenic may enter the human body through drinking water, inhalation, and diet, but above all drinking water probably possesses the greatest menace to human health. Drinking water contains arsenic at various concentrations depending on the source. However, ground water holds the highest concentration of arsenic owing to various natural and human activities.

1.5.1 Aqueous Chemistry of Arsenic

Arsenic is ubiquitously present in the aquatic environment with concentrations near 0.62 µg/L in river water and 0.5–3.0 µg/L in ocean water [25]. Based on redox conditions and pH, arsenic can exist in all possible oxidation states +5, +3, 0, and −3, among which As(III) and As(V) are the most dominating in aqueous systems [26]. Various forms of arsenic are distributed in the environment; the most common species are listed in Table 1–4.

Arsenic readily forms oxyanions in both oxidation states As(III) (H_3AsO_3) and As(V) (H_3AsO_4), and becomes mobile in the pH range of 6.5–8.5 usually found in ground water.

Table 1–4 List of Various Arsenic Species and their Chemical Formulae

Name	Abbreviation	Chemical Formula
Arsenite, arsenous acid	As^{III}	$As(OH)_3$
Arsenate, arsenic acid	As^{V}	$AsO(OH)_3$
Monomethylarsonic acid	MMA^{V}	$CH_3AsO(OH)_2$
Monomethylarsonous acid	MMA^{III}	$CH_3As(OH)_2$
Dimethylarsinic acid	DMA^{V}	$(CH_3)_2AsO(OH)$
Dimethylarsinous acid	DMA^{III}	$(CH_3)_2AsOH$
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$
Trimethylarsine	TMA^{III}	$(CH_3)_3As$
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2COO^-$
Arsenocholine	AsC	$(CH_3)_3As^+CH_2CH_2OH$
Tetramethylarsonium ion	Me_4As^+	$(CHM_3)_4As^+$
Dimethylarsinoyl ethanol	DMAE	$(CHM_3)_2AsOCH_2CH_2OH$

At this pH range, most of the toxic trace metal ions remain in solution form and precipitate or co-precipitate with an increase in pH as an oxide, hydroxide, carbonate, or phosphate. In contrast, oxyanion-forming elements such as arsenic, chromium, and selenium also exist in solution at higher pH values. Among all oxyanion-forming elements arsenic is unique in terms of its relative mobility over a wide range of redox conditions. Other oxyanion-forming elements such as Se, Cr, U, Mo, and V remain mobile under oxidizing conditions only and become immobilized in a reducing environment due to stronger adsorption or reduction to metallic form. On the other hand, arsenic oxyanions remain mobile under both oxidizing and reducing conditions.

Redox potential (Eh) and pH are the most important factors controlling arsenic speciation in aqueous systems. The redox potential of arsenic oxyanions is such that arsenite becomes stable in aqueous form under moderately reducing conditions (+300 mV at pH 4 to − 200 mV at pH 9) while arsenate is stable in oxidized aqueous solutions [18]. Under oxidizing conditions and low pH (less than 6.9), arsenic exists as As(V) and $H_2AsO_4^-$ species dominate, while at higher pH due to ionization of one more proton, $HAsO_4^{-2}$ exists predominantly. In reducing conditions and pH less than 9.2, the neutral arsenite species H_3AsO_3 predominates. The structural configuration of arsenous acid (As^{3+}) reveals that arsenic has three pyramidal bonds with a lone pair of electrons occupying the fourth arm of a tetrahedron (Figure 1–4). It has been suggested spectroscopically that in solution form also, arsenite retains its pyramidal structure [27]. Arsenous acid showing a pKa of 9.23 dominates in natural aqueous solution, while deprotonated forms can exist under alkaline conditions. The geometry of arsenic acid suggests a regular tetrahedron, which can easily donate protons under alkaline conditions to form the stable arsenate $(AsO_4)^{-3}$ anion, which resembles the orthophosphate $(PO_4)^{-3}$ anion and shows competitive chemical behavior in natural water systems. The adsorption and desorption behavior of arsenic on mineral surfaces plays an important role in regulating its aqueous concentration in various water systems. In the presence of reduced sulfur, arsenic exists in solution form as sulfides, while under reducing conditions these sulfides (As_2S_3, AsS) become precipitated. Arsenic species are also affected by temperature, such as in hydrothermal fluids from shallow-water islands; the

dominant species between pH 5 and 7 is trivalent $As(OH)_3$, whereas at higher temperature and pressure in deep-water systems the $H_2AsO_4^-$ arsenate species predominates. Moreover, at high temperature and high concentration of Fe and S, orpiment and claudetite preferentially precipitate. Thio-arsenic species are also possible in sulfide-rich hydrothermal systems; such thioarsenates were reported in the geothermal waters of the Yellowstone National Park [28].

1.5.2 Redox-Dependent Mobilization of Arsenic

Redox reactions are of great significance for controlling the behavior of many chemical species in natural water systems, including arsenic. Thermodynamic equilibrium suggests that under strongly reducing conditions As(V) should dominate over As(III); however, this is not necessarily followed in all natural systems where other redox couples also influence arsenic oxidation/reduction. In oxygenated water, the As(V)/As(III) ratios should be of the order of 10^{15}–10^{26}, which remain stable for a period of days or weeks due to slow oxidation reactions. It has been suggested that the As(V)/As(III) ratio can be used as a reliable redox indicator in groundwater systems [29]. Experimental studies suggested that oxidation of As(III) becomes slow under acidic conditions (pH 5), while at pH 8–12.5 the reaction accelerates. The rate of As(III) oxidation increases in the presence of manganese oxide, which is evident by the fact that it can reduce the half-life of As(III) from 1–3 years to 10–20 minutes. Oxidation reaction is independent of the concentration of dissolved oxygen; however, it is controlled by surface reactions such as photochemical oxidation and reduction triggered by titanium-containing particles [30].

1.5.3 Microbial-Dependent Mobilization

Redox reaction of As(III)/As(V) is also catalyzed by microorganisms; thus sterile water samples are less susceptible to speciation changes. Arsenite in geothermal water in the southwest USA was found to oxidize rapidly with a rate constant of less than 0.3 hours. Arsenate reduced rapidly in Mono Lake, CA, in the presence of bacteria with rate constant ranging from 0.02 to 0.3 per day [31]. Methylated arsenic species also readily oxidized chemically and biologically.

1.6 Sources of Arsenic in the Biosphere

Arsenic is a natural constituent of Earth's crust and ranks 20th in abundance in relation to the other elements; it is found in approximately 320 mineral forms in rocks. Arsenic can enter terrestrial and aquatic environments via both natural geologic processes and anthropogenic activities. Natural occurrence of arsenic depends upon various processes such as weathering of rocks and sediments, hydrothermal and geothermal activities, dissolution of sulfide minerals, forest fire, and wind-blown dust [32]. Anthropogenic sources of arsenic include mineral processing, glass manufacturing, wood preservation, pesticide production and uses, leaching through landfill or waste, and production of coal and petroleum products and their processing. Anthropogenic sources of arsenic are responsible for its release into the atmosphere via emission due to copper smelting and coal combustion and into the hydrosphere and lithosphere through all types of waste [33]. Major natural and anthropogenic sources of arsenic

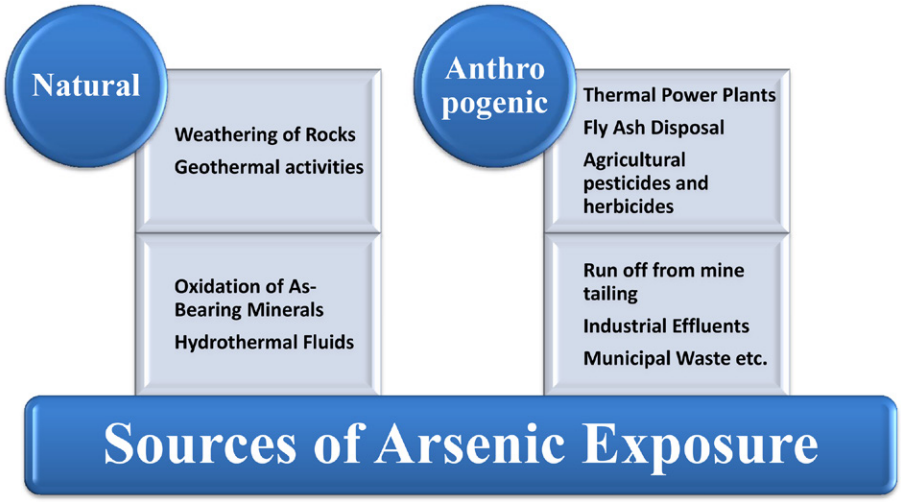

FIGURE 1–7 Major natural and anthropogenic sources of arsenic entry in to the environment.

are depicted in Figure 1–7. The detailed process of arsenic release from various sources is discussed in subsequent sections.

1.6.1 Arsenic in Rocks and Soils

Historically, the concentration of arsenic in Earth's crust was reported to be 2 mg/kg and subsequently it was estimated in various forms of rocks and soils. Indisputably, the arsenic burden has increased at a tremendous rate on planet Earth and varies according to the types of rocks, sediments, and soils. In igneous rocks, arsenic concentrations vary from 1.5 to 5.9 mg/kg with the major contributor being volcanic material generating high–arsenic water [34]. Metamorphic rocks generally show less than 5 mg/kg arsenic with the exception of pelitic rocks containing unusually high concentrations, 18 mg/kg. In sedimentary rocks, arsenic concentrations range between 5 and 10 mg/kg. Argillaceous deposits have a high arsenic concentration (13 mg/kg) probably owing to enhanced sulfide mineral, oxides, clays, and organic matter. Iron-rich rocks, phosphorites, coal, and bituminous deposits are enriched with exceptionally high arsenic concentration ranges of between 100 and 900 mg/kg [35].

Marine deposits due to higher sulfur content contain arsenic at greater concentrations compared to non-marine deposits. Historically, the average concentration of arsenic in deep-sea sediments was estimated to be approximately 40 mg/kg. The most important arsenic minerals in the oceanic crust are cobaltite (CoAsS), loellingite-safflorite ($FeS_2(Co,Fe)As_3$), luzonite (Cu_2AsS_4), tennantite ($Cu_{10}(Fe,Zn)_2As_4S_{13}$), claudetite, and elemental arsenic, principally intergrown with elemental gold [36]. In oceanic sediments, arsenic is usually concentrated in fine-grain sediments, particularly those rich in organic matter, sulfide minerals, phosphate, or iron oxides [37]. Elevated arsenic concentrations were found in river sediments in India, from the

Ganges plain ranging between 1.2 and 2.6 mg/kg, from the Brahmaputra river (between 1.4 and 5.9 mg/kg), and from the Meghna river (between 1.3 and 5.6 mg/kg) [38].

Availability of metals to organisms is not only dependent upon the total amount of a metal present in the soil, but also on the nature of chemical bonding of the metal with the soil particles. Arsenic concentration in soil also varies between 5 and 10 mg/kg, with peats and bog soils having higher values (13 mg/kg) due to increased pervasiveness of sulfide minerals under reduced conditions. The presence of arsenic in soils primarily depends upon geological reactions. However, it is also greatly enhanced by smelting and fossil fuel combustion, mining activity, and use of pesticides and phosphate fertilizers. Previously in orchard soils, the concentration of arsenic was reported in the range of 366–732 mg/kg due to excessive application of arsenical pesticides. Arsenic occurs in soils mainly as arsenate, AsO_4^{3-}, under oxic conditions. This form of arsenic is strongly adsorbed onto clays, oxides/hydroxides of Fe and Mn ($AlAsO_4$ and $FeAsO_4$), and organic matter. In alkaline and calcareous soils, the main form is $Ca_3(AsO_4)_2$. Arsenic leaching from surface soil becomes higher in sandy soil due to weak sorption. The amount of sorption depends upon arsenic concentration, contact time, and concentration of Fe and manganese in the soil. The mobility of As in soils is increased under reducing conditions such as flooded soils. An increase in pH also increases the mobility of the arsenic apparently by bringing about a change from Al and Fe arsenates to calcium arsenate. Bacteria are also important in increasing the mobility of arsenic as they increase the bio-methylation of arsenic, the compounds formed being comparatively mobile forms of arsenic. Various factors regarding the mobilization of arsenic in the soil are presented in Figure 1–8. The natural occurrence of arsenic-bearing minerals may significantly enrich the soil concentration of arsenic. Soils in the vicinity of gold mines as well as gold processing wastes often contain elevated concentrations of arsenic [39]. As mentioned above, arsenic accumulation in soil not only occurs

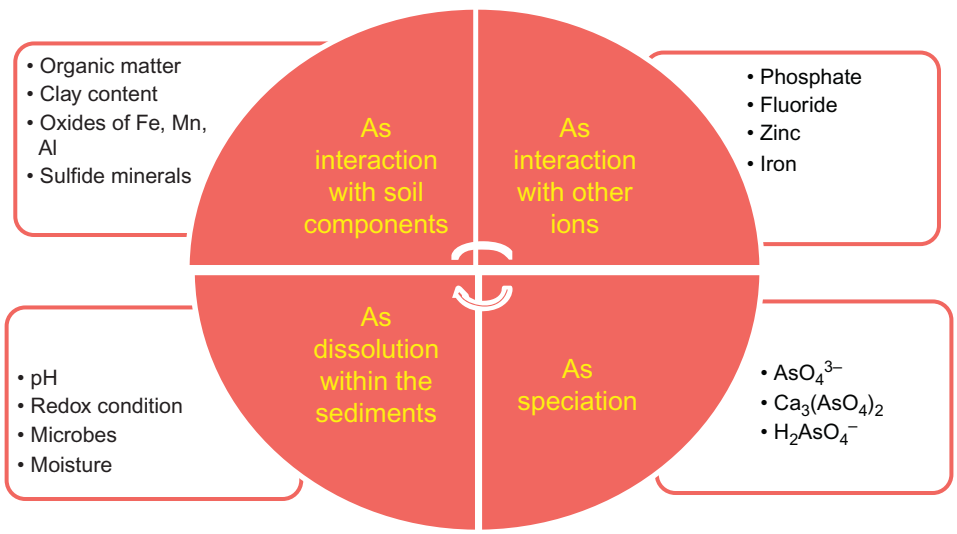

FIGURE 1–8 Various factors affecting arsenic mobilization in soil.

via natural sources but also from anthropogenic sources such as mining, airborne emissions, waste discharges from metal processing facilities, burning of fossil fuels, use of wood preservatives, and agricultural application of arsenical pesticides [40].

Since the beginning of the industrial revolution, the most common human-contributing sources leading to the introduction of trace metals into the soil have included disposal of industrial effluents, sewage sludge, mining, landfills, and use of agricultural chemicals [41]. Arsenic contamination of soils may cause loss of vegetation, groundwater pollution, and arsenic toxicity in plants, animals, and humans [42]. The chemistry and behavior of arsenic is determined by arsenic speciation rather than its total amount. Conversion of the arsenic forms is also affected by different soil factors, such as soil reaction, oxidation–reduction state, microbial activity, organic matter content, etc. Wood impregnation releases considerable amounts of arsenic into the soil in the vicinity of the impregnation plant. To carry out the processing, chiefly arsenic pentoxide (As_2O_5) is used, which is easily dissolved in water (65.8 g/L). Another method of wood impregnation is by pressure treatment with chromated copper arsenate (CCA). In many countries use of CCA for wood preservation has already been banned.

The bio-availability of arsenic in soil depends upon physical and chemical properties of soil and water such as pH, redox conditions, presence of precipitating, complexing, and sorbing agents, and mineralogical composition of the soil and its grain size. In addition, climatic, hydrogeological, and geomorphological activities also influence the mobility and availability of arsenic. Arsenic leached in the pentavalent form is able to form anions, such as $H_2AsO_4^-$, of a strong acid, with pKa values of 2.24, 6.94, and 11.5, which adsorb most effectively at low pH. Consequently, its mobility is fairly low in acidic soils with high clay or oxide content.

1.6.2 Arsenic in the Atmosphere

In 1936 James Svoboda first described mass eradication of honeybee families (Tisin's disease) as a most sensitive ecological indicator of arsenic pollution in air [43]. The phenomenon was observed in the vicinity of various smelters and power plants that burned arsenic-containing coal. In the atmosphere, arsenic concentration increases from smelting, fossil-fuel combustion, and volcanic activity.

Arsenic emission depends upon its concentration in raw material, quality of fuel or waste, production procedure, temperature, pressure, gas composition, and gas cleaning equipment. Due to specific evaporation–condensation processes at high temperatures, arsenic partially or entirely evaporates into the atmosphere. The diameter of arsenic particles dispersed in the atmosphere was typically found to be 1 μm, suggesting efficient movement with wind and air [44]. Combustion of fossil fuels releases arsenic to the atmosphere with typical levels of 0.1–80 mg/kg (dry) for coal and biomass fuels, and up to 500 mg/kg (dry) for sewage sludge.

Airborne arsenic is transferred to water bodies by wet or dry precipitation and increases aqueous concentration. Atmospheric arsenic arising from coal burning has been cited as a major cause of lung cancer in parts of China and India; exposure is known to be lethal from direct inhalation from domestic coal fires [45].

1.6.2.1 Arsenic Emission due to Burning of Coal

Coal is a natural source of arsenic and primarily responsible for the release of arsenic into the air, via smoke stacks during coal combustion, and into soil and water via combustion of products, mainly fly ash. India has the world's fourth largest reserves of coal and the third largest coal excavator, after the United States and China, serving the electricity demands of the nation. Electricity generation is mainly dependent upon coal-based thermal power plants. In eastern India, coal combustion is one of the major sources of anthropogenic arsenic emission into the biosphere. Coal contains both inorganic and organic forms of arsenic; however, its enrichment is related to sulfide mineralization, either syngenetic or epigenetic [46]. The arsenic-bearing sulfides are epigenetic and contain high concentrations of arsenic, whereas pyrites are syngenetic containing very low arsenic levels [47]. Release of arsenic during coal combustion depends upon various factors such as initial concentration in the parent coal, design and operating conditions of the furnace, and combustion temperature. During low temperature combustion (1000–1200°C) AsO and As_2O_3 escape in the gaseous phase, while at high temperature (1200–1600°C) only As_2O_3 is released.

1.6.2.2 Arsenic Dissipation from Fly Ash

Fly ash is produced in substantial amounts around the world and contributes as a potential and significant anthropogenic source of arsenic [48]. During combustion of coal in thermal power plants, the main part of the initial arsenic evaporates into the atmosphere in the gaseous phase. Most of the organic arsenic, pyrite, and some micro-minerals also escape into the atmosphere in the gaseous phase and only a minor part of arsenic remains in bottom ash. The escaping arsenic is captured by fly ash and collected by an electrostatic precipitator. This suggests that the atmospheric emission of arsenic in solid and gaseous phases is rather minor and most of the arsenic ends up with fly ash. Upon cooling, the combustion gases are adsorbed onto fly ash particles and their concentration becomes comparatively higher in particles less than 10 μm due to high surface area. Particles of less than 1 μm when inhaled may be deposited in the pulmonary tissue of the respiratory tract and enter the blood. Release of arsenic from fly ash produces air, soil, and water pollution. Excessive amounts of arsenic over permissible levels have been reported in the soil, tubewell, and surface water near thermal power plants. Arsenic associated with fly ash ends up in ground water through leaching and adversely affects water quality. Reports suggest that arsenic is highly leachable in fly ash as it occurs as a surface precipitate [49]. Arsenic concentration increases with time in ground water from acidic to alkaline fly ash leaching. However, under alkaline conditions, after attaining a maximum level concentrations start declining with time. Many factors such as pH, concentration of Ca/Mg, reducing or oxidizing conditions, leaching time, temperature, and anionic constituents (sulfate and phosphate) strongly influence leaching of arsenic from fly ash [50].

The extent of the arsenic in fly ash depends on both the mineralogy and particle size distribution of the coal and combustion temperature. Arsenic concentrations in fly ash generally range from 2 to 440 mg/kg and sometimes can be as high as 1000 mg/kg depending upon the concentration in the original coals, the combustion methods, and combustion temperature [49]. The toxicity of arsenic when present with fly ash depends upon its state, mobility, and

Table 1–5 Various Forms of Arsenic Present in Fly Ash

Name of Species	Formula
Arsenopyrite	FeAsS
Arsenic trioxide	As_2O_3
Calcium orthoarsenate	$Ca_3(AsO_4)_2$
Scorodite	$FeAsO_4 \cdot 2H_2O$
Thallian arsenian pyrite	$(Fe,Tl)(S,As)_2$

availability in the biosphere. Arsenic concentration was found to be higher in hollow spherical particles (low density) than in compact spherical particles (high density) [51]. The concentration of arsenic in fly ash also depends upon sulfur content of the coal and is found higher in those feed coals with high sulfur content. The pH of fly ash also influences concentration and species of condensed arsenic. In an acidic medium, fly ash contains more Si, thus occurring as $Fe_3[AsO_4]_2$, while in alkaline medium due to an increase in Ca concentration it is present as $Ca_3[AsO_4]_2$. Arsenic is captured by calcium-bearing minerals and hematite, and forms a stable complex with Ca or Fe in fly ash. It has been reported that arsenic is largely present in Ca-rich fly ash as $Ca_3(AsO_4)_2$ and $Ca_2As_2O_7$ because Ca can react with arsenic vapor and capture the metal in water-insoluble forms as As(V) [52]. Fly ash may contain various forms of arsenic depending upon pH condition, particle size, and presence of other minerals, as summarized in Table 1–5.

1.6.3 Arsenic in Various Water Resources

1.6.3.1 Rain Water

Arsenic enters the atmosphere in various ways including volcanic emissions, marine aerosols, burning of fossil fuels, and industrial pollution, and is then reversed in the form of precipitation. It is estimated that anthropogenic sources of atmospheric arsenic emission contribute to about 30% of global arsenic release. Concentrations of arsenic in rainfall were found to be very high: approximately 0.5 µg/L in the areas affected by smelting operation, power plants, soil dust, and burning of coal. In copper smelting areas, the average arsenic concentration was found to be 16 µg/L. Nevertheless, rainfall contributes little arsenic to surface and groundwater bodies. In rain water the most dominant species of arsenic are As_2O_3 near smelters and coal burning areas, AsH_3 near landfills, and reducing soils and arsenate near marine aerosols. The reduced form of arsenic undergoes oxidation in the presence of atmospheric SO_2 and O_3.

1.6.3.2 River and Lake Water

Arsenic concentration in river water depends upon bedrock lithology, base flow contribution, surface recharge, and industrial or sewage effluents. Increased concentrations of arsenic are found in areas where water flows into surface water from surrounding rocks and increases the pH and alkalinity of resultant water. A high concentration of arsenic was found in the Loa River Basin of northern Chile ranging from 190 to 21,800 µg/L, which correlated well with high salinity of water [53]. Increased arsenic concentrations of 114 µg/L was also reported in river waters

in central Argentina owing to increased pH conditions [54]. A higher concentration of arsenic (1100 μg/L) has been observed in Sugar Creek, South Carolina, downstream of an industrial complex. Arsenic concentration also increases in river water due to mining activities such as mine waste and mill tailing. A high arsenic concentration (200–300 μg/L) was found in surface water affected by tin and gold mining activities [55].

Due to high evaporation rate, weathering of volcanic rocks, and geothermal activities, high arsenic concentrations are also found in lake water. Arsenic concentrations of 10–20 mg/L in Mono Lake in California, USA, was reported below the pH range of 9.5–10 [56]. Thermal stratification in lake water also affects arsenic concentration and is found to be increased at depths up to 10 m due to increasing ratio of As(III) to As(V) and influx of mining contaminated water. The concentration of As(III) becomes higher in summer due to depleted dissolved oxygen levels and increased biological productivity in the deeper lake water. In lake and river waters, As(V) is generally the dominant species, varying according to seasonal variation, redox conditions, temperature, and biological activity. During summer As(III) can be detected in water due to biological reduction of As(V). In the thermally stratified Mono Lake, under hypersaline and hyperalkaline conditions, As(V) was found in the upper oxic layer and As(III) in the lower reducing layer [31]. The ratio of organic arsenic species also increases in summer due to enhanced methylation reaction catalyzed by microbial activity, and the most common organic forms that exist in water are DMA^V and MMA^V [57].

1.6.3.3 Sea and Estuarine Water

In open sea water the average arsenic concentration varies around 1.5 μμg/l; however, in estuaries it is more variable (0.1–4 μg/L) due to various river inputs, salinity, and redox gradient. Increased arsenic concentration was observed in the Krka Estuary in Croat with increased salinity from 0.13 to 1.8 μg/L dominated by As(V) [58]. In sea water arsenate (As(V)) dominates over arsenite (As(III)) and their ratio exists in the range of 10–100 μg/L. At seawater pH (7.5–8.2), As(V) exists as $HAsO_4^{-2}$ and $H_2AsO_4^-$, while As(III) exists as the neutral species H_3AsO_3. Organic arsenical also exists in seawater as a result of methylation reactions by phytoplankton. In seasonally anoxic estuarine waters, variations in the relative proportions of As(III) and As(V) can be large. In marine and estuarine waters, organic forms are usually less abundant but are often detected and depend upon temperature and variation in aquatic biota [59].

1.6.3.4 Ground Water

Concentrations of arsenic in water bodies depends upon the source of arsenic and local geochemical conditions varying more than four orders of magnitude. Among all the sources of arsenic the greatest range and highest concentrations are found in groundwater due to considerable mineral water interactions and favorable conditions for arsenic mobilization and accumulation. Governments, external agencies, academic institutions, and private research and development centers are making determined efforts to identify increasing concentrations of arsenic worldwide. There are many countries in the world where the arsenic concentration in drinking water was found to be more than the World Health Organization (WHO) permissible value (10 μg/L) [60]. Over the past two or three decades, the occurrence of high concentrations of arsenic in drinking

water has been recognized as a major public health concern in various parts of the world; apparently, there are areas where the concentration still needs to be recognized. Early identification of arsenic-affected areas can serve as a milestone for the remediation program and to provide safe drinking water. In ground waters, the ratio of As(III)/As(V) varies to a great extent due to variation in redox conditions, sources of water, and environmental conditions. Arsenic mobility in surface and ground water is dependent upon five factors: (1) redox reactions; (2) adsorption and desorption; (3) competitive adsorption (ion exchange); (4) solid phase precipitation and dissolution; and (5) biological activity. These processes are regulated by redox potential, pH, chemical composition of the system, competing anions, aquifer minerals, and reaction kinetics [61].

1.6.4 Arsenic in Dietary Products

1.6.4.1 Plants and Crops

The presence of arsenic in soil adversely affects growth of plants and the effects depend upon the chemical form and availability of As. The toxicity of arsenic varies in the following order as per its oxidation state and form: AsH_3 > As(III) > As(V) > organic As. Arsenic availability to plants depends upon soil texture and is found to be high in coarse-textured soils with less colloidal material and little ion exchange capacity, while the concentration is low in fine-textured soils that are high in clay, organic material, iron, calcium, and phosphate. The average arsenic concentration naturally occurring in the soil worldwide is 10 mg/kg. In arsenic-contaminated soil, the uptake of arsenic by plants was found to significantly increase, particularly in vegetables and edible crops [62]. Uptake of arsenic follows a different mechanism for the two oxidation states, As(III) and As(V). As(V) is taken up via a phosphate uptake system in plants due to the similarity of As(V) to phosphate [63]. Therefore, phosphate supplementation has been suggested to reduce arsenic uptake because of competition between phosphate and As(V).

1.6.4.2 Rice and Other Food Items

It is reported that 40% of arsenic in the human body comes from the food chain [64]. Arsenic intake via food varies from 17 to 291 µg/day in different countries. Seafood accounts for 60–96% of the total dietary intake of arsenic, mostly in the form of arsenobetaine and arsenosugars, relatively non-toxic forms of arsenic [65]. Other food sources are vegetables, mushrooms, grains, milk, chicken, and beef, which account for inorganic arsenic consumption [66]. In non-arsenic endemic regions, the principal sources of inorganic arsenic in the diet are rice and chicken, which results in the accumulation (55–97 ng/g) of methylated arsenic compounds [67]. Herbal medicines and seaweed are also important sources of arsenic, e.g., Hijiki seaweed has high levels of inorganic and organic arsenic [68].

Rice is the major crop in India and covers about 23% of the country's total irrigated land for its production. Nearly 42% of the total food grain production is rice in India and West Bengal is one of the major rice producing states covering an area of 5,900,000 ha. In West Bengal, a huge amount of the population is dependent upon water for irrigation purposes but it is found to be contaminated with arsenic. For the cultivation of rice during lean periods an enormous amount of ground water is used, which leads to oxidative decomposition of pyrite to form Fe^{+2}

Table 1–6 Concentration of Arsenic in Different Rice Varieties

Rice Variety	Origin	Total As (μg/g)
Basmati white	India	0.05
Long grain	America	0.26
Long grain	Bangladesh	0.13
Long grain	Europe	0.18

and Fe^{+3} sulfate and sulfuric acid, responsible for arsenic mobilization. In South East Asian countries, rice is grown mostly under waterlogged lowland conditions in which the physical and chemical condition of the soil affects the mobilization of arsenic and its uptake by rice [69].

In West Bengal, villagers living in arsenic-contaminated areas largely depend upon rice for their calorific intake [63]. The concentration of arsenic varies according to rice species and is summarized in Table 1–6. Due to the high arsenic levels found in paddy rice, such rice has been considered an important source of arsenic intake and risk is increased if it is cooked in arsenic-polluted water due to chelation of arsenic to the rice starch/bran [70]. According to WHO, the permissible limit of arsenic in rice and other food items has been reported as between 1.0–2.0 mg/kg and 2 mg/kg [71].

1.6.4.3 Tobacco

Cigarette smoking is a well-established risk factor for carcinogenesis. The chemical agents associated with cigarette smoke are classified as group I carcinogens and include benzene, polycyclic aromatic hydrocarbons (PAHs), cadmium, arsenic, nickel, chromium, 2-naphthylamine, vinyl chloride, 4-aminobiphenyl, and beryllium [72]. In the United States, arsenic levels in tobacco rose from 12 mg/g in the 1930s to over 50 mg/g in the 1950s. By the 1970s, in response to policy efforts, arsenic levels reduced to 1 mg/g of tobacco, which further declined in subsequent years [73]. Arsenic exposure due to tobacco smoking has been prevalent in Asian countries due to the smoking of handmade cigarettes, especially bidis [74].

1.7 Arsenic in Hydrothermal and Geothermal Fluids

1.7.1 Arsenic Occurrence in Hydrothermal Fluids

The concentration of arsenic in the world's oceans is usually high and governed by various factors such as riverine input, weathering and sedimentation on the sea floor, water exchange between atmosphere and sea, inputs of volcanic gases, and hydrothermal fluids. The dwelling time of arsenic in the oceans is assumed to be between 32,000 and 63,000 years with an average concentration of 1.7 μg/L.

In the past 25 years, active hydrothermal systems, polymetallic mineral deposits, and robust biological communities have been discovered including sediment-free and sediment-covered ocean ridges, the East Pacific rise, mid-Atlantic ridges, rifted continental margins,

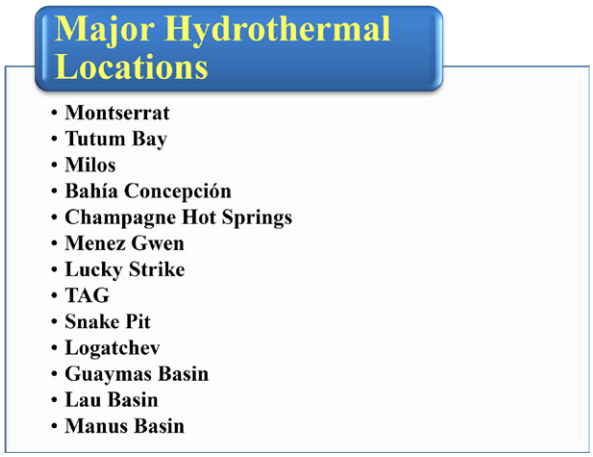

FIGURE 1–9 Major hydrothermal locations distributed worldwide contributing significantly to the environmental arsenic burden.

Table 1–7 Comparison between Shallow and Deep Water Systems

Property	Shallow Water	Deep Water
Depth	Less than 200 m	More than 200 m
Photosynthesis rate	High due to benthos and phytoplanktons	None takes place
Water source	Meteoric and sea water	Sea water
Temperature	Up to 100°C	Above 400°C

volcanic arcs, back-arc basins, and seamounts. Major hydrothermal locations worldwide are listed in Figure 1–9. Chemical analyses of hydrothermal fluids and suspended particles from submarine systems reveal that arsenic and other metals are effectively mobilized from the oceanic crust and sedimentary, transported to the sea floor, and concentrated in surface mineral deposits. Hydrothermal fluids emerging at the sea floor are known to contain considerable amounts of dissolved transition metals and toxic elements due to water/rock interaction at elevated temperatures and potentially magmatic degassing. Arsenic concentrations in hydrothermal fluids are 2–500 times higher as compared to seawater concentrations.

The chemistry of hydrothermal fluids is chiefly governed by physicochemical variables, phase separation, depth and temperature, rock lithology, water/rock interaction, and heat gradient [75]. Cold and dense water percolates downward and is heated up to 400°C, and hot water rises upwards due to resilience. Hydrothermal systems can be divided into shallow and deep water based on their depth, and systems at a depth of 200 m are considered deep water systems [76]. Comparison of shallow and deep water systems is summarized in Table 1–7.

In shallow water systems, photosynthetic organisms can absorb arsenate from the surrounding sea water owing to its similarity to phosphate [77]. Arsenate is converted to arsenite

and then further to methylated species, which are again converted and detoxified to different arsenosugars. These arsenosugars are further converted to the less toxic arsenobetaine in higher marine organisms.

1.7.2 Arsenic Concentration in Shallow and Deep Hydrothermal Systems

Some shallow and deep water hydrothermal systems are listed in Figure 1–7. Montserrat Island in the Caribbean, located at the northern end of the inner arc consisting mainly of andesitic rocks, showed a large increase in shallow water hydrothermal discharge near its western coast after a volcanic eruption in July 1995 [78]. The springs showed alarmingly high concentrations of arsenic (3600 µg/L) after the eruption, followed by a steady decline.

Another shallow-water hot springs in Tutum Bay located 150 m offshore along the southwest part of Ambitle Island (Papua New Guinea), shows temperatures up to 98°C in 5–10 m of water. The sediments in this region consist mainly of feldspar, hornblende, pyroxene, magnetite, and weathering product coated with hydrous ferric oxide [79]. Arsenic concentrations in the water range from 750 to 1050 µg/L, while in ferric oxide they reach up to 50,000 mg/kg.

Milos, another hydrothermal site situated in the central Hellenic volcanic arc, showed arsenic concentrations as high as 2900 µg/L in Paleochori Bay, whereas concentrations were even higher (up to 5850 µg/L) in Spathi Bay, known to be the highest in a hydrothermal system. At Milos, hydrothermal fluids have been divided into two groups, one with high chlorine containing less arsenic and the other with low chlorine but supplemented with more arsenic [80].

Bahia Concepcion located in the Gulf of California, covered by a marine and continental sedimentary layer, contains hydrated ferric oxide enriched with high concentrations of arsenic up to 50,000 mg/kg [81]. Champagne hot springs located near the island of Dominica in the Caribbean consist mainly of medium-K calk-alkaline andesites and hydrothermal hydrated ferric oxide, augmented with arsenic concentrations up to 1880 mg/kg [82].

Various shallow water systems differ in terms of temperature, pH, H_2S content, redox state, fluid pressure, iron concentration, and gas fugacity, which seem to be responsible for leaching of arsenic from host rock and its transportation in hydrothermal fluid. Aquifers with high temperature, low pH, and high H_2S content show high concentrations of arsenic, perhaps due to higher leaching capacity and phase separation. Phase separation generates a low chlorine vapor phase in which arsenic partitioning is high pertaining to dominance of uncharged arsenic species and SH^- complexation [83].

The Mid-Atlantic Ridge (MAR) is a deep hydrothermal region occupied by two hydrothermal systems, Menez Gwen and Lucky Strike, which are both similar in rock composition and fluid geochemistry. The systems were found to be poor in chlorine and iron, while rich in gaseous components [84]. Menez Gwen hydrothermal discharge, located at a depth of 840–865 m with a temperature around 285°C, had an arsenic concentration found to be 18.5 µg/L, while Lucky Strike at a temperature range of 170–324°C had an arsenic concentration of 4.3–24 µg/L [85]. Another hydrothermal field in the MAR region is TAG, discovered in 1972, and, further towards the south, the Snake Pit hydrothermal system was identified. Both systems were found

to be chlorine rich with TAG showing low arsenic (0.8 µg/L) and Snake Pit in the range of 3.2 to 14.2 µg/L [86].

East Pacific Rise spreads 9000 km in the Pacific Ocean with various hydrothermal regions studied in different sections, primarily Guaymas Basin located in the central Gulf of California characterized by 2000 m water depth. The basin is set apart with a temperature of up to 315°C, highly reducing conditions, and arsenic concentration in the range of 21.1–80.5 µg/L [87]. The area possesses a high sedimentation rate of organic and carbonate-rich material, which undergoes pyrolysis at high temperature to release NH_4^+ ions in the fluid [88].

Deep-water hydrothermal springs also occur in back-arc basins with a high temperature gradient and a different chemical composition from those of mid-ocean ridges [89]. The fluid is characterized by very low pH (≈2), high amounts of dissolved metals (Mn, Zn, Cu, and Pb), and the presence of rocks like dacites and andesites [90]. The most common back-arc basin is Lau Basin located between the remnant Lau Ridge and the active Tofua volcanic arc with 1700–2000 m water depth [91]. At low pH and with high chlorine, arsenic concentration lies in the range of 450–825 µg/L.

Another hydrothermal system is Manus Basin located at the New Britain Trench, active in 1650 to 2500 m water depth [92]. Hydrothermal systems from back-arc basins show higher values than those from mid-ocean ridges. Higher arsenic concentration could be due to transportation of high amounts of dissolved iron leading to precipitation and incorporation of arsenic into iron sulfides.

1.7.3 Arsenic Occurrence in Geothermal Systems

High temperature geothermal systems occur throughout the world generally in one of three tectonic plates. The edge of the Pacific plate is defined by geothermal fields in New Zealand, Papua New Guinea, the Philippines, Indonesia, Japan, Kamchatka, Alaska, western USA, Mexico, Central America, and Chile. Other geothermal systems are Earth's "hot spots," such as in Hawaii, Yellowstone, and the Azores. The presence of arsenic in geothermal fluids has been known since the mid-19th century. Geothermal fluid arsenic concentrations are usually three orders of magnitude higher than those in uncontaminated surface and ground waters. Arsenic concentrations in the thermal fields of Yellowstone National Park range from 0.1 to 10 mg/kg, a range that is observed in most active geothermal fields. On the other hand, extremely high arsenic concentrations (more than 20 mg/kg) were found in geothermal well fluids. Arsenic is a ubiquitous component of geothermal systems and occurs together with other elements such as mercury, antimony, selenium, thallium, boron, lithium, and fluoride [93]. In geothermal areas, arsenic contamination occurs via two main processes: (1) natural contamination where the geothermal waters reach the surface as natural springs and then mix with surface water flows or shallow groundwater bodies used by humans for both irrigation and drinking water supply; and (2) human exploitation of geothermal waters as an energy resource causing mobilization of arsenic and other heavy metals contained in the geothermal waters to reach the surface and then contaminate surface and shallow groundwater bodies.

Arsenic concentrations in natural geothermal systems exceed permissible limits, and in some parts such as West Yellowstone, the concentration reached as high as 2000 μg/L. The population in these areas shows symptoms of chronic arsenic poisoning such as skin lesions and malignant growth [94]. River systems receive geothermal fluids that accumulate arsenic in aquatic plants, a notable source of exposure.

Geothermally active areas came into existence due to the emergence of hot water and steam at the Earth's surface and development of high geothermal gradient. The elevation in temperature is related to volcanic or magmatic activity, metamorphism, faulting, and radioactivity. Deeply circulating hot fluids are of low density and rise through the host rock, while cold water from the water table moves downward to recharge ground water. The chemical composition of geothermal fluid is regulated by fluid temperature and host rock composition. At high temperature, water behaves as an excellent solvent and can dissolve various ionic crystalline minerals forming new hydrated materials. At high temperature due to enhanced water/rock interaction, various elements such as Li, Rb, Cs, B, and Cl leach into solute. The concentration of dissolved gases such as CO_2 and H_2S are also a function of mineral solubility, especially carbonate and sulfide minerals. When geothermal fluid rises through the crust, pressure decreases and fluid separates into two phases, steam and water. Geothermally active areas can be divided into two groups: (1) hot water springs with neutral pH, rich in chloride and silica; and (2) steam vents of acidic pH, which are sulfate rich and susceptible to microbial oxidation of sulfuric acid. Geysers and carbonate-rich springs are also hot water discharges, but include steam heating and steam-phase mixing, respectively.

The development of geothermal fields for power generation increases the rate and volume of geothermal water reaching the surface. Geothermal sites used for power generation require substantial amounts of water to be extracted. During the separation of steam and water in geothermal power generation, arsenic is retained in the waste bore water and creates a number of problems in its disposal [95]. It becomes essential to re-instill thermal waste to its reservoir in order to prevent contamination of cold water. The geothermal springs are also used as swimming pools and hot spas, both for local residents and for tourists.

1.7.4 Arsenic Speciation and Deposition in Geothermal Fluids

According to water chemistry, geothermal waters can be classified into two main categories: (1) neutral Cl-SO_4^{-2} water; and (2) acid-SO_4^{-2} water. The former system has a high concentration of chlorine (263–1337 mg/L) with arsenic concentration ranges from 1 to 890 μg/L while the latter has a low concentration of Cl (1.19 to 6.9 mg/L) and a low concentration of arsenic (0.8–660 μg/L) [96].

The behavior of arsenic in geothermal systems and surface water environments can be interpreted using thermodynamic data. In most hydrothermal fluids, arsenic is expected to be transported as arsenous acid (H_3AsO_3), which forms by the dissolution of orpiment at acidic to neutral pH and a wide range of temperatures [97]. Orpiment solubility increases with temperature, however, independent of pH under acidic or acid neutral conditions and then increases with pH.

In sulfide-rich hydrothermal fluids, arsenic exists as thioarsenite complexes, a group of covalently bonded arsenic–sulfide complexes that may or may not include oxygen. Orpiment solubility increases with pH and sulfide concentration but is less affected by temperature changes below 200°C. When rising geothermal fluid is exposed to atmospheric oxygen or mixed with another oxidizing fluid, such as a shallow ground water, arsenous acid (As^{+3}) oxidizes to arsenate ($H_2AsO_4^-$ or $HAsO_4^{-2}$). Oxidation to arsenate occurs very rapidly in hot spring overflows and receiving streams and rivers. Various geothermal waters such as found in Yellowstone National Park, on the island of Dominica, in the Valles Caldera (New Mexico), and Massive Central (France) are likely to contain mostly As(V), due to strong oxidizing conditions. Hot springs formed from reservoir fluids contain mainly As(III), whereas that with acid sulfate and bicarbonate is enriched with As(V).

1.7.5 Biogeochemical Fate of Arsenic in Hydro- and Geothermal Systems

The biogeochemical fate of arsenic at submarine hydrothermal sites is less well known. Average arsenic concentrations in soft tissues of mussels (40 ppm) from the Mid-Atlantic Ridge are higher than those of mussels (2–20 ppm) from coastal environments. In contrast, shrimp recovered from the Mid-Atlantic Ridge have a lower arsenic content (13 ppm) compared to shrimp (15 ppm) from surface waters and coastal zones [98]. The high levels of arsenic (up to 70 ppm) in gill tissues and digestive tracts of these organisms were found mostly in the form of arsenobetaine and arsenosugars, suggesting assimilation of arsenic through the activity of symbiotic bacteria and by filtration of Fe oxyhydroxide particles. The presence of arsenic-bearing sulfosalt minerals proustite (Ag_3AsS_3) and pearceite ($Ag_{16}As_2S_{11}$) in filamentous bacteria from northern Gorda Ridge located off the coast of Oregon and northern California is a further indication that microorganisms play a key role in the chemistry of arsenic in submarine hydrothermal environments. Thermophilic bacteria and cyanobacteria in hot spring bio-films also facilitate the formation of silica, silicates, carbonates, and oxide minerals in sinter deposits and indicate enrichment of arsenic along with other elements such as Sb, P, and Zn [99].

1.8 Arsenic Release from Mining and Mineral Processing

1.8.1 Oxidation of Arsenic Sulfide and Industrial Ore Leaching

High concentrations of arsenic and other heavy metals such as Cd, Fe, Pb, Ni, and Zn are commonly present in acid mine drainage (AMD) effluents chiefly due to oxidation of arsenopyrite. AMD is polluted water, and contains high levels of iron, aluminum, and sulfuric acid. Arsenic is one of the priority pollutants associated with AMD, especially from gold mining operations [100]. Mining activities accidentally accelerate physical weathering of pyrite by grinding up the

ore and then oxidation of pyrite takes place. The presence of pyrite in sulfide-rich coals also plays a major role in the generation of acid rain and AMD.

$$FeAsS + 13Fe^{+3} + 8H_2O \leftrightarrow 14Fe^{+2} + SO_4^{-2} + 13H^+ + H_3AsO_4 \quad (1.7)$$

Fe^{2+} and H^+ are released after oxidation of pyrite and move to the surface and ground water, where Fe^{2+} is oxidized to Fe^{3+} in the presence of dissolved oxygen in water. Fe^{3+} can further hydrolyze in water and precipitate as $Fe(OH)_3$, which releases more H^+ into the water. In addition, iron-(oxy)hydroxysulfates often precipitate in iron-rich, acid-sulfate waters, under acidic conditions [101]. Under such conditions, soluble Fe^{3+} reacts readily with more pyrite and oxidizes it due to the action of acidophilic chemolithotroph bacteria, particularly *Acidithiobacillus ferrooxidans* [102].

The dissolution of the most common arsenic sulfide minerals orpiment and arsenopyrite follows the same mechanism and largely depends upon pH. Orpiment dissolution increases linearly from acidic to alkaline conditions [103], whereas arsenopyrite dissolution is minimal at near neutral pH and increases towards acidic and alkaline conditions [104]. Orpiment dissolution is controlled by weak van der Waal forces. At alkaline pH, due to absorption of hydroxide ion (OH^-) at the mineral surface, van der Waals forces becomes weak and two species can be formed, S-As-S and S-As-S-OH [105]. These species release arsenite and sulfide followed by recombination to thioarsenites and oxidation to thioarsenates in solution. In sulfide-deficient solutions, arsenite dominates at pH 2–8; however, in anoxic, sulfide-rich solutions, thioarsenite ($AsO_xS_x^{-3}$) has been proposed to pre dominate [106]. The formation of mono- (AsO_3S^{-3}), di- ($AsO_2S_2^{-3}$), and trithioarsenate ($AsOS_3^{-3}$) was proposed upon dissolution of orpiment at neutral pH [107].

Dissolution of arsenopyrite releases iron along with arsenic and sulfur, which also influence arsenic mobility and formation of thioarsenic species. Initially, Fe^{+2}, S^{-2}, and As^- species are released from the mineral surface and undergo oxidation at alkaline pH to form iron-arsenic oxide or hydroxide surface coating to prevent further release of arsenic [108,109]. The presence of iron and rapid formation of iron sulfides leave no or little free sulfide for thioarsenate formation.

Under acidic conditions, neither mineral forms thioarsenate because at low pH thioarsenic species transform quantitatively to arsenite or precipitate as amorphous arsenic sulfide minerals [110]. At highly alkaline conditions, thioarsenite transforms to arsenite due to competitive ligand exchange of SH^- vs. OH^- groups. Monothioarsenate is stable towards reduction; however, di- and trithioarsenate are reduced to arsenite (As(III)). It has been postulated that thioarsenates once released by mineral dissolution might have higher mobility compared to arsenites and arsenates and may increase arsenic burden in natural or mining-impacted environments.

Release of arsenic from arsenopyrite is also enhanced in the presence of hydrogen peroxide (H_2O_2), a common constituent of rain water [111]. Hydrogen peroxide is a stronger oxidizing agent compared to molecular oxygen. It can also generate more potential oxidizing agent

hydroxyl radical via Fenton's reaction. Hydrogen peroxide can decompose readily and generate dissolved oxygen as per the following chemical equation:

$$2H_2O_2 + O_2 \rightarrow 2H_2O + 2O_2 \tag{1.8}$$

Release of arsenic from the mineral surfaces in the presence of oxygen can be illustrated as:

$$FeAsS + \frac{11}{4}O_2 + \frac{3}{2}H_2O \rightarrow 2Fe^{+2} + 2H_3AsO_3 + 2SO_4^{-2} \tag{1.9}$$

H_3AsO_3 can be further oxidized by molecular oxygen to produce As^{5+}:

$$H_3AsO_3 + \frac{1}{2}O_2 \rightarrow H_2AsO_4^- + H^+ \tag{1.10}$$

Oxidative decomposition of arsenopyrite releases Fe^{2+}, and thus it may react with H_2O_2 to generate the hydroxyl radical, which can convert As^{3+} into As^{5+}:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{+3} + {}^{\bullet}OH + OH^- \tag{1.11}$$

$$As^{+3} + 2\,{}^{\bullet}OH \rightarrow As^{+5} + 2OH^- \tag{1.12}$$

Arsenic concentrations were noted from 180 samples of mine drainage from the USA in the range of 1 to 340,000 µg/L, much higher when compared to arsenic concentrations in natural water bodies, 0.5–5000 µg/L [112].

1.8.2 Chemistry of Arsenic Within Mining Wastes

Arsenate generally predominates under oxidizing conditions in mining waste water, while arsenite predominates when conditions become sufficiently reducing. It has been suggested by thermodynamic calculations and experimental results that at high redox levels and pH > 10, arsenate predominates, while under reduced conditions and pH < 8, arsenic is the dominating species. Arsenite oxidation is a slow process in distilled and demineralized water, whereas at the mineral surface oxidation occurs rapidly and increases the environmental burden of arsenic. Arsenite oxidation is also enhanced in the presence of manganese oxides in sediments, aquifer materials, river and lake water, and by biogenic manganese oxide. It has also been postulated that the oxidized species of couples with positive redox potential can oxidize the reduced species of couples with negative redox potential at specified pH. It has been suggested that manganese hydroxides are responsible for arsenite oxidation under near-neutral to alkaline conditions (pH 6–12), while iron hydroxide behaves as an oxidizing agent under acidic conditions in general.

Redox reactions also regulate stability of most arsenic-bearing sulfide and hydroxide minerals, which are sources and sinks of arsenic species. Sulfide minerals are generally stable under reducing conditions but oxidize under oxidizing conditions to release arsenic into the biosphere.

These minerals are generally stable in the neutral to high pH range but are dissolved in acidic or highly alkaline solutions due to their amphoteric nature. Under highly reduced conditions, arsenic accumulates at redox boundaries in sediments and associates with sulfide [113].

1.8.3 Arsenic Release by Artisanal Mining

Artisanal mining is becoming progressively more common in many parts of the world, with more than 30 million active artisanal miners in more than 55 countries. Artisanal mining activities are strenuous in rural areas due to lack of proper employment and the individuals undertaking informal mining generally lack education, training, management skills, and essential equipment for safe mining practices. Arsenic, a constituent of gold ore, is a very common occupational pollutant resulting from pervasive environmental contamination. These mining sites create great menace as most of them are run without due consideration given to environmental, occupational, or community exposures. Moreover, environmental monitoring and waste management in artisanal gold mining areas are not appropriate. People living in proximity to artisanal mining areas are vulnerable to arsenic exposure, especially pregnant women, their fetuses, and young children. Women in these gold mining regions may engage in geophagy, or earth-eating behaviors, which are common in artisanal mining areas because of poor nutritional status and cultural acceptance of the practice [114]. Consumption of 50 g of sikor (a molded soil sold in the local market, from an arsenic-contaminated area) per day is known to be equivalent to ≈370 μg of arsenic [115].

1.9 Global Occurrence of Arsenic in Ground Water

1.9.1 Arsenic Release and Mobility in Natural Water

Arsenic release from naturally-occurring sources, principally reductive dissolution of iron oxides and hydroxides, desorption of arsenic from mineral surfaces, and oxidation of arsenic-bearing sulfide minerals, is the dominant cause of elevated arsenic concentrations in groundwater [16]. These processes usually occur simultaneously and can be abiotic and microbially mediated. Redox chemistry of iron greatly influences release and mobilization of arsenic in ground water. Under reductive conditions iron oxide is dissolved and releases adsorbed arsenic to subsurface waters [116]. The reduction reactions accelerate in anaerobic surroundings via consumption of oxygen and release of more CO_2 through the decomposition of organic matter in water bodies. Major reduction reactions are as shown below:

$$NO_3^- \rightarrow NO_2 + N_2O + N_2 \tag{1.13}$$

$$MnO_2 \rightarrow Mn^{2+} \tag{1.14}$$

$$Fe_2O_3 \rightarrow Fe^{2+} \tag{1.15}$$

$$SO_4^{-2} \rightarrow S^{-2} \tag{1.16}$$

$$As^{+5} \rightarrow As^{+3} \tag{1.17}$$

Release and mobilization of arsenic in water is also influenced by oxygen concentration and growth of microorganisms. The US Department of Natural Resources (DNR) issued guidelines for maintenance of domestic wells in arsenic-sensitive areas suggesting disinfection with a low dose of chlorine solution as high doses of chlorine can increase arsenic concentration by maintaining oxidizing conditions. The release of arsenic from soils and sediments is associated with iron oxide dissolution. A speedy increase in pore water arsenic concentration (about 30 μg/L) has been observed with the increase in dissolved iron [117]. Gotkowitz et al. [118] demonstrated effects of strongly oxidizing conditions on mineral transformations and arsenic release, and suggested that microbially induced reduction of arsenic-bearing iron oxides releases significant quantities of arsenic in to water.

The majority of the population in India and other developing countries depends upon ground water as a source of drinking water, which has been found to contain elevated levels of arsenic. In developed countries such as the USA however, treated water enters through urban drinking water distribution systems, which contain arsenic below its permissible limit, i.e., 10 μg/L [119]. Arsenic has been well reported in pipe scales and corrosion products [120] suggesting its pervasive prevalence within drinking water. Arsenic has been found in various types of pipe scales including iron corrosion products and lead scales reaching maximum levels up to 14 mg/g pipe scale [121].

1.9.2 Natural and Anthropogenic Occurrence Globally

Analysis of groundwater samples from various parts of the world, including Argentina, Australia, Bangladesh, Cambodia, Chile, China, Hungary, West Bengal (India), Inner Mongolia, Mexico, Nepal, New Zealand, the Philippines, Taiwan, Thailand, Vietnam, and the United States, has identified the presence of arsenic levels above 10 μg/L, the WHO guideline value and new US maximum contaminant level. The situation in Bangladesh, West Bengal, and Vietnam is extremely bad with 40 million people estimated to be exposed to arsenic concentrations above 50 μg/L. Dermal absorption during bathing and hand-washing is also an important exposure route. Most health studies investigate the effects of drinking arsenic-contaminated water. However, there is a scarcity of data regarding the differential health effects of arsenite and arsenate. Evidently, cultured human keratinocytes exhibit greater permeability to arsenite as low as 10 μg/L resulting in cytotoxicity and inhibition of DNA and protein synthesis [122]. *In vivo* studies with rhesus monkeys revealed dermal absorption at arsenic concentrations as low as 14 μg/L [123].

1.9.2.1 India, Bangladesh, and Nepal

In India, groundwater arsenic contamination has been identified in many states, especially West Bengal, Jharkhand, Bihar, Uttar Pradesh, Assam, Manipur, and Rajnandgaon village in Chhattisgarh, with concentrations above the permissible limit of 50 μg/L. All the arsenic-affected river plains have their river routes originating from the Himalayan region. The arsenic problem in ground water from the alluvial and deltaic aquifers of Bangladesh and West Bengal represents the most serious calamity identified globally. Groundwater arsenic contamination

has exceeded 2000 μg/L in some areas of India and Bangladesh [124]. Arsenic occurs widely in ground water of the Bengal Basin in West Bengal and Bangladesh beneath the flood plains of the rivers Ganges, Brahmaputra, and Meghna [125]. Among Asian countries, the large population in West Bengal consumes arsenic-contaminated drinking water and many suffer from arsenicosis [126]. Ninety-five percent of the population in this region relies on ground water for drinking and domestic use, and for irrigation purposes. It has been estimated that out of a total population of 125 million people, 35 million people were exposed to arsenic levels in water above 50 μg/L and 57 million people to that in water above 10 μg/L. The biggest arsenic calamity in the world has been reported from the seven districts of West Bengal, namely, Malda, Murshidabad, Burdwan, Nadia, Hooghly, and 24-Parganas (North and South), covering an area of 37,493 km^2 and having a population of about 34 million. It has been estimated that 27% of tubewells used for domestic water supply in this region contain arsenic above the Bangladesh national permissible limit of 50 μg/L and 47% exceed the WHO recommended limit of 10 μg/L. It has been reported that the 8–9 million people of West Bengal are affected by arsenic toxicity due to use of arsenic-contaminated ground water for drinking purposes [42]. The average arsenic concentration in the drinking water is about 200 μg/L reaching as high as 3700 μg/L. Water samples from shallow and deep tubewells in Bangladesh were found to contain arsenic concentrations up to a maximum of 1660 μg/L [127].

A positive correlation has been found from the reports of Taiwan and Bangladesh between high arsenic exposure and several dermatologic and vascular diseases such as blackfoot disease, hypertension, and cerebrovascular disease. Studies have also suggested an association between arsenic in drinking water and diabetes, infant mortality, and reduction in birth weight. Arsenic groundwater contamination and its health effects in the Rajnandgaon district of Chhattisgarh have been reported recently [128]. In this area, wells located in the granitic terrain with pegmatitic intrusions were found to have arsenic concentrations above WHO [129] and BIS (Bureau of Indian Standards) standards, with the highest being found in a well with more than 250 μg/L of arsenic. Buragohain and Sarma [130] presented a comprehensive analytical and spatial analysis of arsenic distribution in ground water of five blocks of Dhemaji district, Assam. They reported that the majority of samples were found to contain arsenic concentrations exceeding WHO permissible limits while a few were approaching them. The most probable source of arsenic in ground water in this area was assumed to be heavy deposition of sediments due to surface erosion from surrounding hills.

The Department of Water Supply and Sanitation of Nepal in collaboration with the WHO surveyed three districts of eastern Terai for the first time in 1999 and found that arsenic concentrations exceeded the 10 ppb guideline recommended by WHO. Over 90% of the Terai population draws ground water from tubewells for drinking, household use, and irrigation. Of the 18,635 tubewells tested, 23.7% have arsenic contents above the WHO limits of 10 ppb, and 7.4% were above the Nepal interim standard of 50 ppb.

1.9.2.2 Latin America

The native population of Latin America may have suffered arsenicism 7000 years ago caused by high arsenic exposure due to consumption of arsenic-contaminated food and water [131].

At the beginning of the 21st century, high arsenic concentrations were found in water bodies from Latin American countries and continued to increase sharply. As of now, 15 of the 20 Latin American countries, such as El Salvador, Nicaragua, Brazil, Bolivia, Cuba, Ecuador, Honduras, Uruguay, Colombia, and Guatemala [132], show high arsenic concentrations in surface and ground water. Castro de Esparza [133] estimated that in Latin America 4.5 million people are exposed to drinking water with more than 50 μg/L of arsenic and 14 million people are exposed to 10 μg/L arsenic concentrations. Studies from Latin American countries revealed that smoking, gender, long latency period, exposure at early life, and incomplete metabolism of arsenic significantly increase the risk of cancer [134].

1.9.2.3 Argentina, Mexico, and Chile

From the beginning of the 19th century to the 1960s, a number of reports suggested arsenic contamination of water from Argentina, Chile, and Mexico. Argentina was the first country in Latin America where ground water was found contaminated with appreciable amounts of arsenic. The first cases of human arsenic poisoning were described from the locality of Bell Ville in the Chaco-Pampean plain in Argentina, related to arsenic-contaminated drinking water. During the years 1913–1917 initial adverse effects of arsenic on human health were reported [135] and further it was found that the effects were predominantly from the northern part of the Chaco-Pampean plain. This region is reported to be the largest identified area in the world with high arsenic concentrations in ground water [136]. The predominating species was found to be As(V) due to moderately reducing to oxidizing conditions.

Mexico is one of the world's major arsenic producers, generating 1946 t in the year 2002. In the Lagunera area, wells were found to contain arsenic concentrations up to 0.624 mg/L. The Lagunera region, located at the northern part of the country, was identified in 1958 as an arsenic-contaminated zone [137]. Thirteen out of 31 states in Mexico, namely Durango, Coahuila, Zacatecas, Morelos, Aguascalientes, Chihuahua, Sonora, Puebla, Nuevo Leon, Jalisco, Oaxaca, Guanajuato, and San Luis Potosi, receive arsenic in supplied drinking water. From these locations, approximately 450,000 people are reported to be exposed to arsenic concentrations of more than 50 μg/L [138]. About 400,000 people were determined as being exposed to arsenic concentrations greater than 50 μg/L in Comarca Lagunera, one of the important cattle-raising areas of Mexico. Sources of groundwater arsenic in these regions were assumed to be due to oxidation of arsenopyrite and dissolution of scorodite. Zimapan mining district was also reported to contain high arsenic content in soil due to smelting and tailings [139]. Arsenic concentrations were also determined in lake water and sediments of Chihuahua state with ranges from 9.11 to 16.62 mg/kg in sediments, while 6 to 170 μg/L were found in water samples [140].

The northern Chile region is characterized by mountainous landscape and a semi-arid climate with highly variable precipitations. The Elqui watershed covers an area of about 9800 km^2, and its main river (the Elqui) constitutes one of the few water resources of this hilly, semi-arid region of Chile. Epidemiological investigations from arsenic-endemic areas of Chile and Argentina have linked high levels of arsenic exposure with elevated risk of bladder and lung cancer mortality [141]. In northern Chile arsenic in river water is largely released from volcanic rocks and weathering of sulfide ore deposits at the Andean volcanic chain and

mobilized by snow melt and rain to the rivers and springs. Geothermal springs are also potential sources of arsenic in this region. Cases of first skin lesions were detected at the beginning of the 1960s, especially in children. During that period approximately 500,000 people were exposed to arsenic-contaminated drinking water. The population of Esquina consumes drinking water from small waterfalls (As—12.2–74.0 μg/L), whereas the population of Illapata consumes drinking water from the Camarones river (As—48.7 μg/L) and waterfalls (As—1252 μg/L). Further, arsenic concentrations in water, urine, hair, and nails were studied in people from the same regions; arsenic was found to be contained therein, in water localities [142]. In this study, the arsenic was found in drinking water (50 and 1090 μg/L), urine (0.20 and 1.10 μg/L), hair (0.30 and 3.8 mg/kg), and nails (3.2 and 11.2 mg/kg), respectively. Another study in northern Chile is from the region of the Elqui valley, a drain area with important hydrothermal alterations and epithermal ore deposits, including arsenic-rich copper channels. Arsenic in the sediments of the valley ranges from 55 to 485 mg/kg in river sediments and from 1.19 to 2.344 mg/kg in Holocene lake sediments, generally associated with iron oxides and iron oxyhydroxides [143].

1.9.2.4 Peru

This region contains arsenic in high concentrations predominantly of geogenic origin, which are released in the Andean region by natural weathering and mining activities. In 2000, it was estimated that around 250,000 people were drinking water with arsenic concentrations exceeding 50 μg/L [133]. The rural population in Peru that depends upon untreated water for drinking and irrigation is mostly exposed to high arsenic. The Rimac river basin situated east of Lima is also contaminated with arsenic predominantly from mining activities in the middle and upper parts of the basin and from natural leaching of Andean volcanic rocks and sulfidic ore deposits. The presence of arsenic adversely affects the quality of water, soils, and vegetables in the lower part of the basin, and is a severe threat to human health. In 1994, the first investigation was performed in 53 drinking water samples from rivers, wells, and springs and it was found that 84.9% of the samples exceeded the limit recommended by the WHO.

1.9.2.5 California

The presence of high concentrations of arsenic in ground waters of the Tulare Basin, California, USA, has been well documented. In this region, shallow groundwater quality is generally poor with high salinity, pesticide residues, and toxic elements such as arsenic, boron, molybdenum, selenium, and uranium present. In 1989, the US Geological Survey investigated the areal distribution of dissolved arsenic in shallow ground water in the southern San Joaquin Valley from areas near Hanford to Lost Hills and extending over to areas near Bakersfield, and reported arsenic concentrations in the range 1–2600 μg/L [144]. Elevated arsenic concentrations exceeding 50 μg/L were also reported to the south of the Tulare Lake Bed in the Kern Fan Element (KFE) west of Bakersfield.

1.9.2.6 Central America

High levels of arsenic were detected in surface and ground water, from several locations in Central America, used especially for drinking and irrigation purposes. The origin of arsenic

in these regions is natural due to the tectonic setting of the Pacific coast, leaching from volcanic rocks and hydrothermal settings, geothermal fluids, and mining and metallurgical processing [145]. In June 2007, there were several announcements about the presence of arsenic in ground water in different areas of the municipality of Mixco in the department of Guatemala with average arsenic concentrations of 15 μg/L [146]. Goff et al. [147] studied the geochemistry of springs in seven geothermal sites in Honduras and detected arsenic in four of them, namely Azacualpa, Pavana, Platanares, and Vanes Caldera, with arsenic concentrations of 70, 110, 1260, and 1160 μg/L, respectively. High concentrations of arsenic have also been detected in surface and ground waters in El Salvador. In this area, water samples from three lakes were studied for arsenic contamination and found to contain arsenic in variable amounts: Coatepeque Lake 90 to 3090 μg/L, Ilopango Lake 290 to 780 μg/L [148], and Olomega Lake 4210 μg/L, the highest arsenic concentration determined in surface waters in El Salvador. In Nicaragua, the contamination of ground water with arsenic was detected in May 1996 in water of an artesian well from the community of El Zapote in the Sebaco valley, containing arsenic at 1320 μg/L, which was further confirmed by different agencies and institutions [149–151]. The largest water quality study (1488 wells) was carried out by UNICEF and the Pan American Health Organization (PAHO) during 2004–2005, suggesting that 18 of the 46 studied municipalities' water supplies had arsenic concentrations higher than 10 μg/L and reaching up to 161 μg/L [151]. Cienfuegos Bay, also in Central America, has a long history of heavy metal contamination due to industrial development in the close vicinity. The contamination became severe in 2001 due to spillage of a high pH solution containing inorganic oxides of arsenic from a nitrogen fertilizer plant.

1.10 Methods of Arsenic Removal from Water

1.10.1 Removal via Precipitation

Arsenic from mining and metallurgical effluents is removed with other metals by precipitation or non-precipitation methods. In precipitation methods, lime is used to precipitate As(III) and As(V) both as calcium arsenite/arsenate, while Fe(II) is used to precipitate arsenic as basic ferric arsenates or crystalline scorodite [152]. Alum and iron precipitations are also common methods used in water treatment for arsenic removal; however, they are not generally believed to be the major arsenate removal mechanisms. Scorodite ($FeAsO_4{\cdot}2H_2O$) is widespread in arsenic-bearing ore deposits and its solubility regulates the concentration of arsenate in natural waters with low pH [153]. As(III) does not precipitate in salt form with metal ions; however, it precipitates in the form of orpiment (As_2S_3) in reducing and sulfide-rich environments.

Along with direct precipitation as pure minerals, arsenic may also co-precipitate with oxides and hydroxides of iron, aluminum, and manganese. Co-precipitation of arsenate with iron and aluminum is assumed to be the chief arsenic removal mechanism in water treatment plants. It has been reported that if the Fe/As weight ratio exceeds 5:1 at elevated pH then two mechanisms, surface complexation and electrostatic attraction, lead to co-precipitation of arsenate. Mamtaz and Bache [154] reported Fe(III) to be more efficient than Fe(II) at removing arsenite from groundwater via co-precipitation due to the lower rate of oxidation of Fe(II). Meng et al. [155]

reported that Fe/As weight ratios greater than 40 can be beneficial in reducing arsenic levels to less than 50 μg/L in Bangladesh well water due to the presence of elevated phosphate. Fuller et al. [156] performed kinetic studies of arsenate adsorption and co-precipitation and showed that the initial arsenate uptake by co-precipitation was significantly greater than by adsorption.

1.10.2 Removal via Adsorption

Arsenic adsorption on different minerals has been reported. Fe oxide minerals are considered to be most important because of their strong binding ability to arsenic. Therefore, the interactions of arsenic with various Fe-bearing minerals such as hydrous ferric oxide, goethite, ferrihydrite, Fe oxide-coated sand, and red mud have been studied. Hydroxides of iron are considered to be most commonly involved minerals in adsorption of arsenic under both acidic and alkaline conditions, while other adsorbents perform the function under acidic conditions only. Arsenic adsorption on aluminum oxides such as alumina, gibbsite, kaolinite, illite, and montmorillonite has also been studied extensively [157]. Adsorption of arsenic in two different oxidation states, As(III) and As(V), was not identical under the same conditions such that As(V) adsorption was high under acidic conditions, while As(III) adsorbs under basic conditions. Several solid materials such as clay minerals, fly ashes, metal oxide mixtures, and specially hydrated lime [$Ca(OH)_2$] have been tested as sorbents for retaining arsenic and other metals. Lopez-Anton et al. [158] reported that activated carbons are able to capture arsenic and other elements present in coal combustion and gasification flue gases to a certain extent.

Natural products are also used as adsorbents because of their good performance and low cost for the removal of heavy metals including arsenic [159]. The most widely adsorbent used is activated carbon manufactured from bituminous coal, agricultural wastes, wood, and petroleum. Efficacy of the adsorbents can be significantly enhanced by physical or chemical activation. The three-pitcher filtration system has been employed in Bangladesh at the household level to remove arsenic from ground water by adding Fe filings for arsenic adsorption [160]. In this method, an upper pitcher is used to reduce arsenate to arsenite, a middle pitcher contains charcoal for adsorption of arsenic species, and a lower pitcher collects arsenic-free water. Natural red earth can adsorb both species of arsenic, and thus by using this adsorbent three pitcher systems can be reduced to two pitcher systems.

It has also been reported that significant arsenic adsorption occurs on the surfaces of metal sulfides [161]. Arsenite and arsenate primarily adsorb at the surface of Fe oxides due to their wide occurrence, high point of zero charge, and high affinity for arsenic. Two commonly occurring minerals of Fe, ferrihydrite and goethite, are considered important due to their large surface area. Major soil constituents such as phosphate, silicic acid, sulfate, and natural organic matter also strongly affect arsenic adsorption. Numerous studies have shown that organic acids such as humic substances also compete with arsenic for adsorption sites on oxide surfaces due to the anionic nature of organic acids and strong affinity of their carboxylic and phenolic groups to the oxide surface [162].

Arsenic removal efficiency can also be improved by pretreatment of oxidized As(III) to As(V). The oxidation methods used include ozone and oxygen, hydrogen peroxide, manganese

oxides, UV/Fe, and TiO_2/UV. It has been suggested that ferric ions assisted by UV/TiO_2 could significantly accelerate the oxidation process of As(III) in comparison to other oxidizing agents [163]. Ferrate [Fe(VI)] is one of the environmentally friendly reagents used in water and wastewater treatment as it can be used as both an oxidant and a coagulant.

Adsorption of arsenic is a complex phenomenon and depends upon properties of the solid surface, hydration, soil pH, changes in cation coordination, isomorphous substitution, crystallinity, concentration, and species of arsenic and competing ions. Adsorption of arsenite and arsenate on mineral surfaces can be of two types: non-specific and specific. In non-specific adsorption, electrostatic attraction lies between a charged surface and an oppositely charged ion in solution in which the adsorbed ion resides at a certain distance from the mineral surface. On the other hand, in specific adsorption a coordination complex is formed between the ion and the mineral surface, which is stronger as compared to non-specific adsorption. It has been suggested that arsenate adsorbs mainly through non-specific sorption (electrostatic attraction), while arsenite is adsorbed via specific sorption (inner-sphere complexation). Adsorption of arsenite and arsenate on various adsorbents under different pH ranges is depicted in Table 1–7. Decreasing pH leads to increased arsenic retention because of the shift in mineral surface charges from negative to more positive; consequently, arsenic species become less mobile. At a very low pH range (less than 1) when nearly all arsenic species exist in neutral form, arsenic is no longer sorbed and becomes mobile again. At low pH, oxide mineral is dissolved in water and arsenic is released into the aqueous phase, with a resultant very high arsenic input to downstream water bodies. Precipitation of Fe^{3+} ions as hydroxides effectively scavenges most of the arsenate from water through sorption. However, arsenite sorption causes comparatively less contamination of downstream water bodies. The sorbed arsenate is also released into the water bodies under acidic conditions due to dissolution of iron hydroxide and under basic conditions due to the change in the surface charge of iron hydroxide.

Anions that are present with similar or higher charge densities such as phosphate (PO_4^{-3}) and sulfate (SO_4^{-2}) may compete for the sorption site of arsenic on mineral surfaces. The priority sequence of various anion adsorptions over activated alumina under the pH range 5.5–8.5 follows the order [164]:

$$OH^- > H_2AsO_4^- > Si(OH)_3O^- > F^- > HSeO_3^- > SO_4^{-2} > CrO_4^{-2} > HCO_3^- > Cl^- > NO_3^- > Br^- > I^-$$

Phosphate, which has similar chemical properties and behavior to arsenate, strongly competes with arsenate for sorption sites on metal oxide surfaces. Significant reduction in arsenate and arsenite adsorption by phosphorus and sulfate on various adsorbents such as amorphous iron oxide goethite, gibbsite, kaolinite, montmorillonite, aluminum oxide, and quartz has been reported [165]. It has been suggested that arsenic pollution of ground water in the Bengal Basin, Bangladesh, is due to competitive exchange of phosphate anions from overusage of fertilizers with arsenic thereby releasing arsenic into water bodies [166].

Yean et al. [167] observed a considerable decrease of arsenic sorption to magnetite nanoparticles in the presence of natural organic matter. Redman et al. [168] suggested that natural

organic matter affects arsenic redox and complexation speciation by delaying formation of sorption equilibrium.

1.10.3 Phyto-Remediation

In the main, extraction of arsenic from polluted soil involves use of physical and chemical techniques which are neither economical nor environment friendly. In addition, they cause further deterioration of the land in the locale and render it unusable. A promising technology for arsenic extraction is phyto-remediation, in which plants known as hyperaccumulators are grown in arsenic-polluted sites. Plants extract large amounts of pollutant from the soil via their roots and concentrate it in aboveground tissues [169]. A Chinese brake fern (*Pteris vittata*) shows excellent arsenic hyperaccumulation properties in contaminated soil. It concentrates arsenic in aboveground biomass, which is a primary requirement for efficient phyto-remediation. Several other ferns (*Pityrogramma calomelanos* L., *Pteris cretica*, *Pteris longifolia*, and *Pteris umbrosa*) have also been reported as arsenic hyperaccumulators [170]. Other plants such as Indian mustard and tomato have also been reported to accumulate arsenic, principally in roots. It has been reported that *P. vittata* stores arsenic in epidermis and mesophyll tissues, and the xylem strands of the pinnae ultimately reach the leaves via transpiration stream [171].

Detoxification of heavy metals by plants is based upon biochemical mechanisms specific for plant species and metals. In general, the mechanism includes formation of intracellular metal-chelating ligands such as amino acids, organic acids, and two classes of peptides: phytochelatins and metallothioneins. Organic acids such as malic and citric acid are known for chelating Ni and Zn, whereas proteins and phenolics detoxify Cu. The thiol-rich phyto-chelatins are specific for the chelation of arsenic via bonding to thiol groups [172]. Arsenic toxicity can also be reduced by applying sulfates of Zn, Fe, and Al to the soil. The arsenic reacts with these sulfates and forms their insoluble salts.

$$\text{Arsenate} + \text{FeSO}_4/\text{ZnSO}_4 \rightarrow \begin{array}{l}\text{Fe/Zn-arsenate} \\ \text{(insoluble and unavailable to the plant)}\end{array} \tag{1.18}$$

1.11 Conclusions

This chapter has provided a global scenario of current status of research on arsenic in the biosphere. The mitigation of arsenic-related problems is a huge challenge worldwide. The foremost requirement to plan advanced treatment and removal technologies is to understand the chemistry behind this unique element. A brief overview has been presented in this chapter pertaining to various sources of arsenic exposure and its mobilization into the environment, which serves as a critical information resource to individuals involved in studying arsenic environmental chemistry and epidemiology and its removal from water bodies [173]. A arsenic for the most part releases naturally from the leaching of volcanic rocks, sulfidic ores, hydrothermal and geothermal alterations, and volcanic exhalations. In addition, mining activities, metallurgical processes, and coal-based thermal power plants contribute significantly to the release of arsenic into air, water, and soil. The presence of arsenic and its mobilization are

highly dependent upon geographical conditions, environmental factors, redox conditions, and pH. Thus, the information gained from a consideration of all these factors will contribute to the exploration and demarcation of high and low risk areas suffering from arsenic contamination.

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2

Ground Water Arsenic Contamination and Its Health Effects in Bangladesh

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CHAPTER OUTLINE

2.1 Introduction

Arsenic contamination of ground water in Bangladesh was first reported in 1993, followed by official reporting of arsenicosis patients in 1994 [1]. It is estimated that more than 95% of the people in Bangladesh drink tubewell water and more than 50 million people are estimated to be ingesting arsenic-contaminated water [1–4]. Approximately 27% of shallow (<150 meter deep) wells in Bangladesh contain more than 50 μg/L arsenic [2]. In Bangladesh, concentrations of arsenic in ground water as high as 4.0 mg/L have been reported from Chatkhil of Noakhali district [2,4]. The worst-affected area is in the southeast of Bangladesh, whereas in some districts more than 90% of tubewells are affected [2,5,6]. Arsenic contamination of ground water tapped through tubewells had been reported from 62 out of 64 districts of Bangladesh [3]. In surveys covering 57,482 villages of 2934 unions in 270 upazillas, 29.12% of the 4,946,933 tubewells were found to be arsenic contaminated. About 15% (8540) of the villages had more

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than 80% of their tubewells contaminated and these villages are identified as "hot spots." Among 66,034,962 individuals residing in 12,001,665 households in these upazillas, a total of 38,430 individuals had been reported as arsenicosis patients [5]. Many of the health problems known to be related to arsenic toxicity have been reported in Bangladesh [1,7–19].

In the early 1970s, when people in Bangladesh mainly relied on surface water (ponds and rivers) and subsurface (dug-well) water sources, diarrheal diseases and cholera were widely prevalent. This had prompted the search for a microbially safe water source. This search had led to mass use of tubewells. With the help of a hand-pump and pipes with strainers sunk a few meters into the ground, tubewells yielded water that was reasonably safe from the point of microbial contamination that causes diarrheal diseases. Moreover, this means of obtaining water became relatively cheap and the water was easy to collect, and in subsequent years hundreds of thousands of tubewells were installed on the basis of personal, governmental, and NGO initiatives. The tube-well installation initiative to supply safe water in Bangladesh was almost a total success, and increased the percentage of people with access to safe water from 77.6% in 1991 to 91.3% in 1994. Though the tubewell installation initiative provided safe water, a new problem regarding groundwater contamination surfaced. Tube-wells were found to yield water containing arsenic at levels not acceptable for consumption even by the Bangladesh water quality standard. The maximum allowable concentration of arsenic in drinking water in Bangladesh is 0.05 mg/L, which is five times higher than the World Health Organization provisional guideline value (0.01 mg/L) for arsenic in drinking water [1,2,6,9,20].

Arsenic contamination of tubewell water in Bangladesh was reported in 1993 by the Department of Public Health Engineering; and in 1994 the Department of Occupational and Environmental Health, National Institute of Preventive Medicine (NIPSOM), identified eight arsenicosis patients in Bangladesh [1,3]. The contaminated tubewells and patients were detected in the western part of Bangladesh in the village of Chamagram of Nawabganj District. Surveys for detection of arsenic in tubewells throughout the country showed a widespread distribution of contaminated tubewells. Regarding identification of arsenicosis patients in Bangladesh, up to 1997, 1625 arsenicosis patients had been identified and in 2008 the number of identified arsenicosis patients increased to 24,389. In 2012, 56,758 arsenicosis patients were identified by the Director General of Health Services (DGHS), Bangladesh [1,3].

2.2 Arsenic Contamination in Bangladesh Ground Water

Ground water is available at very shallow depths all over Bangladesh where the major aquifers are the Holocene alluviums and fan deposits and Pliocene fluvio-deltaic (Dupi Tila) sediments. Mio-Pliocene Tipam sands form minor aquifers in the hilly areas. The aquifers are highly transmissive and generally multilayered. The aquifer conditions vary from unconfined to leaky confined in the shallow alluvial deposits (Holocene alluviums) and are confined in the Dupi Tila and in deeper alluvial deposits. In the Ganges delta area the thickness of the recent sediment is higher and the Dupi Tila sandstone lies at greater depths. In the southern

part (often called coastal plain) the thickness of the alluvial deposit is highest compared to other parts of the country. The aquifer systems in Bangladesh are geologically controlled and depend on the sedimentary characteristics, depositional environments, and other related parameters. Arsenic contamination in ground water is reported to be greater in the Holocene alluviums and fan deposit areas of Bangladesh [2,9,21].

The Bengal Basin is one of the largest sedimentary basins in the world. Bangladesh occupies most of the present-day delta in the Bengal Basin. The Bengal Basin is bounded on the west and northwest by the Rajmahal Hills (Trap). The northeast is bounded by the Garo, Khasi, and Jayantia hills (west to east), which stretch for about 97 km from north to south and 240 km from east to west. In the far northeast, Shillong or Assam Plateau acts as a boundary. Generally, the Bengal Delta is often referred to as the "Ganges-Brahmaputra-Meghna Delta" (GBMD), which is still active. It is reported that sediments from the Himalayas, adjoining India and Burma, had contributed to the development of the Bengal Basin, and the Ganges-Brahmaputra-Meghna river system in addition to tectonic activity, climatic changes, and accompanying sea level changes had played a significant role. The Ganga-Brahmaputra river system had mainly contributed to the buildup of the Bengal Fan. This river system had carried enormous volumes of sediments from the Himalayan belt. The sediments are derived from the upland Himalayan catchments, the Indo-Burman ranges, and from basement complexes of the northern and western parts of West Bengal (Rajmahal Hills, Choto Nagpur Plateau, Shillong Plateau). Along with sediments from those areas many weathered minerals including arsenic had entered the basin and had been deposited in the delta over thousands of years. The high arsenic content areas are found in the catchments of the Ganges, Brahmaputra, and Meghna rivers [22–31].

A variety of anthropogenic sources have been proposed as the cause of particular occurrences of elevated arsenic concentration in ground water in the Bengal Basin, including industrial pollution and the use of agrochemicals and wood preservatives; only the mineralogical source within the sediments of the Bengal Basin is consistent with the full regional extent and magnitude of arsenic occurrence as observed. It is now widely accepted that arsenic in ground water of the region has a source within the sediments of the Bengal Basin originating possibly from the Himalayan region; it is of a natural origin (geogenic in nature) mainly from the GBM river system, especially in the Holocene period [29–35].

2.2.1 Reasons for Leaching Arsenic in the Ground Water of Bangladesh

The reasons behind leaching arsenic in the ground water in Bangladesh are not clear. Three mechanisms have been proposed to explain arsenic pollution of ground water in the GBMD [2,32,33,36–42]:

1. **Pyrite oxidation:** It has been proposed that arsenic is present as arsenical pyrite in the alluvial sediments. Due to heavy withdrawal of ground water through shallow and deep tubewells for irrigation and domestic purposes, a vacuum is created that leads to entry of atmospheric oxygen into the aquifer subsequent to aquifer drawdown, which in turn leads to oxidation of arsenical pyrite and as a result arsenic is released.

2. **Anion (competitive) exchange of sorbed arsenic with phosphate from fertilizer:** According to this hypothesis arsenic anions sorbed to aquifer minerals are displaced into solution by competitive exchange of phosphate anions derived from overapplication of fertilizer to surface soils. Phosphate derived from excessive use of phosphate fertilizer, from latrines, and from the fermentation/decay of buried peat deposits and other natural organic materials may leach into the aquifer and cause displacement of arsenic from sorption sites on aquifer minerals as a result of competitive (anion) exchange, resulting in arsenic pollution in the aquifer. However, it has been suggested that competitive exchange with phosphate generated *in situ* may contribute to arsenic pollution but this contribution is believed to be small.
3. **Reductive dissolution of FeOOH and release of sorbed arsenic to ground water:** The most widely accepted hypothesis is that, under anoxic conditions, reduction of iron oxyhydroxides (FeOOH) takes place, which results in release of sorbed arsenic to solution. Reduction of FeOOH is driven by microbial metabolism of organic matter.

2.2.2 Reasons for Variation of Arsenic Concentrations Between the Aquifers

The reasons for the distinction between groundwater arsenic concentrations in the shallow and deep aquifers of the Bengal Basin are not yet well understood. Differences between the sediments at depth may occur in terms of absolute arsenic concentrations and in the oxidation states and binding properties of the arsenic to the sediments. However, it is also possible that the history of groundwater movement and aquifer flushing in the Bengal Basin has been important in generating the differences in dissolved arsenic concentrations between the shallow and deep aquifers. Older, deeper sediments have been subject to longer periods of groundwater flow, aided by greater hydraulic heads during the Pleistocene period when glacial sea levels around the Bangladesh landmass were up to 130 m lower than today. Flushing of the deeper older aquifers with ground water is therefore likely to have been much more extensive than in the Holocene sediments deposited during the last 5000–10,000 years. Hence, much of the arsenic in the deep sediments may have previously been flushed away [2,9,21,43].

2.3 Extent of Arsenic Contamination in Bangladesh

A nationwide sample survey of tubewells, mostly from the Department of Public Health Engineering (DPHE)-installed tubewells located in 433 upazillas (eight samples per upazilla) from 61 districts (excluding the three hill districts), found that the arsenic concentrations ranged from less than 0.00025 to 1.670 mg/L. The median and mean arsenic concentrations in the samples were 0.004 mg/L and 0.055 mg/L respectively. Among the surveyed "shallow" tube-wells (wells less than 150 m deep), 27% of them exceeded the Bangladesh standard for arsenic in drinking water (0.05 mg/L) and 1% "deep" wells (more than 150 m deep) exceeded the Bangladesh standard. About 9% of the tubewells exceeded 0.20 mg/L, 1.8% exceeded 0.50 mg/L, and 0.1% exceeded 1.0 mg/L [1,2,44].

Deep wells tapping depths of more than 150–200 m have, almost invariably, low arsenic concentrations, less than 5 µg/L and usually less than 0.5 µg/L. Wells from the older Plio-Pleistocene sediments of the Barind and Madhupur Tracts have low arsenic concentrations. The worst-affected areas of Bangladesh are south and east of Dhaka where in some villages more than 90% of the wells have arsenic concentrations above 50 µg/L. The ground waters are predominantly reducing as evidenced by geo-chemical analysis. Arsenic speciation studies have revealed a large range in the relative proportions of dissolved arsenate and arsenite. The modal proportion of arsenite appears to be between 50 and 60% of the total arsenic. Reducing arsenic-rich ground waters from Bangladesh have As^{III}/AsT ratios varying between 0.1 and 0.9 but are typically around 0.5–0.6 [2,4,45,46].

2.4 Arsenic in Different Environmental Media of Bangladesh

Different environmental media information relating to arsenic in the soil, rivers, and foods of Bangladesh are reasonably accessible. However, this is not the case for information on arsenic concentration(s) in air in Bangladesh, which was not able to be retrieved.

2.4.1 Soil

Significantly high concentrations of arsenic have been found in agricultural soil irrigated with arsenic-contaminated ground water. In Bangladesh the average concentration of arsenic in alluvial sand and mud/clay has been reported to be 2.9 mg/kg and 6.5 mg/kg, respectively. Arsenic concentrations varying from 1.5 to 19 mg/kg have been found in Samta village of Jessore, higher concentrations being found in the top layers of the soil. Arsenic concentrations as high as 51 mg/kg and 83 mg/kg have been reported in soils of Faridpur and Comilla districts, respectively [9,47]. Arsenic contents in different depths of soils of Bangladesh have been reported to be less than 10 mg/kg. Table 2–1 shows arsenic contents in soils collected from different areas of Bangladesh and at different depths [48].

2.4.2 River

Arsenic concentrations in water samples from seven rivers in Bangladesh have been reported in the range <0.5–2.7 µg/L but with one sample having an exceptionally high concentration of 29 µg/L. The arsenic content of surface water of these rivers is extremely low and ranges from negligible to 2.25 ppb. The average arsenic content in the sediment from the River Ganges was found to be 2.0 mg/kg (1.2–2.6 mg/kg), that from the Brahmaputra River 2.8 mg/kg (1.4–5.9 mg/kg), and that from the Meghna River 3.5 mg/kg (1.3–5.6 mg/kg). The total arsenic in the sediments of these rivers ranges from 1.021 to 3.525 mg/kg in the pre-flood period (Table 2–2) and from 4.067 to 5.466 mg/kg in the post-flood period (Table 2–3) indicating the transport of arsenic from upstream. Higher levels of arsenic were detected in clay (3.52–6.48 mg/kg) than in silt (1.86–3.94 mg/kg) or sand (1.00–2.98 mg/kg) [2,9,49].

Table 2–1 Arsenic in Water and Corresponding Arsenic in Soils of Different Depths

Location	Water As (mg/kg)	Soil Depth (cm)	Soil As (mg/kg)
Sharsha	0.041	0–15	13.67
Sirjdikhan	0.544	15–30	10.66
Alamdanga	0.021	15–30	11.82
	0.021	0–15	16.65
	0,191	0–15	11.91
Meherepur	0.016	15–30	28.22
	0.163	0–15	33.91
Laksham	0.037	15–30	16.97
	0.658	15–30	39.11
	0.729	0–15	18.13
	0.261	0–15	28.0
Chandina	0.160	15–30	19.27
	0.380	15–30	19.27
Sonargaon	0.860	15–30	14.83
	0.860	15–30	13.67
	0.682	0–15	38.93
	0.860	0–15	22.87
Bancharampur	0.092	0–15	17.15
	0.115	0–15	11.32
Nagarkanda	0.064	15–30	26.56
	0.077	0–15	81.25

Source: Huq et al. (2003) [48].

Table 2–2 Arsenic Concentrations of Sediments from Side and Middle of River Bed during Pre-flood Period

River	Location	Arsenic Concentration (mg per kg)
Jamuna	Bhuapur side	3.53
	Middle	2.67
Padma	Bheramara	3.16
	Middle	1.02
	Pakshi	1.02
Meghna	Daudkandi	3.38
	Middle	3.34
	Gajaria	1.08

Source: Chowdhury et al. (2003) [49].

Table 2–3 Arsenic Concentrations in Suspended Sediment and River Bed Sediments of Padma, Meghna, and Jamuna Rivers during Post-flood Period

		Arsenic Concentration (mg per kg)			
River	**Location**	**Suspended Sediment**	**Riverbed (0–5 cm)**	**Top Riverbed Core**	**Bottom Riverbed Core**
Jamuna	Bahadurabad	4.87	4.41	3.20	3.29
	Jagannathganj	4.07	2.65	2.87	3.54
	Bhuanpur	4.34	4.17	4.19	3.72
	Nagarbari	5.09	3.05	2.92	2.68
Padma	Rajshahi (Kutibari)	4.32	3.65	3.78	3.13
	Pakshi	4.32	3.40	2.46	5.81
	Aricha-Paturia	4.34	3.30	3.37	3.20
	Mawa	4.69	3.91	2.71	4.64
	Tongibari-Digirpar	5.47	3.01	2.88	4.68
Meghna	Sohagpur	–	4.31	5.84	3.53
	Zia fertilizer factory	–	4.73	5.08	2.04
	Daudkandi	–	4.74	3.31	3.87
	Chandpur	4.99	3.24	3.80	4.23
	Heimchar	4.16	3.29	3.33	4.27

Source: Chowdhury et al. (2003) [49].

2.4.3 Food Chain

Arsenic has been found in foodstuffs as a mixture of inorganic and organoarsenical species. High arsenic content in foodstuffs has been reported from many areas of Bangladesh but the arsenic species in most of the food stuffs are not known. High arsenic content in vegetables and crops was found in the areas receiving arsenic-contaminated irrigation and in areas with arsenic-contaminated soils (Table 2–4). Further, it has been found that the arsenic concentration in crops and vegetables that grow in the Gangetic alluvium is high compared to those growing in the Teesta alluvium [50–55]. Studies conducted in arsenic-contaminated areas found more organic arsenic compared to inorganic arsenic in rice and vegetables. The proportions of inorganic arsenic, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in raw rice were 33.6%, 25.2%, and 41.1%, respectively, and in vegetables 27.9%, 21.5%, and 50.6%, respectively. The average total arsenic concentration in raw rice was 882.2 µg/kg. There are wide variations of arsenic content in vegetables; the high arsenic content vegetables include arum lati (1143.4 µg/kg), arum leaf (1134.4 µg/kg), arum root (1130.9 µg/kg), eggplant (893.3 µg/kg), etc. and the low arsenic content vegetables include kalmi leaf (111.5 µg/kg), potato (448.5 µg/kg), amaranth leaf (458.2 µg/kg), etc. (Table 2–5). In arsenic-affected areas of Bangladesh the average daily intake of arsenic through food and drink by an adult was 1017.9 µg of which 54.3% was from rice and vegetables. The remaining 45.7% of arsenic intake was from drinking water. Considering the total intake of inorganic arsenic, rice and vegetables contributed only 27.4% [55]. It is well known that in the human body arsenite is more toxic compared to arsenate [1,7].

Table 2–4 Arsenic Contents in Different Crops of Contaminated and Non-contaminated Areas of Bangladesh

Crops	No. of Samples	Arsenic Content (mg/kg)			
		Contaminated		Non-contaminated	
		Range	Mean	Range	Mean
Tomato	3	0.016–0.049	0.030	0.001–0.025	0.011
Potato	14	0.000–0.390	0.078	0.000–0.284	0.049
Brinjal	3	0.042–0.063	0.049	0.028–0.063	0.045
Lalshakl	4	0.132–0.606	0.321	0.072–0.240	0.163
Amaranth	8	0.093–2.791	0.605	0.060–0.370	0.168
Kataua data	4	0.060–0.333	0.226	0.092–0.163	0.120
Cabbage	3	0.031–0.042	0.06	0.000–0.059	0.047
Indian spinach	5	0.096–0.387	0.205	0.000–0.228	0.111
Okra	2	0.034–0.046	0.040	0.016–0.031	0.031

Source: Farid et al. (2003) [52].

Table 2–5 Arsenic in Rice and Vegetables from Arsenic-exposed Areas of Bangladesh

Foodstuffs	Number of Samples	Speciation of Arsenic in Foodstuffs (μg/kg)			Total Arsenic (μg/kg)
		Inorganic	MMA	DMA	
Raw rice	75	296.3	222.5	363.4	882.2
Amaranth stem	39	166.0	144.4	309.0	619.4
Arum stem	40	229.7	323.1	410.1	962.9
Dhundal	9	214.7	241.7	182.2	638.6
Egg plant	42	252.6	191.6	449.1	893.3
Lady's finger	42	211.6	155.9	318.0	685.5
Papaya (green)	45	177.9	220.5	282.5	680.9
Pumpkin	2	0	331.4	433.5	764.9
Ridge gourd	11	141.2	89.3	486.0	716.5
Snake gourd	22	270.3	121.6	294.7	686.6
Arum lati	34	377.6	232.3	533.5	1143.4
Arum root	16	274.4	149.8	706.7	1130.9
Amaranth leaf	34	39.1	134.3	284.8	458.2
Arum leaf	45	369.3	230.6	534.4	1134.3
Halancha leaf	22	196.0	130.2	348.1	674.3
Indian spinach	73	227.7	157.5	386.0	771.2
Jute leaf	10	212.1	98.4	321.8	632.3
Kalmi leaf	3	62.3	0	49.2	111.5
Pumpkin leaf	26	225.3	193.4	365.9	784.6

Source: Misbahuddin et al. (2007) [55].

2.5 Health Effects of Arsenic Toxicity in Bangladesh

Chronic exposure to arsenic has been linked to adverse health effects in human populations. Arsenic is a known carcinogen and has potentialities of producing cancers at multiple sites, notably in the skin, bladder, kidneys, prostate, and lungs [56–58]. Arsenic is known to have both cancer and non-cancer health effects [56–58]. Many of the health problems related to arsenic exposure, ranging from the classical dermatological signs such as melanosis (Figure 2–1), keratosis (Figures 2–2 and 2–3), and leukomelanosis (Figure 2–4) to respiratory problems, anemia, weakness, conjunctival congestion, diabetes mellitus, hypertension, hepatopathy, peripheral neuropathy, non-pitting edema of lower limbs (Figure 2–5), adverse reproductive outcomes, gangrene (Figure 2–6), Bowen's disease (Figure 2–7), cardiovascular and cerebrovascular diseases, peripheral vascular disease, and skin cancers (Figures 2–8 and 2–9), are already evident among the arsenic-exposed population in Bangladesh [9–19].

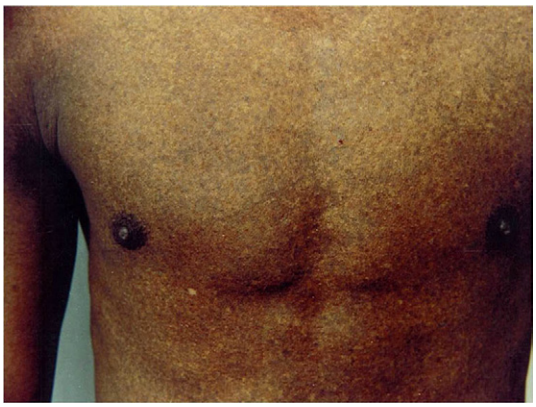

FIGURE 2–1 Melanosis.

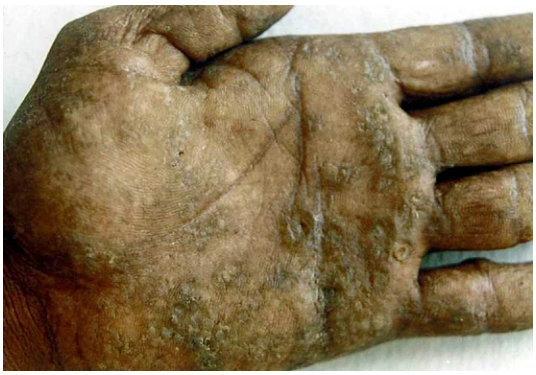

FIGURE 2–2 Keratosis of palm.

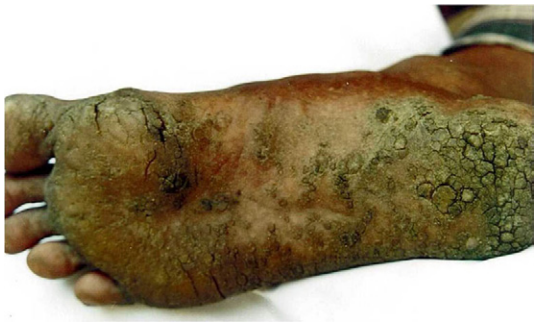

FIGURE 2–3 Keratosis of sole.

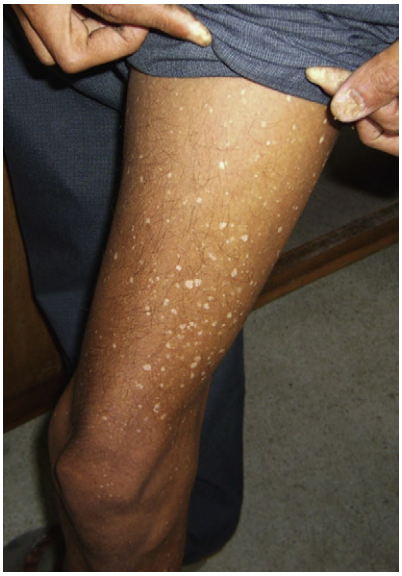

FIGURE 2–4 Leukomelanosis.

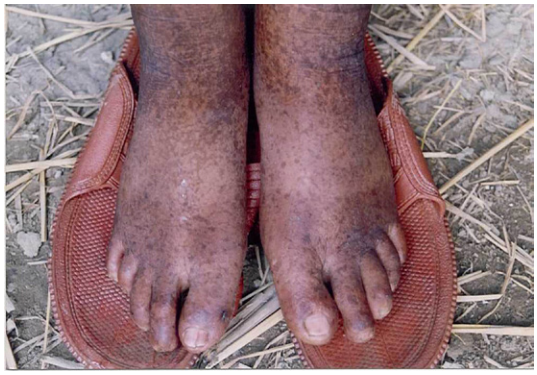

FIGURE 2–5 Bilateral non-pitting edema.

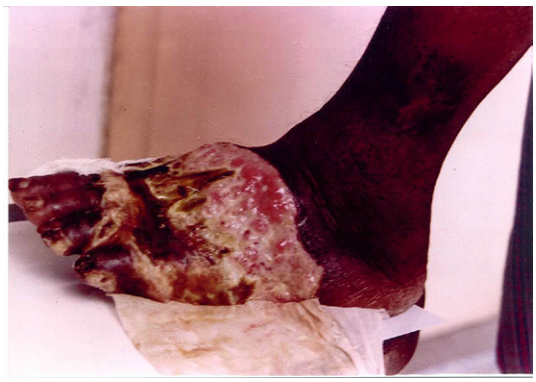

FIGURE 2–6 Gangrene of foot.

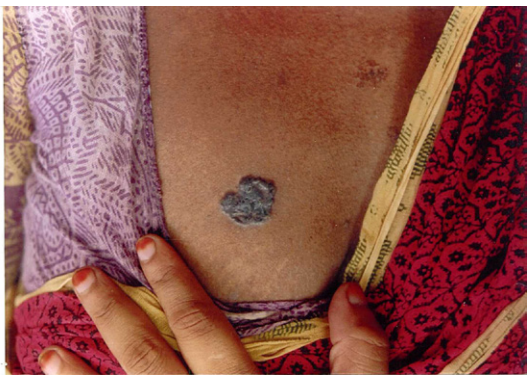

FIGURE 2–7 Bowen's disease.

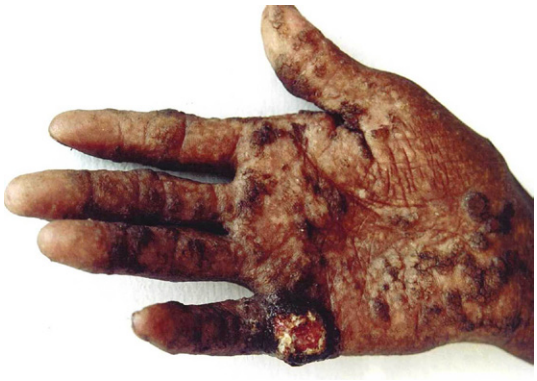

FIGURE 2–8 Squamous cell carcinoma of palm.

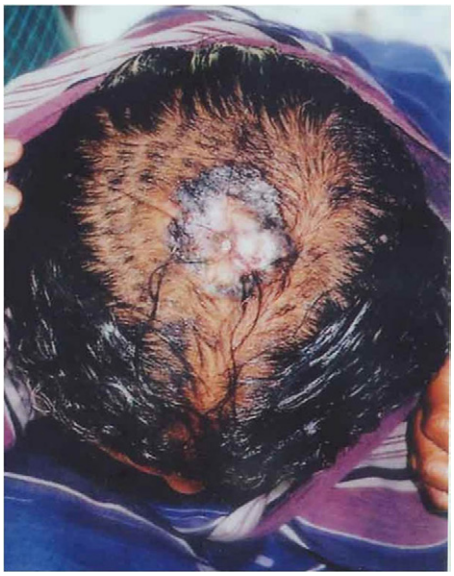

FIGURE 2–9 Squamous cell carcinoma of scalp.

In Bangladesh, the disease due to chronic arsenic toxicity is known as arsenicosis. For identification and diagnosis of arsenicosis cases the WHO case diagnosis and management protocol is usually followed. The disease is usually first diagnosed on the basis of skin manifestations. According to WHO protocol [59], arsenicosis has been defined as a chronic health condition arising from prolonged ingestion of arsenic above the safe dose for at least 6 months, usually manifested by characteristic skin lesions of melanosis and keratosis, occurring alone or in combination, with or without the involvement of internal organs. Two major diagnostic criteria, namely: (1) the presence of pigmentary and/or keratotic skin lesions, and (2) evidence of exposure to elevated levels of arsenic established by history of intake of arsenic-contaminated water (red tubewell water), or by significant arsenic concentrations in urine, hair or nails, are followed during diagnosis. A reliable history of consuming drinking water with an elevated concentration of arsenic (red tubewell water) for at least 6 months is considered to establish arsenic exposure in humans. In the absence of adequate information regarding a subject's exposure history, the finding of elevated levels of arsenic in a subject's urine, hair or nails is taken as presumptive evidence of elevated arsenic exposure. In Bangladesh, the categorization of arsenicosis patients is carried out on the basis of the WHO algorithm (Figure 2–10). The diagnostic algorithm labels individuals as suspected case, probable case, clinically confirmed case, laboratory confirmed case, and non-arsenic case.

A "suspected case" is a subject who shows characteristic skin lesions or pigmentary changes or keratosis on first presentation and who has not undergone in-depth medical examination.

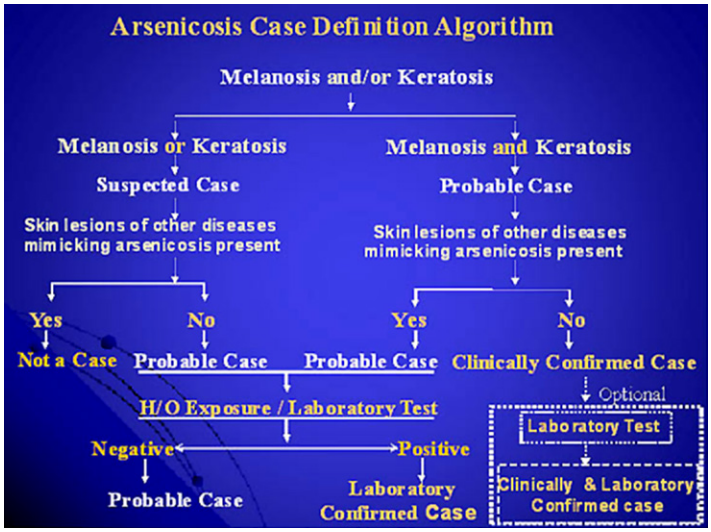

FIGURE 2–10 Arsenicosis case definition algorithm. *Source:* WHO (2005) [59].

The classification of "suspected case" is temporary. It is reclassified as "probable," "confirmed" or "non-arsenic" after further clinical examination and/or history of exposure to arsenic.

A "probable case" is a suspected case that has undergone further clinical examination and belongs to one of the two categories below:

1. Suspected case showing melanosis and bilateral keratosis involving palms and soles.
2. Suspected case showing melanosis or keratosis after excluding other skin lesions mimicking arsenicosis.

A probable case whose history of exposure to arsenic is not available or in whom tests for arsenic in body tissue are found to be negative, maintains the status of probable case.

A "clinically confirmed case" is a "probable case" in which the presence of other arsenicosis-simulating skin lesions has been ruled out by differential in-depth skin examination by either a trained dermatologist or an arsenic expert.

A probable case classified on the basis of differential skin diagnosis becomes a "laboratory confirmed case" when subsequent laboratory tests for arsenic in water or human tissue prove to be positive.

A "non-arsenic case" is a "suspected" or "probable" case in which the medical specialist finds that the patient's skin condition is due to a cause other than arsenic exposure.

The present case definition algorithm for non-cancerous skin lesions shows acceptable sensitivity (>80%) and specificity (>80%) for prevalent arsenic-associated skin lesions.

In different studies (Table 2–6) it was reported that almost 100% of arsenicosis patients had melanosis. Keratosis was found in about 58.8 to 80% of the reported cases. Other common

Table 2–6 Common Manifestations of Chronic Arsenic Toxicity Patients

Arsenicosis Manifestations	*n* = 110	*n* = 96	*n* = 363	*n* = 250	*n* = 116	Total
Melanosis	100.0	98.9	99.5	100.0	100.0	99.7
Keratosis	58.8	92.7	68.9	80.0	79.3	75.5
Conjunctival congestion		9.4	15.7	12.0	25.0	15.3
Chronic cough	33.8	25.8	23.7	20.0	26.7	21.8
Weakness	88.2			100.0	93.0	93.7
Pedal edema	4.4		2.8		6.9	4.7
Skin cancer	2.9	1.04	0.8	1.0		1.4

Source: [7,14,15,60,61].

Table 2–7 Prevalence of Some Non-communicable Diseases (NCDs) Among the Arsenic-Exposed Population

	Exposed to Arsenic			Unexposed			
NCDs	Yes	No	Total	Yes	No	Total	Statistical Test
Diabetes	21	142	163	25	829	854	CPR 4.4
	12.9%	87.1%	100.0	2.9%	97.1%	100.0	(95%, CI 2.5–7.7)
Hypertension	198	1283	1481	9	105	114	CPR 1.7
	13.4%	86.6%	100.0	7.9%	92.1%	100.0	(95%, CI 0.8–3.3)
Respiratory	29	65	94	13	111	124	CPR 2.9
effect	30.8%	69.2%	100.0	10.5%	89.5%	100.0	(95%, CI 1.6–5.0)

Source: [7–19].

manifestations were chronic cough (20.0 to 33.8%), conjunctival congestion (9.4 to 25.0%), and pedal edema (2.5 to 6.9%). Pedal edema is a complication of arsenicosis, usually bilateral, and is mainly reported from Bangladesh and West Bengal, India. However, one of the major complications of the arsenicosis patient is weakness and in Bangladesh weakness was found among 88.2 to 100% of arsenicosis patients [1,10,14,15].

Occurrence of non-communicable diseases such as hypertension and diabetes mellitus is higher among the arsenic-exposed population compared to the non-exposed population. Studies conducted on a Bangladesh population had demonstrated increased occurrence of hypertension, diabetes mellitus, and chronic respiratory disorders in terms of chronic cough and asthma among arsenicosis patients compared to those having no exposure to arsenic [17–19,62–65]. Studies revealed (Table 2–7) that the crude prevalence ratio for diabetes mellitus among arsenicosis patients was 4.4 (95%, CI 2.5–7.7). The crude prevalence ratio for hypertension was 1.7 (95%, CI 0.8–3.3) and that for respiratory disorders was 2.9 (95%, CI 1.6–5.0).

A study conducted in Bangladesh compared ECG findings among the arsenicosis, non-arsenicosis, and non-exposed groups of respondents, and revealed that overall, abnormal ECG findings were high (58%) among arsenicosis cases and highly significant

Table 2–8 Adverse Pregnancy Outcomes Among Exposed and Non-exposed Population

Adverse Pregnancy Outcomes	Arsenic		*P*-value
	Exposed	Non-exposed	
Abortion	68.8	23.7	$z = 2.65$, $p = 0.008$
Still birth	53.1	23.7	$z = 2.0$, $p = 0.046$
Pre-term birth	68.8	27.1	$z = 2.45$, $p = 0.014$

Source: [11].

($p < 0.001$). Prolonged corrected QT was found among 46%, 18%, and 8% of the arsenicosis, non-arsenicosis, and non-exposed groups of respondents, respectively, and was significantly different. Abnormal QRS complex was found among 14%, 8%, and 2% of the arsenicosis, non-arsenicosis, and non-exposed groups, respectively [66]. Another study revealed that mortality due to cardiovascular diseases among the arsenic-exposed population was high in Bangladesh: it was found that 43% of total arsenic-related deaths was due to such disease [65].

An extreme manifestation of lower extremity arterial disease (LEAD) is gangrene, which has been reported in a number of studies focusing on the health effects of arsenic in Bangladesh [1,14]. A study found that the prevalence and risk of LEAD was higher among the population whose drinking water source(s) contained arsenic in excess of 0.05 mg/L than among those whose drinking water source(s) did not contain excess arsenic (<0.05 mg/L). An a normal ankle brachial systolic pressure index (ABSPI) was found more frequently in the arsenic-exposed (13.3%) than in the non-exposed (2.5%) group. Further, the study found that the prevalence and risk of LEAD was higher if patients had developed signs of arsenicosis (melanosis ± keratosis) [67].

For arsenic-exposed mothers, adverse pregnancy outcome has been reported from various parts of the world. In Bangladesh, adverse pregnancy outcome has also been evident in studies [11,68,69]. A study (Table 2–8) that compared pregnancy outcomes in terms of live birth, stillbirth, spontaneous abortion, and pre-term birth among arsenic-exposed and non-exposed women revealed that spontaneous abortion, stillbirth, and pre-term birth rates were significantly higher among those women who had been exposed to arsenic-contaminated water than among those who had not been exposed ($p = 0.008$, $p = 0.046$, and $p = 0.018$, respectively). The participants of the study had been matched for age, socio-economic status, education, and age at marriage [11]. In another study, the odds ratios for spontaneous abortion, stillbirth and neonatal deaths were found to be 2.5 (95% CI 1.5-4.3), 2.5 (1.3-4.9) and 1.8 (0.9-3.6) respectively in mothers whose drinking water arsenic content was greater than 50 µg/L compared to that in mothers whose drinking water arsenic level was 50 µg/L or less [69].

It is known that arsenic is neurotoxic; studies conducted in Bangladesh showed reduced mental development among children aged 1–5 years. In that study 18% of the children who were exposed to arsenic-contaminated water (arsenic levels >50 µg/L) showed abnormal mental development and reduced intellectual function compared to the children who did not drink

arsenic-contaminated water [70]. Another study conducted among children aged 8–11 years revealed an inverse association of arsenic exposure through drinking water with lower developmental scores of the children [71].

Arsenic is a known carcinogen and has the potential to produce cancers at multiple sites, notably in the skin, bladder, kidneys, prostate, and lungs [56–58]. In Bangladesh, skin cancer, lung cancer, and renal cancer have been reported in different studies [12,72,73]. However, skin cancers were more common and the types of skin cancers are squamous cell cancer and basal cell cancer. Bowen's disease is a precancerous skin lesion and is evident among the arsenicosis patients of Bangladesh [7,12,74]. It is estimated that there will be a doubling of the potential lifetime mortality risk from cancer in Bangladesh due to arsenic in drinking water and it is indicated that the overall lifetime mortality risk due to cancer of the lung, liver, and bladder resulting from such exposure will be 229.6 per 100,000 population [72].

2.6 Epidemiology of Arsenicosis in Bangladesh

Arsenicosis is prevalent in the rural areas of Bangladesh, and is associated with age, sex, socio-economic conditions, and nutritional status of the host. In Bangladesh, most arsenicosis patients are between 20 and 40 years of age. People are being exposed to arsenic mainly through consuming arsenic-contaminated tubewell water. So far, no arsenicosis patient has been found among villagers who consume tubewell water having arsenic levels less than 0.082 mg/L. Arsenicosis has been found to be more common among males (53.7%) in comparison to females (46.3%). Female arsenicosis patients are found to be associated with low concentrations of arsenic in tubewell water. A significantly low dose of arsenic intake was found among females (1.321 mg) compared to males (1.734 mg). These findings indicate that females are more susceptible to the toxic effects of chronic arsenic exposure. Arsenicosis patients are mostly from low socio-economic backgrounds and suffering from malnutrition. The majority of these patients are in a mild or moderate stage, and severe cases or cases with complications are few. Mild and moderate cases are found to improve quickly on cessation of further intake of arsenic-contaminated water and by taking protein and vitamin A, E, C-rich food [7,12,14,75,76].

2.7 Management of Arsenicosis Patients in Bangladesh

To date there is no specific treatment for chronic arsenic toxicity in humans available in Bangladesh. Consumption of safe water both for drinking and cooking purposes is key to arsenicosis patient management. In Bangladesh, the current practice of patient management continues to be stoppage of further intake of arsenic-contaminated water; increased intake of vitamin A (β-carotene), E, and C through food and medicinal supplement; increased intake of locally available proteins; and application of keratolytic ointment to remove keratotic lesions of the palm and sole [1,59,77]. These measures are helpful in the early recovery from

symptoms of arsenicosis patients. Mild and moderate arsenicosis cases are found to recover from illnesses using the above management regimen. However, severe arsenicosis cases or cases with complications take longer to recover [1,74,77]. Studies carried out on arsenicosis patient management with different treatment regimens such as spirulina, selenium, and folic acid in Bangladesh found that none of these regimens is very effective [61,77,78], However, the vitamin A, E, and C regimen is still commonly used for arsenicosis management in Bangladesh [74].

2.8 Socio-Cultural Aspects of Arsenicosis in Bangladesh

Arsenic contamination in ground water and thereby the occurrence of arsenicosis is not only a major public health problem but leads to many social problems and economic setbacks in rural Bangladesh. Studies found that poor people are more affected than others by arsenicosis. Skin lesions are higher among men compared to women, and women are more socially damaged than men. The majority of rural people believe that arsenicosis is a contagious disease and could spread from person to person through physical contact, or through contact with objects touched or used by individuals with arsenicosis [75,79,80].

In Bangladesh, women suffering from arsenicosis are ignored in society and may be divorced or abandoned, and unmarried women find difficulty getting married. Female arsenicosis patients are less likely to receive treatment and face further problems getting treatment from a healthcare facility. Social and cultural values make it difficult for them to attend to their own health care and travel to service providers. Other concerns for female patients needing treatment are long waiting times, discrimination in service delivery, and inadequate separate facilities for female patients [79,80].

Arsenic is a poison; in Bangla, poison is “Bish” and rural people know that ingestion of Bish may have many serious consequences, even death. But ingesting arsenic through drinking water does not cause such acute problems, which creates confusion and ignorance among rural people regarding drinking arsenic-contaminated tubewell water. In a study it was found that almost one-third of the respondents knowingly use arsenic-contaminated tubewell water [80].

Besides social problems, there are some economic consequences of arsenicosis to consider. Many arsenic victims who are ill are found to be too weak to work, lose their jobs, or are refused work because of fears of contagion. In a study, the majority (58.6%) of the respondents said they faced various economic setbacks [79,80].

Regarding access to a healthcare facility for arsenicosis treatment, about half (50.7%) of arsenicosis patients face difficulties getting treatment. On the other hand, the length of time needed to recover from symptoms of arsenicosis lead to a loss of faith in the efficacy of treatment, which extends to laxity in seeking health care. Study findings reveal that a significant proportion (79.9%) of arsenicosis patients are found to access alternative health care, which includes homeopathy, village doctors, and *kabiraj* [79].

2.9 Conclusions

Considering the nature of the problem, arsenic contamination in Bangladesh has been recognized as a major public health issue. Arsenic-contaminated tubewells are widely distributed throughout Bangladesh, except in three hilly districts. About 50 million people are at risk of arsenic toxicity.

Besides common skin manifestations, increased risks of non-communicable diseases (NCDs), adverse pregnancy outcomes, and skin cancers among arsenicosis patients are evident and could become a big public health problem.

The most important concern is increased incidence of cancers due to long-term exposure to low dose arsenic—which could overburden the economy and health system of Bangladesh.

The environmental health crisis stemming from arsenic contamination of ground water is currently one of the world's greatest environmental health crises. Millions of people continue to be exposed to arsenic through water and food. The situation is especially worse among rural populations with poor nutrition. Although the full spectrum of arsenic-related morbidity and mortalities in Bangladesh remains to revealed, lessons learnt from other areas, especially Taiwan, Argentina, and Chile, are ominous. The population in Bangladesh will be confronting the consequences of arsenic contamination in water supply, agriculture, and health sectors for generations to come. There is an urgent need to look for alternative sources of water for irrigation, drinking, and cooking. Surface water could be harnessed and used in agriculture. But to find a source of safe water for cooking and drinking both in terms of chemicals and pathogens would be challenging and needs to be given serious thought. Many arsenic removal technologies and systems to remove pathogens have been field tested in rural Bangladesh, but have yielded limited success. In many cases, they were not only expensive, but were either cumbersome or required regular sustained attention. As for health issues, there is a need to introduce programs to enhance the nutritional status of the rural population and surveillance programs for NCDs and cancers. All exposed individuals, not only those having dermatological signs of arsenicosis, should be brought under the protection of a surveillance program.

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3

Arsenic and Fluorescent Humic Substances in the Ground Water of Bangladesh: A Public Health Risk

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CHAPTER OUTLINE

3.1 Introduction

Drinking water should be thoroughly purified to ensure the quality of the water and to protect the health of consumers. Consumption of surface water for drinking purposes has been a primary source of waterborne disease in developing countries like Bangladesh. Over the past few decades, ground water has become an important alternative source for the inhabitants of the Ganges-Brahmaputra-Meghna (GBM) floodplains. Unfortunately, much of the water extracted from the alluvial aquifers contains naturally occurring As (arsenic) from the alluvium sediments deposited in these floodplains [1–3]. Arsenic contamination in ground water of deltaic aquifers of Bangladesh represents the most serious public health concern in terms of global population density [3–5]. Resultant health problems were first identified in West Bengal in the 1980s but the first diagnosis in Bangladesh was not made until 1993 [5–7].

In ground water, arsenic is primarily found in its inorganic forms, either As(III) or As(V). Both inorganic forms are toxic for the human body, where in As(V) is reduced to As(III). The mechanisms of causing toxic effects are based on the inhibition of various mitochondrial enzymes by As(III) and the uncoupling of oxidative phosphorylation. The affinity of As(III) for

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sulfhydryl groups of enzymes and the chemical similarity of As and phosphorus, which allows PO_4^{3-} to be replaced by AsO_4^{3-}, lead to these toxic effects [8]. Several cases of arsenic-related skin lesions were observed in Bangladesh [9]. Since the daily use of groundwater for drinking purposes has become popular in Bangladesh only during the last 20–30 years, it is expected that there will be many more victims of chronic arsenic poisoning in the near future.

Regardless of source, ground water contains dissolved humic substances (HS). HS consists of a complex mixture of organic compounds originating from the degradation of terrestrial and aquatic organisms [10,11]. However, little is known about the actual chemical composition of this material as it consists of a vast number of compounds at very low concentrations, presenting a considerable analytical challenge. Fluorescent HS has been implicated as a contributory factor for blackfoot disease, which is an endemic peripheral vascular disease, occurring on the As-contaminated southwest coast of Taiwan [12–15]. The clinical symptoms include numbness or coldness of one or more of the extremities, resulting in black discoloration, ulceration, or gangrenous changes to the extremities [16–18]. An epidemiological study revealed a causal association between drinking well water and the occurrence of blackfoot disease [18]. In the 1980s, it was reported that the artesian well water in the area of Taiwan contained a high level of fluorescent compounds that induced blackening of the tail and feet of experimental mice [19,20].

Although extensive research has been carried out on arsenic contamination in ground water in Bangladesh by many national and international organizations (e.g., Department of Public Health Engineering (DPHE) Bangladesh; British Geological Survey (BGS), 2001) as well as individual researchers [2,6,9,21,22] during the last decade, the mobilization process of As in the GBM aquifers had still not been properly identified and characterized, and no systematic study had been carried out on fluorescent HS distribution in the ground water in Bangladesh. In addition, as the mobility and poisoning of As in the ground water were controlled by its association with humic substances through different biogeochemical processes [20], it is important to study the linkage between the humic substance properties of ground water and the state of As presence in order to elucidate the mobilization process and health risk. Therefore, the aim of this study was to provide a comprehensive overview of arsenic contamination of ground water and major food composites of Bangladesh, the fluorescence property of ground water, and the qualitative assessment of the health risk of arsenic and fluorescent HS.

3.2 Materials and Methods

3.2.1 Geologic and Demographic Overview

The Ganges, Brahmaputra, and Meghna river systems transport a huge amount of sediments and converge at the lower reaches to form the great delta complex—the GBM delta (Figure 3–1). Bangladesh, located at the head of the Bay of Bengal, occupies most of the Bengal Basin, one of the largest sedimentary basins in the world. The climate is monsoonal humid and tropical, with an average temperature of 27–30°C. The rainy season lasts from May to September. Ground water occurs at very shallow depths all over the country where the major aquifers are the Holocene alluviums and fan deposits and Pliocene fluvio-deltaic (Dupi Tila)

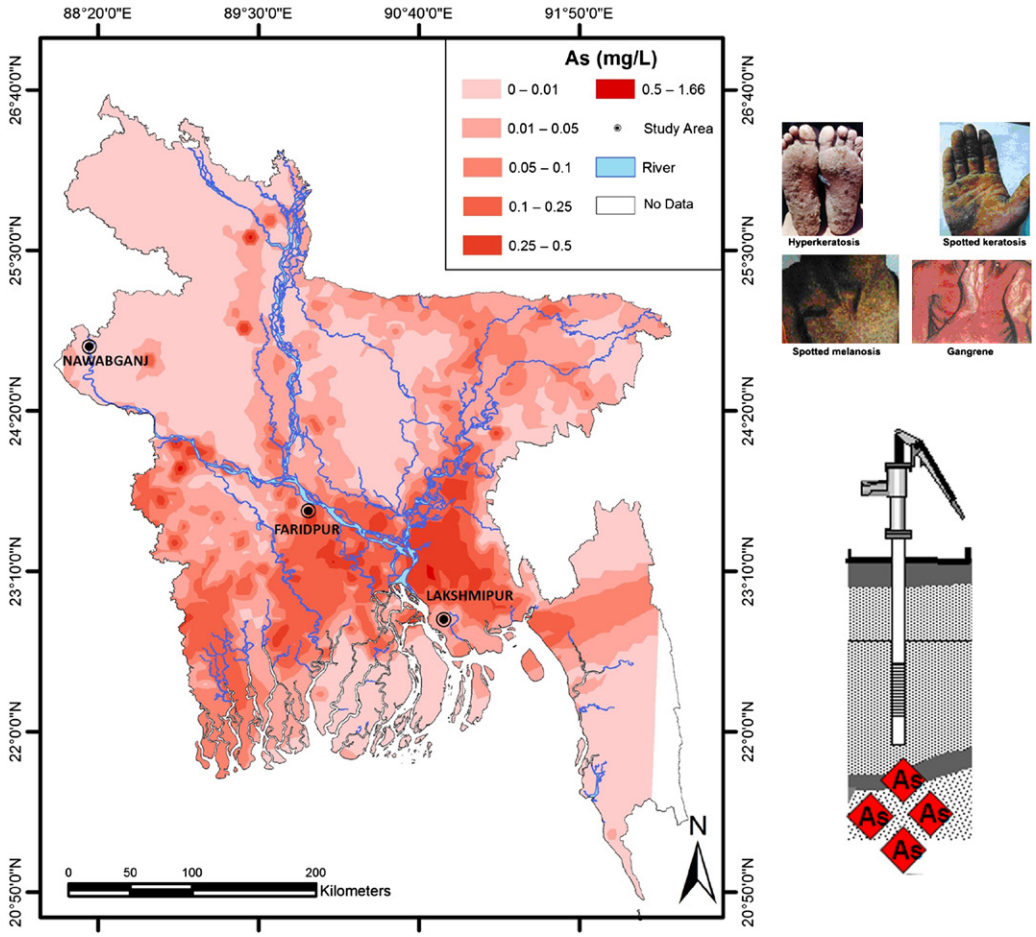

FIGURE 3–1 Geochemical map of arsenic concentration in ground water in Bangladesh. The input data are laboratory analyses from the regional survey by DPHE-BGS [21]. Also shown are dermatological effects of arsenic poisoning in drinking water and locations of three intensive survey areas.

sediments. Water levels lie within a few meters of the ground surface and fluctuate with the annual dry and wet season conditions. The aquifers are recharged during the monsoon season (July–September) when the area receives more than 80% of its annual precipitation (around 2000 mm). Huge amounts of annual flood water standing on a large part of the country for a considerable period also contribute to the recharge process. Annual fluctuations in ground water levels are controlled by the local hydrogeological conditions and withdrawal of ground water for irrigation. In general, the fluctuations are more pronounced in the northwestern part of the country as compared to the coastal plains.

Bangladesh, a developing country, is overburdened with one of the highest population densities, and one of the highest rates of water-related diseases, low per capita income, malnutrition

problems, and natural disasters in the world. Bangladesh has 15.6 million people living in a 144,000 km^2 area. More than 80% of the population depends on agriculture for their livelihood. Historically, clean, uncontaminated water ran through forests and soils that filtered naturally to form clean ground water safe for human and animal use. Nowadays, 90% of rural peoples of Bangladesh depends on ground water for drinking purposes because much of the surface water of Bangladesh is microbially unsafe to drink. Before the 1970s, people usually used surface water (e.g., pond, lake, and river) for drinking and domestic purposes, but severe microbial diseases, especially cholera, were very common at that time. As a poor, developing country, Bangladesh has many health-related problems caused by poverty and insufficient distribution of services, e.g., safe drinking water. As the population grows 1.59% each year, health-related risks increase and the rural/urban gap widens with high health risk from drinking water. Health and social problems caused by environmental issues are heavily impacting on the demography and development.

3.2.2 Sampling

We selected three study areas, namely, Faridpur, Lakshmipur, and Nawabganj, for intensive survey along the side of the main rivers (Figure 3–1). In order to estimate the effect of arsenic on a few other worst-affected districts (Chandpur, Satkhira, Barisal, Pabna, Rajshahi, and Jessore), we have used the data from the DPHE-BGS [21]. Groundwater samples were collected from 100 tubewells in the Faridpur, Lakshmipur, and Nawabganj areas. At least three well bore volumes of ground water were pumped before sampling. Some physicochemical parameters, e.g., pH/Eh, total dissolved solids (TDS), and dissolved oxygen (DO) were also measured *in situ* using portable meters during sampling. Samples for As and other metals analysis were collected in prewashed polyethylene bottles. At each well, bottles were rinsed three times with the respective tubewell water just before sampling. Water was filtered through a Millipore GF/F filter (0.7 μm pore size) prior to acidifying with ultra-pure concentrated HNO_3 to bring the pH to 2. This was done to prevent precipitation of dissolved iron as well as adsorption of trace metal onto the container surface. Samples were stored at 4°C and immediately transported to the laboratory for analysis.

Samples collected for dissolved organic carbon (DOC), fluorescence, and high-performance size exclusion chromatography (HPSEC) analyses were filtered through a pre-combusted Millipore GF/F filter (0.7 μm pore size). GF/F filters were combusted for 4 hr at 450°C to remove organic material prior to use. After filtration, samples were kept in the dark and transported in a cooler box and finally stored in a freezer to prevent microbial degradation and/or other changes before analysis. In addition, a number of vegetables as well as rice samples were collected from the study areas in both Aman and Boro seasons (the two main harvesting seasons in Bangladesh) for measuring total As content.

3.2.3 Analysis

Arsenic was determined by graphite furnace atomic absorption spectrometry (GF-AAS), with a subset of samples also analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

Dissolved Fe and Mn were determined by ICP-MS. The instrument was linearly calibrated from 1.0 to 50.0 µg/L with custom multi-element standard (SPEX Industries) yielding a minimum r^2 of 0.999. The lower detection limit of the instrument was 1 µg/L. All samples were diluted several times to adjust to the operation range and analyzed. Arsenic content in rice samples was measured after digestion. Cation and anion analysis was performed using the ion chromatography (Dionex) technique while HCO_3^- concentration was determined by titration method. DOC was measured by the high temperature catalytic oxidation method (HTCO) with a TOC 5000A (Shimadzu, Japan) using potassium hydrogen phthalate (KHP) as a standard. Samples and standards were acidified to pH 2 with 6 M HCl and sparged with carbon dioxide-free carrier gas for 10 min at a flow rate of 100 mL/min to remove inorganic carbon prior to injection. A non-dispersive infrared detector measured carbon dioxide gas from the combusted carbon. Each sample was injected 4–6 times. The average relative standard deviation for all the samples was 5%.

Fluorescence properties of samples were determined by a spectrofluorometer (F-4500; Hitachi, Tokyo, Japan). Bandwidth was 5 nm for excitation and emission, scanning speed was 1200 nm/min, and scanning range was 225–500 nm for excitation (Ex) and 240–600 nm for emission (Em). Fluorescence data were collected every 5 nm of excitation wavelength and every 2 nm of emission wavelength. The corrections of spectra were performed with rhodamine B solution for excitation wavelength and with a light diffuser for emission wavelength, according to the instrument operation manual. Fluorescence data were obtained in triplicate for each sample and averaged. EEM data were calibrated by normalization to water Raman scattering [23]. To calculate the Raman area of the samples, the Raman signals were corrected by the baseline and integrated over the entire Raman peak for each excitation wavelength. Then, the fluorescence intensities were divided by the Raman area for the corresponding excitation wavelength to obtain a Raman unit (RU, nm^{-1}). The advantages of Raman normalization are suitable for samples with wide ranges of concentration and different properties of DOM [24].

The HPSEC analysis followed the method of Yamada et al. [25] using an HPLC system (Shimadzu LC-10AD) equipped with a column of Superose 12 10/300 GL (Pharmacia Biotech, 10 mm ID–300 mm), and 0.01 M NaOH solution was used as an eluent at a flow rate of 0.40 mL/min. Separated humic molecules were detected by both UV (280 nm) and fluorescence (Fl, Ex: 340 nm, Em: 435 nm) detectors. Natural organic matter (NOM) purchased from the International Humic Substance Society (IHSS) was used as a standard to quantify humic substance carbon. Sodium polystyrene sulfonates (PSS) were used as apparent molecular weight (MW) calibration standards.

3.3 Results and Discussion

3.3.1 Groundwater Quality

The groundwater quality of Bangladesh has been studied extensively during recent years [9,21,22]. Groundwater pH is predominantly near neutral to slightly alkaline (pH 6.5–7.6) with low DO. The Eh values (+0.594 to −0.444 V) suggest a mildly oxidizing to moderate/strongly

reducing character of the aquifers. Before the discovery of arsenic contamination, the quality of ground water of the Holocene floodplains was assumed to be generally good [26], although shallow ground water is vulnerable to contamination by bacteria [27]. Iron was known to be a widespread problem, and salinity is a constraint in the shallow aquifers of the coastal area. Subsequently, in addition to arsenic, the DPHE [21] has identified manganese and boron as common, naturally occurring constituents, present in places at concentrations above the World Health Organization (WHO) health-related guidelines for drinking water, 0.5 mg/L in both cases. Ground water is generally of the Ca-HCO_3 or Ca-Mg-HCO_3 type, although Ca-Na-HCO_3 type and Na-Cl type water are also found locally in several parts of the country, mostly near the coast [28]. The ion composition is dominated by HCO_3^- (169–848 mg/L) and appears to vary according to depth and lithology [28]. There are elevated bicarbonate levels, together with the high dissolved iron and other indications of reducing conditions. Concentrations of SO_4^{2-} (<2.50 mg/L) and NO_3^- (<0.70 mg/L) are generally low, except for some local variations [21]. Dissolved iron is typically present at around 5–10 mg/L. Manganese commonly exceeds 0.50 mg/L. Considerable variability is noted in the levels of total As (range from 0.02 to 2.40 mg/L), total Fe (range from 0.40 to 11.30 mg/L), and Mn (range from 0.01 to1.86 mg/L) in the groundwater samples as a function of both depth and region. Although As(V) is prevalent in some wells, As(III) is the dominant species, representing about 50–90% of total As in the ground water [28,29].

High HCO_3^- concentrations correlate with the levels of DOC (1.15–14.7 mg/L) in groundwater. DOC levels in the analyzed groundwater indicate distinct trends of variation with both total As and Fe concentrations. Although DOC characteristics in Bangladesh's groundwater are not investigated in detail, organic matter in the Holocene sediments can be considered as an active source for DOC in groundwater [30]. Sulfate and NO_3^- have no significant correlation with As. Microbial degradation of the organic matter in the aquifers results in the reduction of both SO_4^{2-} and NO_3^-, thereby increasing the concentrations of sulfide (2 mg/L) and NH_4^+ (up to 13.2 mg/L) in groundwater [31]. Little is known about the concentrations of pesticides in the ground water in Bangladesh, although they are widely used for agriculture practices, and surface water has been found to contain chlorpyriphos, carbofuran, and carbaryl (commonly used pesticides) at concentrations ranging from 0.54 to 0.89, 0.95 to 1.67, and 0 to 0.19 µg/L, respectively [32].

3.3.2 Arsenic Poisoning in Ground Water

A nationwide survey by DPEH-BGS showed that As concentrations ranged from less than 0.01 mg/L to more than 1.60 mg/L. Figure 3–1 shows clear differences in As concentrations in different parts of Bangladesh with the greatest number of high As wells in the south and southeast of the country. However, superimposed on this regional pattern is considerable well-to-well variability over the scale of a few kilometers. The high As content region in the south and east of Bangladesh is clear from this map. The survey showed that 25% of all the tubewells sampled contain in excess of 0.05 mg/L As, the Bangladesh drinking-water standard. In addition, 9% of the tubewells had As levels exceeding 0.20 mg/L, 1.8% exceeding 0.50 mg/L, and

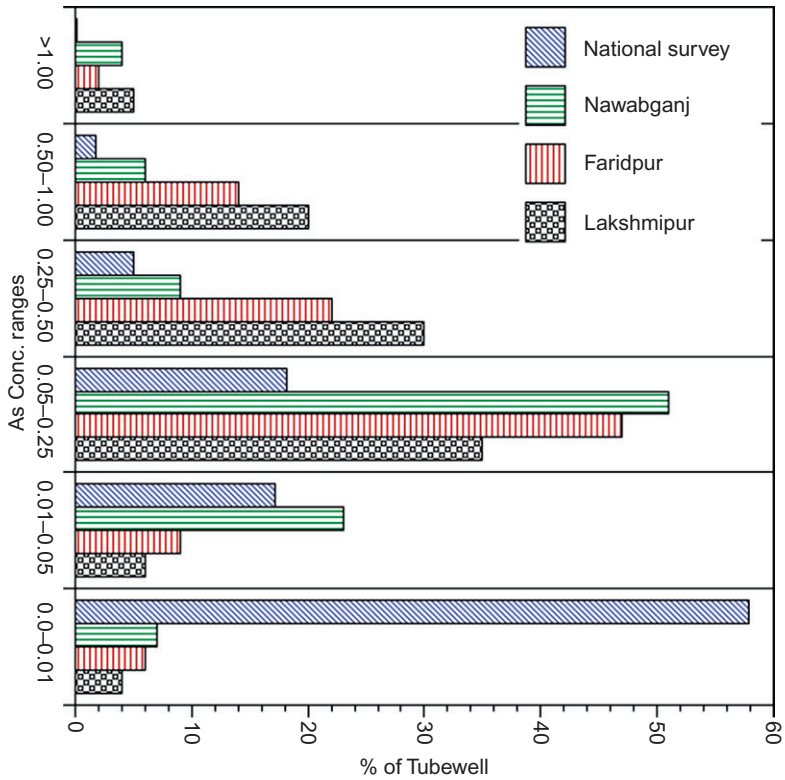

FIGURE 3–2 Percentage of contaminated wells at different levels of arsenic concentration in ground water in Faridpur, Lakshmipur, Nawabganj, and the whole of Bangladesh.

0.1% exceeding 1.00 mg/L. Few shallow ground waters from the south of the country were arsenic free. They are also typical of the deep aquifer and the water derived from aquifers in the older sediments of the Madhupur and Barind tracts. The mean concentration was about 0.05 mg/L. This value depends to some extent on the concentration of arsenic assumed to be in the large number of wells containing less than the detection limit.

Intensive survey of the three study areas, namely Faridpur, Lakshmipur, and Nawabganj, showed that the level of arsenic in the well with the highest percentage of contamination is in the range of 0.05–0.25 mg/L (Figure 3–2). A comparison of these results with the magnitude of pollution throughout the country by using the DPHE-BGS national database shows a similar pattern of pollution with different orders of magnitude, although the DPHE-BGS country-wide data were not built on intensive survey or based on severely contaminated zones. The majority (76%) of the tubewells yielded arsenic concentrations above the current WHO guideline of 0.01 mg/L and the concentration varied greatly (Faridpur: 0.02–2.25 with an average value of 0.55 mg/L, Lakshmipur: 0.02–2.05 with an average value of 0.35 mg/L, and Nawabganj: 0.06–2.40 mg/L with an average value of 0.62 mg/L) within the present study areas. Arsenic concentrations in 71% of the studied wells in the Faridpur area, 73% in the

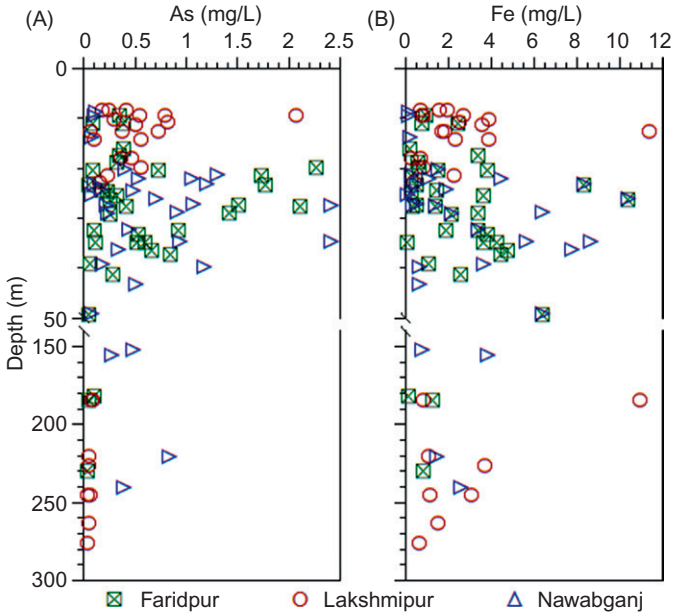

FIGURE 3–3 Vertical distributions of arsenic (As) (A) and iron (Fe) (B) in ground water in Faridpur, Lakshmipur, and Nawabganj, Bangladesh.

Lakshmipur area, and 81% in the Nawabganj area exceeded the Bangladesh standard of 0.05 mg/L.

The deeper aquifer water of the study area appears to be less contaminated than the overlying alluvial aquifer with respect to arsenic (Figure 3–3A). Ground water from depths of more than 150 m in all the areas typically had low As concentrations. Water from very shallow hand-dug wells also had low As concentrations. Variation in Fe concentration with depth was neither systematic nor correlated with As concentration. Iron concentration varied in wide ranges of 2.22–10.3, 0.15–11.3, and 0.01–10.3 for Faridpur, Lakshmipur, and Nawabganj, respectively. The concentration of iron in small number of shallow wells (20–50 m) was very low while water of some of the deep wells nearby contained considerable amounts of Fe in both Lakshmipur and Nawabganj (Figure 3–3B), suggesting Fe occurrence depends on the fluvial-sedimentary history.

Figure 3–4 shows the spatial distribution of arsenic in groundwater in Faridpur, Lakshmipur, and Nawabganj. The spatial distribution of arsenic in the shallow aquifer forms no systematic pattern, although there is some tendency for acute arsenic enrichment within and immediately towards the meander of the rivers. In many areas adjacent to the Lower Meghna Estuary more than 80% of wells exceed the 0.05 mg/L concentration limit. For example, Jakariya and Bhattacharya [33] have reported that 93% of 12,000 wells in Hajiganj Upazila in southeast Bangladesh exceed the limit. The probability of encountering extreme As concentrations, above 0.25 mg/L, is also highest in the south and southeast (Figure 3–1 and Figure 3–4). High concentrations of arsenic are found in the lower catchments of all three

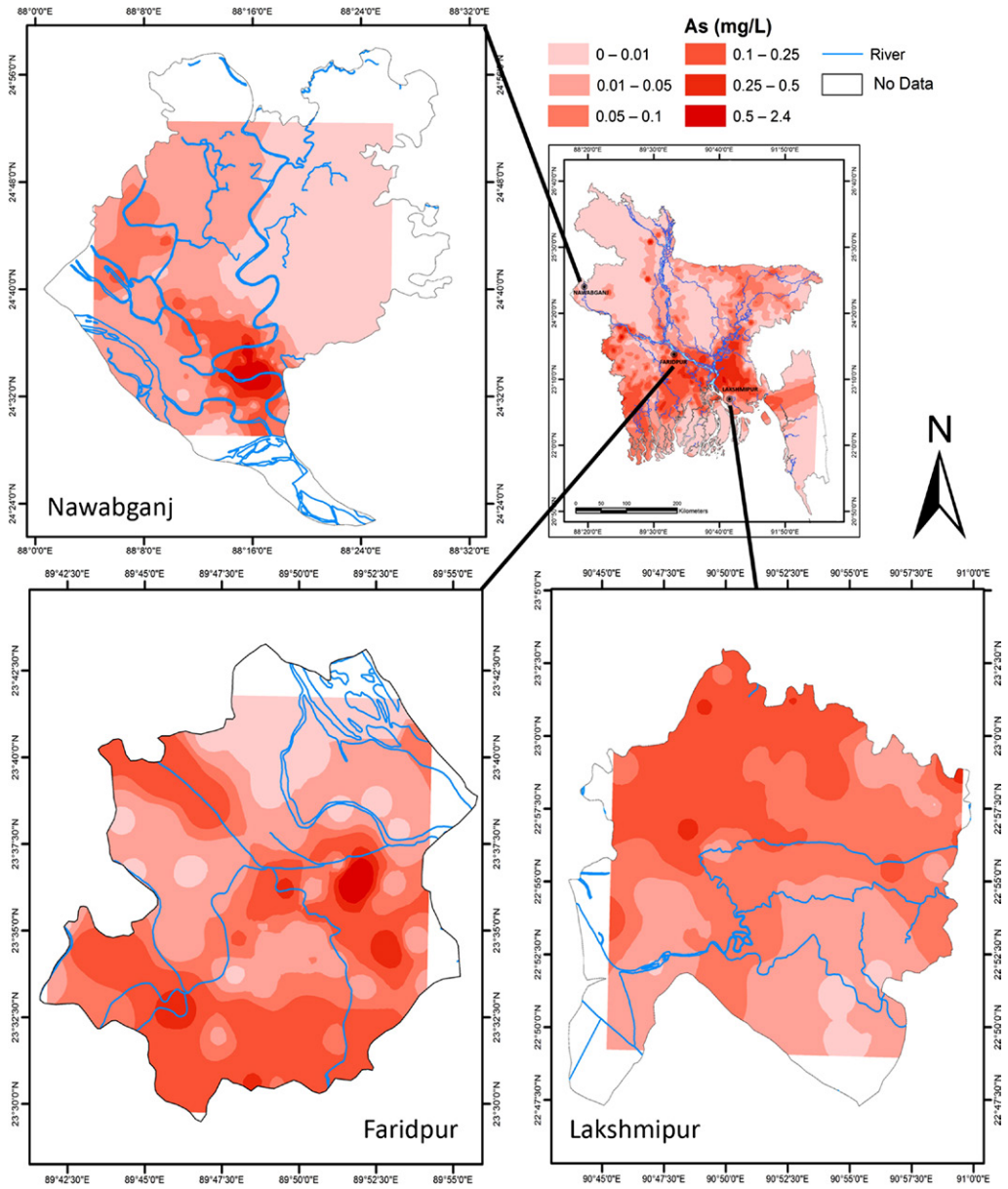

FIGURE 3–4 Spatial distributions of arsenic (As) in ground water in Faridpur, Lakshmipur, and Nawabganj, Bangladesh.

major rivers of the GBM system, indicating the existence of multiple source areas and the likelihood of related mechanisms of mobilization across the whole of Bangladesh. Arsenic speciation showed that the median percentage of As(III) was close to 50% but there was a wide range of As(III) to As(V) ratios and little relationship with other measured parameters, which

confirms earlier experience in Bangladesh and West Bengal [2,3,6,7,23]. The more detailed chemical data from the DPHE-BGS report confirm that the waters are anoxic with high dissolved ammonium concentrations in Faridpur and Lakshmipur (but not Nawabganj), and low nitrate concentrations everywhere except where surface pollution was suspected.

3.3.3 Arsenic in Rice and Vegetables

The pattern of arsenic bioaccumulation and its transfer from one trophic level to another is important. The immediate and long-term impact of using As-contaminated water for irrigating paddy soils is a burning concern as As can transfer from water to soil and plants and several studies have proven this phenomenon. Meharg and Rahman [34] predicted that soil arsenic levels could be raised by 1.00 mg/kg per annum due to irrigation with As-contaminated water. Alam and Sattar [35] showed that As contained in soils was positively correlated with As content in water. In the unaffected areas, where irrigation water contained little As (<1 μg/L), As concentrations of rice field soils ranged from 1.5 to 3.0 mg/kg and did not vary significantly with either depth or sampling time throughout the irrigation period. In the As affected areas where the irrigation water contained elevated As (0.08 to 0.44 g/l), As concentrations of rice field soils were much higher compared to those in the unaffected areas and varied significantly with both depth and sampling time [36].

Arsenic is not an essential element for plants and animals. Food crops such as rice and vegetables can become a route by which As may enter in the food chain, because they can reflect the levels of As that exist in the environment in which they are cultivated (soil and irrigation water). So, the accumulation of As in rice field soil and its introduction to the food chain through uptake by the rice plant and vegetables are of major concern (Figure 3–5).

There are two seasons for rice cultivation: aman and boro. The aman cultivation period is in the rainy season when no irrigation is required but the boro cultivation phase (in the dry season) is completely dependent on irrigation. About 86% of total ground water withdrawn in Bangladesh is utilized in the agricultural sector, especially in rice cultivation in the dry season. A total of 925,152 shallow and 24,718 deep tubewells were used for irrigation during the 2004 dry season [37] and groundwater irrigation covered about 75% of the total irrigated area. It is thought that surface soil of agricultural land accumulates arsenic from contaminated water due to its high affinity with metal oxides/hydroxides in the soil.

Concentrations of arsenic in rice of both amon and boro seasons collected from Faridpur, Lakshmipur, and Nawabganj in Bangladesh were investigated and compared with As concentrations in rice in other regions (Table 3–1). It was observed that the maximum concentrations of arsenic was observed in boro rice in Faridpur (0.51 ± 0.07 mg/kg), followed by Lakshmipur (0.42 ± 0.08 mg/kg), Satkhira (0.38 ± 0.03 mg/kg), and Nawabganj (0.34 ± 0.05 mg/kg). Boro season rice contained more As than was found in aman season rice in all regions (Table 3–1). Williams et al. [38] showed that rice obtained from districts with contaminated waters (>0.05 mg/L) had clearly more elevated As levels than those found in rice from less contaminated or uncontaminated (<0.05 mg/L) districts. A high level of As in rice grain (ranging

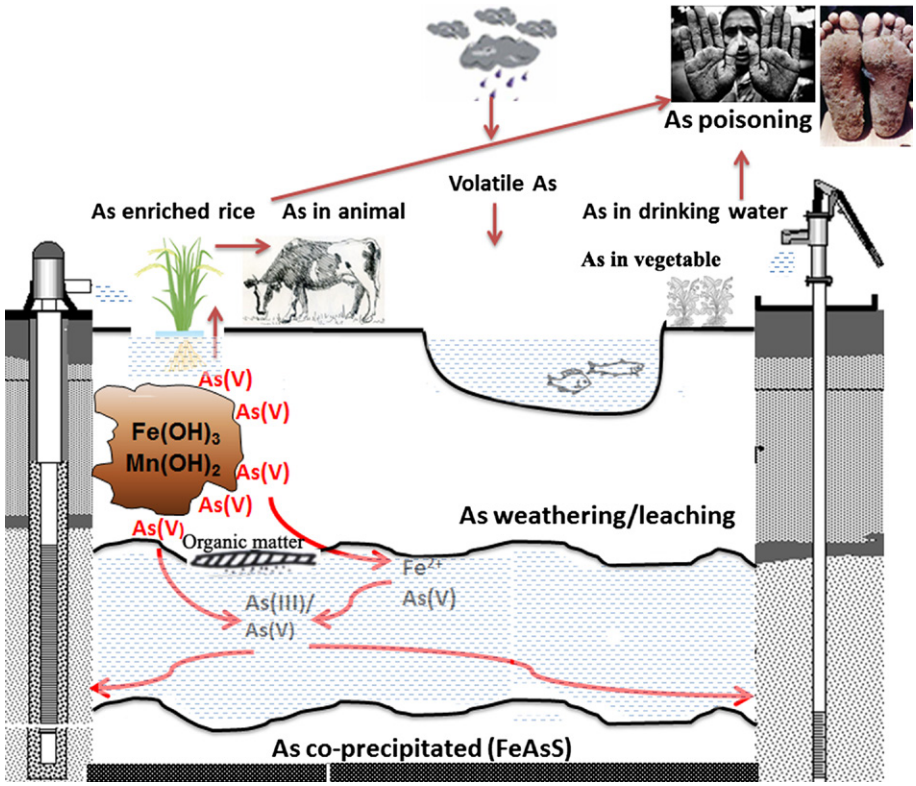

FIGURE 3–5 Possible routes of arsenic exposure to humans through the water-soil-vegetation-animal food chain in Bangladesh.

Table 3–1 Arsenic Content in Rice Grain and Corresponding Water and Soil in Different Districts of Bangladesh

Location (district name)	Aman Season Rice (mg/kg dry wt.)	Boro Season Rice (mg/kg dry wt.)	As in Soil (mg/kg dry wt.)	As in Ground Water (mg/L)
Faridpur	0.34 ± 0.06	0.51 ± 0.07	2.53–16.78	0.55
Lakshmipur	0.36 ± 0.04	0.42 ± 0.08	1.24–11.25	0.35
Nawabganj	0.29 ± 0.03	0.34 ± 0.05	3.17–18.31	0.18
Barisal[a]	0.16 ± 0.01	0.25 ± 0.06	–	0.09
Chandpur[a]	0.22 ± 0.02	0.28 ± 0.09	–	0.37
Dhaka[a]	0.11 ± 0.02	0.12 ± 0.23	–	0.04
Khulna[a]	0.12 ± 0.01	0.17 ± 0.02	–	0.04
Satkhira[a]	0.36 ± 0.04	0.38 ± 0.03	–	0.13

Data are presented as mean ± SD (n = 3).
[a]Data obtained from [37].

between 0.34 and 0.51 mg/kg) in Faridpur and Nawabganj has been found. Duxbury et al. [39] found that arsenic concentrations in rice could varied from 0.01 to 0.42 mg/kg in dry conditions. On the other hand, the study of Abedin et al. [40] showed that no samples of rice grain had arsenic concentrations more than the recommended limit of 1.0 mg/kg in different regions of Bangladesh. Arsenic accumulation in rice grain depends on the variety of rice [38]. Thus, the magnitude of As levels in rice grain is related to the magnitude of As levels in irrigated water and soil as well as variety of rice species. It appears that As present in irrigation water and soil results in higher levels of As in rice plant root, leaf, and stem. A very recent study of Khair [40] on the distribution of As in rice plant showed that the order of As accumulation in the rice plant was root > leaf > grain and they detected levels of As up to 248 ± 65 mg/kg in root tissue whereas 1.25 ± 0.23 mg/kg was detected in the grain. It can be predicted that As-contaminated irrigation water could easily increase the As level in rice grain, straw, and other parts of the rice plant. Arsenic contents in boro rice could be 1.3 times higher than for aman rice (Table 3–1). However, accumulation of As by rice largely depends on redox potential in plant and soil phosphate concentration, rhizosphere iron plaque formation, microbial activity, and rice variety [34]. The precise mechanisms controlling the translocation of As to grain are yet to be determined.

Several studies have indicated that the concentration of As in edible parts of most plants is generally low but long-term ingestion of As-enriched rice grain could be dangerous for human health. In Bangladesh, the majority of the residents depend on rice for their caloric intake (about 70% of total), suggesting that rice is an important dietary source of arsenic for the Bangladesh population (Figure 3–5). The average daily rice consumption by an adult in Bangladesh is between 400 and 650 g raw rice grain [39]. Therefore, intake of arsenic from rice and its potential impact on human exposure should not be ignored. Use of contaminated groundwater for drinking and cooking may exacerbate the overall situation.

Arsenic concentrations in 14 different vegetables collected from homestead gardens of some arsenic-prone villages in Bangladesh were investigated and the data are presented in Table 3–2. It was observed that the maximum concentrations of As was observed in string beans (*Vigna sesquipedalis*) (1.26 ± 0.06 and 0.88 ± 0.0.04 mg kg^{-1}), followed by chilli (*Capsicum melongena*) (0.87 ± 0.48 mg kg^{-1}), mint (*Mentha viridis*) (0.56 ± 0.04 and 0.59 ± 0.07 mg kg^{-1}), and beans (*Lablab niger*) (0.40 ± 0.21 and 0.44 ± 0.02 mg kg^{-1}), in Lakshmipur and Faridpur areas, respectively; meanwhile, the As concentrations were below the detectable limit (BDL) in okra (*Abelmoschus esculentus*) and bottle gourd (*Lagenaria siceraria*). In light of the current legislation and health considerations, the vegetable products, except for string beans, are safe to consume because the average As concentrations in these vegetables are much lower than the acceptable limit in Bangladesh (1.0 mg/kg). As reported in the literature, the total As contents in vegetable products were <0.004 to 0.303 mg/kg fresh weight [41], which is within the range of values found in the samples in Bangladesh (Table 3–1).

The average arsenic concentration in the vegetables collected from some arsenic-prone areas of Bangladesh was 0.28 mg/kg fresh weight (ranging between 0.25 and 0.38 mg/kg fresh weight), which was higher than that of the United Kingdom, 0.003 mg/kg fresh weight [42]. A Bangladeshi individual, regardless of gender, consumes an average of 130−200 g of vegetables

Table 3–2 Arsenic Content in Common Vegetables Collected from Arsenic-prone Areas of Bangladesh

		Arsenic Content (mg kg^{-1} fresh weight)	
Vegetables	**Scientific Name**	**Lakshmipur Area**	**Faridpur Area**
Chilli	*Capsicum frutescens*	0.22 ± 0.07	0.87 ± 0.48
Sweet gourd (pumpkin)	*Cucurbita maxima*	0.11 ± 0.01	0.12 ± 0.02
Okra	*Abelmoschus esculentus*	BDL	–
Red amaranth	*Amaranthus gangeticus*	–	0.16 ± 0.03
Tomato	*Lycopersicon esculentum*	0.08 ± 0.01	0.54 ± 0.31
Bottle gourd	*Lagenaria siceraria*	BDL	BDL
Bean	*Lablab niger*	0.44 ± 0.02	0.40 ± 0.21
Bitter gourd	*Momordica charantia*	–	0.37 ± 0.05
Brinjal	*Solanum melongena*	0.24 ± 0.01	0.26 ± 0.07
Green papaya	*Carica papaya*	0.08 ± 0.03	–
Mint	*Mentha viridis*	0.59 ± 0.07	0.56 ± 0.04
Potato	*Solanum tuberosum*	0.12 ± 0.07	–
Pumpkin leaf	*Cucurbita maxima*	–	0.41 ± 0.07
String bean	*Vigna sesquipedalis*	1.26 ± 0.06	0.88 ± 0.04

Data are presented as mean ± SD (n = 3). BDL = below detection limit.

per day (leafy and non-leafy) [43]. Thus, the average dietary intake of total arsenic from vegetables by the inhabitants of arsenic-prone areas in Bangladesh was estimated to be 0.036–0.056 mg/day. In another study, Rahman et al. [44] reported that the average dietary intake of arsenic from vegetables by the inhabitants of Bangladesh was 0.015 mg/day. However, the recommended daily dietary intake of vegetables is 200 g/person/day, though the availability of vegetables is only about one-fifth of the suggested intake in Bangladesh [43]. If we consider that every person is able to fulfill the recommended amount of vegetables in their daily diets, the estimated average daily dietary intake of arsenic from vegetables in Bangladesh would be 0.056 mg/day. The average intake of total arsenic from vegetables by an adult has been reported as 0.015 mg/day in the Netherlands [45], 0.0592 mg/day in Canada [46], and 0.160–0.280 mg/day in Japan [47]. From a toxicological point of view, a daily intake of 2 µg of inorganic arsenic per kg body weight should not be exceeded to minimize the health risk [48].

3.3.4 Fluorescence Properties of DOC

All of the groundwater samples collected from three study areas exhibit high fluorescence, implying the presence of fluorescent materials in DOC of groundwater in Bangladesh. The three-dimensional excitation emission matrix (3DEEM) fluorescence spectra of groundwater samples generally exhibited two major fluorescence maxima: humic-like and protein-like fluorescence (Figure 3–6A). The humic-like fluorescence peak occurred at Ex/Em = 335–365/435–480 nm, and the protein-like fluorescence occurred at Ex/Em = 275–290/310–335 nm (Figure 3–6A). The major peaks of the groundwater samples in three study areas were found at Ex/Em = 335–350 nm/440–465 nm with shoulder peaks at Ex/Em = 325–340 nm/425–450 nm

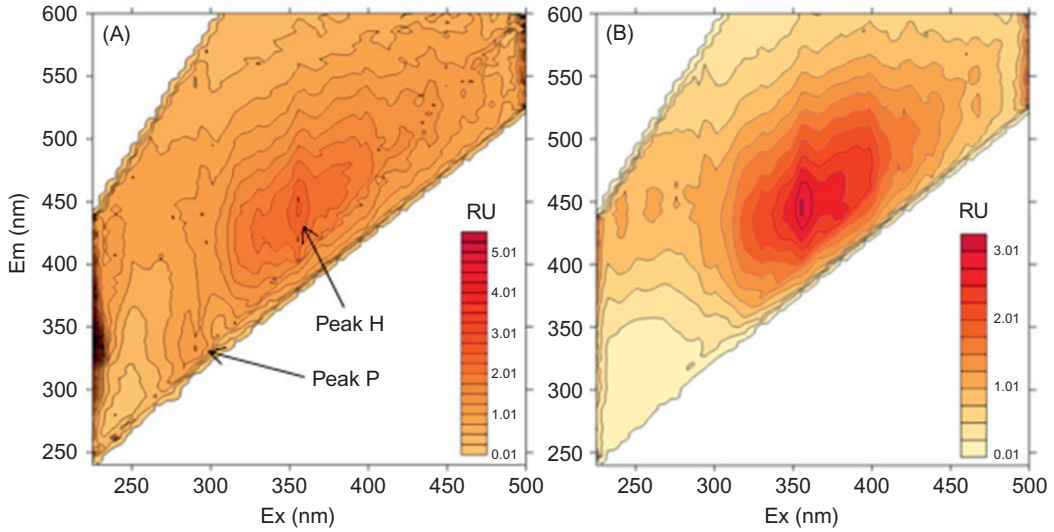

FIGURE 3–6 A representative illustration of 3DEEM fluorescence spectra of ground water from arsenic contaminated (A) and uncontaminated (B) areas of Bangladesh. Fluorescence intensity is expressed in Raman units (nm^{-1}). RU, Raman unit; H, humic-like; P, protein-like.

and 355–365 nm/460–480 nm, respectively. These fluorescence EEMs are typical for humic substances [24]. The EEMs for the majority of the samples show a peak at Ex/Em = 335–350/440–465 nm, which indicates the presence of humic substances derived from soil. The fluorescence spectral patterns of groundwater samples from both As-contaminated and -uncontaminated areas are very similar (Figure 3–6A and B). However, ground water in the As-contaminated area contains both humic-like and protein-like peaks with the highest relative fluorescence intensity. In contrary, ground water from uncontaminated areas has only a humic-like peak with relatively low fluorescence intensity (Figure 3–6B).

Figure 3–7 shows variation in DOC concentration, humic-like peak, and protein-like peak fluorescence intensities of the three study areas. The DOC concentrations in ground water in the three study areas vary from 1.80 to 13.85 mg/L with an average concentration of 6.95 mg/L. All DOC concentrations, humic-like fluorescence intensities, and protein-like fluorescence intensities decrease with the depth of the tubewells. The decrease in DOC concentration during transport has been previously found in other groundwater sites in Bangladesh [49]. The simultaneous increasing DOC concentration and fluorescence intensity in the shallow depth (<50 m) might reflect the infiltration of DOC stored in top soils during the monsoon period. During the monsoon period, comparatively large amounts of less decomposed DOC were washed out from the top soils and infiltrated the shallow aquifer. In addition, temperature was higher in the dry summer event, which may consequently enhance photodegradation and sedimentation of DOC, thus contributing to the increase in fluorescence intensity.

Humic-like fluorescence and DOC concentration were strongly correlated. It appears that the groundwater DOC was mainly allochthonous, and humic substances were the main

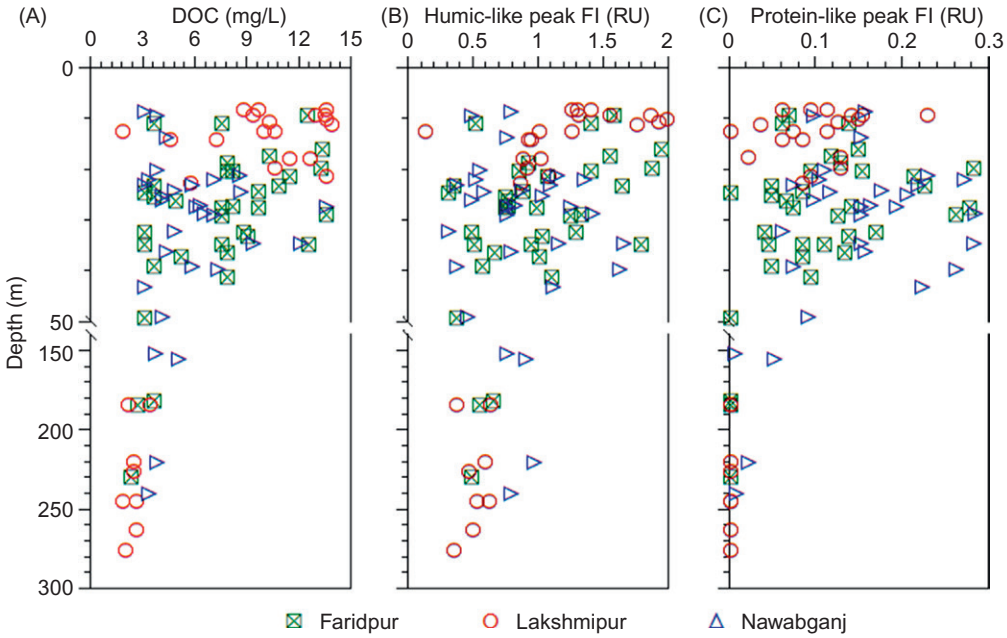

FIGURE 3–7 Vertical distributions of dissolved organic carbon (DOC) (A), humic-like peak (B), and protein-like peak (C) intensity in groundwater in Faridpur, Lakshmipur, and Nawabganj, Bangladesh. Fluorescence intensity is expressed in Raman units (nm^{-1}).

component of DOC. Consistent increases in fluorescence intensity, together with the occurrence of protein-like fluorescence, indicated that autochthonous sources of DOC also infiltrated the shallow aquifer. Protein-like fluorescence materials remained relatively small in the deep aquifer, but occasionally increased in variability (Figure 3–7). Larger variability and higher fluorescence intensities were found in the shallow aquifer. Protein-like fluorescence was often associated with biological activities such as plankton blooms, phytoplankton, and bacteria in aquatic environments [23,50]. The high protein-like fluorescence intensity in the shallow aquifer indicated specific heterotrophic activities in the top section of the groundwater table. The scattered patterns of protein-like fluorescence, as compared to DOC concentration and humic-like fluorescence, imply considerable environmental variability, and indicate that this fluorescing DOC persisted for a period of time in the ground water. However, more detailed sampling and biological investigations are warranted.

The decrease in DOC concentration with depth of well was accompanied by a series of simultaneous changes in DOC quality and biogeochemical process. This may derive from the different remineralization of large molecular weight organic matters (humic substances) from sediment providing the short chain small molecular organic compounds in ground water. The different concentrations of DOC may also be attributed to the precipitation and readsorption of DOC on reactive minerals, strong local variation, dilution, and dispersion of DOC concentration due to groundwater recharge and water flow depending on depth. The microbial

degradation and adsorption by minerals matrix within the aquifers may be responsible for the overall distribution of DOC in ground water.

3.3.5 Molecular Characteristics of Humic Substances

The representative HPSEC chromatograms of humic substances in ground water from both As-contaminated and -uncontaminated areas of Bangladesh, using a fluorescence detector at Ex/Em 350/450 nm and a UV detector at 280 nm, are shown in Figure 3–8. HPSEC separated groundwater humic substances into three completely resolved peaks of different molecular weights with particular retention times. The molecular weight of separated peaks with both the fluorescence and the UV detector were estimated to be 1.81 kDa for peak 1, 1.24 kDa for peak 2, and 0.65 kDa for peak 3 based on the molecular weight calibration of PSS (Figure 3–8). In most of the groundwater samples, 1.24 and 0.65 kDa molecules were predominant, with the presence of 1.81 kDa molecules. On the other hand, surface water in the study areas had 1.24 kDa and 0.65 kDa molecules [49]. One common characteristic of all ground water and surface water was that the largest fractions were almost undetectable, and the predominant fractions were the smallest molecular weight fractions [50].

Relatively high percentages of small molecules in groundwater and surface water DOC indicated that the largest molecular fractions of the DOC were either biologically degraded into smaller molecules or physicochemically adsorbed into soil particles during the recharges of ground water. Degradation of large molecules within the surface by photodegradation and/or biological degradation during infiltration to ground water might be one alternative explanation for such observation. The small molecular fraction of DOC is high in ground water up to 50 m depth in three study areas, and suggested that this is an indication of infiltration of biologically reactive DOC from surface water.

Among three molecular sizes (1.81, 1.24, and 0.65 kDa), the small molecular weight fraction (0.65 kDa) of DOC was the only variable showing significant correlation with As in ground water. Similar results have already been observed in other As-contaminated areas [49]. Temporal changes in the concentration of As were generally related to variations in the availability of low molecular weight fractions (peak 3 of Figure 3–8) of DOC in ground water. This might indicate a potential binding of As with high fluorescence active low molecular weight DOC components, and low molecular weight fluorescent compounds of DOC in the ground water of Bangladesh may be a serious threat to public health.

3.3.6 Health Risks

In Bangladesh, the inhabitants of As-contaminated areas consume a large amount of groundwater (4–6 L/day) as drinking water. Considering that, on average, 5.0 L of water is consumed per day, people who are drinking water containing 0.20 mg/L of arsenic, i.e., 1.0 mg of arsenic per day for a few years, are vulnerable to multiple health hazards [48]. The DPHE-BGS [21] estimated that about 20 million people in Bangladesh are exposed to As concentrations >0.05 mg/L. The long-term consumption of water above that level is harmful and may cause multiple health hazards including damage to the kidneys, liver, and bladder. Anawar

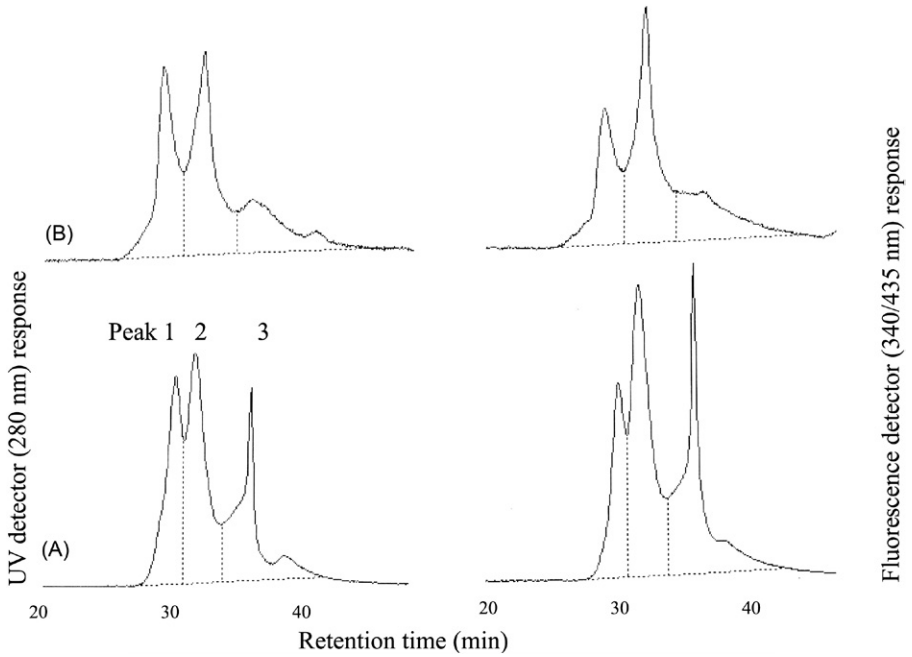

FIGURE 3–8 Representative HPSEC of both As-contaminated (A) and -uncontaminated (B) ground water in Bangladesh for both the UV and the fluorescence detector.

et al. [9] estimated that about 20% of the total population are drinking As-contaminated water above a 0.2 mg/L level and nearly 3.58 million people are potentially affected and at high risk of health hazards. The present study shows that half of the total population of these three As-contaminated districts were drinking As-contaminated water in the ranges of 0.05–0.25 mg/L (Figure 3–2) and nearly 2.25 million people in these districts are at high risk of health hazards.

There are some instances of patients with skin lesions in Taiwan and Chile who are drinking water containing very low concentrations of As [5,18] but of high humic substances [5]. These results demonstrate that arsenic toxicity may be aggravated by humic substances. There is a compelling reason to suspect that the presence of fluorescent humic substances is related to the cause of skin lesions in Bangladesh because most of the As-contaminated samples have fluorescence characteristics (ranging from 0.20 to 5.2 RU nm^{-1} with an average value of 2.5 RU nm^{-1}). It is possible that high As concentrations combined with other trace or minor elements in some cases may enhance the toxicity of these humic compounds to biological systems. High fluorescence intensities and elevated concentrations of fluorescent humic substances in ground water are possible compounding factors with synergistic effects. Humic substances are characterized as phenolic and phenolic carboxylic polymer structures containing both -COOH and -OH as their main functional groups [20]. Both arsenic and fluorescent substances are found in high concentrations in Bangladesh ground water. Therefore, it is highly

possible that HS–metal complexes in the tubewell water play an important role in the pathogenesis of arsenocosis [15,18].

The synergistic effects of arsenic and fluorescent humic substances in ground waters with respect to biological toxicity are not clear yet. The groundwater samples revealed high fluorescence intensities accompanied by elevated levels of DOC in water, which are possible compounding factors or cause synergistic effects. Animal model experiments found some significant relationships between the intensity of blue-green fluorescence and the occurrence of disease. The fluorescent compounds present in well water can induce some toxic effects and crippling, phlegmasia, ulceration, necrosis, and gangrene in the extremities in mice [20]. The complexes composed of humic substance and arsenic show enhanced inhibition of plasma activity as compared with either humic substance or arsenic alone [51]. Both arsenic and fluorescent substances are found in high concentrations in ground water in Bangladesh. Therefore, it is probable that humic substances and a few more governing factors may raise the toxicity of arsenic in ground water.

3.4 Conclusions

The results of the present study show that ground water from the three study areas is characterized by neutral to slightly alkaline pH, low DO, high DOC, and high concentrations of As and fluorescence humic substances. Arsenic concentrations in ground water are highly variable (0.02–2.40 mg/L), and mostly exceed the maximum permissible limits of the WHO (0.01 mg/L) and Bangladesh (0.05 mg/L) for drinking water. The most contaminated aquifers in the three study areas are confined to a depth of around 20 to 50 m. Elevated As concentrations are related to reducing conditions in a shallow aquifer environment with high DOC and HCO_3^-. Drinking As-contaminated water is the main cause of arsenic poisoning in the public health of Bangladesh. Arsenic might be enriched in rice and vegetables due to using As-contaminated ground water for irrigation. Food composites are a major source of arsenic exposure in Bangladesh and elsewhere in regions with subsistence rice and vegetable diets, which can affect human health through the water-soil-vegetation-animal food chain. The bioavailability of arsenic in rice and vegetables must be addressed to understand the importance of arsenic exposure from the food source.

In the past, only arsenic concentrations in ground water were considered as having a direct correlation with the epidemical degrees of arsenicosis including different types of dermatological diseases, but the results of this investigation indicate that fluorescent humic substances may also have some correlation. Groundwater samples in Bangladesh have relatively high fluorescence intensities of humic substances. Both fluorescent compounds and arsenic in well waters therefore have a direct correlation with the epidemical degree of arsenicosis; fluorescent compounds are a form of humic substance, i.e., a polymer with a multiplicity of anions, having a strong chelating ability, which may therefore bind many positively charged metal cations and other compounds. In the process of chelation, arsenic in well water can combine with fluorescent compounds to form a complex. The evidence of a strong correlation between fluorescence intensity of small molecular size fractions and arsenic in ground water supports the

above inference [49]. Therefore, mitigation efforts must be undertaken to provide safe drinking water and these efforts should not be limited to arsenic, which is unquestionably the most significant health risk factor; they should also address fluorescent humic substances.

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4

Arsenic Risk Assessment

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CHAPTER OUTLINE

4.1 Introduction

Risk assessment is an important scientific tool for informing risk management policy decisions made by regulatory agencies such as the United States Environmental Protection Agency (USEPA), the Food and Drug Administration (FDA), the Consumer Products Safety Commission (CPSC), and the United States Department of Agriculture (USDA). Industries and other entities might also apply this tool when evaluating products to support their registration and approval before marketing and other regulatory decisions. Advisory groups and organizations such as the World Health Organization (WHO) might use this tool in guiding other countries in chemical safety. Below, we first present the general principles of risk assessment as background information before discussing the specifics of arsenic risk assessment. This chapter focuses mainly on human health assessment of arsenic compounds.

The scope and focus of a risk assessment depends on the specific needs of various organizations. Risk assessments are context specific. For example, a risk assessment might be used to inform emergency response needs, to derive safe drinking water goals and to set safe air

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exposure levels, to help establish food tolerance levels, or to provide guidance on chemical or microbial contaminant safety.

The risk assessment process consists of the following four components: hazard identification, dose–response assessment, exposure assessment, and risk characterization. The execution of these steps depends on the needs in scoping and problem formulation, of various groups to inform risk management decisions. In hazard identification, whether a particular chemical is causally inked to particular health effects is determined. The dose–response assessment determines the relationship between the magnitude of exposure and the probability that health effects will occur. The dose–response assessment can be based on an apical endpoint or a precursor event mediating the particular health outcome. This component typically culminates in the derivation of a toxicity value such as reference dose or cancer slope factor based on the hazard identification and characterization and on the mode of action conclusions. The associated mode of action in mediating different endpoints or health effects is supported by toxicokinetic information (e.g., absorption, metabolism, distribution, and excretion). In the exposure assessment, the exposure to humans from different sources (e.g., drinking water, food, air) and by different routes (oral, inhalation, dermal) is estimated. In recent years, biomonitoring data such as chemical or metabolite concentrations in urine or blood have been used because they enable chemical exposures to humans to be ascertained at the individual level and thus serve as biomarkers of exposure. In the final step of risk assessment, risks to humans from chemical exposures are characterized by combining reference values with estimates of exposure. In the risk characterization step, various assumptions used in estimating the human health risk are described. The influence of factors such as age, gender, life stage susceptibility (e.g., childhood, pregnancy), and genetic polymorphism, and the impact of lifestyle factors such as smoking and socioeconomic status, is examined. In the risk characterization, the pros and cons of various risk assessment options are provided to risk managers to inform their decision making. In addition gaps in research data are identified that could provide a rationale for development of new data to address uncertainties and to help understand the variability of risk estimates for human populations from exposure to chemical contaminants. The extent to which each of the four components of risk assessment is developed depends on the availability of resources (time, money, expertise) and the needs of the organization.

In this chapter, the authors do not conduct a risk assessment, Instead, they present arsenic-related literature that is pertinent to each component of risk assessment. The chapter presents discussion on arsenic chemistry and metabolism, occurrence and exposure, hazard identification, mode of action risk characterization and susceptibility. The authors also do not present a dose–response assessment but rather review reference levels from various state, federal, and international regulatory and advisory entities. Additionally, this chapter focuses primarily on inorganic arsenic but, does present some information on other arsenicals such as (monomethyl arsenic (MMA) and dimethyl arsenic (DMA)) and arsine. Finally, the chapter highlights the oral route of exposure although reference values also are provided for inhalation exposures of arsenic compounds.

This chapter is not intended to be an extensive review of the literature on various health risk aspects posed by arsenic. References provided at the end of this chapter and the material

presented in other chapters serve as useful resources for in-depth information on specific arsenic-mediated health outcomes. Unless otherwise specified, "arsenic" as used in this chapter refers to inorganic arsenic.

4.2 Arsenic Chemistry

Inorganic arsenic is a naturally occurring element. Arsenic exists in different oxidation states (−3, 0, +3, +5). Arsine (−3) exists as gas. In drinking water, arsenic exists as arsenite (+3) or arsenate (+5) depending upon the oxidation conditions.

4.3 Arsenic Occurrence and Exposure

Arsenic is widely distributed in Earth's crust, which contains about 3.4 ppm arsenic. Because arsenic is naturally occurring, low levels are found naturally in all environmental media. Inorganic arsenic and organic arsenic compounds also have been introduced into the environment through anthropogenic use (e.g., pesticides, pharmaceuticals, glassmaking, and semiconductors) [1–3]. Arsenic has been identified in at least 1149 of the 1684 hazardous waste sites and is included in the EPA National Priorities List (NPL). Mean total arsenic concentrations range from 0.02 to 4 ng/m^3 in remote and rural areas and from 3 to 200 ng/m^3 in urban areas. Near industrial sources such as metal smelters and coal-burning power plants, concentrations greater than 1000 ng/m^3 have been reported. The concentration of arsenic in ambient water sources is less than 10 μg/L, although it can be as high as 5 mg/L near anthropogenic sources [2,4].

The primary route of arsenic exposure for the general human population is via the ingestion of contaminated water or food. When the arsenic exposure via drinking water is low, the contamination of arsenic from food sources (e.g., rice) becomes a significant contributor to the total arsenic exposure in humans. Exposure from air through inhalation also has been reported in occupational settings and could contribute to total arsenic exposure [1–3]. High levels of arsenic contamination in drinking water have been found in southwestern Taiwan, Argentina, and Chile (generally >100 μg/L). Populations from Bangladesh, India/West Bengal, Mexico, northeastern Taiwan, and other countries have been exposed to arsenic in drinking water sources over a wide range of arsenic levels (up to several hundred μg/L or ppb). In the United States, the average drinking water concentration is about 2 μg/L, although higher levels have been reported in the west, midwest, and New England [2].

Arsenic in food can be inorganic and organic. Organic arsenic forms are less toxic than inorganic arsenic. For example, the arsenic in rice is primarily inorganic, and in seafood it is mainly organic (e.g., arsenobetaine and arsenocholine). Total arsenic concentrations can be substantially higher in certain seafood; however, approximately 10% of seafood arsenic is inorganic arsenic [5]. Inorganic arsenic is found in many foods, at concentrations that usually range from 20 to 140 μg/kg [2]. Analyzing a large number of food commodities and drinking water, the EFSA (European Food Safety Authority) estimated the range of average inorganic arsenic intake as to be 0.13 to 0.56 μg/kg bw/day and the 95th percentile intake as to be 0.37 to 1.22 μg/kg bw/day.

Because 98% of occurrence data was reported as total arsenic, several assumptions were made in estimating lower and upper bound ranges for inorganic arsenic intake. High consumers of rice in Europe, such as certain ethnic groups, have a daily dietary intake of inorganic arsenic of about 1 μg/kg bw/day. High consumers of algae-based products have a daily dietary intake of inorganic arsenic of about 4 μg/kg bw/day. For children under 3 years of age, inorganic arsenic intake was reported as two- to three-fold higher compared to adults [6].

Arsenic exposure from drinking water are based on measured concentrations in the water and estimations of the amount of water consumed. Obtaining accurate estimates of human exposure due to this source is difficult because arsenic concentrations in drinking water can change over time and the individuals might not remember the actual amount of water consumed. Biological monitoring provides a better estimate of the absorbed dose in arsenic-exposed individuals. Among the various exposure biomarkers, arsenic levels in the urine are commonly reported in epidemiological investigations and provide information on very recent arsenic exposure (i.e., in the past few days). Some studies report arsenic measurements in nail and hair, and although interpreting these levels poses some challenges due to external contamination; however, the presence of arsenic in these samples indicates longer exposure to arsenic—weeks or several months. Such biomarkers help ascertain individual exposures but, they do not distinguish exposure due to drinking water or food or other sources of exposure. Evidence from the United States National Health and Nutrition Examination Survey (NHANES) suggests that direct exposures in food (e.g., from pesticide residues) or from seafood arsenicals and their metabolites can confound measurements of urinary dimethylated arsenic [7]. Urinary monomethylated arsenic therefore might be a preferable marker of inorganic arsenic exposure via drinking water.

4.4 Hazard Identification

4.4.1 Arsenite and Arsenate

Acute or subacute arsenic exposure at high levels (greater than several milligrams of inorganic arsenic per day) might induce overt gastrointestinal disturbances ranging from mild abdominal cramping and diarrhea to severe life–threatening hemorrhagic gastroenteritis associated with shock. These symptoms are absent under chronic exposure conditions [5].

Chronic exposure to inorganic arsenic is related to many adverse health effects. The most commonly reported are skin lesions, cancers in multiple tissues, cardiovascular diseases, diabetes, pulmonary effects, neurological impairment, and developmental and reproductive toxicity. Other effects reported in cohorts exposed to arsenic include immune effects and hematological and hepatic effects.

4.4.1.1 Skin Lesions

Numerous epidemiological studies conducted in arsenic-contaminated areas around the globe have reported skin lesions. The skin lesions that arsenic exposure causes are one of the earliest detectable health effects in exposed populations and occur prior to other endpoints

such as cancers and cardiovascular diseases. These skin lesions are distinct and characterized by hyperpigmentation and hyperkeratosis; they are observed in individuals exposed to arsenic in drinking water or via inhalation, or in patients treated with Fowler's solution (potassium arsenite) [5]. The hyperpigmentation commonly appears in a finely freckled, "raindrop" pattern that is particularly pronounced on the trunk and extremities but that might also involve mucous membranes such as tongue or buccal mucosa [5]. Arsenic-induced hyperkeratosis is manifested predominantly on the palms and plantar aspects of the feet [5]. Skin lesions are also reported in young children as old as 2 years. Evidence suggests these skin symptoms could serve as markers of susceptibility to other disease outcomes such as cancer and cardiovascular diseases [5]. The dose-dependent association between low dose levels of arsenic and incidence of skin lesions was reported in prospective cohort subjects (HEALS, Health Effects of Arsenic Longitudinal Study) in Bangladesh [8,9]. Increased monomethylarsonic acid (MMA) concentrations in urine are associated with increased risk for skin lesions [10,11].

4.4.1.2 Cancer Effects

The International Agency for Research on Cancer (IARC) first reported in 1987 that there was sufficient evidence of arsenic carcinogenicity in humans 1973. In a recent evaluation, IARC concluded that the evidence in humans is sufficient to indicate that inorganic arsenic causes lung, bladder, and skin cancers. IARC found that the evidence of cancers in other tissues cannot rule out the possibility of chance or bias; the agency concluded, however, that there is a positive association between exposure to arsenic and cancer of kidney, liver, and prostate [12].

The carcinogenic role of arsenic compounds was first noted over 200 years ago by J. Hutchinson when patients treated with arsenicals (Fowler's solution) developed an unusual number of skin tumors. Later evidence from patients treated with arsenicals exhibited cancers in internal tissues (e.g., lung, bladder). Evidence from occupational exposure studies in workers exposed to arsenic in mining and smelting industries also indicated cancers in respiratory tissues. In the 1980s, evidence from several environmental exposure studies in which populations were exposed to arsenic from drinking water for many years revealed increased mortality from cancers in multiple tissue sites.

Many epidemiological studies (ecological, case–control, cohort, cross-sectional) evaluating the association between inorganic arsenic exposure and carcinogenic effects have been conducted in populations exposed to arsenic in drinking water. The earlier ecological studies conducted in southwestern Taiwan, an endemic area for arsenic, reported a prevalence of skin lesions and skin cancers in populations exposed to arsenic over a lifetime mostly at high levels (several hundred μg/L arsenic in drinking water) [13,14]. The skin cancers caused by inorganic arsenic exposure were identified as non-melanoma type (basal cell carcinoma and squamous cell carcinoma). Ecological and cohort studies of these populations subsequently reported increased mortality from internal tissue cancers (lung, liver, bladder, kidney, and other tissues) [15–18]. Similar observations were also made in populations exposed to inorganic arsenic from drinking water sources at high levels in Chile [19], Argentina [20,21], and Japan [22]. In a 12-year follow-up cohort study in northeastern Taiwan, Chen et al. [23,24] found increased risk for lung and urinary tract cancers in populations exposed to arsenic over a wide range of exposure levels including levels <100 μg/L.

Over the last 15 years, epidemiological studies have attempted to evaluate the low dose inorganic arsenic and the occurrence of internal tissue cancers in the United States [25–34], Europe [35–37], and other places such as Chile and Bangladesh [38,39]. The results from these studies are inconsistent. Small sample size, lack of adequate exposure period (a long latency period is needed for development of cancer), and exposure misclassification are some aspects that challenge the ability to detect risk at low arsenic exposures [40,41].

Some evidence suggests that chronic inorganic arsenic exposure produces lung tumors in mice [42,43] and evidence is growing that tumor formation occurs in mice upon short-term arsenic exposure during critical periods such as gestation. For example, *in utero* exposure to arsenic during gestation alone has been shown to produce tumors in liver, lung, adrenal gland, and other tissues of the offspring after 2 years [44]. The development of tumors in rodents gestationally exposed to inorganic arsenic informs findings in epidemiological studies. For example, using Chilean cohorts, Smith et al. [45] demonstrated that early-life arsenic exposure is associated with increased risk for lung cancers. The likelihood of susceptibility to inorganic arsenic has been shown to enhance the carcinogenic effects of other agents such as smoking in humans, UV light and nitrosamines in animal models [3].

4.4.1.3 Diabetes

The National Toxicology Program conducted a comprehensive review of epidemiological and animal evidence on arsenic and diabetes. The review found that the available human data provide limited to sufficient evidence for an association between arsenic and diabetes in populations with relatively high exposure levels in drinking water (≥150 ppb), but insufficient evidence at lower exposure levels in drinking water (<150 ppb). The findings from animal literature investigating the link between arsenic and diabetes are mostly inconclusive [46]. Nevertheless, some human studies that have more fully characterized the exposures and outcomes support an association between moderate arsenic exposure and diabetes [47–49].

4.4.1.4 Cardiovascular Effects

Acute exposure to inorganic arsenic in the range of milligram to grams per day has caused overt cardiovascular manifestations, including hypotension, congestive heart failure, and cardiac arrhythmias (prolonged QT interval).

Many epidemiological studies have demonstrated a dose-responsive association between chronic arsenic exposure in drinking water and various cardiovascular diseases. These cardiovascular disorders include carotid atherosclerosis, impaired microcirculation, prolonged QT intervals and increased QT dispersion in electrocardiography, and clinical outcomes such as hypertension, blackfoot disease, coronary artery disease, and cerebral infarction [50]. Peripheral vascular disease (thromboangiitis, and overt gangrene of the extremities) was noted in German vintners from consumption of arsenic-contaminated wine substitutes [5].

In a systematic review of 13 epidemiological studies (8 from Taiwan, 3 from the United States, and 1 each in Hungary and Spain), increased relative risk for coronary artery disease, stroke, and peripheral arterial disease was reported at high exposure levels [51]. In a subsequent systematic review of 18 additional studies conducted in Taiwan, the United States,

Spain, Japan, Bangladesh, Chile, Inner Mongolia, Pakistan, and Slovakia, the authors found conclusive evidence for a causal association between chronic arsenic exposure and clinical cardiovascular endpoints at high arsenic levels and inconclusive evidence at low–to–moderate arsenic exposures [52].

From meta-analysis of many epidemiological studies, the evidence relating environmental arsenic exposure to hypertension or increased blood pressure is positive in endemic areas and is limited in low exposure regions [53]. The evidence linking arsenic and hypertension in another meta-analysis was inconclusive [54]. The studies included in the two analyses and the methodologies used, however, differed.

4.4.1.5 Neurological Effects

Acute inorganic arsenic poisoning producing initial gastrointestinal or cardiovascular symptoms can be followed by both central nervous system and peripheral nervous system effects. Central nervous system effects occur within 1–5 days of acute poisoning and can range from headache and mild confusion to florid encephalopathy, seizures, and coma. Peripheral neuropathy effects emerge within 1–4 weeks. In subacute or chronic exposure, arsenic occasionally can result in peripheral neuropathy without gastrointestinal or cardiovascular symptoms. The occurrence of peripheral neuropathy is inconsistent in individuals chronically exposed to arsenic in drinking water [5]. The neurological effect of arsenic is characterized by symmetrical sensorimotor neuropathy, often resembling Guillain–Barré syndrome. The predominant clinical features of neuropathy are paresthesias, numbness, and pain. In addition, the affected individuals have reduced nerve conduction velocities, typical of those seen in axonal degeneration [55]. Auditory, visual, and somatosensory impairment have been reported in Mongolian farmers living in the Yellow River Valley, where drinking water is contaminated by arsenic. The arsenic-exposed subjects in Inner Mongolia exhibited reduced pinprick scores and vibration thresholds at arsenic exposure concentrations well below the 1 ppm drinking water level [56].

In addition to acute neurotoxicity and peripheral neuropathy, exposure to inorganic arsenic during brain development might have adverse health outcomes. For example, decreased IQ in children exposed to arsenic has been reported in studies conducted in Araihazar, Bangladesh, and West Bengal, India [57,58]. Hamadani et al. [59] found that pre- or postnatal arsenic exposure did not affect child development (problem solving and motor development) at 18 months in Matlab, Bangladesh. However, Hamadani et al. [60] demonstrated associations of early-life arsenic exposure and decreased verbal IQ and full scale IQ in preschool-aged children (Matlab). In a systematic review, Rodriguez-Barranco et al. [61] reported evidence for an association between increased arsenic levels in urine and decreased intelligence quotient (IQ) in children aged 5–15 years.

Neurobehavioral impairments are also reported in the animal studies at high dose levels [62]. Perinatal exposure at low dose levels, however, also impairs learning and behavior in mice [63].

4.4.1.6 Pulmonary Effects

Studies from India and Bangladesh report increased respiratory symptoms (e.g., cough, shortness of breath, bronchitis) upon exposure to arsenic [64,65]. Impaired pulmonary

function, chronic obstructive pulmonary disease (COPD), and tuberculosis were observed in populations from Bangladesh, West Bengal/India and Chile [45,64,66–70]. In Chile, Dauphine et al. [71] reported that early-life arsenic exposure was associated with pulmonary dysfunction (decreased forced expiratory volume, lower forced vital capacity, and increased breathlessness) in adult subjects but these effects were reported nearly four decades after the peak arsenic exposure. However, urinary arsenic exposure levels in NHANES subjects from the USA did not correlate with prevalence of respiratory diseases (asthma, chronic bronchitis, emphysema) [72].

4.4.1.7 Kidney Effects

Chronic exposure to arsenic in drinking water-from low to high exposure levels- has been shown to increase the risk for kidney effects (e.g., chronic glomerulonephritis, renal failure, decreased glomerular filtration rate) [73–76].

4.4.1.8 Immune Effects

Epidemiological, animal, and *in vitro* studies all support an association between arsenic exposure and suppression of the immune system [3]. Evidence suggests that maternal arsenic exposure impacts the thymus weight and increased the respiratory infection incidence in infants. A positive association between arsenic exposure and pro-inflammatory cytokine levels has been shown in humans. Increased levels of autoimmune markers (antinuclear antibodies, anti-double stranded DNA) in the serum of arsenic-exposed individuals in West Bengal/India suggest that arsenic at moderate levels may induce autoimmune diseases such as rheumatoid arthritis [77].

4.4.1.9 Other Effects

Increased levels of biomarkers of hepatic damage such as aminotransferases, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase, and bilirubin were reported in individuals exposed to arsenic. Increased liver enzyme levels such as those of serum alkaline phosphatase, aspartate transaminase, and alanine transaminase were associated with increased arsenic levels in drinking water in Bangladesh [78]. Das et al. [77] found a similar increase in liver enzymes and elevated bilirubin levels in people who lived in arsenic-contaminated areas compared to people in non-exposed areas in West Bengal/India. Acute and chronic arsenic poisoning might result in anemia, leukopenia, and thrombocytopenia [5]. A linear relationship between total urinary porphyrin concentration and urinary arsenic has been observed in a limited number of human cohorts [5].

4.4.1.10 Developmental and Reproduction Effects

Inorganic arsenic and its methylated metabolites can pass through the placenta in humans [79,80] and mice [81]. Some studies have found that arsenic induces several effects in the fetus, some of which result in fetal loss or growth retardation [88,89]. A growing body of evidence

indicates that some of the changes induced in fetal or infant life lead to detectable adverse health effects later in childhood as well as in adult life [82].

Studies from arsenic endemic areas such as Bangladesh, West Bengal, Chile, and northeastern Taiwan have reported many reproductive and developmental effects including increased risks of spontaneous abortions, stillbirths, infant mortality, preterm birth, low birth weight, and growth restriction [83–92].

4.4.2 Hazard Identification—DMA and MMA

The acid and salt forms of pentavalent monomethylated arsenic (MMA^V) and pentavalent dimethylated arsenic (DMA^V) are used as herbicides. In the environment, the organoarsenicals can be converted to inorganic arsenic, which is more toxic to humans [93]. Further, ingested organoarsenicals are eliminated more rapidly than ingested inorganic arsenic. Evidence from studies of animals administered MMA^V suggests that the large intestine is the target organ. Diarrhea and vomiting were observed in dogs chronically exposed to MMA. No evidence of carcinogenicity following exposure to MMA^V has been observed in rodent bioassays. EPA's Office of Pesticide Programs considers MMA as "not likely" to be a human carcinogen [93]. The thyroid and bladder have been identified as the target organs for DMA. Thyroid lesions, primarily incidence of cuboidal to columnar epithelial cells lining thyroid follicles, were observed in rats upon subchronic and chronic exposures. Long-term exposure to DMA^V has resulted in urinary bladder tumors in male rats but not in female rats or mice [94]. Chronic exposure to MMA^V produced no tumor effects [95]. IARC [12] concluded that there is sufficient evidence of carcinogenicity of DMA^V in experimental animals. More recently, however, IARC classified DMA as "possibly carcinogenic to humans" (Group 2B) [172].

4.4.3 Hazard Identification—Arsine

The predominant effects of arsine poisoning shown in clinical studies are intravascular hemolysis and subsequent passage of dark or bloody urine and jaundice. This hemolytic anemia is accompanied by low hemoglobin values and the presence of reticulocytosis, red cell fragments, and ghost cells. Early death after acute arsine poisoning could result from myocardial failure accompanied by massive pulmonary edema that precedes the onset of renal failure. Animal studies show decreased red cell count and hemoglobin level correlated with increasing arsine concentrations.

4.5 Arsenic Metabolism, Mode of Action, and Physiologically Based Pharmacokinetic Modeling

After entering the body, pentavalent arsenic (As^V) can be reduced to trivalent arsenic (As^{III}) which can then proceed through a series of methylation and conjugation reactions, some of which involve reoxidation of arsenic to As^V. The traditional metabolic pathways proposed

for arsenic are shown in Figure 4–1. Compared to the pentavalent species, the trivalent species are more cytotoxic, more genotoxic, and more potent inhibitors of enzyme activity [96]. In this metabolic scheme less toxic species (i.e., As^{V}, MMA^{V}, and DMA^{V}) can be converted to the more toxic species (i.e., As^{III}, MMA^{III}, and DMA^{III}). The predominant metabolite excreted in humans and animals is DMA^{V}, but some animal species (e.g., rats) further metabolize DMA^{V} through DMA^{III} to trimethylarsine oxide (TMAO).

Methylation is an important factor affecting arsenic tissue distribution and excretion. Humans and most experimental animal models methylate inorganic arsenic to MMA and DMA, with the amounts differing across species, as determined by analysis of urinary metabolites. Historically, the methylated metabolites have been considered less acutely toxic, less reactive with tissue constituents, less cytotoxic, and more readily excreted in the urine than inorganic arsenic [98–105]. The trivalent species MMA^{III} and DMA^{III}, however, are more cytotoxic in a human liver cell line called Chang cells [106,107], Chinese hamster ovary cells [108], and cultured primary rat hepatocytes [109,110] than As^{III}, As^{V}, MMA^{V}, or DMA^{V}.

A small percent of DMA^{III} can be methylated further to TMAO in mice and hamsters (see [111] for a review). 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 [112] or in the urine of mammals exposed orally to inorganic arsenic. The only data available on human metabolism of DMA to TMAO are from a study with a single human volunteer who ingested DMA and excreted 3.5% of the dose as TMAO [111]. Minimal to no detection of TMAO in the urine of animal models or humans could be due to rapid clearance of DMA and MMA from cells [113]; most analytical methods, however, are not optimized for the detection of TMAO.

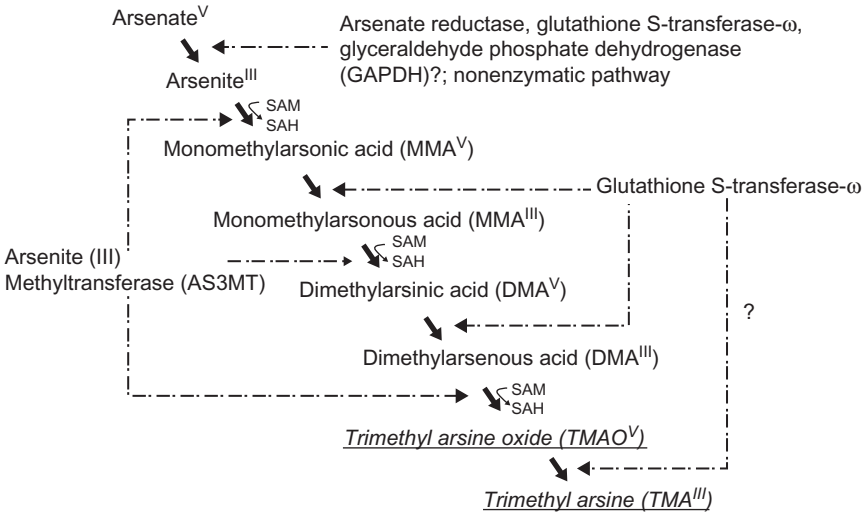

FIGURE 4–1 Traditional metabolic pathways for arsenic [97].

The major route of excretion for most arsenic compounds, including TMAO, by humans is via the urine [114–117].

The US EPA defines the term mode of action (MOA) as "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation or other adverse outcomes" [118]. MOA data can inform the human relevance of data in animal or other model systems, the identification of susceptible populations, and dose–response assessment. Arsenic has been observed to affect numerous key events, but no specific MOA has been identified for arsenic-mediated health effects. Several key events could be common for both cancer and non-cancer endpoints, for example, gene expression changes, oxidative stress, cytotoxicity, signal transduction, interference with hormone function, apoptosis, cell cycle arrest, and enzyme activity inhibition.

Elevated levels of reactive oxygen species (ROS) have been measured as arsenic is metabolized and could be involved in its toxicity; however, the specific mechanism of ROS production is unknown [119]. Arsenic has been observed to produce the following reactive species: superoxide ($O_2^{\bullet-}$), peroxyl radicals ($ROO^{\bullet}$), nitric oxide (NO), hydrogen peroxide (H_2O_2), dimethyl arsenic peroxyl radicals [$(CH_3)_2AsOO^{\bullet}$], and dimethylarsinic radical [$(CH_3)_2As^{\bullet}$]. ROS have been linked to vascular diseases [120,121], diabetes [122,123], and neurological effects [124,125]. Changes in genes and proteins also have been identified as key events relevant in the development of skin lesions [126–128], vascular diseases [129–136], and reproductive effects [137]. Changes in cell proliferation and apoptosis have been linked to skin lesions and effects on the immune response [138–142].

PBPK models for inorganic arsenic are central to developing a biologically based dose–response (BBDR) models which has proven challenging because inorganic arsenic appears to mediate its toxicity through a range of metabolites, the roles of which with regard to specific adverse effects are not clear [143]. Mann et al. [144] developed a PBPK model for exposure to inorganic arsenic (orally, intravenously, and intratracheally) was developed in hamsters and rabbits. The same group [145] then extended the PBPK model for use in humans by adjusting physiological parameters (organ weights, blood flows) and reestimating absorption and metabolic rate constants. Yu [146,147] 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, gastrointestinal and stomach contents, and bile. Gentry et al. [148] adapted the model proposed by Mann et al. [144] to different mouse strains by adjusting physiological parameters (organ weights and perfusion rates).

In 2007, Clewell et al. [143] noted that these PBPK models did not incorporate the most recent available information on arsenic methylation kinetics and suggested several steps for improving them. El-Masri and Kenyon [149] developed a PBPK model incorporating some of the improvements that Clewell et al. [143] suggested (although not the simulation of changes in gene expression). The model predicts the levels of inorganic arsenic and its metabolites in human tissues and urine following oral exposure to As^V, As^{III}, and 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 address the gastrointestinal 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 uses noncompetitive inhibition to incorporate the inhibitory effects of As^{III} on the methylation of MMA^{III} to DMA and of MMA^{III} on the methylation of As^{III} to MMA. This model differs from the other models described above because it provides an updated description of metabolism using biochemical data on the mechanism of arsenic methylation. This model also is an improvement over previous models because it can quantitatively assess impacts of parameter variability arising from genetic polymorphism. 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 it can predict the formation and excretion of trivalent methylated arsenicals. The partition coefficients estimated in the model are comparable to those Yu. [147] developed Model performance was tested against limited human data on urinary excretion; the model should be evaluated in large numbers of subjects for its ability to predict the tissue and urinary concentrations of arsenicals.

4.6 Potential Sources of Susceptibility

Studies have found that individuals with a slower secondary methylation capacity for arsenic (i.e., MMA to DMA), as measured by low urinary DMA concentration or high urinary MMA concentration, have an increased risk of disease [10,150–158]. Children are another subpopulation that is more susceptible to adverse health effects following arsenic exposures. Although children are generally exposed to arsenic through the same sources as adults, behavior and physiology of children might result in higher absorbed doses relative to 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 could result in higher relative doses than in adults. Children are more likely to ingest arsenic-contaminated soil through hand-to-mouth activity. In addition, exposure occurs *in utero* likely at concentrations similar to those occurring in the mothers. Hall et al. [80] measured levels of MMA, DMA, and inorganic arsenic in maternal blood and cord blood and found the levels were similar, indicating that children are likely exposed at similar levels as adults during embryonic development.

Limited data are available 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 indicates that absorption does occur. Data are equivocal on the capacity of children to metabolize arsenic compared to adults.

Sex might present another susceptibility factor for arsenic. Specifically, differences in methylation patterns have been noted between men and women in several studies. Higher MMA:DMA ratios have been observed in men compared to women in a variety of populations tested, including in the USA [20,153,159,160], Taiwan [154], and Bangladesh [150]. Loffredo et al. [161] observed that gender differences (ages were not specified, but subjects were presumably adults) in arsenic methylation varied across populations studied in Mexico, China, and Chile, and sometimes by exposure level.

Age and reproductive status also could affect the male-female differences in arsenic methylation patterns. Concha et al. [162] demonstrated that pregnant women in their third trimester excrete approximately 90% of arsenic as DMA. Engström et al. [163] also observed pregnant women to have an increased proportion of DMA in their urine compared to nonpregnant women in the same population, with increases occurring with gestational age. Lindberg et al. [164] also observed possible hormonal effects on arsenic methylation, noting that women less than 60 years of age, likely premenopausal, generally were more efficient in methylating arsenic than men of the same age, while the difference narrowed considerably in men and women older than 60 years. Lindberg et al. [165] observed that although females of all ages generally methylated arsenic at a higher rate than males, the greatest disparity between the sexes occurred between the ages of 20 and 55 years. Lindberg et al. [164] also observed that selenium, body mass index, and AS3MT (arsenic methyl transferase) gene polymorphism affected the observed proportions of methylated urinary arsenic metabolites in men only. The pattern of arsenic methylation was also altered in men with mutations in one allele of the methylenetetrahydrofolate reductase (MTHFR) gene, but in women, variants in both alleles were required [164].

Although evidence suggests that age and sex play a role in differences in arsenic methylation capacity, data from Bangladesh analyzed by Lindberg et al. [165] suggest that genetic polymorphism is the most important factor affecting the methylation of inorganic arsenic. According to Lindberg et al. [165] only 30% of variation in methylation patterns is attributable to the level of arsenic exposure, gender, and age. Polymorphisms G12390C, C14215T, and A35991G in the AS3MT gene were associated with an increase in urinary DMA. Polymorphisms TT/AA in the MTHFR gene caused an increase in urinary levels of inorganic arsenic and MMA and increased the risk for skin lesions. Polymorphisms in GST (glutathione S-transferase) were associated with different methylation patterns. GST polymorphisms were not associated with risk for skin lesions, but were associated with changes in risk for carotid atherosclerosis. Data in *Drosophila* by Ortiz et al. [166] suggest that genetic variations in the glutathione synthetase gene also might be related to interindividual variability to arsenic.

Nutritional deficiencies could also be related to changes in arsenic methylation and in some cases to increased risk of specific diseases. The risks associated with nutritional deficiencies are not as strong as other risk factors such as gender or genetic polymorphism. Therefore, even though nutritional deficiencies might add to the risk of developing arsenic-induced diseases, their role is minimal compared to the arsenic exposure.

Cigarette smokers, both current and former, have been observed to have a significantly decreased secondary methylation capacity, resulting in increased urinary MMA ($p < 0.01$) and decreased DMA concentrations ($p < 0.05$) [151]. Steinmaus et al. [159] observed 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 smoked and had proportions of MMA in the upper tertile had a two-fold elevated risk (i.e., OR = 2.13; 95% CI = 1.03–4.40) of bladder cancer compared to subjects with proportions of MMA in the lower two tertiles. Therefore, the conclusion was that individuals who smoke had an increased susceptibility to arsenic toxicity. Lindberg et al. [167] also observed a relationship between impaired arsenic methylation in male smokers compared to non-smokers and increased risk

of skin lesions (population included all individuals older than 4 years, and one category examined effects in individuals 18 years old or younger).

Lindberg et al. [168] reported that the higher prevalence of arsenic-related skin lesions in men was the result of less efficient methylation in men. Melkonian et al. [169] looked at whether an interaction between cigarette smoking and arsenic exposure occurred in the risk for skin lesions in a population in Bangladesh as part of HEALS. These researchers [169] found an additive effect between smoking status and level of arsenic exposure.

4.7 Dose–Response Approaches

A dose–response assessment examines the mathematical relationship between exposure and effects. The outcome of the dose–response assessment can help in the derivation of reference and guideline values. As noted in the introduction, a dose–response assessment is outside the scope of this chapter, but regulatory levels and the reference toxicity values determined for inorganic and organoarsenical compounds by various government agencies and organizations are listed in a subsequent section.

4.8 Risk Characterization

The goal of risk characterization is to summarize and integrate information from the preceding steps of the risk assessment to synthesize an overall conclusion about risk including information on uncertainty and variability. To account for uncertainty and variability, reference values are calculated by applying uncertainty factors to the point of departure. Areas of variability or uncertainty include human variation, animal-to-human extrapolation, adverse-effect level to no-observed-adverse-effect level, subchronic to chronic exposure, and incomplete databases.

4.8.1 Reference Values and Regulatory Standards for Arsenical Compounds

4.8.1.1 US EPA

4.8.1.1.1 INORGANIC ARSENIC

The maximum contaminant level (MCL) is the highest level of a contaminant that is allowed in drinking water based on cost benefit analysis and is enforceable. The maximum contaminant level goal (MCLG) is non-enforceable level at which no known or anticipated adverse effects on the health of persons occur and which allows adequate margin of safety. The US EPA published an MCL of 0.01 mg/L and an MCLG of zero for arsenic on January 22, 2001 [170]. The MCL for arsenic was set at the level at which lung and bladder cancer reduction benefits were maximized at a cost that was justified by the benefits.

The reference dose (RfD) is an estimate of daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. Similarly, the reference concentration (RfC) assesses inhalation risks. The human population addressed in these toxicity values includes sensitive groups, such as asthmatics, or life stages, such as

children or the elderly. The RfD for inorganic arsenic for oral exposure is 0.0003 mg/kg/day, and is based on hyperpigmentation, keratosis, and possible vascular complications in numerous southwestern Taiwanese subjects from the blackfoot disease endemic area for arsenic [171]. A chronic reference (RfC) concentration for arsenic exposure via inhalation was not provided.

The EPA concluded that inorganic arsenic from oral and inhalation exposures is a human carcinogen. Cancer slope factors (CSF) are used to estimate the risk of cancer associated with exposure to a carcinogenic substance. A slope factor is an upper bound, with 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent by ingestion or inhalation. An oral cancer slope factor for inorganic arsenic of 1.5 per mg/kg/day was derived in 1993 based on skin cancers observed in a large Taiwanese cohort exposed to arsenic via drinking water [171]. This cancer risk estimate corresponds to the drinking water arsenic concentration of 0.02 µg/L for a cancer risk of 1 in 1,000,000. Inhalation unit risk is the upper-bound excess lifetime cancer risk estimated from continuous exposure to an agent at a concentration of 1 µg/m^3 in air for a lifetime. The inhalation unit risk was determined as 0.0043 per µg/m^3 based on respiratory cancer mortality observed in smelter workers. This cancer risk estimate corresponds to the air arsenic concentration of 0.0002 µg/m^3 for 1 in 1,000,000 cancer risk.

4.8.1.1.2 ORGANOARSENICALS

The US EPA Office of Pesticide Programs [95] derived a chronic RfD of 0.03 mg/kg/day for MMA based on decreased body weights, diarrhea, body weight gains, food consumption, histopathology of gastrointestinal tract and thyroid in rats, and 0.014 mg/kg/day for DMA based on regenerative proliferation of the bladder epithelial tissue in rats. For carcinogenicity effects, the US EPA Office of Pesticide Programs determined there was "no evidence for carcinogenicity" for MMA and that DMA was "not carcinogenic at doses that do not result in enhanced cell proliferation." More recently, in 2009 IARC classified MMA and DMA as "possibly carcinogenic to humans" (Group 2B) [172].

4.8.1.1.3 ARSINE

The 1994 US EPA Integrated Risk Information System (IRIS) assessment indicates the chronic inhalation reference concentration of 0.05 µg/m^3 based on increased hemolytic effects and spleen changes in animal models [173].

4.8.1.2 ATSDR

4.8.1.2.1 INORGANIC ARSENIC

ATSDR did not evaluate inhalation risk levels for inorganic or organic arsenical compounds. In 2007 the agency [2], however, determined oral toxicity values for potential non-cancer effects associated with exposure to inorganic and organic arsenical compounds. Minimal risk levels (MRLs) are estimates of the daily human exposure to a chemical that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. The acute oral MRL for inorganic arsenic was determined as 0.005 mg/kg/day based on edema of the face, and gastrointestinal effects (nausea, vomiting, diarrhea) observed in humans exposed to arsenic-contaminated soy sauce in Japan. The chronic oral

Table 4–1 Acute Exposure Guideline Levels (AEGLs) for Arsine [175]

Arsine Exposure Time	AEGL 1 (ppm) (Discomfort)	AEGL 2 (ppm) (Impaired escape)	AEGL 3 (ppm) (Life Threatening/Death)
10 minutes	NR	0.3	0.91
30 minutes	NR	0.21	0.63
1 hour	NR	0.17	0.5
4 hours	NR	0.04	0.13
8 hours	NR	0.02	0.06

NR: not reported.

MRL of 0.0003 mg/kg/day was based on skin lesions (hyperpigmentation and hyperkeratosis) observed in southwestern Taiwanese populations exposed to arsenic in drinking water.

4.8.1.2.2 ORGANOARSENICALS

In 2007 ATSDR [2] derived an intermediate oral MRL for MMA of 0.1 mg/kg/day based on diarrhea observed in rats and a chronic oral MRL of 0.01 mg/kg/day based on progressive glomerular nephropathy observed in mice. The chronic oral MRL for DMA was derived as 0.02 mg/kg/day based on vacuolization of the urothelium in the urinary bladder in female mice.

4.8.1.3 RIVM National Institute for Public Health and the Environment

Tolerable daily intake (TDI) refers to the amount of a chemical that has been assessed as safe on a long-term basis, usually whole lifetime. Baars et al. [174] determined the TDI of 1.0 μg/kg/day for chronic oral exposures and the tolerable concentration in air as 1.0 $\mu g/m^3$ for chronic inhalation exposures for inorganic arsenic.

4.8.1.4 Other Standards, Regulations, and Guidelines

As a collaborative effort with public and private sectors in handling emergency situations, the US EPA [175] has derived the Acute Exposure Guideline Levels (AEGLs) for arsine in 2010 presented in Table 4–1. AEGLs are threshold exposure limits for the public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. The recommended exposure levels are also applicable to infants and children, and other individuals who might be susceptible.

4.8.1.5 World Health Organization (WHO)

WHO guidance criteria for arsine was derived as 0.05 $\mu g/m^3$ based on hematological effects observed in animal models [176]. As of 2011 WHO recommends a provisional guideline for arsenic in drinking water of 10 ppb because of scientific uncertainties in the assessment [177].

4.8.1.6 California Environmental Protection Agency

In 2008 the California Environmental Protection Agency (Cal EPA) determined the acute inhalation reference exposure levels as 0.2 $\mu g/m^3$ and the chronic inhalation reference exposure

levels as 0.015 μg/m^3 for inorganic arsenic compounds including arsine [178]. The inhalation unit risk for inorganic arsenic is determined as 0.0033 per μg/m^3. The inhalation slope factor derived in 2009 is 12.0 per mg/kg/day based on lung tumors observed in smelter workers [179].

Disclaimer

This work is not a product of the United States Government or the United States Environmental Protection Agency, and the authors are not doing this work in any governmental capacity. The views expressed are those of the authors only and do not necessarily represent those of the United States or the US EPA.

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Evaluation of Novel Modified Activated Alumina as Adsorbent for Arsenic Removal

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CHAPTER OUTLINE

5.1 Introduction

Arsenic (As) is a persistent contaminant and highly toxic in nature. As is a known carcinogen that can affect the liver, lungs, bladder, and skin. It also causes cardiovascular diseases and hypertension. Further, As is known to interfere with some of the functions performed by enzymes in the body as well as replace phosphate in some processes [1]. Arsenic is found in food and environmental media in various forms; however, the most common route of exposure is via consumption of arsenic-contaminated drinking water [2,3]. Predicting the mobility of As in drinking water sources, including groundwater and surface water, becomes difficult since As mobility depends on the redox properties of the water. Redox conditions in turn determine the chemical properties of As. As(V) is commonly present in surface water while As(III) is present in anaerobic groundwater conditions. As(V) is less toxic and easier to remove than As(III) [4].

The presence of As in drinking water has become a major health concern in the world [5]. The permissible limit of arsenic in drinking water has been recommended as 10 μg/L by the

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World Health Organization (WHO) [6,7]. The limit suggested by the WHO is being enforced in countries such as the United States, New Zealand, Taiwan, and Vietnam [8]. Other countries such as Mexico, Argentina, Bangladesh, China, and Nepal have set their limit at 50 μg/L [8]. Regions like Bangladesh, India, and eastern Croatia are well known for having high levels of As contamination in water. Other regions reported with high probabilities of As contamination include Zambia, Central Australia, and New Zealand. Unhealthy As levels are present in Mexico and in the Latin American countries of Argentina, Chile, Peru, and Nicaragua [4].

In view of the toxic effects of As to human health, it becomes important to remove it from the environment. Some of the most common technologies for arsenic removal include oxidation, coagulation/precipitation, adsorption, lime treatment, ion exchange, electrocoagulation, and membranes [9,10]. Among these removal methods, adsorption has proven to be the best method because of its versatility and affordability [11]. Additionally, adsorbent materials can be regenerated and reused and in this way the amount of generated waste material is considerably reduced [8]. The affordability of adsorbents makes them ideal to implement in third-world countries [12] and in small municipalities seeking to comply with standards.

Some of the most widely used adsorbents for arsenic removal include activated alumina (AA), natural zeolites [13], and granular ferric oxide [14]. Activated alumina was approved by the US Environmental Protection Agency (EPA) as the best available treatment for arsenic removal. Few studies have been reported to remove As using alumina adsorbents [15,16]. Deng and Lin [16] developed a sol-gel method for preparing AA beads that enhanced the surface area in the mesopore structure of the adsorbent material. Substantially higher capacity for arsenic was reported for this material, which was attributed to the unconventional pore structure of the novel sol-gel material [17]. The purpose of this research study was to evaluate the performance of sol-gel activated alumina and to determine the effect of the presence of nanoparticles in the adsorption process.

5.2 Materials and Methods

5.2.1 Preparation of Sol-Gel Activated Alumina

Granular AA adsorbent beads were prepared following the Yoldas [18] sol-gel process. The first stage of this process was synthesis of a stable boehmite sol (γ-ALOOH) by dissolving alumina-trisecondary butoxide in deionized water at 75°C. The obtained solution was peptized with 1.0 M HNO_3 and refluxed at 90–100°C for 10 hours to obtain a stable boehmite sol. Granular boehmite beads with a median diameter of about 2 mm were generated through a glass pipette and a thin layer of paraffin oil. Ammonia solution was used to convert the partially gelated spherical wet-gel beads to firm wet-gel spheres. The wet-gel spheres were then washed, dried, and calcined at 450°C. Reagent grade chemicals and paraffin oil were obtained from Fischer Scientific.

5.2.2 Surface Modifications of Sol-Gel Activated Alumina

Three different modifications were made for preparing the sol-gel AA adsorbent. These modifications included: (1) addition of calcium oxide to the already prepared AA beads; (2) addition of zinc oxide nanoparticle solution during the gelation process; and (3) use of supercritical CO_2 drying

instead of calcination. The purpose of the modifications was to further improve the adsorption properties of the AA adsorbent. Adsorption enhancement is expected to occur due to the increase of pore spaces inside the AA. Supercritical CO_2 will increase the void spaces inside the AA; zinc oxide nanoparticles will enhance the affinity for As to the AA and will provide additional nanopores for adsorption; and calcium oxide will increase the ion-exchange capacity of the AA.

1. CaO-modified AA: The calcium oxide-modified AA (CaO-AA) was obtained by mixing 5 g of prepared Solgel AA beads with 100 mL of a 3M analytical grade $CaCl_2$ solution. The obtained solution was shaken for 24 hours and filtered. The coated CaO-AA beads were then dried in a conventional oven at 100°C for 24 hours and calcined at 450°C for about 12 hours. Afterwards, the coated adsorbent was cooled at room temperature.
2. Nanoparticle-modified AA: The ZnO nanoparticle-modified AA (Nanogel AA) was obtained by suspending 30 mg of ZnO nanoparticles in 200 mL of water in an ultrasonic bath. The resulting suspension was added to 106 mL of 2 M alumina-tri-secondary butoxide during the hydrolysis step of the process. The obtained solution was peptized with 1.0 M HNO_3 and refluxed at 90–100°C for 10 hours. The nano-AA beads were obtained with the help of a glass pipette and a thin layer of paraffin oil as established by the Yoldas method. The wet-gel spheres were then washed, dried, and calcined at 450°C.
3. Sol-gel AA dried with supercritical CO_2 (Aerogel AA): The supercritical-CO_2-dried AA (Aerogel AA) was obtained by drying the wet-gel AA spheres in a supercritical CO_2 reactor at 1200°C and 80 psi for 2 hours. The gas was released from the reactor after 2 hours to send the evaporated water out of the system, and the beads were dried for a second time in the reactor at 1000°C and 90 psi for 1 hour. The beads were then removed from the chamber and calcined at 450°C for 3 hours.

5.2.2.1 Characterization of Adsorbent Materials

Novel sol-gel AA and sol-gel-modified AA adsorbent materials were analyzed for chemical composition and pore textural properties using scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS) (Hitachi, S-3400N) and N_2 adsorption and desorption (Micromeritics® ASAP 2020) analysis. The pore textural properties including BET-specific surface area, pore volume, and pore size distribution were obtained from the built-in software in the ASAP-2020 instrument.

5.2.3 Batch Adsorption Experiments

Batch adsorption experiments were conducted to determine the As adsorption equilibrium properties of the adsorbent materials. These experiments included pH versus time, pH versus concentration, adsorbent amount versus concentration (adsorption isotherms), and breakthrough column experiments. A total of four adsorbent materials was analyzed, namely, Solgel AA, CaO-AA, Nanogel AA, and Aerogel AA.

1. Equilibrium experiments—determination of time required to achieve equilibrium: In order to determine the time required to reach equilibrium, a 100 mL solution and two replicates

were prepared to a concentration of 100 μg/L of arsenic (V). An arsenic standard solution from High-Purity Standards was used as the bases for the preparation of the As solutions. Each solution was adjusted to have a pH between 6.5 and 7.0 by adding drop-wise 0.1 N HCl or 0.1 N NaOH. Then, 1.0 g of the adsorbent was added to each of the bottles containing the solutions. The bottles were placed in a shaker at 100 rpm. The pH of each of the solutions was monitored as a function of time until equilibrium was reached. Equilibrium was reached when the pH did not change significantly. The pH of the solutions as well as the As concentrations were measured at the beginning and end of the equilibrium experiments.

2. Effect of pH on As adsorption: To determine the effect of pH on the adsorption removal of As, solutions of 100 μg/L of arsenic (V) were prepared and adjusted with 0.1 N HCl or 0.1 N NaOH to an initial pH of 2, 4, 5, 6, 7, 8, and 10. Each solution was prepared in a 200-mL plastic bottle and to each solution 1.0 g of AA adsorbent was added. Once the adsorbent was added to the As solution, the bottle was covered and placed in the automatic shaker at 100 rpm until the solution reached equilibrium. For each pH an original and a replicate were prepared. Both pH and As concentration were measured at the beginning and end of the experiment.
3. Effect of As concentration on adsorbed quantity: To determine how the initial As concentration affects the amount of As adsorbed, the two best adsorbents from the previous pH vs. concentration experiment were selected and tested in an equilibrium experiment. The experiment was conducted by preparing 100 mL solutions with As concentrations of 5, 10, 25, 50, 75, 100, and 200 μg/L. The pH of the solutions was adjusted to the selected pH using 0.1 N HCl or 0.1 N NaOH. One gram of the adsorbent was added to the solution, and the solution was shaken for a specific period of time. The pH and As concentration of the solutions were measured at the beginning and end of the experiment. The results obtained from these experiments were modeled using Freundlich and Langmuir isotherms.

5.2.3.1 Breakthrough Column Experiments

Breakthrough (BT) column experiments were conducted to determine the time needed by the two selected adsorbents to reach saturation. Breakthrough experiments were performed on a 12″ PVC column with an outside diameter of 3/8″ (1.72 cm) and an inside diameter of 0.460″ (1.17 cm). A glass-wool plug was placed at the bottom of the column to prevent loss of the adsorbent. The adsorbent was carefully added to the column, and the entrapped air was removed by fluidizing the column with deionized (DI) water. The system was run in upflow mode by gravity. Arsenic (As) solutions with an initial concentration of 500 μg/L of arsenic (5 +) were used to run these experiments. The pH of the solutions was adjusted with 0.1 N HCl or 0.1 N NaOH to a pH between 7.0 and 7.5. The amount of adsorbent used depended on the height of the column with which each experiment was to be conducted. Two different column heights or empty bed volume (EBV) were selected. The As solution was fed to the column continuously without recirculation, and sample solutions were taken periodically at the exit of the column to measure the changes in the As concentration. An inductively coupled plasma

optical-emission spectroscopy (ICP-OES) instrument was used to measure the As concentrations. Release of As at the exit of the column meant that the saturation of the adsorbent material had already started.

5.2.3.2 Adsorbent Regeneration

Regeneration of selected adsorbent materials was made by passing a 2% NaOH (0.5 M) solution (Reagent Chemical) through the BT column. The column contained the adsorbent material already exhausted or saturated with As. The 2% NaOH solution was passed by gravity in down-flow mode through the column. Solution samples were collected at the exit of the column and were analyzed for As until the As concentration was minimal. Once the regeneration process was finished, the regenerated adsorbent material was washed with DI water and was ready for reuse.

5.3 Results and Discussion

5.3.1 Adsorbents Properties

Figure 5–1 shows the nitrogen adsorption and desorption isotherms of Solgel AA and modified AA adsorbents. The mesoporous nature of the adsorbent can be observed by the shape of the adsorption/desorption hysteresis. Solgel AA and Nanogel AA hysteresis suggests a wide range of pore sizes in the surface. The shape of Aerogel AA hysteresis suggests that drying the wet-gel spheres with supercritical CO_2 causes the pores inside of the surface to become larger. Pores in CaO-AA tend to be of much smaller diameter. This is confirmed by the BJH and H-K average pore size measured by the ASAP instrument (Table 5–1). The values of BET-specific

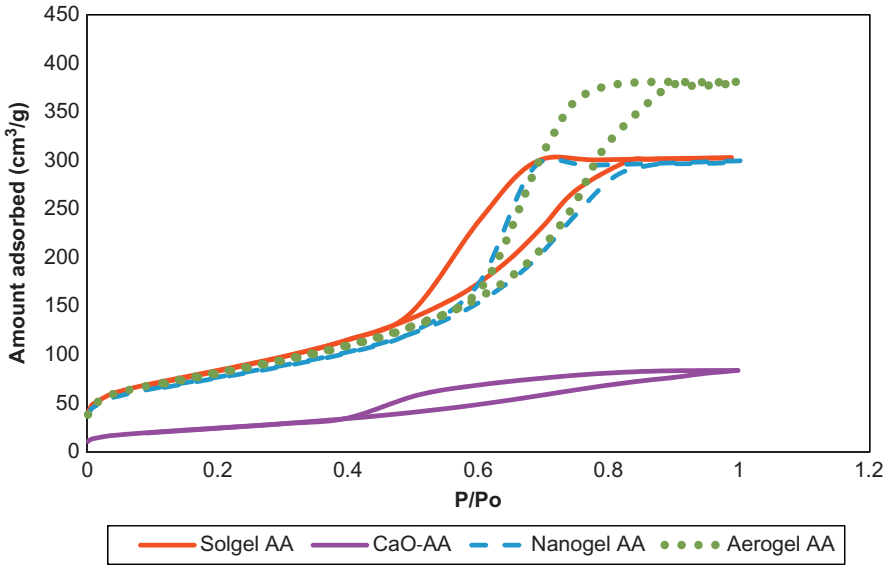

FIGURE 5–1 Nitrogen adsorption/desorption isotherms on Solgel AA and modified AA adsorbents.

surface area and pore volume for the adsorbents are also presented in Table 5–1. Based on the BET-specific surface area the adsorbent materials follow the order Solgel AA > Aerogel AA > Nanogel AA > CaO-AA. Even though the BET surface area of Nanogel AA and Xerogel AA are smaller than the surface area of their precursor (Solgel AA), their pore size and pore volume are bigger. This behavior may play an important role in the adsorption capability of the adsorbents. Based on the H-K pore size the adsorbents follow the order Aerogel AA > Nanogel > Solgel AA > CaO-AA.

SEM images for Solgel AA and modified AA adsorbents are presented in Figure 5–2. Small amorphous clusters can be seen in the SEM image of Solgel AA. The main components of these adsorbents are alumina and oxide.

Table 5–1 Surface Properties of Adsorbent Materials Produced in the Laboratory

Property	Solgel AA	CaO-AA	Nanogel AA	Aerogel AA
BET surface area (m^2/g)	308.01	92.77	278.11	297.19
BJH average pore size (Å)	48.70	48.09	53.67	63.14
BJH average pore volume (cm^3/g)	0.474	0.129	0.463	0.589
H-K average pore size (Å)	59.84	55.59	68.26	87.90
BJH average pore volume (cm^3/g)	0.468	0.129	0.459	0.589

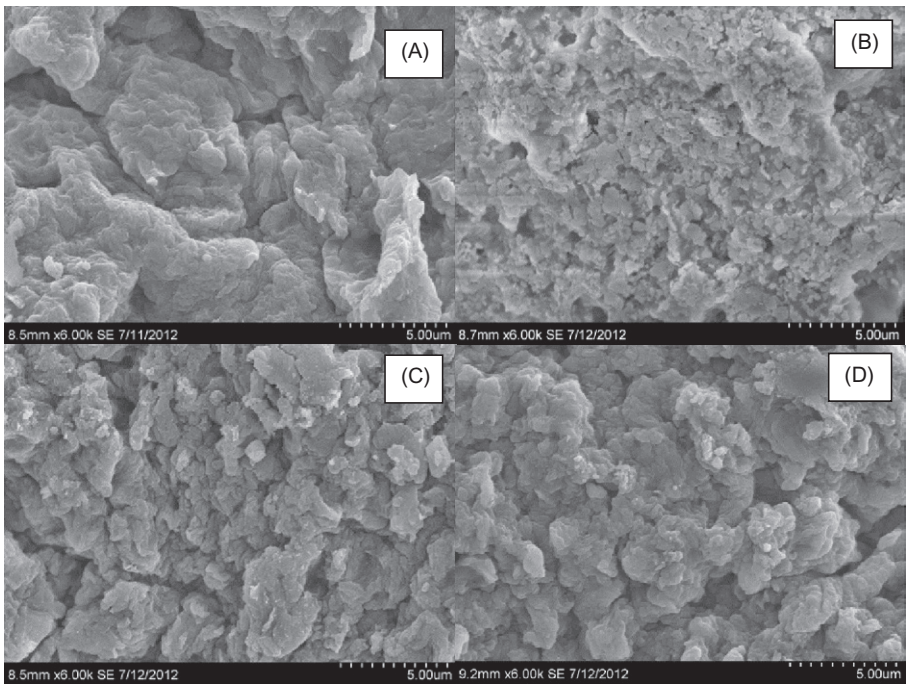

FIGURE 5–2 SEM image of (A) Solgel AA, (B) CaO-AA, (C) Nanogel AA, (D) Aerogel AA. (Scale bar = 5μm.)

SEM images of modified Solgel AA adsorbents reveal smaller and less heterogeneous clusters than the clusters in Solgel AA. This observation is particularly notorious in CaO-AA adsorbent. Zinc oxide crystals are randomly distributed in Nanogel AA. Homogeneous distribution of clusters is evident in Aerogel AA. This is possible because air was completely extracted from the pores and void spaces of the adsorbent by drying it with supercritical CO_2. The slope of the Aerogel AA's hysteresis in Figure 5–1 is characteristic of the presence of mesopores in the adsorbent.

5.3.2 Arsenic Adsorption Equilibrium

5.3.2.1 Solution pH as a Function of Adsorption Time

Analysis of pH as a function of adsorption time was conducted to determine the minimum time required for obtaining equilibrium adsorption. Figure 5–3 displays the experimental data for arsenic solutions with an initial concentration of 100 µg/L adsorbed onto one gram of Solgel AA and modified AA adsorbents. Solgel AA showed an initial pH of 6.68. Even though the Solgel AA's pH seemed to stabilize during the first 10 hours, it completely stabilized after about 50 hours. Observed behavior demonstrated that this adsorbent may have high alkalinity or internal buffer capacity, which helped to maintain the small pH changes during the first hours. After 72 hours the final pH of Solgel AA was 6.58.

During the first 10 hours, the initial pH of CaO-AA adsorbent decreased from 6.7 to 6.5 (Figure 5–3). It then increased continuous and steadily throughout the experiment. After 50 hours CaO-AA had a pH of about 10.0. After 72 hours the pH for this adsorbent was about 10.6 and had not completely stabilized.

Variations in pH for Nanogel AA and Aerogel AA (Figure 5–3) were minimal with respect to their precursor Solgel AA (Figure 5–3). The initial pH for these two adsorbents was about 6.8; the final pH after 72 hours was slightly higher than 6.0; at this time they were completely stabilized. From the four adsorbents produced in the laboratory, Solgel AA, Nanogel AA, and

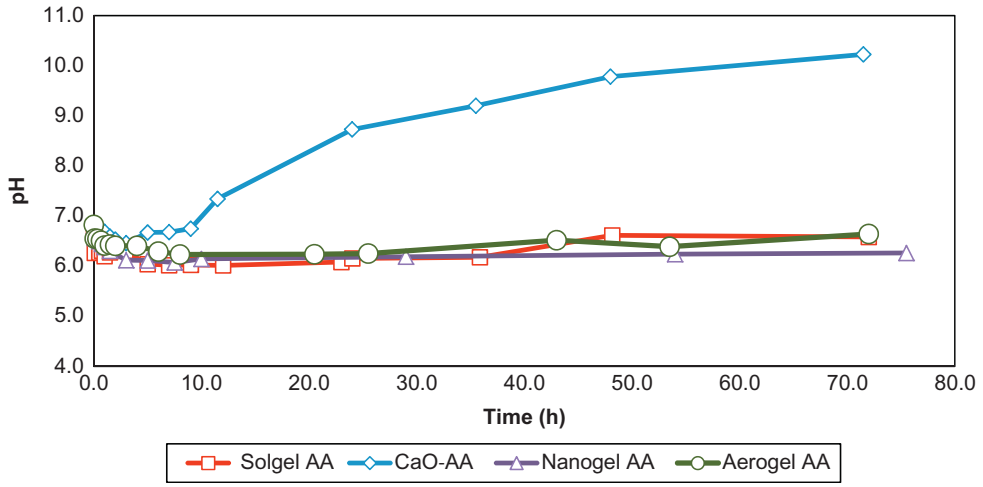

FIGURE 5–3 Time to reach adsorption equilibrium in Solgel AA and modified AA adsorbents.

Aerogel AA showed that they were more stable materials in the presence of As solution. This may be an important factor to consider when treating drinking water, because the pH of the treated water would not need to be adjusted after treatment. Based on the time to reach equilibrium, 60, 72, and 72 hours were considered the appropriate number of hours for Solgel AA, Nanogel, and Aerogel, respectively, to conduct the next set of adsorption experiments.

5.3.2.2 *As Adsorbent Amount as a Function of pH*

Experiments on the adsorption equilibrium of As as a function of pH were conducted on Solgel AA and modified AA adsorbents to determine the optimum pH at which the highest As adsorption occurs. An initial As concentration of 50 or 100 µg/L was used for all the experimental runs. The initial and final pH was monitored for each of the arsenic solution concentrations tested. The As adsorbent amount (µg As/g adsorbent) of the adsorbent materials was determined by mass balance using the equation:

$$q_e = (C_o - C_e) * V/m \tag{5.1}$$

where C_o is the initial As concentration (µg/L), C_e is the concentration at equilibrium (µg/L), V is the volume of As solution (mL), and m is the mass of adsorbent (g).

The variation of the quantity of As adsorbed with final pH is illustrated in Figure 5–4. In all cases covering pH from 2.0 to 10.0, the equilibrium concentrations were lower than the initial solution concentrations of 50 or 100 µg/L, suggesting that removal of As by the adsorbent materials occurred.

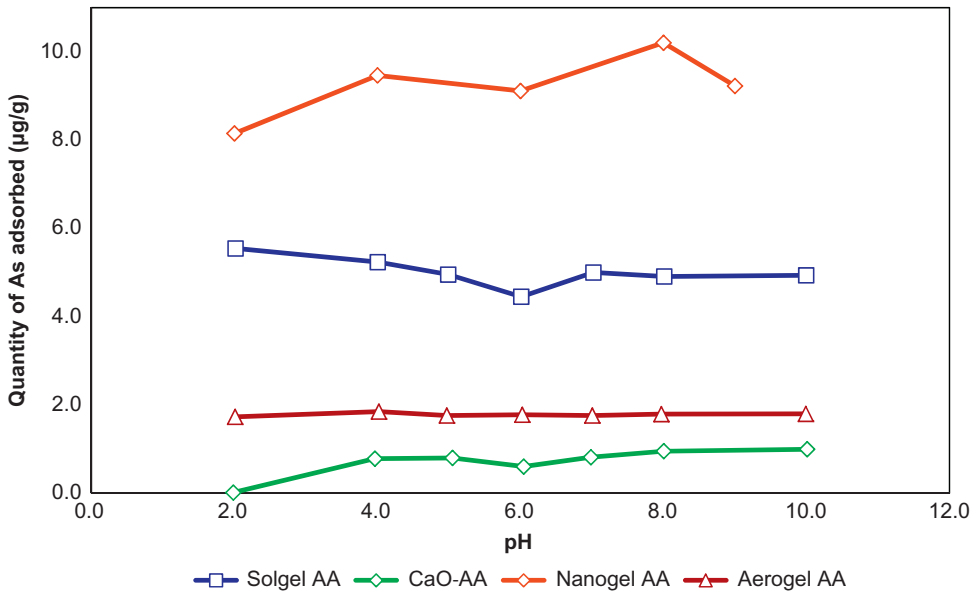

FIGURE 5–4 Effect of pH on quantity of As adsorbed by AA adsorbents.

The maximum As adsorption capacity obtained by precursor Solgel AA was 5.4 μg/g at pH 2.0. The minimum adsorption capacity of 0.01 μg/g was obtained by CaO-AA at pH 2.0. Nanogel showed an average As adsorption capacity of 9.0 μg/g at any of the pH values tested, except at pH 2.0. Its adsorption capacity was more than two times that of Solgel AA. In general, even though the adsorption capacity of most of the adsorbent materials was pH independent, Solgel AA and Nanogel AA showed superior performance at almost any pH value. The buffer capacity of these two adsorbents may be a key component that reflects in their performance. Based on the observed adsorption capacity, the adsorbent materials followed the order Nanogel AA > Solgel AA > Aerogel > CaO-AA. The poor performance observed in CaO-AA may be explained by the high pH at which it equilibrates (Figure 5–4). Based on the observed pH, it may be assumed that CaO-AA becomes a good adsorbent material at pH higher than 10.0. However, most of the existing environmental conditions are at a pH between 5.0 and 8.0. Therefore using CaO-AA as adsorbent will require the addition of chemicals to increase the pH of the system to at least 10.0.

The observed results demonstrated that As removal capacity depends mostly on the pH properties of the adsorbent materials and less on the surface area properties of the material. This can be explained by the amphoteric properties of As, the solubility of which depends mainly on the protonated species that are present at the specific pH of the solution. At a pH of 5.0–8.0, which is typical of natural waters, arsenate, As(V), is predominantly present in the form of $H_2AsO_4^-$ and $H_2AsO_4^{2-}$ species, while arsenite, As(III), remains fully protonated and is present as the $HAsO_2$ neutral species. At a pH of 2.0, As(V) is present in the form of H_3AsO_4 neutral species and $H_2AsO_4^-$ species and As(III) is present in the form of $HAsO_2$ species. At a pH of 10.0, As(V) is present in the form of $H_2AsO_4^{2-}$ species and As(III) is present in the form of $H_2AsO_3^-$ and $HAsO_3^{-2}$ species [4,13]. Results indicate that the addition of zinc oxide nanoparticles during the hydrolysis step in the Yoldas process enhances the adsorption properties of the resulting adsorbent. This is turn contributes to increase the adsorption capacity of the new material. Even though chemical modification of already produced Solgel AA helps to increase its surface area, an improvement of the Yoldas process by itself, i.e., by incorporating nanoparticles during the process of producing the adsorbent, may be even more convenient to help increase the adsorption capacity of this material.

Considering a typical sample of drinking water with neutral or slightly basic pH and the advantage of having an adsorbent material that is pH independent, the three adsorbents selected for showing the best properties for maximum adsorption capacity were Nanogel AA > Solgel AA > Aerogel AA. The fact that Nanogel AA adsorbed more As than Solgel AA suggests that the novel sol-gel method to produce AA is important and successful. The high As adsorption capacity of Nanogel AA can be attributed to the enhanced pore textural and surface properties but mostly to its relatively high pH independency.

5.3.2.3 Effect of Initial Concentration on As Removal Efficiency

The effect of initial concentration on the adsorption of As by Solgel AA and modified adsorbents was analyzed by calculating the percentage of As removal at different initial concentrations. The removal efficiency was calculated based on the difference of initial and final As

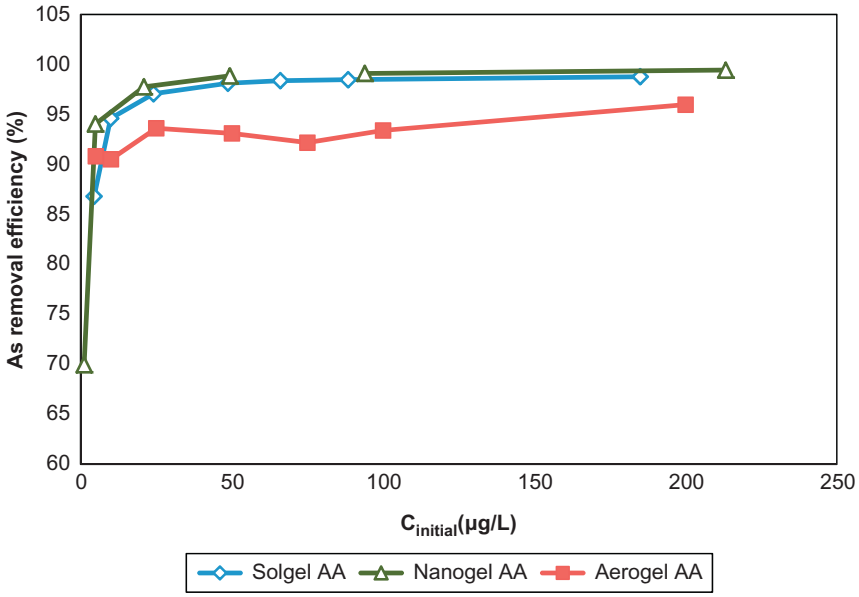

FIGURE 5–5 Removal efficiency of As for produced adsorbents.

concentration at the end of the equilibrium process. Figure 5–5 displays the As removal efficiency as a function of initial As solution concentration.

For all the adsorbents, As removal efficiency increased with the increase of initial As concentration and reached a maximum at high initial As concentrations. Nanogel AA removed almost 100% As from solutions with high As concentrations; it removed 95% As from an As solution of 10 µg/L. Performance of Nanogel AA may be improved by increasing the concentration of ZnO nanoparticles during the beads' formation process. Solgel AA removed up to 98% and 97% from a solution containing an initial As concentration of 50 µg/L and 10 µg/L, respectively. This corresponded to an adsorption capacity of 4.5 µg/g and 1.0 µg/g, respectively (Figure 5–5). Only 3% As remained in the solution containing 10 µg/L As, which is the MCL for As in drinking water. Aerogel removed 90% As from a 10 µg/L As solution. These equilibrium experimental results demonstrated that the Solgel AA and modified Solgel AA adsorbents are promising adsorbent materials for As removal.

5.3.2.4 Adsorption Equilibrium

The As adsorption equilibrium data for selected adsorbents are presented in Figure 5–6. Results were obtained at selected pH for solutions with initial As concentrations varying from 5 to 200 µg/L. Freundlich and Langmuir adsorption isotherm models were used to correlate the experimental data. The Freundlich and Langmuir isotherms are two-parameter models that provide useful information to predict the adsorption capacity of the adsorbent materials at given feed concentrations. Correlation of the equilibrium data provide important parametric information that can be used to optimize the design of the adsorption system for the removal of As.

Table 5–2 presents the Freundlich and Langmuir equations as well as the estimated adsorption isotherm parameters for the three AA adsorbents produced in the lab. In the two models, q_m (mg/g) is the amount of arsenic adsorbed per weight of adsorbent at equilibrium, and C_e (mg/L) is the equilibrium concentration of the adsorbate in the solution. In the Freundlich model, K is the Freundlich constant representing the relative adsorption affinity of the adsorbent toward the adsorbate molecules, and n represents the heterogeneity of the adsorbent (Table 5–2). In the Langmuir model, b (L/mg) is the Langmuir constant that characterizes the adsorbent–adsorbate interaction, and a (mg/g) represents the monolayer adsorption capacity (Table 5–2).

The Freundlich adsorption isotherm fitted well the As adsorption on the three AA adsorbents. The R-squared values for Solgel AA, Nanogel AA, and Aerogel AA are 0.9582, 0.9717, and 0.9731, respectively. Figure 5–6 shows the fitted model for each of the adsorbents tested.

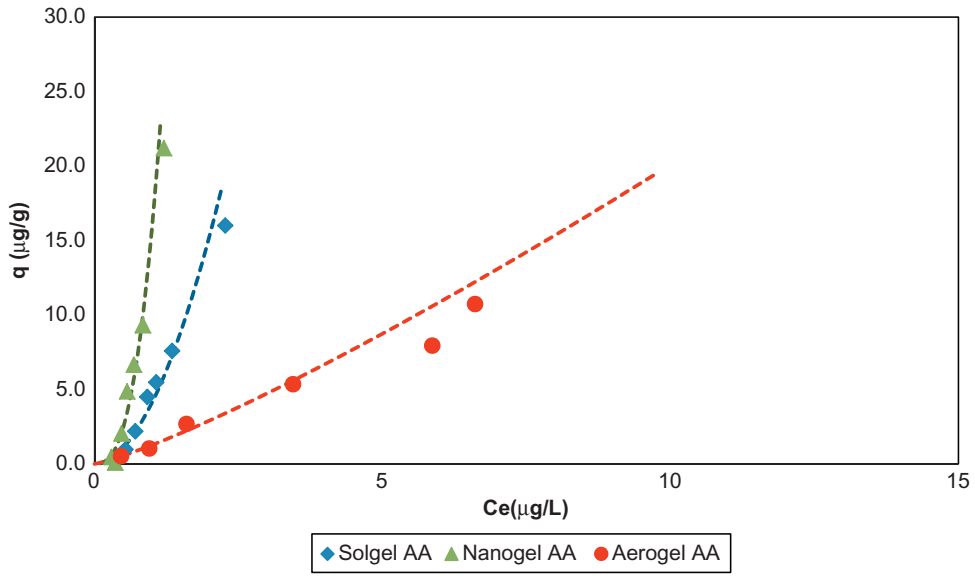

FIGURE 5–6 Effect of concentration on quantity of As adsorbed for the three adsorbents studied (dashed lines represent the Freundlich model of the adsorption process).

Table 5–2 Freundlich and Langmuir Isotherm Constants for As Adsorption on Tested Adsorbents

	Freundlich Isotherm $q_e = k * C_e^{1/n}$			Langmuir Isotherm $q_e = a*b*C_e/(1 + b*C_e)$		
Adsorbent	***k***	***n***	R^2	***a* (mg/g)**	***b* (L/mg)**	R^2
Solgel AA	4.13505	0.52815	0.9582	2.6702	0.56088	0.8763
Nanogel AA	15.9809	0.37127	0.9717	1.0086	0.84911	0.8812
Aerogel AA	1.26921	0.83381	0.9731	14.144	12.9165	0.9591

Although the Langmuir isotherm model has been reported as a good model to represent the adsorption of As with a variety of adsorbent materials, it failed to correlate the data for As adsorption by the AA adsorbents tested. This may be attributed to changes that occurred to the Solgel AA adsorbent during the different modifications made to the Yoldas process. It has to be taken into consideration that the correlation coefficient accounts only for the linearity of the adsorption process.

5.3.3 Breakthrough Column Analysis

Breakthrough (BT) column experiments were conducted using Solgel AA adsorbent. Behavior of As concentration in the BT column was analyzed as a function of the empty bed volume (EBV) in the column. The value of one EBV was obtained by calculating the volume of the column. Table 5–3 shows the conditions under which the experiments were run. Each experiment was run until the As concentration at the exit of the column was approximately the same as the initial As concentration. It was assumed that at this point the column is saturated and therefore not able to adsorb more As. The capacity of the column was obtained by calculating the total bed volumes (BVs) of water treated before the concentration of As exceeded 10 µg/L. The cumulative number of BVs was obtained by adding the volume fed to the column at time "t" to the volume that has been already fed at a specific flow rate and by dividing this accumulative sum by one EBV as shown by the equation:

$$\text{Cumulative BV} = (V_t + (Q * \Delta t))/\text{EBV} \tag{5.2}$$

where V_t is the volume of solution at time t (mL), Q is the flow rate (mL/min), Δt is the interval of time (min), and EBV is the volume of the empty column at the particular height in the column (mL).

During the elapsed time of the BT column experiments, the pH of the effluent water for the two samples increased slowly until it reached a typical pH of drinking water (pH = 7.3). The average pH of the solution fed to the column was approximately 6.7. The total duration of the BT experiment was 208 hours and 178 hours for Solgel 1 and 2, respectively. The column had a small diameter and was not large, and therefore gravity was sufficient to feed the As solution to the column.

The As concentration changes at the effluent of the BT column for Solgel AA are presented in Figure 5–7. The EBV for Solgel 1 was 9; for Solgel 2 it was 19, two times the EBV of Solgel 1.

Table 5–3 Experimental Conditions for BT Column Experiments on Solgel AA Adsorbent

Parameter	Solgel 1	Solgel 2
Initial As concentration (µg/L)	514	504
Mass of adsorbent in column (g)	6.8	15.2
EBV (mL)	9	19
Average flow rate (mL/min)	30.4	37.1
Initial solution pH	6.55	6.8

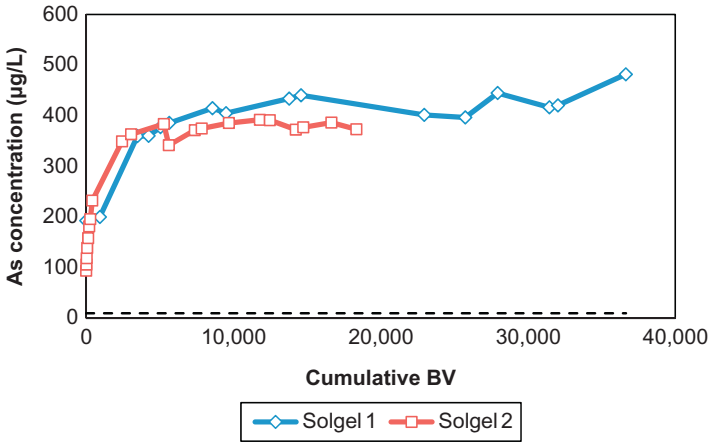

FIGURE 5–7 BK for As removal at different column heights for Solgel AA (dashed line represents MCL of 10 µg/L for As).

This means that Solgel 2 would need two times the BV used by Solgel 1 to reach the same As effluent concentration. Due to the small EBV in the Solgel 1 column, the adsorbent started saturating very rapidly and the breakthrough at 10 µg/L (MCL for As in drinking water, dashed line in Figure 5–7) happened before the first concentration reading was made at the exit of the column (elapsed time less than 5 minutes). The first water sample taken at the effluent already measured an As concentration of 193 µg/L (19 times the MCL for As). Solgel 1 reached saturation, i.e., As effluent concentration equal to As influent concentration of 514 µg/L, after treating about 40,000 BV of water (368 liters of water).

The EBV of 19 in Solgel 2 column was still small and did not allow observing breakthrough at a concentration of As of 10 µg/L. The first As reading made at the effluent of the column was already 93 µg/L (9.3 times the MCL for As) (elapsed time less than 5 minutes). Interestingly, the first As concentration reading in the Solgel 2 column (193 µg/L) was about two times the first As concentration reading in the Solgel 2 column (93 µg/L), as expected. Based on the 1:2 ratio in both EBV and As concentration at the effluent, it is expected that Solgel 2 will treat a total of about 80,000 BV (736 liters of water) before reaching column saturation (As concentration of 514 µg/L). In order to be able to observe column breakthrough at 10 µg/L of As, under the given process and parametric conditions, the EBV of the Solgel 1 column would have to be two times the EBV of the Solgel 2 column (EBV ≈38).

Additional BT column experiments for Solgel AA with prehydration (to improve removal capacity), different flow rate, EBV, column length, particle size, and adsorbate concentration are needed to determine the optimal process conditions that will extend the good performance of this novel and promising adsorbent material prior to saturation. A combined experimental design will allow combining process and parametric conditions in the laboratory to reduce the number of experiments needed as well as cost. Development of a mathematical model specific for this particular process could also help to optimize the BT column performance for further pilot test-scale experiments.

5.3.4 Adsorbent Regeneration

Regeneration of the adsorbent material is important in relation to the cost-effectiveness and user-friendliness of the process. After adsorbing arsenic for a certain time in the BT column, the Solgel AA adsorbent material became exhausted. The exhausted adsorbent must either be replaced or regenerated to restore its adsorption capacity. The regeneration of Solgel 1 and Solgel 2 was effected by passing a strong base solution (2% NaOH) through the column to wash the adsorbent until no arsenic was detected at the exit of the column. In the regeneration process, desorption of arsenic from the adsorbent took place by increasing the hydroxyl ions in contact with the adsorbent in the column. The high pH of NaOH (pH > 10.5) caused arsenic in the form of the arsenate ion ($HAsO_4^-$) to lose adsorptive attraction to the alumina and become repelled, following the reaction:

$$\text{Alumina.}H_2AsO_4^- + 2NaOH \rightarrow \text{Alumina.NaOH} + NaHAsO_4 + H_2O \tag{5.3}$$

Reuse of the AA adsorbent material was possible after regeneration with 2% NaOH solution. Equilibrium experiments using the regenerated material were conducted following the same procedure. A 100 mL solution with a given As concentration was prepared. The pH of the solution was adjusted to the selected pH. Then 1 g of the adsorbent was added to the solution and the solution was shaken for a specific period of time. The pH and As concentration of the solution were measured at the beginning and end of the experiment. The adsorption capacity of regenerated Solgel AA was not calculated, because the As concentration of the solution after reaching equilibrium was higher than the initial concentration. This may be explained by As from the previous run being still contained in the adsorbent material. This might be possible if As was strongly bonded to Solgel AA. In this case, additional NaOH 2% or NaOH with a higher concentration may be needed to completely wash out the As.

5.4 Conclusions

A novel Solgel AA adsorbent produced in the laboratory and its modifications using CaO (CaO-AA) ZnO nanoparticles (Nanogel AA) and supercritical CO_2 drying (Aerogel AA) were studied to explore the feasibility of applying these adsorbents for arsenic removal from drinking water. Equilibrium and BT column experiments were conducted to determine the removal efficiency and saturation of the adsorbent material. The latter was recovered for reuse.

The following conclusions were drawn from this experimental study:

- The BET specific surface area of the adsorbent tested followed the order Solgel AA > Aerogel AA > Nanogel AA > CaO-AA.
- From the adsorbents tested, Solgel AA, Nanogel AA, and Aerogel AA appeared to be more stable materials in the presence of As solution. This is an important factor to consider when treating drinking water.
- The adsorbents that showed the best properties for maximum adsorption capacity were Nanogel AA > Solgel AA > Aerogel AA. The high As adsorption capacity of Nanogel AA

is attributed to the enhanced pore textural and surface properties as well as to the pH independency of the adsorbent.
- For all the adsorbents produced in the laboratory, As removal efficiency increased with the increase of initial As concentration.
- The Freundlich adsorption isotherm fitted well the As adsorption on the AA adsorbents tested.
- Additional BT column experiments for Solgel AA with different flow rates, EBVs, column lengths, particle sizes, and adsorbate concentrations are needed to determine the optimal process conditions that will extend the performance of the adsorbent prior to saturation.

Acknowledgments

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6 Health Effects Chronic Arsenic Toxicity

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CHAPTER OUTLINE

6.1 Introduction

Arsenic, a metalloid, occurs naturally, being the twentieth most abundant element in Earth's crust and is a component of more than 245 minerals. The inorganic forms are toxic to human health and consist mostly of arsenite and arsenate compounds. Humans are exposed to arsenic primarily from air, food, and water. Drinking water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits, or improperly disposed arsenical chemicals. Arsenic in drinking water has been recognized as a major public health problem in several regions of the world. Reports of arsenic contamination in ground water are emerging from more than 20 countries as and when cases of chronic arsenic toxicity are reported. Major countries affected are Bangladesh, India, Taiwan, China, Mexico, Argentina, Chile, and the USA.

Human health effects of chronic arsenic toxicity are designated by the term arsenicosis, which was first coined by our group [1] and later used by the World Health Organization (WHO) [2] to imply a chronic disease caused by prolonged exposure of arsenic in humans. Previously, the

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condition was described as arseniasis, arsenism, arsenicism, etc. Most of the reports of chronic arsenic exposure in humans focus attention on skin manifestations because of their diagnostic specificity. However, data derived from population-based studies, clinical case series, and reports relating to intake of inorganic arsenic in drinking water, medications or occupational and environmental exposure show that chronic arsenic exposure adversely affects multi-organ systems of the human body. The symptoms of chronic arsenic toxicity (arsenicosis) are insidious in onset and are dependent on the magnitude of the dose and duration of its exposure. The duration of the patient's arsenic exposure with the date of onset of symptoms does not follow a particular timeframe. It is even the case that not all members of an affected family show clinical effects. The reason for such variation of disease expression is an enigma. Further, there is a wide variation in the incidence of chronic arsenicosis in an affected population. It needs to be mentioned that a few epidemiological studies in the USA reported that none of the population exposed to environmental arsenic showed any clinical manifestation of chronic arsenic toxicity [3,4].

Over and above dermatological manifestations, chronic arsenic exposure in humans produces various non-specific symptoms owing to the fact that arsenic affects multi-organ systems in the body. However, most of the reports of chronic arsenic exposure in humans focus attention on skin manifestations because of their diagnostic specificity. In a field guide for detection, management, and surveillance relating to arsenicosis, the condition has been defined by WHO as "a chronic health condition arising from prolonged ingestion of arsenic above the safe dose for at least six months, usually manifested by characteristic skin lesions of melanosis (hyperpigmentation) and keratosis, occurring alone or in combination with or without involvement of internal organs" [2]. The appearance of lesions usually follows temporal progression, beginning with hyperpigmentation, and then progressing to palmar-plantar keratosis, which is dependent on the magnitude of the dose and the duration of exposure.

6.2 Dermatological Manifestations

The specific skin lesions of chronic arsenic toxicity are characterized by hyperpigmentation, depigmentation, keratosis, and Bowen's disease.

6.2.1 Hyperpigmentation

The hyperpigmentation of chronic arsenic poisoning commonly manifests as a finely freckled, "raindrop" pattern of pigmentation or depigmentation that is particularly pronounced on the trunk and extremities and has a bilateral symmetrical distribution. This occurs anywhere in the body, and is particularly marked in non-exposed parts of the body, such as trunk, buttocks, and thighs. Pigmentation may sometimes be blotchy and involve mucous membranes such as the undersurface of the tongue or buccal mucosa [1,5–11]. The raindrop appearance results from the presence of numerous rounded hyperpigmented or hypopigmented macules (typically 2–4 mm in diameter) widely dispersed against a tan-to-brown hyperpigmented background [7]. Hyperpigmentation is often present combined with hypopigmented spots [5,12]. Other patterns include diffuse hyperpigmentation (melanosis) [5,9], and localized or

patchy pigmentation, particularly affecting skin folds [7,13,14], although less commonly seen. Pigmentation is not histopathologically related to arsenical hyperkeratosis, nor is it a direct precursor of cancer [15].

6.2.2 Hypopigmentation

Hypopigmentation or so-called leukoderma or leukomelanosis [9,10] is found in chronic arsenic exposed people in which the hypopigmented macules take a spotty, white appearance. This follows the same distribution as hyperpigmentation. These spots may be present in the absence of hyperpigmentation [8]. Hypopigmentation, which was described as part of the raindrop-like appearance [16] and as a separate entity named leukoderma [17], was considered the earliest cutaneous sign of chronic arsenic toxicity [8]. However, others suggest that leukomelanosis appears to occur in an arsenicosis patient following stoppage of drinking arsenic-contaminated water for some duration [9,15]. (See Figures 6–1–6–5.)

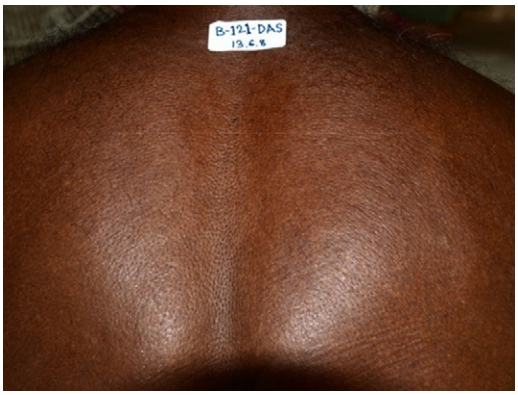

FIGURE 6–1 Melanosis (diffuse pigmentation) in the back.

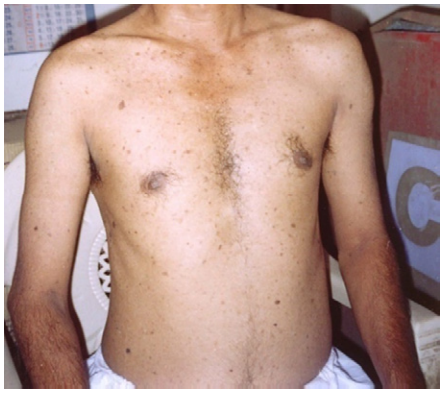

FIGURE 6–2 Pigmentation (spotty, mild).

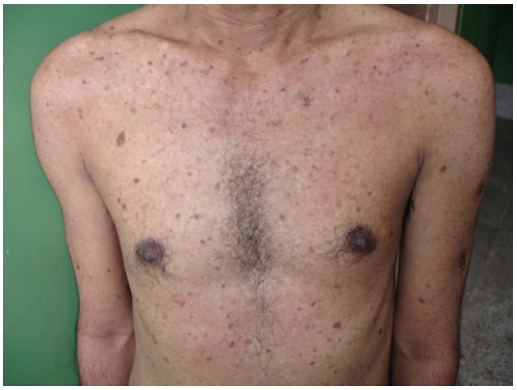

FIGURE 6–3 Pigmentation (moderate).

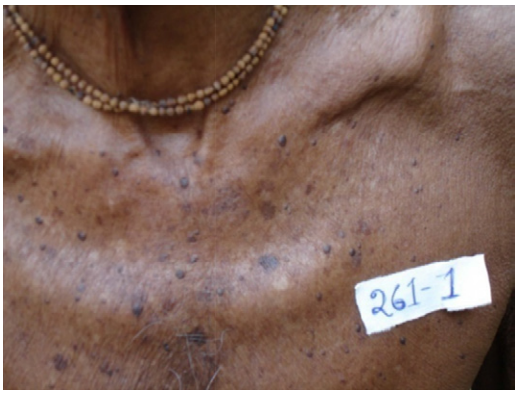

FIGURE 6–4 Pigmentation (severe).

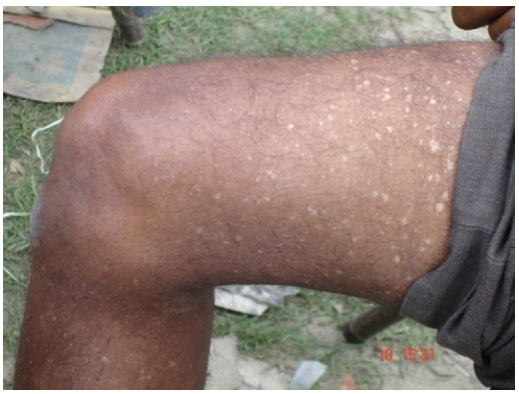

FIGURE 6–5 Leukomelanosis.

6.2.3 Hyperkeratosis

Arsenical hyperkeratosis appears predominantly on the palms and the plantar aspect of the feet, although involvements of the dorsum of the extremities and the trunk have also been described. They are found most frequently on the thenar eminence and the lateral borders of palms and fingers, and on the soles and heels and toes. In the early stages, the involved skin might have an indurated, grit-like character that can be best appreciated by palpation; however, the lesions usually advance to form raised, punctate, 2–4 mm wart-like keratoses that are readily visible [7]. Occasional lesions might be larger (0.5 to 1 cm) and have a nodular or horny appearance occurring in the palm or dorsum of the feet. In severe cases, the hands and soles present with diffuse verrucous lesions. Cracks and fissures may be severe in the soles [1,5–9,17–22]. A scoring system has been developed for assessing the severity of skin lesions of pigmentation and keratosis (Table 6–1) [23]. (See also Figures 6–6–6–9.)

Table 6–1 Dermatological Criteria and Gradation of Skin Lesions of Chronic Arsenic Toxicity (Scoring System) [23]

Pigmentation (Score)		
Mild (1)	**Moderate (2)**	**Severe (3)**
Diffuse melanosis, mild spotty pigmentation, leukomelanosis	Moderate spotty pigmentation	Blotchy pigmentation, pigmentation of undersurface of tongue, buccal mucosa
Keratosis (score)		
Slight thickening or minute papules (<2 mm) in palm and soles	Multiple raised keratosis papules (2 to 5 mm) on palm and soles with diffuse thickening	Diffuse severe thickening, large discrete or confluent keratotic elevations (>5 mm) on palm and soles (also dorsum of extremity and trunk)

Maximum total skin score = 6.

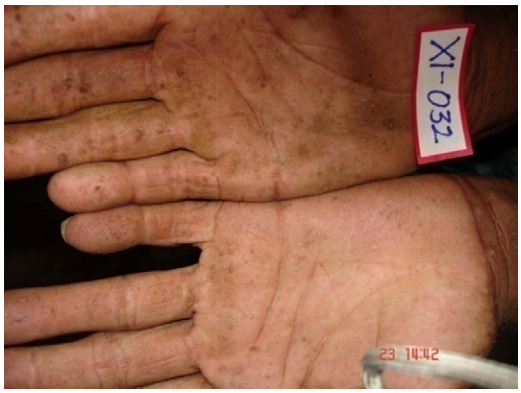

FIGURE 6–6 Mild keratosis.

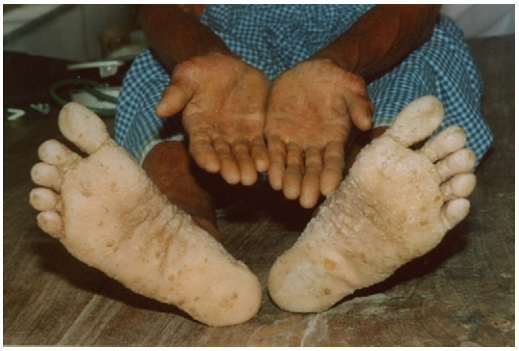

FIGURE 6–7 Moderate keratosis.

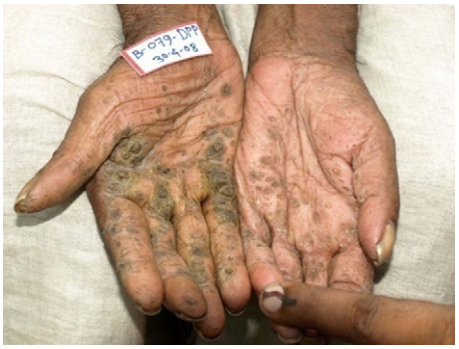

FIGURE 6–8 Severe keratosis.

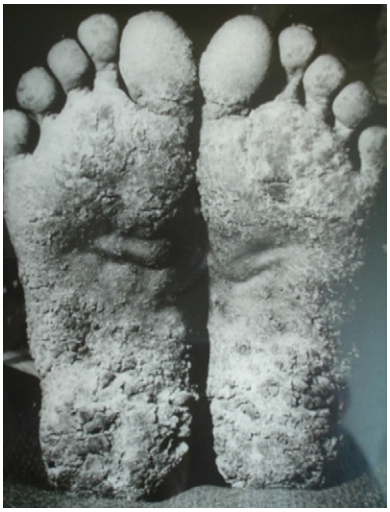

FIGURE 6–9 Severe verrucous keratosis.

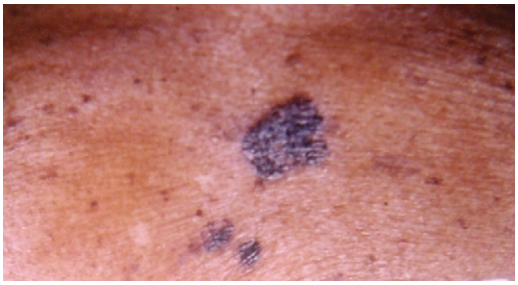

FIGURE 6–10 Bowen's disease in the back.

Histological examination of skin biopsy material of a keratotic skin lesion shows extensive hyperkeratosis and parakeratosis. Parakeratosis is more prominent along either side of the sweat duct orifice. The epidermis is markedly acanthotic with irregular downward prolongation of enlargement of the rete ridges [5,7]. Varying degrees of squamous cell hyperplasia, without any basophilic degeneration of the dermis, are usually seen. In some cases, there might be evidence of cellular atypia, mitotic figures, in large vacuolated epidermal cells [24–26]. Yeh [6] classified two types of arsenical keratosis, a benign type A, further subdivided into two groups, one with no cell atypia and the other with mild cell atypia, and a malignant type B [15].

6.2.4 Bowen's Disease

The skin lesion of Bowen's disease appears as sharply demarcated round plaques or has an irregular polycyclic lenticular configuration. The lesions are usually erythematous, pigmented, crustated, fissured, and keratotic. Some may be nodular, ulcerated, or eroded. The diameter of the lesions may vary from 0.8 to 3.5 cm. The lesions are firm and indurated and rough on palpation [6]. Clinically, arsenic-induced Bowen's disease can be distinguished from non-arsenical Bowen's disease by its multiple and recrudescent lesions and predominant occurrence on the sun-protected areas of the skin [27,28]. The disease is generally believed to be an intradermal carcinoma from its onset [29,30]. Histologically, Bowen's disease can be divided into hypertrophic and atrophic types. Common features in both varieties are windblown appearance of the epithelial cells, with multinucleated giant cells, vacuolated cells, and dyskeratosis; abnormal mitotic figures may be present at all levels of the epidermis. In classical Bowen's disease, the epidermis is composed of atypical cells haphazardly arranged at all levels. In the hypertrophic forms, papillomatosis and acanthosis are marked. In atrophic forms, areas of keratosis are usually thin and dense: papillomatosis, although present, is not prominent, and sometimes even acanthosis is not significant [6]. (See Figure 6–10.)

6.3 Epidemiological Study of Dermatological Manifestations

The first large epidemiological study was carried out to ascertain the prevalence of arsenical skin lesions in a population of 40,421 out of a total population of 103,154 in 37 villages of southwest Taiwan where people had been drinking arsenic-contaminated artesian well water for more than 45 years. In the villages surveyed, the arsenic content of the well water examined ranged from 10 to 1820 μg/L. The overall prevalence rates for keratosis and hyperpigmentation were 7.10 and 18.35 per 100, respectively. The male to female ratio for both conditions was 1.1:1. The prevalence rates for hyperpigmentation increased steadily with age for males but for females a peak appeared at age group 50–59, followed by a gradual downturn in rates. The prevalence rates for keratosis for both sex increased up to age 70 and then declined. However, arsenic levels in drinking water were reported by villages in Taiwan. The youngest patient with hyperpigmentation in this study was aged 3 years and with keratosis, 4 years [19].

Another epidemiological study was carried out in the Región Lagunera, Mexico, where ground water in wells was found to be contaminated with arsenic ranging from 240 to 1000 μg/L. Arsenic was tested by use of chemical methodology. For this study, 296 exposed and 318 unexposed participants were selected from two towns having well water arsenic concentrations of 410 μg/L and 5 μg/L respectively. Every member of each family of both exposed and unexposed households of the respective towns were selected by systematic sampling beginning at a random point, every third house being surveyed. In the exposed population, 64 out of 296 (21.6%), while in the unexposed control population 7 out of 318 (2.2%), showed at least one of the signs of arsenical skin lesioning. The discovery of such lesioning in the control population did not match with observations of two earlier studies in Chile and Taiwan where there were no affected people in the control populations chosen. The authors did not find any sex difference in regard to the prevalence of either hyperpigmentation or keratosis. The shortest time of exposure, after which lesions were detected, was 8 years for hypopigmentation, 12 years for hyperpigmentation and palmoplantar keratosis, and 25 years for popular keratosis [8].

The first population-based survey with individual arsenic exposure data from drinking water sources was carried out in the district of South 24 Parganas, West Bengal, India, on 7683 participants (4093 female and 3590 male) out of a total population of 150,457 to assess the dose–response relationship and arsenical skin lesioning [12]. Water samples were collected from all recruited households of the participants and tested for the presence of arsenic by flow-injection hydride generation atomic absorption spectrophotometry. The tubewell arsenic concentration ranged from non-detectable to 3200 μg/L. The overall prevalence rates for keratosis and hyperpigmentation were 3.64 and 8.82 per 100, respectively. Prevalence of keratosis and hyperpigmentation was examined by water arsenic level. A clear relationship was apparent between water levels of arsenic and keratosis and hyperpigmentation. Age-adjusted prevalence of keratosis and hyperpigmentation was strongly related to water arsenic levels, rising from zero and 0.3 in the lowest exposure level (<50 μg/L), to 8.3 and 11.5 per 100 respectively for females drinking water containing >800 μg/L arsenic, and increasing from 0.2 and 0.4

per 100 in the lowest exposure category to 10.7 and 22.7 per 100 respectively for males in the highest exposure level (>800 μg/L). The prevalence of skin lesions was also examined by daily dose per body weight (μg/kg/day). This showed that men had roughly two to three times the prevalence of keratosis and pigmentation compared to women apparently ingesting the same dose of arsenic from drinking water. Subjects who were below 80% of the standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratosis, suggesting that malnutrition might play some role in increasing susceptibility. A nested case–control study was further carried out using detailed lifetime (at least 20 years) exposure assessment among the above-mentioned study population [31]. Lifetime water arsenic exposure data could be obtained in a total of 192 cases having definite evidence of arsenical skin lesions and 213 age- and sex-matched controls. The lowest peak level of arsenic ingested by a confirmed case was 115 μg/L. Strong dose–response gradients with both peak and average arsenic water concentrations were also observed [31].

The arsenic crisis in Bangladesh is another example of a severe case of environmental toxicity. Fifty out of 65 districts, involving nearly 85% of the total land area of Bangladesh, have arsenic present in ground water. It is suspected that about 25 million people are exposed to arsenic-contaminated ground water [32]. A cross-sectional study was conducted in Bangladesh in which arsenic-related skin lesions were assessed in 1481 subjects (903 males and 578 females) in four rural villages with arsenic exposure data (BDL [below the detectable limit] to 2040 μg/L) related to drinking water in take by the individuals [33]. A total of 430 subjects had skin lesions (keratosis, hyperpigmentation, or hypopigmentation). Individual exposure assessment could only be estimated by present levels and in terms of a dose index, i.e., arsenic levels divided by individual body weight. The crude overall prevalence rate for skin lesions was 29/100. After age adjustment to the world population, the prevalence rate was 30.1/100 for males and 26.5/100 for females. In the males in the lowest exposure category (<150 μg/L) the age-adjusted prevalence of any arsenic-associated skin lesion was 18.6/100 and increased to 37.0/100 in the highest exposure category (>1000 μg/L) (chi-square dose for trend $p < 0.001$). The corresponding rates for females were 17.9/100 in the lowest exposure category and 24.9 in the highest exposure category (test for trend, $p < 0.02$). There was a significant trend for the prevalence rate both in relation to exposure levels and to dose index ($p < 0.05$), regardless of sex. This study shows a higher prevalence rate of arsenic skin lesions in males than females, with a clear dose–response relationship [33]. To ascertain the degree of severity of skin lesions in the arsenic-affected population a cross-sectional study was carried out in an arsenic-affected district of West Bengal (Nadia). Out of 10,469 participants examined, 1616 (15.43%) people showed clinical features of arsenical skin lesions. Mean arsenic concentration in drinking water among the cases was 103.46 [standard deviation (SD) ± 153.28] μg/L while among the controls (participants without skin lesions) it was 73.18 (SD ± 115.10) μg/L. Duration of arsenic exposure of the former was 12.47 (SD ± 7.39) years while that of the later was 12.43 (SD ± 6.59) years. A scoring system was adopted to classify the degrees of severity of skin manifestations (see Table 6–1). Skin score was mild in 87.5%, while moderate in 11.5%, and severe in 0.87% of the people with arsenical skin disease. Out of a population of 0.84 million people suspected to be exposed to

Table 6–2 Clinical features of 156 Cases of Chronic Arsenicosis—Hospital-Based Study in West Bengal, India [11]

Symptoms	No. of Cases	%	Signs	No. of Cases	%
Weakness	110	70.5	Pigmentation	156	100.0
Headache	32	20.5	Keratosis	96	61.5
Burning of the eyes	69	44.2	Anemia	74	47.4
Nausea	17	10.9	Hepatomegaly	120	76.9
Pain in the abdomen	60	38.4	Splenomegaly	49	31.4
– epigastric	39	25.0	Ascites	5	3.0
– paraumbilical	21	13.4	Pedal edema	18	11.5
Diarrhea	51	32.6	Sign of lung disease	45	28.8
Cough	89	57.0	Sign of polyneuropathy	21	13.4
– with expectoration	53	33.9			
– without expectoration	36	23.1			
Hemoptysis	8	5.1			
Dyspnea	37	23.7			
Paresthesia	74	47.4			

arsenic-contaminated water in the district, 0.14 million people were estimated to be suffering from arsenical skin lesions [23].

6.3.1 Systemic Manifestations

Prolonged ingestion of arsenic-contaminated water is reported to produce not only skin manifestations (pigmentation and keratosis), but also many systemic features such as weakness, anaemia, chronic lung disease, conjunctival congestion, hepatomegaly and non-cirrhotic portal fibrosis, polyneuropathy, chronic diarrhea, dyspepsia, solid edema of limbs, and gangrene of toes. This will be evident from the report of a hospital-based study of the clinical features in 156 cases chronically drinking arsenic-contaminated water in West Bengal, India (Table 6–2) [11]. Further, it is to be noted that the frequency of occurrence of various systemic disease manifestations was found to be significantly higher in people exposed to arsenic but having no skin lesions when compared to those not exposed to arsenic [34].

The global health impact due to arsenic exposure was considered in an epidemiological study carried out in the district of Nadia, one of the severely arsenic-affected districts of West Bengal, India. Out of 10,469 arsenic-exposed participants examined, 1616 patients showed clinical features of arsenical skin lesions and various systemic disease while 8853 participants had features of systemic disease but no skin lesions. Chronic lung disease was found in 207 (12.81%) subjects with skin lesions and in 69 (0.78%) without skin lesions ($p < 0.001$). Peripheral neuropathy and abdominal pain were found in 257 (15.9%) and 67 (4.15%) subjects in the former group while in 136 (1.5%) and 67 (0.87%) subjects in the latter group, respectively ($p < 0.001$). Other systemic features found in significantly higher numbers of cases with skin

Table 6–3 Characteristics of Systemic Disease Manifestation in an Arsenic-exposed Population with and Without Skin Lesions Nadia District, West Bengal, India [23]

	Skin Lesions Present (n = 1616)		**Skin Lesions Absent (n = 8853)**		***p*-value**
Mean peak tubewell As concentration (μg/L)	103.4 ± 153.3		73.2 ± 115.1		<0.001
Duration of exposure to peak concentration (years)	12.5 ± 7.4		12.4 ± 6.6		<0.001
Age (mean + SD)	53.36 ± 15.60		33.74. ± 15.99		
	n	**%**	**n**	**%**	
Sex					
Male	934	57.80	3213	36.29	<0.001
Female	682	42.20	5640	63.71	NS
Arsenicosis skin score					
Mild (1–2 score)	1415	87.56			
Moderate (3–4 score)	187	11.57			
Severe (>4 score)	14	0.87			
Disease symptoms					
Lung disease	207	12.81	69	0.78	<0.001
Chronic cough	127	7.86	52	0.59	<0.001
Dyspnea	146	9.03	39	0.44	<0.001
Neuropathy (limb pain/tingling, numbness)	257	15.90	136	1.54	<0.001
Pain in abdomen	67	4.15	77	0.87	<0.001
Chronic diarrhea	19	*1.18*	15	*0.17*	<0.001
Liver—palpable	5	*0.31*	0	*0.00*	<0.001
Ascites	3	*0.19*	1	*0.01*	<0.05
Pallor (anemia)	1	*0.06*	7	*0.08*	>0.05
Non-pitting edema of limbs	4	*0.25*	2	*0.02*	<0.01

lesions compared to those without such lesions are chronic diarrhea, hepatomegaly, ascites, and non-pitting edema of the limbs (Table 6–3). Thus, systemic disease manifestations occur in arsenic-exposed people in a higher number of cases in whom skin lesions are present as opposed to those who do not have such lesions [23].

Individual disease manifestation associated with chronic arsenic exposure is presented below.

6.3.2 Respiratory Disease

Symptoms of lung disease were reported from Kolkata, West Bengal, in a study of 156 cases of chronic arsenic toxicity with arsenical skin lesions associated with drinking of arsenic-contaminated ground water. Symptoms of chronic cough with and without expectoration and chest signs of lung disease (crepitations and rhonchi) were reported in 89 (57%) and 45 (28.5%) cases, respectively. Lung function tests carried out on 17 patients showed features of

restrictive lung disease in nine (53%) and combined obstructive and restrictive lung disease in seven (41%) cases [11]. In another hospital-based study carried out in Kolkata on 107 arsenic-exposed (arsenic level in drinking water, mean ± SD: 550 ± 470 μg/L) and 52 control subjects (arsenic exposure <50 μg/L), 33 cases (30.8%), and four controls (7.6%) had symptoms of lung disease ($p < 0.01$). Lung function study showed obstructive, restrictive, and mixed obstructive and restrictive patterns in 20 (68.9%), 1 (3.7%), and 8 (27.6%) cases, respectively. Bronchiectasis was diagnosed in three cases on the basis of chest X-ray and HRCT (done on four cases) [35]. Pulmonary disease characteristic of bronchitis was also reported in 86 (23.7%) out of 360 people who were found to have arsenical skin lesions in a village in Bangladesh having an average level of arsenic in drinking water of 240 μg/L [22].

6.3.2.1 Epidemiological Study

Earlier, epidemiological studies carried out in Antafagosta, Chile, from survey data collected between 1968 and 1972 reported that the prevalence of cough among 398 children correlated with mean drinking-water arsenic concentration. In addition, the prevalence of reported cough declined from 38% to 7% following the installation of an arsenic removal plant in Antafagosta ($p < 0.001$) [36]. Further, the prevalence of bronchiectasis was found to be greater among children with arsenic-affected skin lesions living in Antafagosta compared to children living in the rest of Chile [36]. Autopsies were conducted on five children, with evidence of arsenical skin lesions on the body, who had died between 1968 and 1969 in Antafagosta. Lung tissue was examined in four of these children, with abnormalities being found in all four. Interstitial fibrosis was detected in two of the cases [37]. A cross-sectional survey was also carried out in Antafagosta in which 144 school children with arsenic-induced skin lesions were examined. Bronchopulmonary disease occurred 2.5 times more often in these children (15.9%) compared with children with normal skin (6.9%) [20].

A large cross-sectional epidemiological survey was carried out during 1998–1999 on 6864 participants (all non-smokers) in the district of South 24 Parganas, West Bengal, India, to ascertain the relationship between chronic arsenic exposure and occurrence of lung disease. The participants were clinically examined and interviewed, and the arsenic content of their current drinking water source was measured (arsenic concentration ranged from non-detectable to 3200 μg/L). Among males and females, the prevalence of cough, shortness of breath, and chest sounds (crepitation and/or rhonchi) in the lungs rose with increasing arsenic concentration in drinking water. These respiratory effects were most pronounced in individuals exposed to high levels of arsenic in drinking water who also had skin lesions. Prevalence odds ratio (POR) estimates were markedly increased for participants with arsenic-induced skin lesions who had high levels of arsenic in their drinking water (≥500 μg/L) compared with individuals who had normal skin and who were exposed to low levels of drinking-water arsenic (<50 μg/L). In participants with skin lesions, the age-adjusted POR estimates for cough were 7.8 for females (95% confidence interval (CI): 3.1–19.5) and 5.0 for males (95% CI: 2.6–9.9); for chest sounds, POR for females was 9.6 (95% CI: 4.0–22.9) and for males 6.9 (95% CI: 3.1–15.0). The POR for shortness of breath in females was 23.2 (95% CI: 5.8–92.8) and in males 3.7 (95%

CI: 1.3–10.6). These results add to the evidence that long-term ingestion of inorganic arsenic can have respiratory effects [38].

A prevalence comparison study of respiratory effects among subjects with and without arsenic exposure through drinking water was conducted in Bangladesh. Exposed participants were recruited through a health awareness campaign program. Unexposed participants were randomly selected, where tubewells were not contaminated with arsenic. A total of 218 individuals participated (94 exposed individuals exhibiting skin lesions; 124 unexposed individuals without skin lesions). The arsenic concentrations ranged from 136 to 1000 μg/L. Only non-smokers without any history of asthma or tuberculosis were recruited and data on respiratory symptoms and signs were collected. The crude prevalence ratios for chronic bronchitis were found to be 1.6 (95% CI: 0.8–3.1) and 10.3 (95% CI: 2.4–43.1) for males and females, respectively. These results also showed respiratory effects on long-term arsenic exposure [39].

A case–control study was carried out during 2000–2003 to ascertain the relationship between respiratory symptoms, lung function, and exposure to arsenic in drinking water in West Bengal among a cohort of 287 participants selected among a source population of 7683 subjects surveyed for arsenic-related skin lesions during 1995–1996. Cases were selected on the basis of their having skin lesions, and primary drinking water sources contained arsenic at levels of 50–500 μg/L, while controls were selected who did not have arsenic-related skin lesions and whose main tubewell water source contained an arsenic concentration of <50 μg/L. For each case, one control matched on age (within 5 years) and sex was randomly identified from all eligible non-cases. The study was confined to those participants of at least 20 years of age who completed the pulmonary function testing and for whom information on smoking was available. In this study 132 and 155 subjects fulfilled the criteria of cases and controls, respectively. Respiratory symptoms were increased in men with arsenic-caused skin lesions (versus those without lesions), particularly "shortness of breath at night" [odds ratio (OR) = 2.8, 95% CI: 1.1, 7.6] and "morning cough" (OR = 2.8, 95% CI: 1.2, 6.6) in smokers and "shortness of breath ever" (OR = 3.8, 95% CI: 0.7, 20.6) in non-smokers [40].

Pronounced decrements in lung function were observed in males with skin lesions (both non-smokers and smokers) as compared to those without skin lesions. Male smokers had, on average, lower mean residual values for spirometric parameters than male non-smokers. The decreases in FEV_1 and forced vital capacity (FVC) in male non-smokers with skin lesions as compared with non-smoking men without skin lesions were 157.3 mL (95% CI: −24.7, 339.2) for FEV_1 and 188.5 mL (95% CI: 0.6, 376.3) for FVC. In male smokers, the decreases were 271.1 mL (95% CI: 158.0, 384.2) for FEV_1 and 304.1 mL (95% CI: 180.1, 428.1) for FVC. Among women, the respective reductions were 63.2 mL (95% CI: −31.8, 158.2) for FEV_1 and 101.5 mL (95% CI: −8.8, 211.8) for FVC. Decreases in FEV_1 and FVC related to increased water arsenic concentration were observed among men; reduction in mean values from low exposure (arsenic level <100 μg/L) to high exposure (arsenic level ≥400 μg/L) were 194.7 mL (95% CI: 35.5, 353.9) for FEV_1 and 83.8 mL (95% CI: −93.8, 261.5) for FVC in non-smokers and 226.1 mL (95% CI: 45.2, 407.0) for FEV_1 and 247.6 mL (95% CI: 58.3, 436.9) for FVC in smokers. Among women,

the respective reductions were 28.5 mL (95% CI: −71.3, 128.2) for FEV_1 and 7.5 mL (95% CI: −122.4, 137.5) for FCV [40].

In the multivariate linear regression analyses stratified by sex and adjusted for age, height, and smoking, lung function was significantly decreased for signs of arsenic-related skin lesions among men, with a reduction in FEV_1 of 256.2 mL (95% CI: 113.9, 398.4, *p* value: <0.001) and in FVC of 287.8 mL (95% CI: 134.9, 440.8, *p* value: <0.001) . To further investigate the effects of ingested arsenic on flow, the FEV_1/FVC ratio and the forced expiratory flow between 25 and 75% of forced vital capacity ($FEF_{25–75}$) were investigated and significant reductions related to the presence of skin lesions consistent with the findings for FEV_1 and FVC in men were found. Reductions were also observed related to smoking in FEV_1 (156.4 mL; 95% CI: −3.2, 316.0, *p* value: 0.055) and FVC (119.7 mL: −52.0, 291.4, *p* value: 0.2) but the effect size was smaller than for presence of skin lesions. Using arsenic levels in water as a measure of exposure instead of skin lesions, significant decreases were found in FEV_1 of 45.0 mL (95% CI: 6.2, 83.9, *p* value: 0.02) and in FVC of 41.4 mL (95% CI: −0.7, 83.5, *p* value: 0.054) in men per 0.1 mg/L increase of arsenic. Potential confounders such as weight, type of house, education, and occupation were assessed in the multivariate models but did not change the estimates for skin lesions or arsenic in water. Interestingly, in women estimates for skin lesions or arsenic in water did not indicate a strong relation with lung function. In this study, consumption of arsenic-contaminated water was found to be associated with respiratory symptoms and reduced lung function in men, particularly among those with skin lesions [40].

To ascertain the incidence of bronchiectasis in the population, the data on 108 subjects with arsenic- caused skin lesions and 150 subjects without skin lesions from a population survey of over 7000 people of an endemic region of West Bengal carried out during 1995–1996 were analyzed. The median highest level of arsenic in drinking water was 330 μg/L [standard deviation (SD) ± 881 μg/L] in subjects with skin lesions compared with 28 μg/L [(SD) ± 147 μg/L] in those without such lesions.

Subsets of both the groups who reported chronic cough (more than 3 months per year for at least 2 years) were referred to a tertiary referral center in Kolkata for high resolution computed tomography (HRCT). The severity of bronchiectasis in each lobe was ranked on a scale of 0 to 4. Briefly, a five-point grading system was employed: 0 = no bronchiectasis; 1 = mild bronchiectasis (non-tapering cylindrical internal bronchial diameter 1.5–3 times the diameter of the accompanying artery); 2 = moderate bronchiectasis (non-tapering cylindrical internal bronchial diameter more than 3 times the diameter of the accompanying artery); 3 = varicose bronchiectasis; and 4 = cystic bronchiectasis. The lungs were divided into six lobes (considering the lingual as a separate lobe) and a score was assigned to each lobe. A single bronchiectasis severity score was assigned to each subject by summing the bronchiectasis scores from each lobe of each lung.

Thirty-three (31%) subjects with skin lesions and 18 (12%) subjects without lesions had reported chronic cough for more than 2 years (OR = 3.2; CI = 1.7–6.1). Of these, 27 subjects with skin lesions and 11 without lesions agreed to travel to Kolkata for HRCT. Overall, the participation rate was 82% in subjects with skin lesions and 61% in subjects without skin lesions. For those subjects who underwent HRCT, the average bronchiectasis severity score was 3.4 (SD ± 3.6) in 27 subjects with skin lesions and 0.9 (SD ± 1.6) in 11 subjects without lesions.

In subjects who reported chronic cough, CT evidence of bronchiectasis was found in 18 (67%) participants with skin lesions and 3 (27%) subjects without skin lesions. Only one (9%) of the 11 subjects without skin lesions had a bronchiectasis severity score greater than 2, while 14 (52%) of the 27 skin lesion subjects had bronchiectasis severity scores greater than 2.

The unadjusted odds ratio for bronchiectasis was 10 (95% CI = 2.9–35) in subjects with arsenic-caused skin lesions compared with subjects having no lesions. The corresponding adjusted odds ratio was 10 (2.7–37). The adjusted odds ratio was 13 (2.6–62) in men and 6.1 (0.6–62) in women [41].

This study was the first investigation of HRCT findings in a population-based study of people exposed to high levels of arsenic in drinking water. In this study, the authors found that persons with arsenic-caused skin lesions have a 10-fold higher rate of bronchiectasis on HRCT. Given the large magnitude of the relative risk estimate they identified, this association is not likely to be due to chance. The highly characteristic skin lesions diagnosed in this study are known to result from the consumption of arsenic-contaminated drinking water. The findings therefore provide evidence that long-term ingestion of arsenic results in increased risks of non-malignant pulmonary disease, in particular, bronchiectasis [41].

6.3.2.2 Mortality Studies

In a blackfoot disease-prone area of Taiwan (arsenic in drinking water: median = 780 μg/L) an ecological study of mortality showed that the standardized mortality ratio (SMR) for "bronchitis" increased significantly relative to a nearby reference population (SMR = 1.53; 95% CI = 1.30–1.80) during the period from 1971 to 1994. The authors observed that it was unlikely that the differences in the rate of smoking account for the increased bronchitis mortality [42].

Further, a retrospective study on mortality due to bronchiectasis, carried out (between 1988 and 2000) in Antafagosta, Chile, suggested that exposure of arsenic in drinking water during early childhood or *in utero* has a pronounced pulmonary effect, greatly increasing subsequent mortality in young adults from the condition. For the birth cohort born just before the high exposure period (1950–1957) and exposed in early childhood, the SMR for bronchiectasis was 12.4 (95% CI: 3.3–31.7; $p < 0.001$) while for those born during the high exposure period (1958–1970) with probable exposure *in utero* and early childhood, the corresponding SMR was 46.2 (95% CI: 21.1–87.7; $p < 0.001$) [43].

6.3.3 Gastrointestinal Disease

Symptoms of dyspepsia were observed in 60 out of 156 (38.4%) cases of chronic arsenic toxicity studied in West Bengal [11]. However, in an epidemiological study carried out in the affected population there was no difference in the incidence of abdominal pain among people drinking arsenic-contaminated water and the control population (27.84% vs. 31.81%) [44]. Gastroenteritis was reported in a study of 1447 cases of chronic arsenicosis caused by drinking arsenic-contaminated water (0.05–1.8 mg/L) in the Inner Mongolian autonomous region of China [45]. Many investigators variously reported symptoms like nausea, diarrhea, anorexia, and abdominal pain in cases of chronic arsenic toxicity [1,8,17,20,21].

6.3.4 Liver Disease

Exposure of inorganic arsenic compounds has been found to be associated with the development of chronic pathological changes in the liver. Several authors reported cases of liver damage following treatment of patients with arsenic as Fowler's solution [46,47]. All these patients developed features of portal hypertension with signs of liver fibrosis. Typical cutaneous signs of long-term arsenic exposure were also observed in some of the patients. There have also been case reports on liver cirrhosis following medication with inorganic arsenic compounds [37,48].

Portal hypertension associated with periportal fibrosis was reported in nine patients who were found to have high arsenic levels in their liver in Chandigarh, India. Two of those patients had been found to be drinking arsenic-contaminated water (549 and 360 μg/L) [49]. From a population-based study in West Bengal, hepatomegaly was reported in 62 out of 67 members of families who drank arsenic-contaminated water (200–2000 μg/L), while only in six out of 96 people who took safe water (<50 μg/L) from the same area. Thirteen arsenic-exposed patients who had hepatomegaly were further investigated in a hospital in Kolkata. All showed various degrees of portal zone expansion and fibrosis on liver histology. Four of the five patients who had splenomegaly showed evidence of increased intrasplenic pressure (30–36 cm saline) suggesting portal hypertension. Splenoportography done in those cases showed evidence of intrahepatic portal vein obstruction. Although routine liver function tests were normal in all these cases, the bromsulphthalein retention test done in three patients was abnormal. The arsenic level in liver tissue (estimated by neutron activation analysis) was found to be elevated in 10 out of those 13 cases (As levels: Cases 0.5 to 6 mg/kg; control 0.10 ± 0.04 mg/kg) [1]. In another study from West Bengal, hepatomegaly was found in 190 out of 248 cases of chronic arsenicosis investigated in the same hospital. Arsenic levels in drinking water varied from 50 to 3200 μg/L. Evidence of portal zone fibrosis on liver histology was found in 63 out of 69 cases of hepatomegaly. Liver functions tests carried out on 93 such patients showed evidence of elevation of ALT, AST, and ALP in 25.8%, 6.3%, and 29% of cases, respectively. Serum globulin was found to be high (>3.5 gm/dL) in 19 (20.7%) cases [50].

Liver function tests were studied in arsenic-exposed subjects in three towns of Leguneara, Mexico. Significant elevation of bilirubin and alkaline phosphatase levels was observed among people living in the town having the highest (239 ± 88 μg/L) arsenic exposure category compared to those living in the lowest (14 ± 3.1 μg/L) exposure group. Serum transaminases and albumin level were, however, normal in all the groups [51].

A cross-sectional epidemiological study was carried out on 7683 people in an arsenic-affected district of West Bengal. Out of these, 3467 and 4216 people consumed water contaminated with arsenic below and above 50 μg/L, respectively. Prevalence of hepatomegaly was significantly higher in arsenic-exposed people (10.2%) compared to controls (2.99%, $p < 0.001$). The incidence of hepatomegaly was found to have a linear relationship proportional to increasing exposure of arsenic in drinking water in both sexes ($p < 0.001$) [44].

Liver enlargement was also reported by other workers in people drinking arsenic-contaminated water in, respectively, Bangladesh, Inner Mongolia, and West Bengal [22,45,52].

6.3.4.1 *Experimental Model for Liver Fibrosis*

In an experimental study, BALB/C mice were given water contaminated with arsenic (3.2 mg/L) *ad libitum* for 15 months, the animals being sacrificed at 3-month intervals. In the experimental animals, progressive reduction of hepatic glutathione and enzymes of an antioxidative defense system were found to be associated with lipid peroxidation. Liver histology showed fatty infiltration at 12 months and hepatic fibrosis at 15 months [53]. In another experimental study, increasing the dose and duration of arsenic exposure in mice was found to cause progressive increase of oxy-stress associated with elevation of cytokines, interleukin (IL)-6, and tumor necrosis factor (TNF)-α with increasing collagen content and arsenic levels in liver [54].

All these studies show that prolonged drinking of arsenic-contaminated water is associated with hepatomegaly. The predominant lesion of hepatic fibrosis appears to be caused by arsenic-induced oxy-stress.

6.3.5 Cardiovascular Disease

Blackfoot disease (BFD), a form of peripheral vascular disease, has been reported to be one of the important complications of chronic arsenic toxicity in Taiwan. It is a unique peripheral arterial disease characterized by the severe systemic arteriosclerosis as well as dry gangrene and spontaneous amputations of affected extremities at end stages. Histologically, BFD can be divided into two reaction groups: arteriosclerosis obliterans and thromboangiitis obliterans, particularly affecting small vessels [55]. Clinically, the disease begins with patients' subjective complaints of coldness or numbness in the extremities (usually in the feet) and intermittent claudication, progressing over the course of several years to ulceration, gangrene, and spontaneous amputation [56]. The prevalence of BFD has been reported to be 8.9 per 1000 among 40,421 inhabitants studied in Taiwan [19]. Comparable peripheral vascular disorders with varying degrees of severity including Raynaud's syndrome and acrocyanosis have also been reported by other researchers, among people drinking arsenic-contaminated water [11,20,22,37,45,57]. It needs to be emphasized that there are differences in the prevalence of peripheral vascular disease causing gangrene and amputation among different populations exposed to arsenic, the incidence being high in Taiwan, while low in Chile, India, and Bangladesh while there is no report from Mexico and Argentina [58].

An epidemiological study reported an increased prevalence of hypertension among residents in the endemic area of BFD and a dose–response relationship between ingested inorganic arsenic and prevalence of hypertension [59]. The investigators studied a total of 382 men and 516 women residing in arsenic hyperendemic areas in Taiwan. They observed 1.5-fold increases in age- and sex-adjusted prevalence of hypertension compared with residents in non-endemic areas. The higher the cumulative arsenic exposure, the higher was the prevalence of hypertension. The dose–response relationship remained significant after adjustment for age, sex, diabetes mellitus, proteinuria, body mass index, and serum triglyceride level. Increased prevalence of hypertension was also reported in 6.2% patients affected with arsenic-induced skin lesions compared to none in those without skin lesions in Antafagesta, Chile [20]. Association of cumulative arsenic exposure

in drinking water was also found to be associated with increased risk of hypertension in a study of 1595 people in Bangladesh [60]. Further study also showed evidence of increased association of hypertension in individuals resident in arsenic endemic regions compared to those from a non-endemic region in West Bengal, India. There were increased odds ratios for hypertension [adjusted OR, 2.87 (95%, CI = 1.26–4.83)] in participants with arsenic exposure compared to participants living in arsenic non-endemic regions. There was a dose–effect relationship seen with increasing cumulative arsenic exposure, with higher arsenic levels in hair and an increased incidence of hypertension in participants living in arsenic endemic regions [61].

Mortality rates from ischemic heart disease (IHD) with endemic arsenicosis (from 1973 through 1986) were correlated with arsenic levels in drinking water among residents of 60 villages in Taiwan [62]. Based on 1,355,915 person-years and 217 IHD deaths, the cumulative IHD mortalities from birth to age 79 years were 3.4%, 3.5%, 4.7%, and 6.6%, respectively, for residents who lived in villages in which the median arsenic concentrations in drinking water were <0.1, 0.1 to 0.34, 0.35 to 0.59, and ≥0.6 mg/L. A cohort of 263 patients affected with BFD and 2293 non-BFD residents in the endemic area of arsenicosis were recruited and followed up for an average period of 5.0 years. There was a monotonous biological gradient relationship between cumulative arsenic exposure through drinking artesian well water and IHD mortality. The relative risks were 2.5, 4.0, and 6.5, respectively, for those who had a cumulative arsenic exposure of 0.1 to 9.9, 10.0 to 19.9, and ≥20.0 mg/L-years compared to those without the arsenic exposure after adjustment for age, sex, cigarette smoking, body mass index, serum cholesterol and triglyceride levels, and disease status for hypertension and diabetes through proportional hazards regression analysis [63].

A significant biological gradient between the risk of IHD and the duration of consuming high arsenic artesian well water was reported from Taiwan. Further, a community-based health survey in an arsenic endemic region was carried out to determine whether there was any association between arsenic-related IHD and serum antioxidant micronutrient levels. A total of 74 patients affected with IHD, who were diagnosed through both electrocardiography and Rose questionnaire interview, and 193 age- and sex-matched healthy controls, were selected for the examination of serum levels of micronutrients by high performance liquid chromatography (HPLC). A significant reverse dose–response relationship with arsenic-related IHD was observed for serum levels of alpha- and beta-carotene, but not for serum levels of retinol, lycopene, and alpha-tocopherol. Multivariate analysis showed a synergistic interaction on arsenic-related IHD between duration of consumption of artesian well water and low serum carotene levels. An increased risk of arsenic-related IHD was also associated with hypertension and elevated body mass index, but not with serum lipid profile, cigarette smoking, and alcohol drinking. The authors suggested that arsenic-related IHD has a pathogenic mechanism, which is at least partially different from that of IHD unrelated to long-term exposure to arsenic [64].

Whether long-term arsenic exposure could be associated with IHD was ascertained in a community-based study in the blackfoot disease-endemic villages of Taiwan. A total of 462 subjects characterized by long-term arsenic exposure from drinking artesian well water were studied. IHD was diagnosed by coding the resting electrocardiograms with the Minnesota code. History of arsenic exposure was estimated through information obtained from a

personal interview according to a structured questionnaire and the arsenic content in artesian well water of the villages. Cumulative arsenic exposure (CAE) was calculated as the sum of the products multiplying the arsenic concentration in artesian well water (mg/L) by the duration of drinking the water (years) in consecutive periods of living in the different villages. Among the subjects, 78 cases (16.9%) were diagnosed as having IHD. The prevalence rates of IHD for the age groups of 30–39, 40–49, 50–59, and ≥60 years were 4.9%, 7.5%, 16.8%, and 30.7%, respectively ($p < 0.001$). For those with a CAE of 0, 0.1–14.9, and ≥15 mg/L-years, the prevalence rates of IHD were 5.2%, 10.9%, and 24.1%, respectively ($p < 0.001$). The odds ratios (95% confidence intervals) for IHD were 1.60 (0.48, 5.34) and 3.60 (1.11, 11.65), respectively, for those with a CAE of 0.1–14.9 and ≥15.0 mg/L-years, when compared with those lacking drinking water exposure to arsenic after multivariate adjustment. The authors concluded that IHD is associated with long-term arsenic exposure. However, the limitation of the paper was that cumulative arsenic exposure was measured on the basis of group level by village. Further prospective studies with individual arsenic data and in other geographic regions are needed to establish the cause–effect relationship [65].

Increased prevalence of microvascular diseases, including neurological and renal disorders, were reported to be associated with arsenic ingestion. Microvascular diseases in relation to arsenic exposure level have been studied in a total of 28,499 subjects living in the study area of Taiwan, from information obtained through their medical records from the National Health Insurance database in 1999–2000. The arsenic concentrations of artesian well water in the villages of the study area were utilized as indices of previous ingestion level. Both stratified analysis and unconditional logistic regression were used to examine mainly neurological and renal disease in relation to arsenic exposure taking into account diabetes status. The age-adjusted and gender-adjusted prevalence of microvascular diseases was 7.51% (95% CI: 7.50–7.51) for an arsenic level of 100 μg/L, and then increased from 6.59% (6.59–6.60) for arsenic concentrations of 100–290 μg/L to 8.02% (8.02–8.03) and 11.82% (11.81–11.83) for those of 300–590 μg/L mg/L and ≥600 μg/L mg/L in non-diabetic subjects. For diabetic patients, the prevalence was 16.41% (95% CI: 16.37–16.45), 15.85% (15.8–15.9), 21.69% (21.6–21.8), and 28.31% (28.2–28.4) for arsenic levels of <100, 100–0.290, 300–590, and ≥600 μg/L, respectively. The prevalence of microvascular diseases increased significantly with arsenic exposure, especially at higher levels, and the relationship is stronger in diabetics than in non-diabetic subjects. The results for neurological disease are very similar, and the patterns are the same for renal disease [66]. In the report, the authors acknowledged that the precise level of arsenic exposure at the individual level, as a consequence of well-water consumption, was rather difficult to assess without individual lifetime histories. The consequences of exposure misclassification might have some limitation in the observed relationship of arsenic exposure and microvascular disease.

6.3.6 Diseases of the Nervous System

There are many reports of the occurrence of peripheral neuropathy due to chronic exposure of arsenic through drinking water [8,9,22,45,67,68]. Peripheral neuritis characterized

by paresthesia (tingling, numbness, limb weakness, etc.) was present in 74 (47.4%) out of 156 patients with chronic arsenicosis due to drinking of arsenic-contaminated water (0.5–14.2 mg/L) in West Bengal, India. Objective evaluation of neuronal involvement, done in 29 patients, showed abnormal electromyography (EMG) in 10 (30.8%) and altered nerve conduction velocity and EMG in 11 (38%) cases [69]. Abnormal EMG findings, suggestive mostly of sensory neuropathy, were reported in 10 out of 32 subjects drinking arsenic-contaminated well water (range 0.06–1.4 mg/L) in Canada [70]. In another electrophysiological study carried out on 88 patients of arsenicosis in West Bengal, sensory neuropathy was found in 24 (27.3%), motor neuropathy in 13 (14.7%), and abnormal EMG in 5 (5.7%) cases [71].

A cross-sectional study of nerve conduction velocity was carried out in Taiwan on 130 students aged 12–24 years exposed to arsenic from drinking arsenic-contaminated water. After adjustment of gender and height, a significant odds ratio of 2.9 (95% CI: 1.1–7.5) was observed for development of slow conduction velocity of sural sensory action potential (SAP) among the study subjects with cumulative arsenic exposure of >100 mg. The authors concluded that chronic arsenic exposure might induce peripheral neuropathy [72].

Increased prevalence of peripheral neuritis was reported in a cross-sectional study from an arsenic endemic district (Nadia) of West Bengal. Out of a population of 10,469 arsenic-exposed participants selected from all 17 affected blocks of the district by multistage sampling, 1616 (15.43%) cases had arsenical skin lesions while 8853 control subjects had no skin lesions. Mean arsenic content in drinking water among the cases was 103.46 (SD ± 153.28) μg/L while among the controls it was 73.18 (SD ± 115.10) μg/L. Peripheral neuropathy characterized by tingling, numbness, and limb pain was found in 257 (15.90%) cases and in 136 (1.54%) controls ($p < 0.001$) [23].

Peripheral neuritis, sleep disturbances, weakness, and cognitive and memory impairment have been reported in residents of Byan College Station, Texas, exposed to arsenic trioxide in air and water used to produce defoliants from an Atochem plant [68]. Headache has been reported to occur in people drinking arsenic-contaminated water in Mexico [8] and in West Bengal [11]. Irritability, lack of concentration, depression, sleep disorders, headache, and vertigo were reported in people with arsenicosis showing features of neuropathy in West Bengal [71].

Increased prevalence of cerebrovascular disease and ingestion of inorganic arsenic in drinking water was reported in a cross-sectional study in Taiwan. A total of 8102 men and women from 3901 households were recruited in this study. The status of cerebrovascular disease of study subjects was identified through home visit, personal interviews, and by review of hospital medical records according to WHO criteria. Information on consumption of well water, sociodemographic characteristics, cigarette smoking, and alcohol consumption habits, as well as personal and family history of disease, was also obtained. A significant dose–response relationship was observed between arsenic concentration in well water and prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The biological gradient was more prominent for cerebral infarction, showing multivariate-adjusted odds ratios of 1.0, 3.4, 4.5,

and 6.9, respectively, for those who consumed well water with an arsenic content of 0, 0.1 to 50.0, 50.1 to 299.9, and >300 μg/L [73]. However, there were limitations in the methodology described in the study. Subjects were recruited from a registry of all adult residents within 18 villages, but criteria for village selection, methods of recruitment, and percentage participation were not stated.

Other studies from the region had not produced similar findings. Increased mortality was not observed due to cerebrovascular accidents when mortality and population data were obtained from the local household registration offices and Taiwan Provincial Department of Health during 1973–1986 and correlated with arsenic levels in well water determined in 1964–1966 in 42 study villages in Taiwan [74].

However, a recent study from Taiwan again showed that exposure to arsenic in drinking water was associated with a higher risk of cerebrovascular disease (CVD). A study of (CVD) mortality was carried out with the national death registry data from two areas in Taiwan from 1971 to 2005. The arsenic levels in the drinking water in the BFD area were generally higher than those in the unexposed area. SMRs adjusted for gender and age were calculated using the whole population in Taiwan and the population in the unexposed area as the reference population, respectively. The SMR of CVD in Taiwan decreased from 2.46/100 in 1971 to 0.63/100 in 2005. The exposed group had higher SMRs of CVD in comparison with the reference populations, with SMRs from 1.06 to 1.09 in men and 1.12 to 1.14 in women. The BFD endemic area had higher mortality rates of CVD than the unexposed area, with SMR = 1.05 in men and SMR = 1.04 in women [75].

Recently, an increased mortality was reported due to CVD with low dose range of arsenic in ground water in the USA. An SMR analysis was conducted in a contiguous six county study area of southeastern Michigan to investigate the relationship between moderate arsenic levels and several selected disease outcomes. Arsenic data were compiled from 9251 well water samples tested by the Michigan Department of Environmental Quality from 1983 through 2002. The six county study area had a population-weighted mean arsenic concentration of 11.00 μg/L and a population-weighted median of 7.58 μg/L. Michigan Resident Death Files data were amassed for 1979 through 1997 and sex-specific SMR analyses were conducted with indirect adjustment for age and race; 99% CI were reported. Elevated mortality rates were observed for both males (M) and females (F) for cerebrovascular disease (M SMR, 1.19; CI: 1.14–1.25; F SMR, 1.19; CI: 1.15–1.23) [76].

6.3.7 Hematological Effects

Hematological abnormalities have been reported in acute and chronic arsenic poisoning [15]. A characteristic pattern of anemia, leukopenia, and thrombocytopenia was found in 55 individuals exposed to arsenic in drinking water in Niigata Prefecture in Japan for approximately 5 years, half of the subjects having arsenical skin lesions [77]. In one study in West Bengal, anemia was reported in all the 13 people exposed to arsenic-contaminated groundwater (0.2–2 mg/L) [1]. A further study in West Bengal on 156 people exposed to

arsenic-contaminated water (0.05–14.2 mg/L) showed the incidence of anemia in 47.4% of cases [11]. However, no association of anemia was found in people drinking well water (mean 0.22 mg/L) in Alaska [3] and in two towns in Utah, USA (arsenic exposure 0.18 and 0.27 mg/L) [78].

6.3.8 Diabetes

The association of diabetes mellitus with chronic arsenic exposure has been variably reported. A dose–response relation between cumulative arsenic exposure and prevalence of diabetes mellitus was observed in Taiwan following a study on 891 persons living in arsenic endemic areas (arsenic levels in artesian well water, 700 to 930 μg/L). Cumulative lifetime ingestion of arsenic in drinking water could be determined for 718 subjects aged over 30 years. The status of diabetes mellitus was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. The researchers observed a dose–response relation between cumulative arsenic exposure and prevalence of diabetes mellitus. The relation remained significant after adjustment for age, sex, body mass index, and activity level at work using a multiple logistic regression analysis giving a multivariate adjusted odds ratio of 6.61 (95% CI: 0.86–51.0) and 10.05 (95% CI: 1.30–77.9), respectively, for those who had a cumulative arsenic exposure of 0.1–15.0 mg/L-year and greater than 15.0 mg/L-year compared with those who were unexposed. However, the study had a few limitations, one being the prevalence of diabetes observed in 108 unexposed subjects as only 0.9%, much lower than the 5.1% background prevalence cited by the authors [79].

From Bangladesh a significantly increased prevalence of diabetes mellitus was reported due to drinking arsenic-contaminated water among 163 subjects with arsenical keratosis living in seven villages having groundwater contamination when compared with the prevalence of diabetes mellitus among 854 subjects recruited from an arsenic-uncontaminated region. Diagnosis of diabetes mellitus was made from history of symptoms, previously diagnosed disease, glycosuria, and blood glucose after oral glucose challenge. Using control population's age- and sex-adjusted prevalence as a reference (1.0), the prevalence ratios for diabetes mellitus were calculated to be 2.6, 3.9, and 8.8 for time-weighted average exposure categories of less than 500 μg/L, 500–1000 μg/L, and greater than 1000 μg/L respectively [80]. The lack of a comprehensive, systematic, long-term sampling of the water supplies in the study area is a limitation of the study because directly measured individual exposure data over time would have been desirable. However, these results suggest that chronic arsenic exposure may induce diabetes mellitus in humans.

High mortality attributed primarily to diabetes mellitus was reported from arsenic-contaminated water (arsenic levels in artesian well water, 250–1400 μg/L) in four townships in Taiwan from the early 1900s until the mid- to late 1970s. Observed mortality between 1971 and 1994 was compared with age- and sex-specific expected mortality based on data from (1) a local reference group similar to a study group derived from nearby counties and (2) all of Taiwan [42].

A prospective cohort follow-up study was carried out on 446 subjects who agreed to participate in investigation for detection of diabetes mellitus by estimating fasting blood sugar and

glucose tolerance test in 1991 and 1993. The participants were drawn from 632 non-diabetic subjects, enrolled in 1970 from three villages in Taiwan having a history of arsenic exposure (700–930 μg/L) from artesian well water. The incidence of diabetes mellitus in the study population was calculated as the total number of incident cases divided by the sum of follow-up person-time in all subjects. Data on each subject included age, sex, body mass index, and index of lifetime CAE. CAE (in units of milligrams per liter-years) was calculated as the product of median arsenic concentration of the well water in every village that a subject inhabited at some point in his or her life multiplied by the length of time they consumed well water in that village. Incidences for diabetes mellitus in the study population were compared with those reported for a demographically similar population that was studied contemporaneously. During the follow-up period, which included 1499.5 person-years, 41 out of 446 subjects developed diabetes mellitus (all non-insulin-dependent diabetes mellitus). The incidence for new cases was particularly increased in subjects 55 years of age or older (50.8 per 1000 person-years). The relative risk for developing diabetes mellitus among those more than 17 mg/L-years CAE compared with those with less than 17 mg/L CAE was 2.1(995% CI: 1.1–4.2), adjusted for age, sex, and body mass index in a multivariate Cox proportional hazard model. When considered as a continuous variable, the CAE was associated with an adjusted relative risk of developing diabetes mellitus of 1.03 for every 1 mg/L-year of exposure ($p < 0.05$) [81].

Though earlier reports from Taiwan and Bangladesh showed an association between the development of diabetes mellitus and exposure to relatively high concentrations (>500 μg/L) of arsenic in drinking water, the risk of diabetes following exposure to lower levels of As (<300 μg/L) has not been conclusively demonstrated. A population-based cross-sectional study was done on 11,319 participants using baseline data in the Health Effects of Arsenic Longitudinal Study in Araihazar, Bangladesh, to evaluate the associations of well water arsenic and total urinary arsenic concentration and the prevalence of diabetes mellitus and glycosuria. The investigators also assessed the concentrations of well water arsenic, total urinary arsenic, and urinary arsenic metabolites in relation to blood glycosylated hemoglobin (HbA1c) levels in subsets of the study population. More than 90% of the cohort members were exposed to <300 μg/L of arsenic in drinking water. The authors reported no association between arsenic exposure and the prevalence of diabetes and no association between arsenic exposure and prevalence of glycosuria. The adjusted odds ratios for diabetes were 1.00 (referent), 1.35 (95% CI: 0.90–2.02), 1.24 (0.82–1.87), 0.96 (0.62–1.49), and 1.11 (0.73–1.69) in relation to quintiles of time-weighted water arsenic concentrations of 0.1–8, 8–41, 41–91, 92–176, and ≥177 μg/L, respectively, and 1.00 (referent), 1.29 (0.87–1.91), 1.05 (0.69–1.59), 0.94 (0.61–1.44), and 0.93 (0.59–1.45) in relation to quintiles of urinary arsenic concentrations of 1–36, 37–66, 67–114, 115–204, and ≥205 μg/L, respectively. No association between arsenic exposure and prevalence of glycosuria and no evidence of an association between well water arsenic, total urinary arsenic, or the composition of urinary arsenic metabolites and HbA1c level were observed in the study. The study population in this report was generally lean and was of low socioeconomic and nutritional status. The findings, therefore, may not be generalized to other study populations, given the possible different distribution of risk factors for diabetes that may influence the effect of arsenic exposure [82].

6.3.9 Eye Disease

6.3.9.1 Conjunctivitis

Symptoms of eye disease are not infrequently found to be associated with chronic arsenic exposure. In a hospital-based study in West Bengal, eye symptoms characterized by burning of the eye and conjunctival congestion were found in 69 (42.2%) out of 156 patients with chronic arsenicosis associated with arsenical skin lesions and a history of prolonged drinking of arsenic-contaminated water [11]. Conjunctivitis associated with chronic arsenic ingestion had also been reported from Bangladesh in 57 (15.7%) out of 363 arsenic-exposed subjects [22]. Increased incidence of conjunctivitis was reported from a study of 1482 arsenicosis patients living in six of 496 upazilas (subdistricts) of Bangladesh, who were identified through household screening. The investigators observed increased duration of arsenic-exposure symptoms and older age to be significantly associated with increased occurrence of conjunctivitis [83]. Papillary conjunctivitis was reported in two members of a family with dermatological manifestations and a history of drinking arsenic-contaminated water for 15 years in Bangladesh. Levels of arsenic in the nails and hair of both patients were very high. Histopathological examination of conjunctival tissue confirmed the inflammatory response of a papillary type; however, an arsenic estimation in conjunctival tissue was not possible. There were no inclusion bodies in conjunctival smears stained with hematoxylin and eosin (H&E) stain. There was no response to the usual treatment for papillary conjunctivitis, which only subsided along with a regression of dermatological changes, when patients were treated with the chelating agent dimercaprol, and multivitamin preparations, as well as no longer drinking the contaminated water [84].

6.3.9.2 Pterygium

Chronic exposure to arsenic in drinking water was reported to be associated with the occurrence of pterygium in Taiwan. Pterygium is a fibrovascular growth of the bulbar conjunctiva and underlying subconjunctival tissue that may cause blindness. Eye examination and a questionnaire interview of 223 participants from three exposure villages and 160 participants from four comparison villages were carried out and photographs taken. Subsequent grading of pterygium status was done by an ophthalmologist. After adjusting for age, sex, working under sunlight, and working in sandy environments, the authors found that cumulative arsenic exposure of 0.1–15.0 mg/L-year and ≥15.1 mg/L-year was associated with increased risks of developing pterygium. The adjusted odds ratios were 2.04 (95% CI: 1.04–3.99) and 2.88 (95% CI: 1.42–5.83), respectively [85].

6.3.9.3 Cataract

Increasing prevalence of posterior subcapsular cataract with an increase in exposure to ingested arsenic was reported from Taiwan. The study was carried out on a total of 349 residents living in arseniasis hyperendemic villages of southwestern Taiwan with recording of cumulative arsenic exposure and determination of different types of lental opacity. The cataract surgery prevalence was 10% for the age group of 50 or more years. Cortical opacity was most common (35%), while nuclear and posterior subcapsular opacities were observed in 24%

and 22% of subjects, respectively. Diabetes mellitus was a significant risk factor for all types of cataract. Occupational sunlight exposure was associated with cortical and posterior subcapsular opacities in a dose–response relationship. The cumulative exposure to arsenic from artesian well water and the duration of consuming artesian well water were associated with an increased risk of all types of lens opacity. But statistically significant dose–response relations with the cumulative arsenic exposure and the duration of consuming artesian well water were observed only for posterior subcapsular opacity ($p = 0.014$ and $p = 0.023$, respectively) after adjustment for age, sex, diabetes status, and occupational sunlight exposure [86].

6.3.10 Non-Pitting Edema of Limbs

A study carried out in Mexico showed that edema of the legs occurred at a higher frequency (18 out of 296; 6.1% of arsenic-exposure cases through drinking water (>50 μg/L)) compared to unexposed controls (four out of 318; 1.3%, $p < 0.01$) [8]. However, whether the edema was pitting or not was not mentioned in the paper. Non-pitting edema of the legs was first reported in 23 (9.3%) cases out of 248 patients with chronic arsenicosis associated with arsenical skin lesions and a history of prolonged drinking of arsenic-contaminated water (50–14,200 μg/L) in West Bengal, India, in a hospital-based study [50,87]. Non-pitting edema of the limbs has also been reported in 10 (2.5%) cases out of 363 cases of arsenicosis in Bangladesh with a history of drinking arsenic-contaminated water (82–1371 μg/L) [22]. Further, an increased incidence of non-pitting edema of the limbs (hand and/or leg) was found in a population-based cross-sectional study carried out in all the arsenic-affected 17 blocks of the Nadia district of West Bengal. Non-pitting edema of the limbs was found in four out of 1616 (0.25%) cases (arsenic level in drinking water: mean, 103.46; SD ± 153.28 μg/L) with arsenical skin lesions while in two out of 8853 (0.02%) control subjects (arsenic level in drinking water: mean,73.18; SD ± 115.10 μg/L) without skin lesions ($p < 0.01$) [23].

6.3.11 Miscellaneous

6.3.11.1 Weakness

Many reports are available where weakness and fatigue were described as occurring in people exposed to arsenic from the chronic drinking of arsenic-contaminated water [1,11,17,26,88]. Prevalence of weakness was studied in a large cross-sectional survey on 6864 arsenic-exposed (arsenic in drinking water, 0.3–3400 μg/L) participants in West Bengal, India. The age-adjusted prevalence of weakness increased strongly with arsenic concentrations (lowest, <50 μg/L and highest, ≥800 μg/L) in both sexes (from 1.7 to 11.9 per 100 among women, $p < 0.0001$, and from 0.9 to 9.5 per 100 among men, $p < 0.0001$) [38].

6.3.11.2 Erectile Dysfunction

A report from Taiwan suggested that chronic arsenic exposure has a negative impact on erectile function. A study had been conducted on 177 males ≥50 years of age through health examinations conducted in three hospitals in Taiwan through assessment of risk factors associated with erectile dysfunction (ED), such as aging, sex hormone levels, hypertension,

cardiovascular diseases, and diabetes mellitus. The investigators used a questionnaire (International Index of Erectile Function-5) to measure the level of erectile function and determined sex hormones, including total testosterone and sex hormone-binding globulin radioimmunoassay. Another standardized questionnaire was used to collect background and behavioral information (e.g., cigarette smoking; alcohol, tea, or coffee drinking; and physical activity). The prevalence of ED was found to be greater in the arsenic endemic area (83.3%) than in the non-arsenic endemic area (66.7%). Subjects with arsenic exposure >50 μg/L had a significantly higher risk of developing ED than those with exposure ≤50 μg/L, after adjusting for age, cigarette smoking, diabetes mellitus, hypertension, and cardiovascular disease (OR = 3.4). Results also showed that the risk of developing severe ED was drastically enhanced by arsenic exposure (OR = 7.5), after adjusting for free testosterone and traditional risk factors of ED [89].

6.3.11.3 Proteinuria

Adverse effects of arsenic exposure from drinking water on the risk of proteinuria have been reported from a study in Bangladesh, the effects being modifiable by recent changes in arsenic exposure. Proteinuria was detected by urinary dipstick tests at baseline and at 2-year intervals on 11,122 participants in the Health Effects of Arsenic Longitudinal Study (HEALS). At baseline, well arsenic was positively related to prevalence of proteinuria; PORs for proteinuria in increasing quintiles of well arsenic (≤7, 8–39, 40–91, 92–179, and 180–864 μg/L) were 1.00 (ref), POR 0.99 (95% CI: 0.77–1.27), POR 1.23 (95% CI: 0.97–1.57), POR 1.50 (95% CI: 1.18–1.89), and POR 1.59 (95% CI: 1.26–2.00) (p for trend <0.01). Hazard ratios for incidence of proteinuria were POR 0.83 (95% CI: 0.67–1.03) and POR 0.91 (95% CI: 0.74–1.12) for participants with a decreasing level of >70 and 17–70 μg/L in urinary arsenic over time, respectively, and were POR 1.17 (95% CI: 0.97–1.42) and POR 1.42 (95% CI: 1.16–1.73) for participants with an increasing level of 16–68 and >68 μg/L in urinary arsenic over time, respectively, compared with the group with relatively little change in urinary arsenic as the reference group (urinary arsenic 15 μg/L) [90].

6.4 Pregnancy Outcome

No conclusive information on pregnancy outcome and infant mortality in relation to arsenic levels in drinking water is available in the literature as few studies have included individual assessment of arsenic concentrations in all water sources used during each pregnancy. In an ecological study carried out in Chile, stillbirths (rate ratio 1.7; 95% CI: 1.5, 1.9), neonatal and post-neonatal infant mortality rates were found to be increased in the high arsenic exposure city of Antofagasta as compared with the low exposure city of Valparaiso [91]. A study conducted in Bangladesh showed an increased risk for stillbirth for women with current arsenic levels of ≥100 μg/L, although the risk estimates were smaller (OR = 2.5; 95% CI: 1.5, 5.9). The authors further reported increased effects on spontaneous abortions (OR = 2.5; 95% CI: 1.5, 4.4) [92]. However, no information was available on arsenic exposure during pregnancy, and high exposure levels of 200 μg/L and more were not considered separately in this study. One

earlier cross-sectional study from Bangladesh compared rates of spontaneous abortions, stillbirths, and preterm delivery between 96 women in one village who were exposed to ≥100 μg/L arsenic to rates in 96 women in another village who were exposed to less than 20 μg/L, and showed two to three times higher rates among exposed women [93]. Both Bangladesh studies reported a relation to overall duration of women's exposure without taking into account exposure during the actual time period of pregnancy.

A retrospective study of pregnancy outcomes and infant mortality was conducted in West Bengal, India, among 202 married women selected from a source population of 7683 between 2001 and 2003. Reproductive histories were ascertained by structured interviews. Arsenic exposure during each pregnancy was assessed based on all water sources used, involving measurements from 409 wells. Odds ratios for spontaneous abortions, stillbirth, and neonatal and infant mortality were estimated with logistic regressions based on the method of generalized estimating equations. High concentrations of arsenic ≥200 μg/L during pregnancy were associated with a six-fold increased risk for stillbirth after adjusting for potential confounders (OR = 6.25; 95% CI: 1.59, 24.6, $p = 0.009$). Arsenic-related skin lesions were found in 12 women who had a substantially increased risk of stillbirth (OR = 13.1, 95% CI: 3.17, 54.0, $p = 0.002$). The odds ratio for neonatal death was 2.03 (95% CI: 0.57, 7.24). No association was found between arsenic exposure and spontaneous abortion (OR = 0.90; 95% CI: 0.36, 2.26) or overall infant mortality (OR = 1.18, 95% CI: 0.38, 3.64). This study adds to the limited evidence that exposure to high concentrations of arsenic during pregnancy increases the risk of stillbirth. However, there was no indication of increased rates of spontaneous abortion and overall infant mortality [94].

6.5 Arsenicosis and Cancer

The evidence of carcinogenicity in humans from exposure to arsenic is based on epidemiological studies of cancer in relation to arsenic in drinking water. The working group of the International Agency for Research on Cancer (IARC) [95] evaluated data from ecological studies, cohort studies, and case–control studies from many countries.

6.5.1 Skin Cancer

Skin cancer is a commonly observed malignancy related to drinking of arsenic-contaminated water. The working group of the IARC [95] evaluated ecological studies from Taiwan, Mexico, Chile, and the USA, cohort studies from Taiwan, and a case–control study from the USA. Numerous cases of skin cancer have been documented from communities with arsenic-contaminated drinking water. A prevalence study was conducted on a population of 40,421 in an arsenic endemic area of Taiwan where 238 cases of skin cancer have been clinically detected. Prevalence rates for inhabitants residing in low (<300 μg/L), medium (300–600 μg/L), and high (>600 μg/L) arsenic-contaminated areas represented over an eight-fold difference in occurrence of skin cancer from the highest to the lowest category of arsenic exposure [19]. In an ecological study, sex- and site-specific cancer mortality for 1989–1993 in Region II of Chile

with national mortality rates was compared. The SMR for skin cancer was 7.7 (95% CI: 4.7–11.9) among men and 3.2 (95% CI, 1.3–6.6) among women [95].

Skin cancer due to chronic arsenic exposure occurs as Bowen's disease (intraepithelial carcinoma, or carcinoma *in situ*), basal cell carcinoma, and squamous cell carcinoma. Skin cancer might arise in the hyperkeratotic areas or might appear on non-keratotic areas of the trunk, extremities, or hand [6,18]. Features of Bowen's disease have been described earlier. Arsenic-related basal cell carcinoma appears to be deep ulcerative or superficial type as those of ordinary type of basal cell carcinoma. Histologically, the cells have scanty and ill-defined cytoplasm. Nuclear atypy and giant cells are not ordinarily found. The size and gross appearance of epidermoid carcinoma varied greatly, some forming fungating masses measuring up to 5 by 5 cm and some causing large crater-like ulcers with elevated margins measuring up to 6 cm in diameter. Histologically, the grade of differentiation of epidermoid carcinoma varies [6].

6.5.2 Urinary Bladder Cancer

The working group of the IARC [95] evaluated ecological studies in Taiwan, Chile, Argentina, and Australia, cohort studies from Taiwan, Japan, and the USA, and case–control studies in Taiwan, the USA, and Finland and found evidence of increased risk for urinary bladder cancer associated with arsenic in drinking water. The first report of bladder cancer associated with drinking arsenic-contaminated water was published from the province of Cordoba in Argentina where 11% of cancer deaths were caused by this cancer [96]. An ecological mortality study for bladder cancer was conducted in Chile in Region II (arsenic endemic region) and in Region VIII (non-arsenic endemic area) for the period 1950–1992 and the SMR was found to be 10.2 (95% CI: 8.6–12.2) [97]. In Taiwan, the evidence of increased occurrence of bladder cancer due to arsenic was supported by case–control and cohort studies within the exposed communities that demonstrated evidence of a dose–response relationship with levels of arsenic in drinking water. There was also evidence of increased risks of bladder cancer from a small cohort study in Japan of persons drinking from wells that had been highly contaminated with arsenic wastes from a factory. The findings of epidemiological studies are consistent with a strong association of arsenicosis with bladder cancer [95].

6.5.3 Lung Cancer

On the basis of ecological studies using mortality data in Taiwan, Chile, Argentina, and Australia, cohort studies in Taiwan, Japan, and the USA, and case–control studies in Taiwan and Chile, a strong association of lung cancer has been observed in populations with high arsenic exposure [95]. In an ecological study, increased mortality from lung cancer was observed in men and women in 1968–1982 in an area endemic for blackfoot disease in Taiwan [98]. There was an exposure–response relationship between the SMR of lung cancer and the prevalence of blackfoot disease. Elevated SMRs (about three) were observed for lung cancer for both sexes in Region II of Chile using national rate as a standard [97].

6.5.4 Other Cancers

Increased risk of liver cancer has been reported in several studies from Taiwan, Japan, and Chile identified from death certificates. There is limitation of interpretation of these findings because of questionable accuracy of the diagnosis of liver cancer on death certificates and potential confounding or modifying effects of hepatitis virus infection or other factors. Ecological studies in Taiwan, Chile, Argentina, and Australia and cohort studies from Taiwan and the USA involving populations with high long-term exposure to arsenic found increased risks for kidney cancer. Relative risk estimates for kidney cancer were generally lower than those for urinary bladder cancer, and no studies have reported dose–response relationships on the basis of individual exposure data. Excess mortality from prostate cancer was found in southwest Taiwan. Inconsistent findings were reported for other cancers [95].

6.6 Diagnosis

The quantity of arsenic dose and the duration of arsenic exposure necessary to cause arsenical skin lesions have been reported by several investigators. The mean arsenic dose in Antofagasta was estimated to be approximately 60 μg per day for subgroups of children aged 3.13 ± 3.33 years but was approximately 20 μg per day for subgroups in their teens and twenties and 6 μg per day for a subgroup in their sixties, indicating an inverse relationship between daily arsenic dose rate/kg body weight and age [37]. The lowest peak arsenic exposure determined by using detailed lifetime (at least 20 years) exposure assessment by a confirmed case of arsenicosis with skin lesions was reported as 115 μg in West Bengal [31]. Prolonged exposure to a non-lethal dose of 5 to 90 μg/kg-body weight/day has been suggested to cause arsenical skin lesions [2]. In a retrospective study of 262 adults treated with Fowler's solution, it was reported that the minimal latency period for hyperkeratosis was 2.5 years following ingestion of approximately 2.2 g of arsenite [99]. Rattner and Dorne reported the development of hyperpigmentation within 6–12 months of the start of treatment with arsenic at a dose of 4750 μg/day [100]. Hyperkeratosis appeared after approximately 3 years. The shortest time of exposure associated with hypopigmentation, hyperpigmentation, and keratosis was 8 and 12 years, respectively, in Mexico with arsenic exposure of 400 μg in drinking water [8]. Arsenical skin lesions were reported to occur in West Bengal, India, after drinking arsenic-contaminated water (50 to 14,200 ppb) for 1 to 15 years [69]. A history of chronic arsenic exposure for more than 6 months is recommended as one of the required diagnostic criteria for the clinical diagnosis of arsenicosis [2].

Although chronic arsenic toxicity produces varied systemic manifestations as well as cancer of skin and different internal organs, dermal manifestations such as pigmentation and keratosis are diagnostic of chronic arsenicosis. For this reason, the field guide of the diagnostic algorithm of arsenicosis of the WHO [2] is based on the presence or absence of characteristic dermatological manifestations of chronic arsenic toxicity. A clinically confirmed case of arsenicosis is a "probable case with pigmentation (diffuse, spotty or blotchy) and/or keratosis (thickening or nodularity or verrucousity) of the skin, distributed bilaterally [and]

Table 6–4 Common Conditions to be Considered for Differential Diagnosis of Non-cancer Skin Lesions

Category	Major Conditions for Consideration
Diffused melanosis	Actinic dermatosis
	Melasma
	Ashy dermatosis
Spotted melanosis	Pityriasis versicolor
	Freckle
	Lichen planus
Leukomelanosis	Idiopathic guttate hypomelanosis
	Pityriasis versicolor
	Pityriasis lichenoides chronica
	Leprosy
Diffuse keratosis	Psoriasis (palms and soles)
	Eczema
	Occupational keratosis
	Tinea pedis
	Pitted keratolysis
Nodular keratosis	Occupational keratosis
	Verruca vulgaris corns/calluses
	Seborrheic keratosis

symmetrically in the body," in whom the presence of other arsenicosis simulating skin lesions has been ruled out. A "clinically and laboratory confirmed case" is a "clinically confirmed case" in whom the arsenic test is also positive by the recommended laboratory criteria. Laboratory criteria for establishing exposure history of arsenicosis cases are: (1) consumption of drinking water with an arsenic concentration in excess of prevailing national standards for at least 6 months (country standard in the Asia Pacific region is 50 μg/L while the WHO standard is 10 μg/L) and (2) an elevated concentration of arsenic in hair (>1 mg/kg of hair) or in nail clippings (>1.5 mg/kg of nails) [2].

Clinical features of hypopigmentation, hyperpigmentation, and keratosis are quite distinctive for the suspicion of diagnosis of arsenicosis, and a history of arsenic exposure through ingestion is helpful in corroborating a diagnosis. However, manifestations such as diffuse melanosis cannot be differentiated from the normal dark complexioning of farmers in the tropics who work in the field bare-bodied under direct sunlight. Similarly, manual laborers and farmers, who work with bare hands and bare feet, might have thickening of the palms and soles. Further, many other dermatological diseases simulate the features of arsenical skin manifestations. Hence, in a probable case of arsenicosis, diagnosis of a clinically confirmed case could be done by excluding other dermatological diseases simulating arsenical skin lesion by differential in-depth skin examination. Common conditions to be considered for differential diagnosis of arsenical skin lesions are given in Table 6–4. The presence of the following features will exclude the diagnosis of arsenical skin lesions: presence since birth, scaling, and erythema surrounding the lesion.

6.6.1 Biomarkers with Special Focus on Diagnosis

The most important biomarkers of internal exposure to arsenic are the urinary excretion of the element and its concentration in hair and nails. Blood concentrations are too low for use in the diagnosis, though it has a place in metabolic and pharmacy-kinetic study. Arsenic measurements in hair and nails are not used as indices of currently absorbed dose. Efforts are needed to develop a standardized procedure to solve the problem of external contamination of samples. Taking advantage of the relationship between arsenic intake through water and urinary excretion of inorganic arsenic and its metabolites MMA and DMA appears to offer a better means of estimation.

The concentration of total arsenic in urine has often been used as an indicator of recent arsenic exposure, because urine is the main route of excretion of most arsenic species [101]. As a parameter of oral intake of arsenic via drinking water in steady-state conditions, the urinary level of arsenic (seafood arsenic excluded) has been reported to be diagnostic by several authors from different countries. Despite possible ethnic and environmental differences, reported results display a quite satisfactory consistency. Most strikingly, an increased excretion rate is observed when the arsenic concentration of the drinking water reaches 100–200 μg/L [102]. The half-life of inorganic arsenic in humans is about 4 days. Average background concentrations of arsenic in urine are generally below 10 μg/L. A urine sample showing more than 50 μg/L may be taken as evidence of recent exposure provided the subject has not consumed seafood during the previous 4 days [2]. Although high arsenic excretion in urine is indicative of continued arsenic exposure, this is not always diagnostic of chronic arsenic toxicity. In West Bengal, India, a significant number of people (nine out of 17) who were drinking arsenic-contaminated water and had high urinary arsenic excretion did not show cutaneous manifestations of chronic arsenic toxicity. On the other hand, many (33 out of 40) of the chronically arsenic-exposed people showing arsenical skin lesions did not have high urinary arsenic excretion [103]. These results might be explained by the fact that all those who are drinking arsenic-contaminated water at a particular point in time may not be showing clinical features of chronic arsenic toxicity, whereas others who might have consumed arsenic-contaminated over a prolonged period in the past and have had skin lesions but have stopped drinking arsenic-contaminated water, might currently have low arsenic levels in urine.

A report from Bangladesh highlighted that urinary arsenic might be a stronger predictor of skin lesions than arsenic in drinking water used by the population. Arsenic levels in drinking water sources and urinary arsenic values had been correlated with the incidence of arsenical skin lesions in residents of three villages in Bangladesh. Arsenic levels in current drinking water source were found to be <50 μg/L in 13 (36.1%) out of 36 subjects with arsenical skin lesions among 167 participants studied. The risk of skin lesions in relation to the exposure estimates based on urinary arsenic was elevated more than three-fold, with the odds ratios for the highest versus the lowest quartiles 3.6 (95% CI: 1.2–12.1) for urinary total arsenic. The risks for skin lesions in relation to exposure estimates based on arsenic in drinking water with the odds ratios for the highest versus lowest quartiles of exposure were 1.7 (95% CI: 0.6–5.1) for current drinking water arsenic and 2.3 (95% CI: 0.7–7.6) for cumulative arsenic index [104].

Arsenic is normally found in higher concentrations in human hair and nails than in other parts of the body. This has been explained by the high content of keratin in these tissues [105]. In people with no known exposure to arsenic, the concentration of arsenic in hair is generally 0.02–0.2 mg/kg [106]. In one study in West Bengal, arsenic content in hair (analyzed by neutron activation analysis) in patients drinking arsenic-contaminated water (220–2000 μg/L) was found to vary from 1.4 to 20 mg/kg [1]. External contamination of the hair by arsenic must be excluded in order to use hair arsenic concentrations to assess toxicity.

Normal arsenic values in nails appear to range from 0.02 to 0.5 mg/kg [107,108]. Arsenic levels in nails (estimated by neutron activation analysis) in arsenic- (220–2000 μg/L) exposed people in West Bengal were found to vary between 16 and 66 mg/kg [1].

There is no correlation between arsenic levels in hair and nails and clinical features of chronic arsenic toxicity. In a village in West Bengal, all the 17 people drinking arsenic-contaminated water had raised hair and nail arsenic levels, but only eight had cutaneous lesions [69]. Further, out of 40 people with arsenical skin lesions in another village of West Bengal with a history of drinking arsenic-contaminated water, normal levels of arsenic in hair and nails were found in 31 and 26 cases, respectively [103].

6.7 Treatment

Chronic arsenicosis leads to irreversible damage to several vital organs and organ systems; arsenic is also an established carcinogen. Despite the magnitude of this potentially fatal toxicity, there is no effective therapy for this disease; once affected, patients may not recover, even after remediation of the arsenic-contaminated water. However, various modalities are used for the management of dermatological manifestations of chronic arsenic toxicity.

6.7.1 Ceasing Drinking of Arsenic-Contaminated Water

Changes in severity of skin lesions were reported among an affected cohort of arsenicosis patients in southern Thailand where interventions to reduce arsenic-contaminated water had been implemented. Over a 10-year period, both regression and progression of lesions occurred, though the majority of the subjects followed up remained the same. Drinking predominantly arsenic-free water increased the probability of regression in subjects with mild stage lesions but not in those with more advanced stage lesions. By contrast, high arsenic content in the household well water, even though it was not used for drinking, decreased the probability of lesion regression among the subjects in more advanced stages but not among milder stage cases. Irrespective of initial stage, a period of absence from the affected area increased the likelihood of lesion regression [109]. Another cohort follow-up study was carried out on 1074 people (arsenic exposed people 623, control population 451) in 2000, 5 years after the original clinical examination done on the same population at South 24 Parganas, West Bengal. Out of 199 people with skin lesions among the arsenic-exposed population who were consuming safe water during the previous 5 years, the skin lesions cleared or decreased in 49.7% of

people. However, out of 306 people who did not have such lesions previously, new skin lesions appeared in 32 (10.5%) [110]. Skin lesions were reported to improve to some extent in cases of arsenicosis in Inner Mongolia, China, after the drinking of low arsenic containing water for 1 year. However, a 5-year follow-up study showed no further significant improvement of skin lesions, while the potential risk of arsenic-induced cancers after cutting off high arsenic exposure was still uncertain and indefinite [111].

From the results of the few studies described above it becomes apparent that significant improvement of mild and moderate dermatological manifestations occurs in many cases of arsenicosis after continuous drinking of arsenic-free water. However, symptoms of severe pigmentation and keratosis may persist in spite of stoppage of consumption of arsenic-contaminated water.

6.7.2 Specific Therapy

Chelation therapy for chronic arsenic toxicity is thought to be the specific therapy for relief of clinical manifestations and reduction of arsenic stores in the body. Chelating agents BAL and d-penicillamine had been recommended for reduction of arsenic levels in the body [112,113]. Piamphongsant reported the efficacy of penicillamine in the management of chronic arsenic toxicity. The dosage used in adults was 100 mg/day for 2–4 months. Follow-up cases after 10 years of therapy showed that a few cases out of several hundred who received the drug still developed Bowen's disease, whereas those who did not receive the agent developed more extensive lesions of Bowen's disease than those who received treatment. However, raindrop pigmentation and white macules remained unchanged in spite of therapy [114].

Specific chelation therapy with DMSA (dimercaptosuccinic acid), a chelating agent related to BAL, in patients suffering from chronic arsenic toxicity has not yielded better results than those seen in control subjects treated with placebo who were studied in a placebo-controlled trial in West Bengal. Twenty-one consecutive patients with chronic arsenicosis were randomized into two groups. Eleven patients (10 males, ages 25.5 ± 8.0 years) received DMSA at 1400 mg/day (1000 mg/m^2) in four divided doses orally in the first week, and then 1050 mg/day (750 mg/m^2) in three divided doses during the next 2 weeks. The same was repeated after 3 weeks during which no drug was administered. The other 10 patients (all males, ages 32.2 ± 9.7 years) were given placebo capsules (resembling DMSA) in the same schedule. The patients were blinded about the nature of treatment being given. The symptoms and signs of dermatological manifestations of patients were evaluated by a scoring system before and after treatment. Therapy with DMSA did not cause any significant clinical improvement of dermatological and systemic score compared with patients treated with placebo. No patient developed any therapy-related side effects. The histological abnormalities in skin biopsy did not show any difference in patients treated with DMSA and placebo before and after therapy [26].

The efficacy of treatment with DMPS (dimercaptopropane sulfonate), another chelating agent, was reported in a single-blind placebo-controlled trial in patients suffering from chronic arsenic toxicity in West Bengal, India. The trial design was similar to that carried out in the DMSA trial described above. DMPS was given via a dosage of 100-mg capsules four times a day

Table 6–5 Clinical Scores of Patients Before and After Therapy

Clinical Features	Drug	Before	After	*p*-value
Pigmentation	DMPS	1.45 ± 0.52	0.90 ± 0.54	0.02
	Placebo	1.60 ± 0.84	1.10 ± 0.87	0.20
Keratosis	DMPS	1.54 ± 0.68	1.09 ± 0.70	0.14
	Placebo	1.40 ± 0.96	1.11 ± 0.87	0.47

for a course of 7 days for four courses, with a 1-week drug-free period between each course. Eleven patients (nine males and two females) received the drug, whereas 10 patients (five males, five females) received placebo capsules. The results of this study indicate that DMPS is more effective than a placebo in improving clinical features of chronic arsenic toxicity including skin score of pigmentation (Table 6–5) [87].

The beneficial effect of oral supplementation with vitamin A (retinol) in the treatment of cutaneous arsenicosis was described as early as 1946 [115]. In that report, oral vitamin A, 150,000 USP units per day for 3 months, resulted in a partial regression of hyperpigmentation and hyperkeratosis of the palms of a 39-year-old male who had taken Fowler's solution (potassium arsenite) for treatment of childhood chorea. The effectiveness of management of chronic arsenicosis in Bangladesh by administering a vitamin A, E, C regimen was further evaluated. Forty-three patients with chronic arsenicosis had been given the following regimen for 6 months: (1) withdrawal of exposure, (2) vitamin A (50,000 IU on alternate days), (3) vitamin E (200 mg daily), (4) vitamin C (250 mg twice daily), and topical application of a keratolytic agent (for arsenical keratosis only). Improvement of melanosis and keratosis was observed in 90.9% and 86.4% of patients, respectively, from among 22 patients who had used safe water and had taken the regimen regularly [116]. However, the characteristics of skin lesions for evaluation of the severity of arsenicosis were not described in the methodology.

A case series of nine patients with cutaneous arsenicosis were treated with oral etretinate, a synthetic aromatic retinoid, for 2–7 months. Clinical and histopathological improvement was noted in arsenical hyperkeratosis, but not in hyperpigmentation [117]. Regression of arsenical keratosis with etretinate treatment was also published [118]. Piamphongsant reported that moderate and severe degrees of palmoplantar keratosis due to arsenicosis improved with treatment with etretinate at a dose of 25 mg twice daily for 3–4 months and then 25 mg/day for 2 months. Keratotic papules and keratoderma usually slough off in 3–4 months. Many of the patients complained of sticky palms due to mucoid sweat [115]. It is noteworthy that etretinate and other retinoids have been reported to have antikeratinizing effects in other disorders of keratinization, such as hereditary palmoplantar keratoderma, pityriasis rubra pilaris, and certain ichthyoses [119].

Surgical excision is usually performed for the management of Bowen's disease. The combination of 5-aminolevulinic acid with photodynamic therapy and 5-fluoroaccil iontophoresis may be an alternative therapy for the condition [114,120].

Supportive treatment could help in reducing many symptoms of these patients. Treatment in hospital with a good nutritious diet has been found to reduce symptoms in many patients and to reduce symptom score in subsets of placebo-treated arsenicosis patients during the course of the DMSA and DMPS trial [26,87]. Presently, the most prevailing practice of symptomatic treatment of keratosis is to apply locally 5–10% of salicylic acid and 10–20% urea-based ointment on keratotic skin lesions [2]. A higher dose (20% salicylic acid) may be used in severe cases of arsenical keratosis.

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7

Changing Concept of Arsenic Toxicity with Development of Speciation Techniques

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CHAPTER OUTLINE

7.1 Introduction

Arsenic (As), a metalloid, is a ubiquitous element that ranks 20th in abundance in Earth's crust, 14th in sea water, and 12th in the human body [1,2]. Since its isolation (as a gray, shiny, metallic-looking element with atomic number of 33 and atomic weight of 74.92) in AD 1250 by Albertus Magnus, this element has been the center of controversy in human history. It is found as a component of more than 245 minerals. These are mostly ores containing sulfide, along with copper, nickel, lead, cobalt, or other metals. Being a group VB element in the periodic table along with N, P, Sb, and Bi, it can exist in different valency states such as −III, 0, +III, and +V. Although it has a metallic allotropic form like the two higher group VA elements, antimony and bismuth, arsenic's chemical behavior is quite similar to that of phosphorus.

Based on different chemical forms and oxidation states, many As compounds exist in nature as well as in biological systems (Table 7–1). Chemical speciation is the determination of the individual concentrations of the various forms of an element that together make up the total concentration of that element. Still, 1970 arsenic species in natural waters were considered to be inorganic forms, such as arsenate [As(V)] and arsenite [As(III)]. Although the Guitzeit method was used initially for qualitative testing of As in aqueous solutions, this method was later improved for a quantification of lower amounts of As in water and other beverages [4,5] over the last 100 years. Until recently the modified Guitzeit technique [6] was used

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Table 7–1 Arsenic Compounds Found in Water Matrix

Name of Species	Abbreviation	Structure of Species
Most often determined		
Inorganic compounds		
Arsenous acid (arsenite)	As(III)	$As(OH)_3$
Arsenic acid (arsenate)	As(V)	$AsO(OH)_3$
Organic compounds		
Monomethylarsonic acid	MMA(V)	$CH_3AsO(OH)_2$
Dimethylarsinic acid	DMA(V)	$(CH_3)_2AsO(OH)$
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2COOH$
Arsenocholine	AsC	$(CH_3)_3As^+CH_2CH_2OH$
Rarely determined		
Trimethyl arsine oxide	TMAO	$(CH_3)_3AsO$
p-Arsanilic acid	*p*-ASA	$C_6H_8AsNO_3$
Trimethylarsoniopropionate	TMAP	$(CH_3)_3AS^+CH_2CH_2COO^-$
Dimethyldithioarsinic acid	DMDTA(V)	$(CH_3)_2As(S)(SH)$
Dimethylmonothioarsinic acid	DMMTA(V)	$(CH_3)_2As(S)(OH)$
Thio-arsenosugar glycerol	Thio-Gly	$C_7H_{14}AsO_3(S)OCH_2CH(OH)CH_2OH$
Thio-arsenosugar sulfate	Thio-SO_4	$C_{10}H_{16}AsO_5(S)OSO_3H$
Thio-arsenosugar phosphate	Thio-PO_4	$C_7H_{14}AsO_3(S)OCH_2CH(OH)CH_2OPO_2 - OCH_2CH(OH)CH_2OH$
Trimethylarsine sulfide	TMAS	$(CH_3)_3As(S)$
Thio-arsenosugar sulfonate	Thio-SO_3	$C_{10}H_{16}AsO_5(S)SO_3H$
Thio-dimethylarsenoacetate	Thio-DMAA	$(CH_3)_2As(S)CH_2COOH$
Thio-dimethylarsinate	Thio-DMA	$(CH_3)_2As(S)(OH)$
Thio-dimethylarsenoethanol	Thio-DMAE	$(CH_3)_2As(S)CH_2COOH$
Phenylarsonic acid	PhAs, PAA	$C_6H_5AsO(OH)_2$
4-Hydroxy-3-nitrobenzenearsonic acid	Roxarson, Roxarsone	$C_6H_5As(O)(OH)_2$
Phenylarsine oxide	PhAsO, PAO	C_6H_5AsO
Dimethylarsinous acid	DMA(III)	$(CH_3)_2As(OH)$
Monomethylarsonous acid	MMA(III)	$(CH_3)_3As(OH)_2$
Dimethylarsinoylacetic acid	DMAA	$(CH_3)_2As(O)CH_2COOH$
Dimethylarsinoylethanol	DMAE	$(CH_3)_2As(O)CH_2CH_2OH$
Tetramethylarsonium ion	TMAs, Tetra, TeMA, TMA	$(CH_3)_4As^+$
Diphenylarsinic acid	DPAA	$(C_6H_5)_2AsO(OH)$

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to quantify total inorganic As (iAs) in water samples. But this method was not reliable for water samples that contained less than 40 µg/L arsenic [7]. In addition, it was also used to quantify As(III) and As(V) in water samples [6]. A recent adaptation of this method is the analysis of the silver diethyldithiocarbamate (SDDC) complex using graphite tube furnace atomic absorption spectrometry (AAS), which gives an improved detection limit of about 10 ng/L [8].

Thereafter the methylated forms, i.e., mono methylarsonic acid [MMA(V)] and dimethylarsinic acid [DMA(V)], have been reported to be the minor species in water [9–12]. In the late

Table 7–2 Summary of Analytical Methods

Techniques	LOD (μm/L)	Sample Size (mL)	Major Equipment Cost (US$)	Analytical Throughput	Comments	Selected Standards
HGAAS	0.05	50	20–100,000	30–60	Single element	ISO 11969 SM 3114
GFAAS	1–5	1–2	40–100,000	50–100	Single element	ISO/CD 15586 SM 3113
ICP–AES	35–50	10–20	60–100,000	50–100	Single element	ISO/CD 11885 SM 3120
ICP-MS	0.02–1	10–20	150–400,000	20–100	Single element	SM 3125 USEPA 1638
HGAFS	0.01	40–50	20–25,000	30–60	Single element	CEN/TC/230/WG1/ TG 12 N 3
ASV	0.1	25–50	10–20,000	25–30	Only free dissolve arsenic	USEPA 7063
SDDC	1–10	100	2–10,000	20–30	Limited to water samples	SM 3500 ISO 6595

1980s and early 1990s several laboratory-based analytical methods were well described for routine analyses of aqueous samples such as hydride generation atomic absorption spectrometry (HGAAS), graphite furnace atomic absorption spectrometry (GFAAS), atomic fluorescence spectrometry with hydride system (HGAFS), inductively coupled plasma atomic emission spectrometry (ICP-AES or ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), anodic stripping voltammetry (ASV), and the silver diethyldithiocarbamate (SDDC)-based spectrometric method using Guitzeit apparatus. The last-mentioned method has been widely used due to its simplicity regarding instrumentation (spectrometry) but it has serious limitations of accuracy and toxicity due to handling of carcinogenic organic solvent chloroform and the highly toxic arsine gas. Also, at least a 100 mL water sample containing a high concentration of As (≈50 μg/L) is needed for a reproducible and accurate result. The detection limits for As range between less than 0.1 and 50 μg/L depending upon the technique employed [13,14]. The limits of detection of the above analytical techniques is summarized in Table 7–2 [15–21].

Arsenic is a known human carcinogen that depends on its chemical forms [12,22,23] as well as oxidation states [24]. Humans are exposed to iAs via air, water, and food. Due to lack of hyphenated techniques for speciation of different arsenicals with respect to the methylation and oxidation states of As, researchers studied only the metabolic pattern of iAs in humans and rodents [25–31]. Rodents and humans can metabolize iAs by consecutive reduction and oxidative methylations after ingestion via drinking water or eating food. Based on the studies of iAs metabolites in rodents and humans [25–37], it was found that approximately 60–75% of the dose was excreted in urine within a few days and the distribution of arsenic species in urine was 10–15% inorganic arsenic, 10–15% MMA(V), and 60–80% DMA(V).

Most mammals methylate iAs to MMA and DMA [38]. Normally, iAs is more reactive to tissue constituents compared to methylated arsenic metabolites, is more toxic, and is less readily

excreted in the urine [25–38]. Inorganic arsenic, especially As(III), is the main form of arsenic interacting with tissues [26,32,39]. Due to lower methylation capacity iAs is associated with higher tissue concentrations [27,28] and leads to a higher risk of toxic effects. Based on these results, the methylation of arsenic was considered a detoxification mechanism in mammalian species and humans.

There are major species differences with respect to arsenic methylation [25–38]. In brief, humans excrete significant amounts of MMA (10–20% of the total metabolite concentration in the urine) following exposure to iAs, whereas mammalian species methylate arsenic more efficiently than humans and the percentage of DMA is greater in urinary arsenic compared to MMA. The lack of ability to methylate iAs in mammalian species such as chimpanzees, marmoset monkeys, squirrel monkeys, tamarins, and guinea pigs [29,30,40–42] results in increased tissue concentrations of arsenic. This lower rate of excretion compared to animal species creates complexity with these species, which ultimately leads to toxicity.

Recently, some investigations have reported different methylation patterns of iAs [30,31,43,44] in arsenic-affected populations. The native Andean people, the Mestizo population in northern Argentina, and the population from West Bengal, India, excrete just a few percent of MMA in urine [30,31,44], whereas a Taiwan study indicates an unusually high percentage of MMA in urine [43]. It was assumed that an inhibition of the enzymes (methyltransferases) involved in the methylation of arsenic may be responsible for this variation in methylation capacity among these populations [45].

Vahter (2000) has reported the biotransformation of iAs and its toxicity among different As-affected populations after comparing results of different studies reported in the literature from the As-affected regions as well as after a single dose of As(V) in different mammalian species [46]. The author attempted to find out the reason for the variation in methylation capacity among the mammalian species (Figure 7–1) and in the As-affected populations

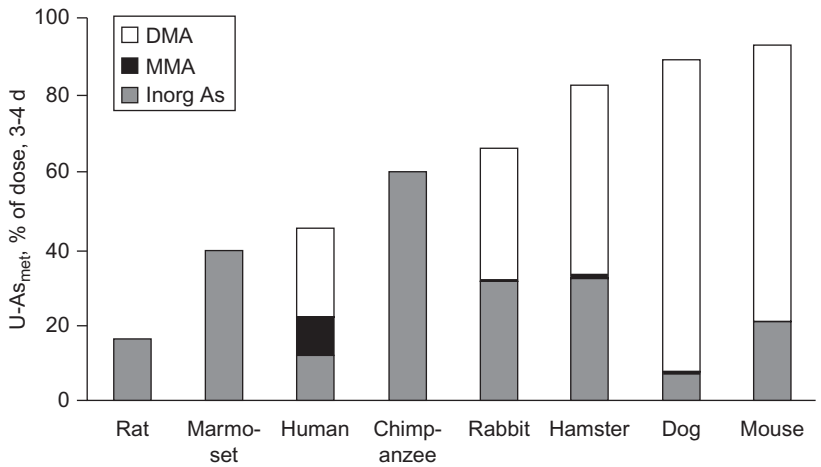


FIGURE 7–1 Species variations in the excretion of methylated arsenic metabolites following a single dose of arsenate. *Reprinted from* [47] *with permission from Elsevier.*

(Figures 7–2–7–4). It is very clear from Figure 7–1 that the marmoset monkey and the chimpanzee do not methylate As(V). Figures 7–2–7–4 show the variation in arsenic metabolites (MMA, DMA, and iAs) in people exposed to arsenic via drinking water. Polymorphism of the genes regulating the expression of arsenic methyltransferases may be responsible for this variation.

Also, the study on the excretion pattern of ingested arsenicals suggests that blood As comes down to a minimum level within a few hours of ingestion, is mostly excreted via urine within 3–4 days, and the remaining part accumulates in keratin-rich biological derivatives of ectoderm such as hair, scales, and nails of the chronically As-exposed population [32,44,45,51,52]. In the past, most investigators measured total As in human nails or hair due to lack of techniques for efficient separation and sensitive detection of particular species [6,52–56].

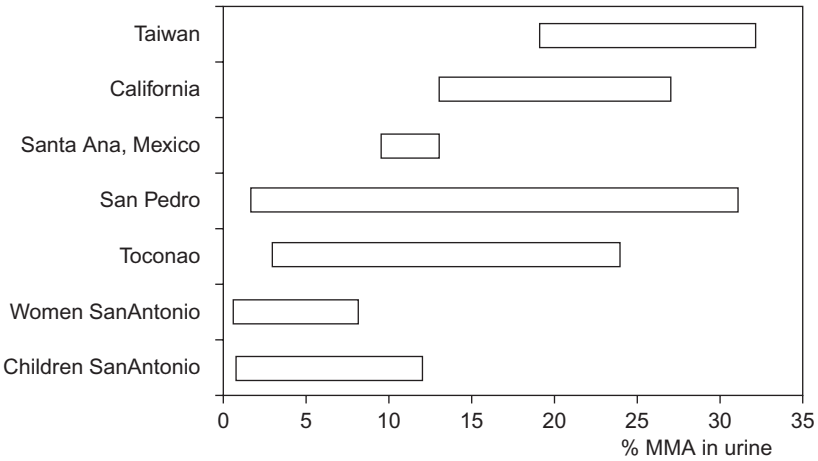


FIGURE 7–2 Inter-individual variation in the fraction of MMA in urine of people exposed to arsenic via drinking water [30,31,42,48–50]. *Reprinted from* [47] *with permission from Elsevier.*

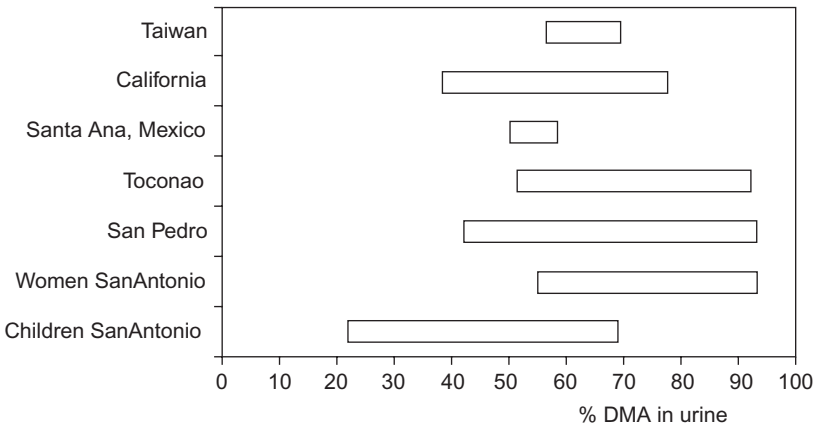


FIGURE 7–3 Inter-individual variation in the fraction of DMA in urine of people exposed to arsenic via drinking water [30,31,42,48–50]. *Reprinted from* [47] *with permission from Elsevier.*

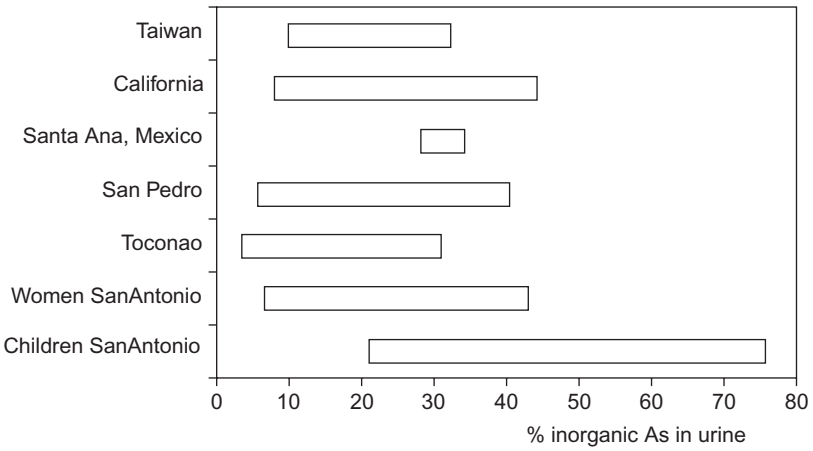


FIGURE 7–4 Inter-individual variation in the fraction of inorganic arsenic in urine of people exposed to arsenic via drinking water [30,31,42,48–50]. *Reprinted from* [47] *with permission from Elsevier.*

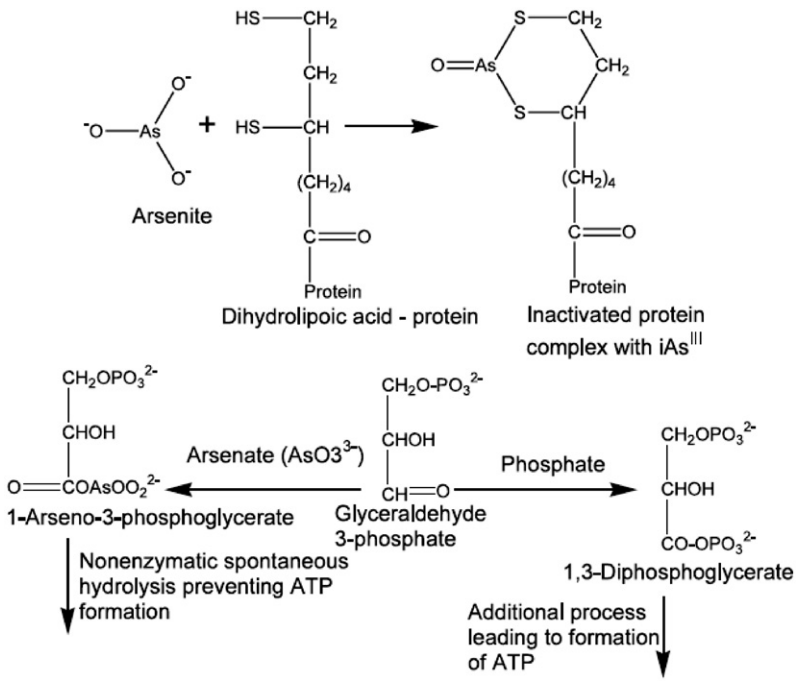
Not only do keratin-rich tissue arsenicals alter the activity of enzymes, which generates cellular energy in the citric acid cycle, but inactivation of pyruvate dehydrogenase by complexation with As(III) is reported, which prevents the generation of adenosine-5-triphosphate (ATP) in the cells. In the presence of As(III), lipoic acid of the protein inside the enzyme replaces two hydrogen atoms from the thiol groups, attaches with a sulfur molecule forming a dihydrolipoylarsenite chelate complex, and prevents the reoxidation of the dihydrolipoyl group (Scheme 7–1). As a result this pivotal enzyme step is blocked and the amount of pyruvate in the blood increases, energy production is reduced, and finally the cell is slowly damaged [57,58]. The strong affinity of As(III) to sulfur is reported when trivalent arsenic forms a stable ringed structure with vicinal dithilols of keratin in hair [59]. Arsenic inhibits enzymes, such as pyruvate oxidase, *S*-amino acid oxidase, choline oxidase, and transaminase. In addition, As(V) can be disruptive by competing with phosphate uncoupling oxidative phosphorylation [60] where reduced nicotinamide adenine dinucleotide (NADPH) is oxidized and reduces ATP production.

$$3ADP + 3H_3PO_4 \rightarrow 3ATP + 3H_2O$$

$$NADPH + H^+ + \tfrac{1}{2}O_2 \rightarrow NADP^+ + H_2O$$

Subsequently, arsenate produces unstable arsenate ester of ADP, which undergoes hydrolysis non-enzymatically and disrupts the production of ATP. This process is termed arsenolysis [61].

Glucose-6-arsenate is produced rather than glucose-6-phosphate, which inhibits the energy metabolism. The clastogenicity of arsenic appears because an arsenodiester bond is weaker than the normal phosphodiester bond after replacement of phosphorus in DNA by



SCHEME 7–1 Interaction of iAs with proteins and blockage of ATP production.

As(V) [62] and inhibits the DNA repair mechanism. More detailed research is needed to establish this concept of incorporation of As(V) into DNA. Due to this affinity to protein, the onset of skin effects (including pigmentation changes, hyperkerotosis, and skin cancers) was linked to the consumption of arsenic in medicines and drinking water in the latter part of the 19th century [63]. Hence, chronic exposure to iAs may give rise to several health issues including effects on the gastrointestinal tract, respiratory tract, skin, liver, cardiovascular system, hematopoietic system, nervous system, etc. [2].

In the first decade of the 21st century several hyphenated analytical techniques were published in different journals which suddenly changed the concept of the metabolism pattern of arsenicals in humans and mammalian species and hence the concept of arsenic toxicity in the arsenic-affected population [64–71]. These techniques separated different chemical species of As with high resolution at lower detection limits. Also, different arsenicals were separated based on their oxidation states of As, which brought about a breakthrough in As metabolism and its toxicity towards humans. Procedures for As speciation in different matrices are schematically presented in Figure 7–5 [72].

Identification and quantification of toxic methylated trivalent arsenicals were carried out by using HPLC hyphenated with HGAAS/HGAFS/HGAFD/ICP-MS/ICP-AFS, and outcomes of simultaneous toxicity and metabolite studies on animals and cell lines created a big question mark over the traditional detoxification concept of biomethylation [52–56,64–80].

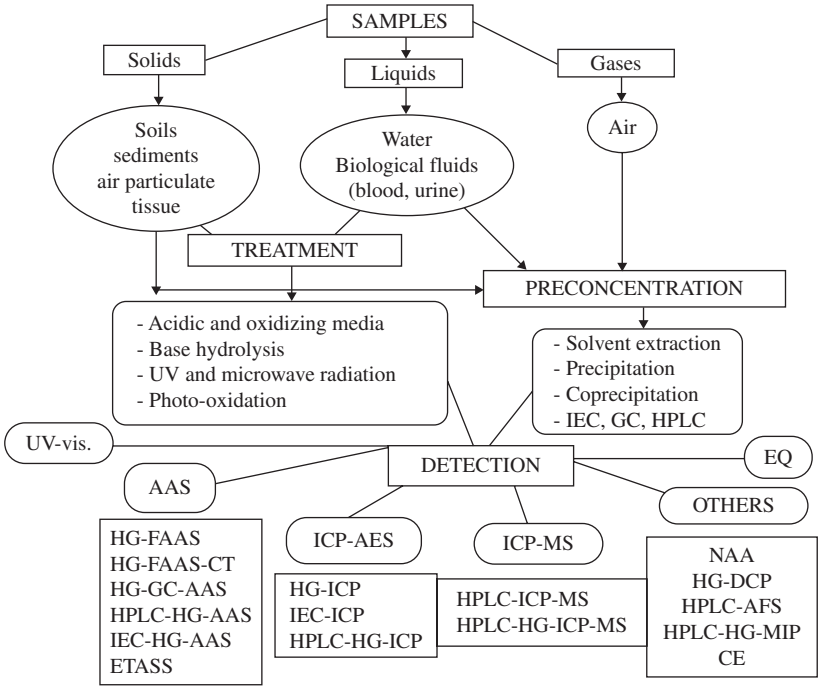


FIGURE 7–5 Procedures for arsenic speciation in different matrixes. UV-vis. = spectrophotometry; EQ = electrochemical methods; AAS = atomic absorption spectrometry; ICP-AES = inductively coupled plasma atomic emission spectrometry; ICP-MS = inductively coupled plasma mass spectrometry; HG = hydride generation; FAAS = flame atomic absorption spectrometry; CT = cryogenic trapping; CC = gas chromatography; HPLC = high performance liquid chromatography; IEC = ion exchange chromatography; ETAAS = electrothermal atomic absorption spectrometry; NAA = neutron activation analysis; DCP = direct current plasma; AFS = atomic fluorescence spectrometry; MIP = microwave induced plasma; and CE = capillary electrophoresis). *Reprinted from* [72] *with permission from Elsevier.*

A representative chromatogram is presented in Figure 7–6 after development of a hyphenated technique coupling HPLC-ICP-MS [64]. At a single run all eight arsenicals, i.e., As(III), As(V), MMA(III), MMA(V), DMA(III), DMA(V), AsC, and AsB, are separated. Using this technique biological samples such as urine (Figure 7–7), nails, and hair samples (Figure 7–8) collected from the As-affected areas of West Bengal were analyzed and quantified. Contents of different arsenicals quantified using the above technique are summarized in Table 7–3 for urine, Table 7–4 for finger nails, Table 7–5 for hair, and Table 7–6 for blood plasma and for red blood cell (RBC) samples collected from the As-affected population of West Bengal. Both DMA(III) and MMA(III) have been detected for the first time directly in urine of some humans exposed to iAs through their drinking water. Of 428 subjects, MMA(III) and DMA(III) were found in 48% and 72%, respectively. MMA(III) was 2–5% and DMA(III) was 4–21% of the total urinary arsenic. These results clearly show that this technique is capable of quantifying submicro levels of arsenicals in different biological samples. Also, Aposhian et al. [70] detected MMA(III) in human urine collected from the arsenic-affected areas of Romania.

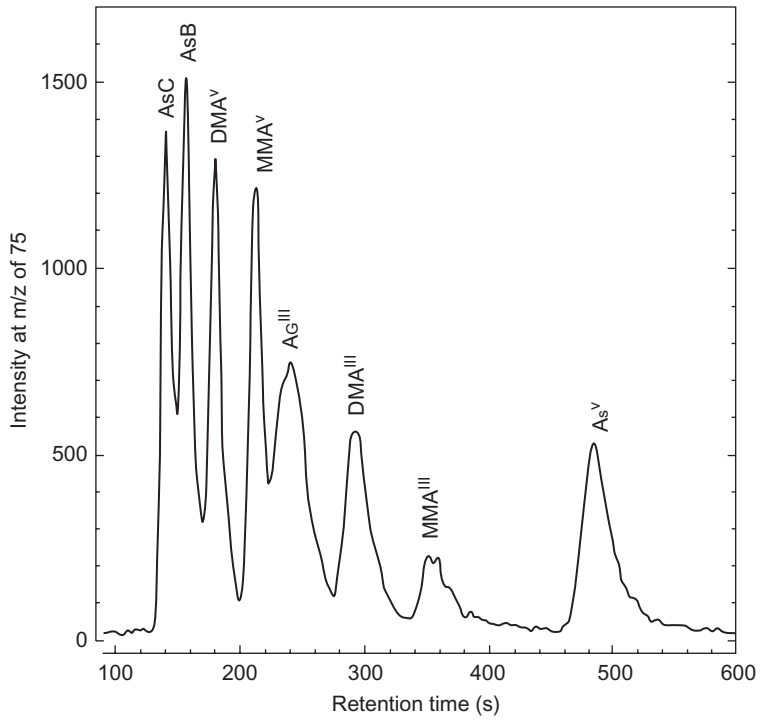


FIGURE 7–6 HPLC/ICP MS chromatogram of a mixture (20 μg of As/L each) of eight authentic arsenic compounds using an anion exchange HPLC/ICP MS system. Separation was carried out on an anion exchange ES-502N 7C column (7.6 mm × 100 mm) with a mobile phase containing 15 mM citric acid. The isocratic pH of the mobile phase was adjusted to 2.0 with 10% HNO_3 at 25°C. The flow rate of the mobile phase was 1 mL/min throughout the study. The injection volume of the sample was 20 μL. The ion intensities at m/z 75 and 77 were recorded with time-resolved analysis software. *Reprinted from* [64] *with permission from American Chemical Society.*

Styblo et al. [81] tried to separate MMA(III) and MMA(V) using thin-layer chromatography, but this is not applicable to biological fluids. Del Razo et al. (2001) used HPLC/hydride generation atomic absorption spectrometry (HPLC-HGAAS) for the separation and quantification of different arsenicals in urine (Table 7–7), metabolites in HepG2 cell lysate after exposure to As(III)-containing arsenicals (Table 7–8), and stability data of As(III)-containing arsenicals in donor urine (Table 7–9) [68]. Thus, analytical techniques based on HG-AAS can also separate and quantify different arsenicals in different matrices. Le et al. used a hydride generation atomic fluorescence detector (HGAFD) coupled with HPLC for the speciation of different arsenicals at submicro levels in different matrices [76,77]. The major challenge of speciation and toxicity studies using methylated trivalent arsenicals is their stability (Table 7–9). However, there is evidence that As(III) undergoes time-dependent oxidation in these matrices [68]. Among the three trivalent species tested [As(III), MMA(III), DMA(III)], As(III) was the most stable species and MMA(III) was more stable than DMA(III). The same authors have tested the stability of these arsenicals [As(III), MMA(III)O, and DMA(III)GS] after spiking in human

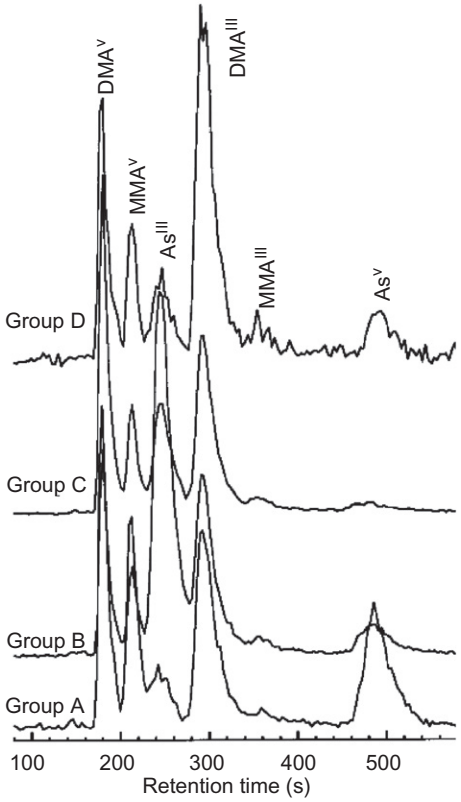


FIGURE 7–7 Elution profile of arsenicals obtained from HPLC/ICP MS. The urine samples collected from groups A–D were diluted four-fold. Their chromatograms represent the peaks of both DMA(III) and MMA(III). Experimental parameters were the same as those described in the legend of Figure 7–6. *Reprinted from* [64] *with permission from American Chemical Society.*

urine and found considerable inter-individual variation in the stability of As(III)-containing species [68]. They speculated that this inter-individual variation may arise due to differences in amounts of oxidants or antioxidants in urine coming from dietary sources. More data on the stability of methylated trivalent arsenicals are urgently needed for *in vitro* and *in vivo* toxicological evaluation of these arsenicals.

Several reports show more toxicity of methylated trivalent arsenicals in different human cell types compared to the pentavalent form [69,73,75]. Methylarsine oxide [MMA(III)O] was more toxic and iododimethylarsine [DMA(III)I] was at least as cytotoxic as As(III) for most of the cell types examined (human normal hepatocytes, epidermal keratinocytes, bronchial epithelial, and urinary bladder UROtsa cells) [69]. Also, several studies have been carried out on human cell lines (such as human hepatocellular carcinoma [HepG2] cells, human bladder transient carcinoma [T24] cells, human acute promyelocytic leukemia [NB4] cells, human monoblastoid [U937] cells, human osteosarcoma [HOS] cells, and human neuroblastoma [SK-N-SH] cells) in assessing cytotoxicity of methylated trivalent arsenicals [74]. It was found

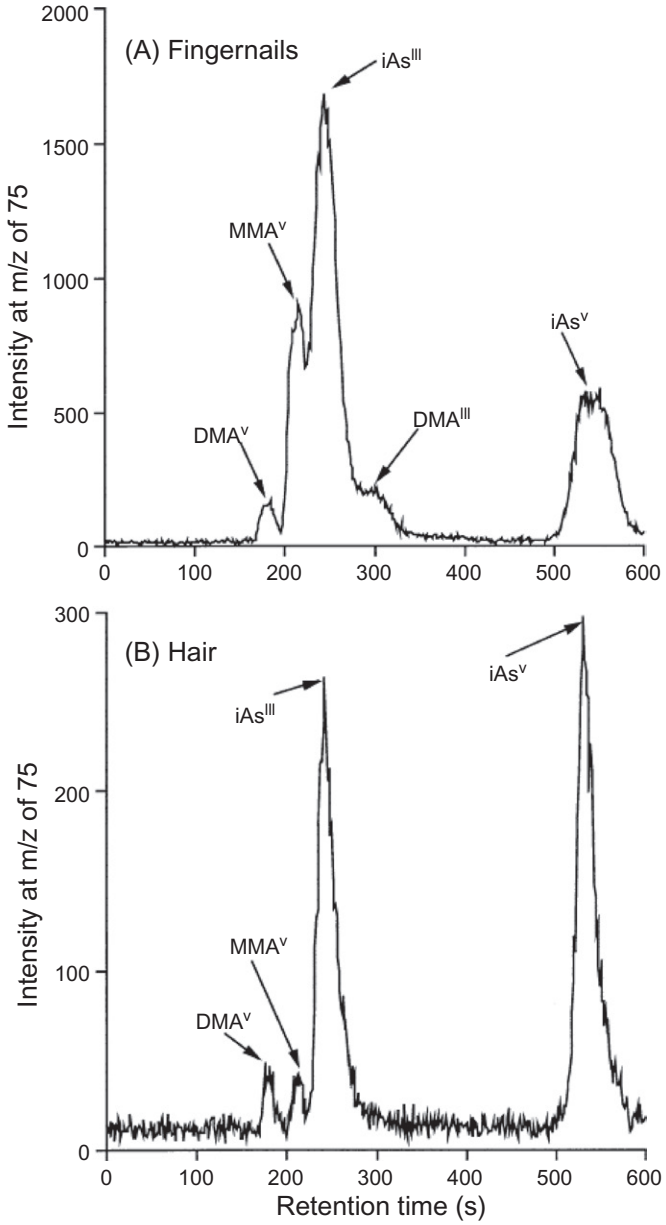


FIGURE 7–8 HPLC-ICP MS chromatograms of As in water extract of nail and hair. (A) Fingernails collected from the As-exposed individual and incubated in water at 90°C for 0.5 h. (B) Hair collected from the As-exposed individual and incubated in water at 90°C for 6 h. Experimental parameters were the same as those described in the legend of Figure 7–6. *Reprinted from* [52] *with permission from Elsevier.*

Table 7–3 Arsenic Concentrations in Human Urine and Distribution of Urinary Arsenicals according to Arsenic Concentrations in Individuals' Drinking Water Relating to the Study Subjects

	Urinary As (μg/L [%]), $n = 41$							
	AsB	**iAs^{III}**	**iAs^{V}**	**MMA^{III}**	**MMA^{V}**	**DMA^{III}**	**DMA^{V}**	**tAs[a]**
Mean	1.08 [1.0]	17.5 [11.3]	15.2 [10.1]	9.39 [6.6]	17.6 [10.5]	21.9 [13.0]	93.9 [47.5]	177
SD	1.02	13.8	9.45	4.09	11.6	30.1	77.6	97.4
Minimum	0.29	4.53	2.36	5.31	2.52	1.24	9.83	50.1
Maximum	3.45	64.3	46.7	22.2	59.0	150	267	390
Water As (μg/L)	**Urinary As (μg/L [SD])**							
29.0 (8)[b]	1.49 (0.9)	9.27 (4.0)	8.58 (3.0)	6.49 (0.9)	8.63 (4.3)	6.31 (3.1)	25.8 (14)	66.5 (12)
55.0 (8)[c]	1.82 (1.7)	11.2 (8.9)	8.94 (3.5)	7.44 (2.5)	10.3 (3.8)	16.8 (7.5)	31.5 (11)	87.9 (13)
130 (12)[d]	1.37 (0.6)	19.9 (15)	17.7 (11)	9.37 (2.9)	14.9 (5.2)	23.1 (9.6)	80.8 (45)	167 (33)
163 (13)[e]	1.18 (0.7)	22.9 (16)	19.3 (9.5)	11.9 (5.2)	28.5 (13)	(32.2) (11)	171 (71)	287 (56)

[a]tAs = concentration of total As after speciation.
[b]No. of subjects drinking water containing 29 μg As/L.
[c]No. of subjects drinking water containing 55 μg As/L.
[d]No. of subjects drinking water containing 130 μg As/L.
[e]No. of subjects drinking water containing 163 μg As/L.
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Table 7–4 Arsenic Concentrations in Human Fingernails and Distribution of Fingernail Arsenicals According to Arsenic Concentrations in Individuals' Drinking Water of the Study Subjects (All Fingernail Samples were Incubated in Milli-Q Water at 90°C for 0.5 h)

	Fingernail As (μg/g [%]), $n = 41$						
	iAs^{III}	**iAs^{V}**	**MMA^{V}**	**DMA^{III}**	**DMA^{V}**	**t_1As[a]**	**tAs[b]**
Mean	1.81 [62.4]	0.59 [20.2]	0.16 [5.7]	0.26 [8.9]	0.08 [2.8]	2.89	4.57
SD	0.82	0.29	0.09	0.13	0.03	1.26	2.12
Minimum	0.76	0.19	0.06	0.09	0.06	1.36	1.95
Maximum	3.95	1.31	0.42	0.63	0.16	5.67	7.83
Water As (μg/L)	**Fingernail As (μg/g [SD])**						
29.0 (8)[c]	0.95 (0.06)	0.27 (0.03)	0.09 (0.02)	0.11 (0.02)	0.04 (0.02)	1.47 (0.06)	2.24 (0.16)
55.0 (8)[d]	1.27 (0.08)	0.41 (0.07)	0.11 (0.02)	0.19 (0.03)	0.07 (0.02)	2.05 (0.15)	3.26 (0.29)
130 (12)[e]	1.71 (0.39)	0.58 (0.15)	0.18 (0.08)	0.27 (0.09)	0.08 (0.02)	2.82 (0.52)	4.52 (0.74)
163 (13)[f]	2.76 (0.65)	0.92 (0.22)	0.21 (0.13)	0.38 (0.12)	0.09 (0.03)	4.36 (0.93)	7.39 (1.77)

SD = standard deviation.
[a]t_1As = concentration of total As after speciation.
[b]tAs = concentration of total As after H_2O_2/HNO_3 digestion.
[c]No. of subjects drinking water containing 29 μg As/L.
[d]No. of subjects drinking water containing 55 μg As/L.
[e]No. of subjects drinking water containing 130 μg As/L.
[f]No. of subjects drinking water containing 163 μg As/L.
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Table 7–5 Arsenic Concentrations in Human Hair and Distribution of Hair Arsenicals according to Arsenic Concentrations in Individuals' Drinking Water of the Study Subjects (All Hair Samples were Incubated in Milli-Q Water at 90°C for 6 h)

	Hair As (μg/g [%]), $n = 41$					
	iAs^{III}	iAs^{V}	MMA^{V}	DMA^{V}	t_1As^a	tAs^b
Mean	0.84 [58.9]	0.46 [34.8]	0.04 [2.9]	0.04 [3.4]	1.43	2.45
SD	0.57	0.30	0.02	0.03	0.83	1.11
Minimum	0.21	0.08	0.02	0.02	0.05	0.07
Maximum	2.64	1.42	0.20	0.13	3.80	4.61
Water As (μg/L)	**Hair As (μg/g [SD])**					
29.0 (8)[c]	0.29 (0.05)	0.26 (0.04)	0.03 (0.02)	0.03 (0.02)	0.59 (0.07)	1.01 (0.24)
55.0 (8)[d]	0.56 (0.23)	0.35 (0.11)	0.04 (0.01)	0.04 (0.02)	0.95 (0.34)	1.77 (0.35)
130 (12)[e]	0.73 (0.21)	0.41 (0.11)	0.04 (0.01)	0.04 (0.02)	1.21 (0.23)	2.11 (0.54)
163 (13)[f]	1.35 (0.63)	0.65 (0.43)	0.05 (0.02)	0.07 (0.02)	2.12 (0.89)	3.53 (0.76)

SD = standard deviation.
[a] t_1As = concentration of total As after speciation.
[b] tAs = concentration of total As after H_2O_2/HNO_3 digestion.
[c] No. of subjects drinking water containing 29 μg As/L.
[d] No. of subjects drinking water containing 55 μg As/L.
[e] No. of subjects drinking water containing 130 μg As/L.
[f] No. of subjects drinking water containing 163 μg As/L.
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Table 7–6 Arsenic Concentrations in Human Blood Plasma and RBCs of the Study Subjects Who Stopped Drinking Arsenic-Contaminated Water 2 years Before Their Sample Collection

	As in Blood Plasma (μg/L [%]), $n = 25$					
	AsB	iAs^{111}	MMA^{V}	DMA^{V}	t_1As^a	tAs^b
Mean	1.28 [16.7]	1.37 [21.1]	1.95 [27.1]	2.56 [35.1]	7.16	7.48
SD	1.64	0.51	1.29	1.45	3.59	5.05
Minimum	0.59	0.81	0.93	0.95	3.56	2.42
Maximum	8.66	2.59	5.32	5.97	19.5	23.3

	As in Blood RBCs (μg/kg [%]), $n = 25$				As in Blood (μg/L [%]), $n = 25$		
	AsB	DMA^{V}	t_1As^a	tAs^b	$Plasma^b$	$RBCs^b$	$Blood^b$
Mean	5.13 [22.5]	25.1[77.5]	30.2	207	7.48 [28]	18.7 [75]	26.3
SD	7.51	51.8	54.5	165	5.05	10.1	13.8
Minimum	2.12	4.45	7.12	84.1	2.42	7.41	10.3
Maximum	37.8	262	276	887	23.3	45.4	65.1

SD = standard deviation.
[a] t_1As = concentration of total As after speciation.
[b] tAs = concentration of total As after H_2O_2/HNO_3 digestion.
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Table 7–7 Speciated Arsenicals in Urines from Individuals Chronically Exposed to Inorganic Arsenic in Drinking Water[a]

Subject	iAs^{III}	iAs^{V}	MAs^{III}	MAs^{V}	$DMAs^{II}$	$DMAs^{V}$	Sum	Total As
1	8.1	4.5	ND[b]	7.1	ND	18.6	38.3	41.8
2	11.2	12.2	1.1	11.2	18.3	52.7	106.6	122.2
3	25.2	16.8	2.3	37.7	18.2	40.1	140.3	169.3
4	14.7	10.1	1.2	19.3	5.7	90.5	141.3	147.4
5	59.4	72.1	5.2	119.8	59.8	408.2	724.5	734.6
6	104.2	122.8	12.3	276.7	114.2	467.2	1097.5	1211.1

[a]First morning void urine samples were provided by three males and three females (11 to 39 years old) residing in Zimapan. As species were determined by a pH-specific HG-AAS; total As was analyzed by HG-AAS after complete digestion of urine samples. Results are the average concentration (ng As per ml) based on duplicate analyses. The variation of values determined for duplicates did not exceed ± 6.5% of the mean.
[b]ND, not detected.
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Table 7–8 Trivalent and Pentavalent Arsenic Metabolites in HepG2 Cells Exposed to As(III)-Containing Arsenicals[a]

Treatment		Sample	Arsenic Metabolites in Cells and Medium[b]					Sum of As in Culture
Arsenical (μM)	ng As/well		iAs^{III}	MAs^{III}	MAs^{V}	$DMAs^{III}$	$DMAs^{V}$	
iAs^{III} (0.1)	15	Cells	2.9	ND[c]	ND	ND	2.5	30.5
		Medium	10.6	ND	2.6	11.9	ND	
iAs^{III} (1.0)	150	Cells	6.6	6.2	ND	ND	3.1	159.0
		Medium	110.6	20.9	ND	9.3	2.3	
iAs^{III} (10.0)	1500	Cells	67.6	10.4	ND	40	ND	1374.3
		Medium	1079.0	67.8	ND	ND	109.5	
MAs^{III} (1.0)	150	Cells	2.3	24.9	ND	ND	ND	175.3
		Medium	6.6	129.6	2.8	ND	9.1	
$DMAs^{III}GS$ (1.0)	150	Cells	2.4	ND	1.4	28.3	ND	165.0
		Medium	9.3	ND	6.1	117.5	ND	
Untreated culture		Cells	2.4	ND	ND	ND	ND	10.0
		Medium	7.6	ND	ND	ND	ND	

[a]HepG2 cells (6×10^6 cells and 2 mL medium per well) were incubated in the presence of inorganic arsenite (iAs^{III}) for 24 h or in the presence of methyloxoarsine (MAs^{III}) or of dimethylarsenic – glutathione complex ($DMAs^{III}$–GS) for 30 min. At the end of exposure, media and cell were collected for As speciation.
[b]Results are the average concentration (ng As per ml) based on duplicate analyses. The maximal variation among duplicates (±13.6% of the mean) was found.
[c]ND, not detected.
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that toxicity of these arsenicals was independent of cell line type and MMA(III)O was the most potent cytotoxin and equivalent to DMA(IIII), but dimethylated trivalent arsenicals are more cytotoxic than As(III) in most cell types [74]. Other studies investigated interaction of MMA(III)O and DMA(IIII) with DNA in intact human peripheral lymphocytes and found that MAA(III)O and DMA(IIII) damaged DNA at a rate 77 times greater and 386 times greater, respectively,

Table 7–9 Stability of As(III)-Containing Arsenicals in Urine from Three Donors

Donors	Arsenical Added	As^{III} Oxidized During a Given Time Interval*		
		1 Day (%)	1 Week (%)	2 Months (%)
a	iAs^{III}	<1	20	32
	MAs^{III}	14	33	30
	$DMAs^{III}GS$	27	47	100
b	iAs^{III}	8	11	21
	MAs^{III}	36	43	100
	$DMAs^{III}GS$	79	86	100
c	iAs^{III}	2	20	30
	MAs^{III}	26	24	43
	$DMAs^{III}GS$	17	33	42

*Percentages calculated from HG-AAS profiles shown in Figure 7–4. Experimental conditions as described in the legend for Figure 7–4.

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than did As(III) [78]. Hence, MMA(III) and DMA(III) are considered to be direct-acting forms of arsenic that are genotoxic. Even exposures to very low concentrations (0.001–0.01 μM) of these arsenicals induced cell proliferation and production of growth promoting cytokines in normal human epidermal keratinocytes [80]. Similarly, studies on human UROtsa, T24, or HepG2 cells and primary human hepatocytes suggest higher potency of MMA(III)O and DMA(IIII) than As(III) in inducing activator protein-1 (AP-1), DNA binding activity, or AP-1-dependent gene transcription (nuclear phospho-c-Jun) [74]. Also, MMA(III) is up to 26 times more toxic than As(III) in Chang human hepatocytes [73] while 100 times more potent than As(III) as an *in vitro* inhibitor of thioredoxin reductase [82]. Therefore, formation of MMA(III) appears to represent toxification of both As(V) and As(III).

Although natural water contains mostly As(III) and As(V), a few investigators reported the presence of small quantities DMA(V) and MMA(V) [71,83–85]. Interestingly, MMA(III) and DMA(III) were also identified in lake water for the first time in 1999 [82]. Both MMA(III) and DMA(III) have been identified in environmental and biological samples such as human urine [64,68,76,77], cultured human hepatocytes [68], and hamster liver tissues [79], whereas only MMA(III) was detected in human urine [64,70] and rat bile [86].

Some recent reports have identified thioarsenicals in biological samples [52,87–93]. Appearance of thioarsenicals in such samples warrants thorough study of arsenical metabolites therein as well as the mechanism of their metabolism in humans. Normally, iAs undergoes reduction followed by oxidative methylation (Figure 7–9) to form DMA(III). In this process, iAs is enzymatically biotransformed to methylated arsenicals including MMA and DMA; these are the end metabolites and the biomarkers of chronic arsenic exposure [64,75,94]. First, glutathione reduces iAs(V) to iAs(III) acting as reducing agent and then the methyl group is transferred to iAs(III) from S-adenosyl methionine to form MMA(V). Next, an intermediate metabolite MMA(III) is produced from MMA(V) during the methylation process followed by a second methylation where MMA(III) is oxidized to DMA(V) [64,75,77]. Glutathione and

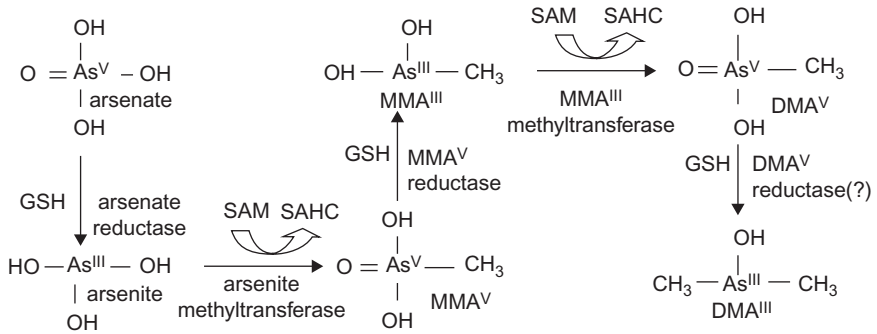

FIGURE 7–9 Putative pathway for biotransformation of inorganic arsenic *in vivo* forming both dimethylarsinous acid [DMA(III)] and methylarsonous acid [MMA(III)]. SAM, *S*-adenosylmethionine; GSH, glutathione; SAHC, *S*-adenosylhomocysteine; DMA^V, dimethylarsinic acid; MMA^V, monomethylarsonic acid. *Reprinted from* [64] *with permission from American Chemical Society.*

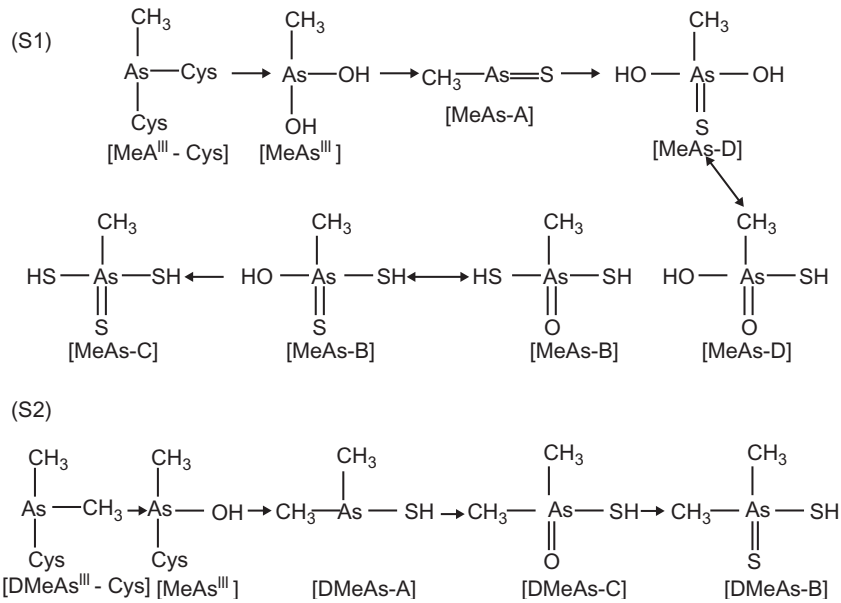

FIGURE 7–10 Proposed reaction pathway for MeAs(V) (S1) and DMeAs(V) (S2) under sulfide-reducing environment. *Reprinted from* [87] *with permission from Elsevier.*

S-adenosyl methionine act as co-substrate [95]. The activity of the first methylation step is monitored by calculating the ratio of iAs/MMA and MMA/DMA. The high ratio value of iAs/MMA indicates poor methylation whereas a low ratio value of MMA/DMA indicates good methylation [47,48]. Children are poor methylators and good excretors in comparison to adults. Thus, children are less susceptible to arsenicism [96].

However, in a sulfide-reducing environment iAs is metabolized to both methylated and thiomethylated metabolites (Figure 7–10) [87]. Figure 7–11 shows HPLC-ICP-MS chromatograms of

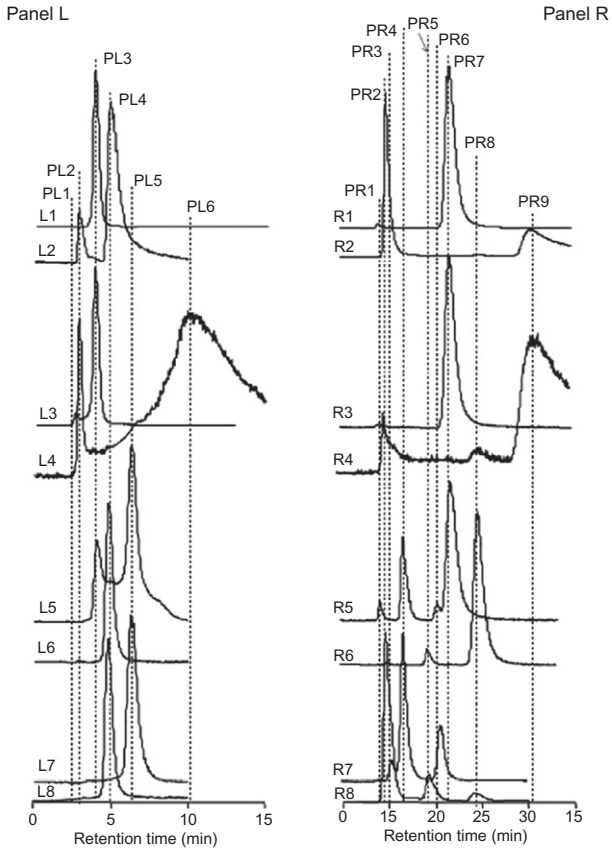

FIGURE 7–11 HPLC-ICP MS chromatograms of reduced standard MeAs^{V} and DMeAs^{V}. In **panel (L):** (L1) a sample (20 μg As L^{-1}) of MeAsIII-I_2 by an anion exchange ES-502N 7C column with a mobile phase containing 15 mM citric acid at pH 2.0 with 10% HNO_3 at 25°C at a flow rate of 1 mL min^{-1}; (L2) aqueous DMeAsIII-I sample; (L3) MeAsIII-Cys; (L4) DMeAsIII-Cys; (L5) reduced std. MeAs^{V} by Reay and Asher; (L6) reduced std. DMeAs^{V} by Reay and Asher; (L7) reduced std. MeAs^{V} by Na_2S and H_2SO_4; (L8) reduced std. DMeAs^{V} by Na_2S and H_2SO_4. In **panel (R):** (R1) an aqueous sample (20 μg As L^{-1}) of MeAsIII-I_2 analyzed by HPLC-ICP MS using a size exclusion GS-220 HQ with 50 mM ammonium acetate buffer (pH 6.5 at 25°C) at a flow rate of 0.6 mL min^{-1}; (R2) aqueous DMeAs^{III}-I sample; (R3) MeAs^{III}-Cys; (R4) DMeAs^{III}-Cys; (R5) reduced std. MeAs^{V} by Reay and Asher; (R6) reduced std. DMeAsV by Reay and Asher; (R7) reduced std. MeAs^{V} by Na_2S and H_2SO_4; (R8) reduced std. DMeAs^{V} by Na_2S and H_2SO_4. The injection volume of the sample was 20 μL. The ion intensities at *m/z* 75 and 77 were recorded with time-resolved analysis software. *Reprinted from* [87] *with permission from Elsevier.*

reduced MMA(V) and DMA(V) under a sulfide-reducing environment [87]. Using this hyphenated technique it is possible to identify and quantify different thioarsenicals in biological samples.

With the development of hyphenated analytical techniques and subsequent toxicity studies of iAs, tri-, and pentavalent methylated arsenicals the toxicity scenario has been changed

Table 7–10 The Currently Accepted National Standards for Arsenic in Drinking Water [103]

Standard	Countries
Standard lower than 0.01 mg/L	Australia (0.007 mg/L, 1996)
Standard of 0.01 mg/L	European Union (1998), Japan (1993), Jordan (1991), Laos (1999), Laos, Mongolia (1998), Namibia, Syria (1994)
Standard lower than 0.05 mg/L but higher than 0.01 mg/L	Canada (0.025 mg/L, 1999)
Standard under consideration for lowering from 0.05 mg/L	United States (1986*), Mexico (1994)
Standard of 0.05 mg/L	Bahrain, Bangladesh (unknown), Bolivia (1997), China (unknown), Egypt (1995), India (unknown), Indonesia (1990), Oman, Philippines (1978), Saudi Arabia, Sri Lanka (1983), Vietnam (1989), Zimbabwe

( ) shows the year standard was established; *new standard value 0.005 mg/L is being proposed and 0.002 mg/L was accepted in 2001.

around the globe. Based on toxicity data from human and mammalian studies, the World Health Organization (WHO) has had a public position on arsenic in drinking water since 1958. Although 0.20 mg/L was considered as an allowable concentration [97], the updated standards in 1963 kept arsenic in the same category and established a lower allowable concentration of 0.05 mg/L without citing any specific reason for this reduction [98]. WHO was updating the progress in research and advancement of arsenicals and an update in 1971 retained arsenic in the toxic substances category reaffirming the allowable value of 0.05 mg/L. Its explanatory notes referred to the fact that figures higher than that quoted are found in a number of Latin American countries and levels up to 0.2 mg/L were not known to have caused difficulties in drinking water [99]. From epidemiological studies in the arsenic-affected areas around the world, especially in Chile and China (province of Taiwan) and data on toxicological studies of different arsenicals, WHO (1984) recommended 0.05 mg/L as a guideline value since a concentration of 0.05 mg of arsenic per liter in drinking water was not associated with any adverse health effects [100].

After development of various hyphenated instrumental analytical techniques the reproducibility and accuracy of determining different arsenicals in water and biological samples were established. Actual data from toxicological studies of arsenicals based on chemical and oxidation states of arsenic were released, which helped and guided WHO to rethink the provisional value of arsenic in drinking water. The last edition of WHO Guidelines for Drinking-water Quality (GDWQ) (1993) established 0.01 mg/L as a provisional guideline value for arsenic in drinking water, because lower levels preferred for health protection were not reliably measurable at that time. In a number of countries, WHO provisional guidelines of 0.01 mg/L have been adopted as the standard [101]. However, many countries have kept 0.05 mg/L as the national standard or as an interim target before tackling populations exposed to lower but still significant concentrations of arsenic in the range of 0.01–0.05 mg/L. Under the 1996 Safe Drinking Water Act (SDWA) amendments [102], the US Environmental Protection Agency (USEPA) proposed a new standard (an MCL) for arsenic in drinking water in June 2000 of 0.005 mg/L (Table 7–10) [103].

This chronological lowering of GDWQ values between 1958 and 2000 is possible due to the outcome of different hyphenated techniques, which have lowered the limit of detection to submicro levels accurately and reproducibly. Also, several toxicological studies have benefited from these techniques to separate, identify, and quantify different chemical species of arsenic in *in vitro* and *in vivo* studies in humans and mammalian species. Consequently, this has helped the policy makers to redraw and reestablish an updated guideline in drinking water, foods, and air.

7.2 Conclusions

The stability of methylated trivalent arsenicals is still a challenge to chemists. Lack of experimental data of methylated trivalent arsenicals on toxicology warrants reliable and authentic reference materials of these arsenicals. Due to the unavailability of reference standard materials of methylated trivalent arsenicals, the generated toxicological data need validation using authentic standard reference materials. Hence, a low cost hyphenated technique with a lower detection limit is sorely needed to quantify arsenicals in different matrices collected from those arsenic-affected populations who consume As-contaminated drinking water at concentrations of less than 0.05 mg/L. In addition, further in-depth research on thioarsenicals is indispensable for the elucidation of metabolic pathways as well as toxicity in humans and other mammalian species.

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8

Mechanism for Arsenic-Induced Toxic Effects

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CHAPTER OUTLINE

8.1 Introduction

Arsenic (As), one of the most widely studied, notoriously poisonous metalloids, is ubiquitous in the environment. It is found in natural and anthropogenic sources, in some abundance in Earth's crust, and in small quantities in rock, soil, water, and air. Arsenic in the environment occurs in both organic and inorganic forms. The major inorganic forms of arsenic include trivalent meta-arsenite (As^{3+}) and pentavalent arsenate (As^{5+}). Among the two states the most toxicologically potent arsenic compounds are in the trivalent oxidation state. This is due to their interaction of trivalent arsenicals with sulfur in proteins and the ability of arsenic to generate oxidative stress. However, humans are exposed to both trivalent and pentavalent arsenicals [1]. Humans are chronically exposed to low levels of arsenic via inhalation in the ambient air and ingestion of food; but the unavoidable source of this poisoning is drinking of arsenic-contaminated groundwater especially in India, Bangladesh, China, and some Central and South American countries [2]. Absorbed arsenic, irrespective of its form, is widely distributed throughout the entire body. Flora [2] recently reported that arsenic concentrations

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in drinking water in Argentina (200 ppb) [3,4], Mexico (400 ppb) [5,6], Taiwan (50–1980 ppb) [7], and the Indo-Bangladesh region (800 ppb) are well above the WHO guidelines' maximum permissible value (10 ppb) [8]. A considerable percentage of the total populations of the world are suffering from chronic arsenicosis due to drinking arsenic-contaminated water [2,9,10]. Understanding the chemistry behind arsenic toxicity and its mode of action depends on several factors and is quite complicated. The major determinants for arsenic to cause toxicity are valence state (trivalent/pentavalent), charge at physiological pH, extent of methylation and electrostatic attraction and repulsion to active sites on important macromolecules, and several pharmacokinetic factors like absorption, distribution, metabolism, protein binding, and excretion [2]. With recent advances in technology and the development of animal experimental models for arsenic toxicity, the understanding of the toxicology of arsenic will continue to improve.

This chapter briefly summarizes the existing literature on toxicology and environmental aspects of arsenicals and describes the molecular mechanisms of arsenic-induced organ pathophysiology and its preventive strategies using antioxidants.

8.2 Biological Consequences of Chronic Arsenic Exposure in Humans

Previous studies suggest that arsenic exposure for a long period of time (chronic exposure) could induce various degrees of toxicities, which might ultimately cause serious organ dysfunctions. Prenatal exposure to arsenic usually results in short- and long-term organ toxicities; both the arsenic and its methylated metabolites can cross the placenta. This exposure can result in impaired fetal growth, fetal loss during pregnancy, or even increased post-birth infant mortality [11]. Besides the development of various organ dysfunctions and illness occurring as a result of arsenic exposure in early life, evidence also shows serious late effects like the development of certain malignancies and other syndromes and diseases [12]. Arsenic is known to be a potent carcinogen; various types of malignancies and solid tumors, including lung, prostate, bladder, cancers, etc. have been reported to develop due to exposure to it [13–17]. Some other reports, however, suggest that malignancies do not develop during low level exposure in some parts of the world (Denmark); it may, instead, reduce the incidence of non-melanoma skin cancer [18]. Combining all this evidence suggests that arsenic-associated carcinogenesis largely depends on the levels of exposure, genetic factors, and geographical locations. Here, it should also be mentioned that malignancies are not the only serious problem resulting from long-term arsenic exposure; other chronic illnesses like hypertension, cardiovascular disease, diabetes, etc. have also been found to be associated with arsenic exposure [11,19]. Further, long-term memory loss and modification of hormonal regulation have also been reported because of chronic exposure to arsenic [11]. Thus, a variety of different chronic illnesses and malignancies can be induced by arsenic exposure, underscoring the diversity of its cellular targets and its ability to deregulate important and diverse cell functions (Figure 8–1).

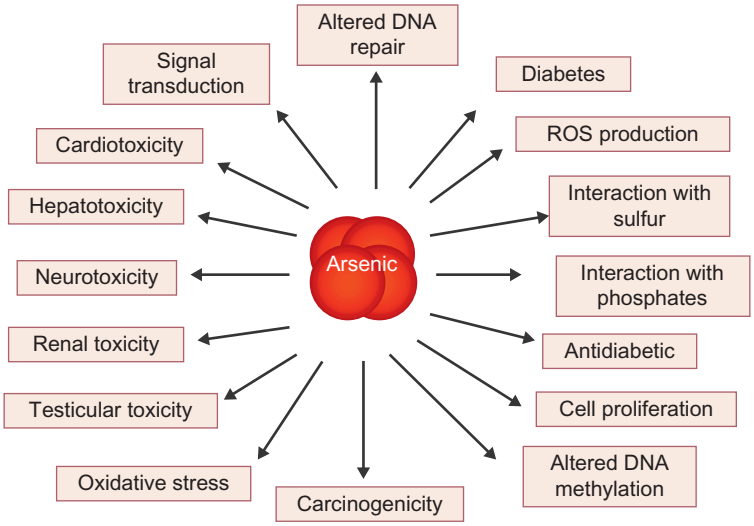

FIGURE 8–1 Biochemical outcomes resulting from arsenic toxicity.

8.3 Arsenic Metabolism

The nutritional requirement for arsenic is not known; however like other toxic metals, the metabolism of arsenic is believed to be associated with the conversion of its most potentially toxic form to the less toxic one, which could either be accumulated in or excreted from the cell, although it does not appear to be biomagnified through the food chain as is found with many toxic metals for example mercury and cadmium [20].

8.3.1 Biomethylation

Since inorganic arsenic-containing molecules are highly reactive and potentially more toxic to humans and other animals, the primary detoxification mechanism of arsenic is considered to be its biomethylation. This idea is also supported by the fact that transformation of inorganic arsenic compounds to the respective methyl derivatives has been observed in the diverse species of yeast, fungi, algae, plants, and animals [21,22]. The organoarsenic species (mostly of methyl derivatives), on the other hand, are widespread in aquatic organisms and it is highly unlikely that consumption of these compounds could constitute any hazard, although there are some exceptions in which certain seafood ingestion has been shown to elevate the urinary arsenic content beyond the expected maximum contaminant level [20,23–25].

Inorganic arsenic is methylated to monomethylarsonic acid (MMA) and finally to dimethylarsinic acid (DMA) by the methyl donor S-adenosylmethionine (SAM) in higher organisms [26]. The transformations are catalyzed by methyltransferases in the presence of glutathione (GSH) and it is found that the trivalent arsenicals are preferred substrates for methylation reactions.

Reduction of pentavalent arsenic to trivalent arsenic might, therefore, be an important step in controlling the rate of arsenic metabolism [27]. It is known that after exposure, methylation of inorganic arsenic reaches a certain level and then begins to decline and results in an increased toxic effect in the species concerned. Evidence shows that DMA excretion reaches its highest level after a few days and at that time the excretion of the inorganic form is substantially increased (while that of MMA is still elevated), suggesting the existence of two successive methylating enzyme activities in this process. In general, arsenic is converted to the less toxic methylated form in the liver and is excreted via urine at 28 h, 59 h, and 9 h (half-lives ranging between 27 and 86 h for different species) following the sequence: As^{5+}<MMA < As^{3+}<DMA [20].

8.4 Pathophysiology of Arsenic Toxicity

8.4.1 Cardiovascular Dysfunction

Cardiac dysfunction such as atherosclerosis, hypertension, ischemic heart diseases, ventricular arrhythmias, peripheral arterial disease, impaired microcirculation, and coronary heart disease are associated with long-term arsenic exposure [28–31]. Arsenic induces cytotoxic effects in cardiomyocytes by reactive oxygen species (ROS) (such as superoxides and hydrogen peroxide) produced by the stimulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase present in the plasma membrane of vascular endothelial cells and vascular smooth muscle cells (VSMCs) [28,32,33]. ROS thus produced cause loss of cardiac actin, reduces size, and damage the nuclei, which coordinates well with disruption of the vascular extracellular matrix and leads to apoptosis via involvement of mitochondria-dependent caspase-3 signaling. It is also reported that arsenic-induced cardiomyocyte death is concentration dependent, with an EC_{50} of 1 mM [2,34,35]. NAD(P)H oxidase-generating ROS couple with nitric oxide (NO) and forms strong oxidant peroxynitrite, which plays a role in the upregulation of inflammatory mediators such as cyclooxygenase-2 [36]. ROS so produced increase the expression of atherosclerosis-related genes such as heme oxygenase-1 (HO-1), monocyte chemo-attractant protein (MCP-1), and interleukin-6 (IL-6) and thus promote the attachment, penetration, and migration of monocytes in VSMC [37]. This also alters VSMCs' focal adhesion proteins, which leads to their proliferation and migration [38]. Singh et al. [28] recently reported that arsenite exposure decreases the activity of endothelial nitric oxide synthase (eNOS) and Akt/protein kinase B, which subsequently decreases nitric oxide (NO) bioavailability, which in turn leads to vascular endothelial dysfunction and associated cardiovascular complications [39,40].

Arsenic exposure causes vasoconstriction of the blood vessels by phosphorylating myosin light chain kinase (MLCK) and increases calcium sensitization, which leads to hypertension [41]. At the same time, it also alters the release of vasoactive mediators in blood vessels leading to elevation of blood pressure [28,42]. Lee et al. [37] showed that sodium arsenite could induce the expression of MCP-1 and IL-6 in vascular smooth muscle cells. Similar results of increased expression of MCP-1 and IL-6 in vascular lesions have also been shown in an *in vivo* model (As-exposed mice). These findings are consistent with the results of some earlier studies in

which Wu et al. [43] showed the increased expression of circulating lymphocyte MCP-1 mRNA and plasma MCP-1 in humans exposed to arsenic. More recent studies by Hsieh et al. [44] suggest that humans exposed to drinking water containing > 10 µg/L of arsenic and carrying two risk genotypes of apolipoprotein E (ApoE) and MCP1 have a > 10-fold risk of carotid atherosclerosis [45]. Moreover, in vascular endothelial cells, arsenic has been shown to increase the synthesis of inflammatory mediators (like leukotriene E (LTE), prostacyclin, tumor necrosis factor-alpha, and nuclear factor kappa B), which induce the pathogenic process of atherosclerosis [28,46,47]. Combining all these observations suggests that As-induced inflammation could act as an important risk factor for atherosclerosis.

Arsenic exposure could also cause enhanced hypertension [2], although this observation is the result of only a few dose–response studies between arsenic ingestion and hypertension [29,48]. Additionally, some previous studies reported increased hypertension prevalence among patients with arsenic-affected skin lesions [49,50]. Signal transduction mechanism studies suggest a close resemblance between arsenic-induced hypertension and that for atherosclerosis, in which vascular redox signaling is suggested to play the major role. Reduced NO bioavailability could also be a major factor in arsenic-induced hypertension [2]. Arsenic trioxide, in addition, could add to the development of ventricular arrhythmia by inducing prolonged Q-T interval and action potential duration [28]. In summary, it may be said that arsenic has the potential to induce cardiovascular complications via oxidative stress accompanied by reduced eNOS activation and enhanced MLCK phosphorylation.

8.4.2 Neurological Disorders

Acute As intoxication is associated with neurological dysfunctions and ranges from neurobehavioral disturbances to memory and cognitive impairments, visual or auditory sensory defects, Parkinson's disease, Guillain–Barré-like neuropathy, verbal miscomprehension, encephalopathy, and peripheral neuropathy [2,28,51–56]. Although the cellular and molecular mechanisms underlying arsenic-induced neurological defects are not clear, involvement of oxidative stress with increased ROS, lipid peroxides along with decreased superoxide dismutase activity, and reduced glutathione levels are considered to be the major reasons behind this [28,57]. After chronic arsenic exposure, numbness of distal extremities (especially the legs), decreased sensibility, ataxia, pain and paresis with nausea, vomiting, etc. are the most common reported symptoms. All these are indicative of systemic peripheral neuropathies [58]. The mechanism for this pathophysiology is, however, not clearly known yet and remains to be elucidated [59–62].

The longer axons of sensory neurons are found to be more affected than motor neurons in arsenic-induced neuropathy. Arsenic exposure (50 µg arsenic/m^3) of smelter workers demonstrated that either demyelination or cytoskeletal defects in neuronal axons are the operative pathologies for the peripheral neuropathy that usually hampers the conduction velocity in peripheral nerves [2]. In addition, arsenic exposure has been shown to alter metabolism of various neurotransmitters (such as monoamines, acetylcholine, gamma amino butyric acid, and glutamate) [63].

Chronic arsenic exposure has been recently reported to cause a significant reduction in monoamines (adrenaline, noradrenaline, dopamine, etc.) in the corpus striatum, frontal cortex, and hippocampal areas of the brain [28]. Signal transduction studies showed that arsenite-mediated neurotoxicity involves the activation of p38 mitogen-activated protein kinase (p38MAPK) and JNK3 pathways and could ultimately induce apoptosis in the cerebral neurons [64]. Moreover, destabilization and disruption of cytoskeletal framework is another adverse effect of arsenic exposure that induces neurotoxicity and eventually leads to axonal degeneration [65]. Thiamine (vitamin B1) deficiency is well known to induce neuronal complications and arsenic could cause this deficiency, which in turn results the inhibition of pyruvate decarboxylase, elevation of blood pyruvate, and ultimately causing encephalopathy [54]. Arsenic-induced oxidative stress causes oxidative DNA damage and subsequent cell death in the brain and leads to the degeneration of dopaminergic neurons resulting in Parkinson-like symptoms [52,53]. Acute exposure of arsenic decreases acetyl cholinesterase activity and causes a cholinergic crisis-like situation with altered mental status and weakness. This can be associated with peripheral neuropathy, neuropsychiatric abnormalities, and extrapyramidal disorders [66]. Moreover, the peripheral nervous system is also affected by disrupting the neuroskeletal integrity because of arsenic toxicity; the nerve conduction velocity in the peripheral nerves is markedly diminished and thus causes peripheral neuropathy [28].

Arsenic and its metabolites (monomethylarsonic acid and monomethylarsonous acid) could suppress the NMDA receptors in the hippocampus, leading to neurobehavioral disorders and cognitive dysfunction as these receptors play a pivotal role in synaptic plasticity, learning, and memory [67,68]. The chronic exposure of arsenic causes morphological changes in axons and nerve fibers of the striatum and thus disturbs central structural organization [28,69]. Arsenic-induced brain encephalopathy involves the disruption of the central nervous system (CNS) [70]. Duration of arsenic exposure is an important factor determining the severity and reversibility of arsenic-induced CNS manifestations and in some cases these may disappear with the cessation of exposure; however, the effects may remain irreversible in some cases [2,71,72].

Calderon et al. [73] reported the adverse effects of arsenic toxicity (such as visual and auditory sensory impairment; memory and cognitive defects) in clinical studies. Many *in vivo* and *in vitro* studies suggest that oxidative stress, induction of thiamine deficiency, and inhibition of pyruvate decarboxylase and acetyl cholinesterase as well as reduction in biogenic monoamines lead to arsenic-induced neurological defects. Also, there are some inconsistent reports on neurotoxicity using animal models and these discrepancies are thought to be due to the varying doses, duration, and route of arsenic exposure used [2,28].

Taken together, these findings act as a summary of the pathophysiological mechanisms involved in arsenic-induced neurological disorders.

8.4.3 Hepatic and Renal Toxicity

Liver is the prime site for arsenic metabolism and considered to be the main target for arsenic to cause toxicity. The highest level of arsenic is detected in the liver [74]. Arsenic-induced

hepatotoxicity is manifested by increased levels of alanine aminotransferase and aspartate aminotransferase. Another primary target organ of arsenic is the kidneys. Arsenite exposure increases ROS production, which consequently enhances the level of lipid peroxidation, other oxidative stress-related biomarkers, and cellular damage in both hepatic and renal tissue [28,75]. Several groups of researchers showed that arsenic-induced hepatotoxicity follows multiple mechanisms, including ROS-mediated oxidative stress, inflammatory response, or metabolic hindrance. This arsenic toxicity depends on the dose; as in acute high-dose arsenic exposure, a number of oxidative stress-related genes (heme oxygenase 1 and metallothionein) are up-regulated [76], although this is not observed after low-dose, chronic arsenic exposure [77]. Moreover, subacute and chronic exposure of arsenic has been reported to enhance ROS generation, and decrease the GSH/GSSG ratio and antioxidant enzyme activities in association with increased lipid peroxidation in hepatic tissues *in vivo* [2,78–81]. Continuous arsenic exposure for long periods of time causes increased hepatic collagen accumulation dose dependently that can be correlated with elevated IL-6 concentrations [82]. An important enzyme of glucose metabolism, pyruvate dehydrogenase (PDH), is susceptible to arsenic-induced ROS generation [83] and its inhibition could cause carbohydrate metabolism disorder resulting in diabetes [84]. An increased level of the oxidative DNA damage biomarker, 8-OHdG, has been found to be associated with arsenic-induced hepatocarcinogenesis [2]. Returning to renal toxicity, arsenic has been reported to cause acute renal failure and chronic renal dysfunction; elevated creatinine levels in the serum have been reported in acute and subchronic arsenic exposure in some cases [85]. The mechanisms of these adverse effects are not very clear. However, because of the direct involvement of kidney in arsenic excretion, hypotensive shock, hemoglobinuric or myoglobinuric tubular injury, or the direct effect of arsenic on tubule cells are thought to be associated with these processes. Studies suggest that sublethal arsenic poisoning is the cause of renal insufficiency and necrosis [86]. Loss of capillary integrity and increased glomerular capillary permeability could be the fundamental lesions in arsenic toxicity resulting in proteinuria. Other injuries in kidney could be due to the presence of hematouria, leukocyturia, and glycouria [2]. In summary, arsenite-induced nephrotoxicity and hepatotoxicity could be thought of as the outcome of oxidative stress, apoptosis, and up-regulation of transcription factors such as AP-1, ATF-2, and Elk-1.

8.4.4 Testicular Toxicity

The effect of sodium arsenite on the male reproductive system is poorly defined. There are some existing reports in which arsenite intoxication is associated with spermatotoxicity [87–89], inhibition of testicular androgenesis, and reduction of the weight of the testes and accessory sex organs [90] in experimental animals. But the actual mechanism causing testicular toxicity from arsenic exposure remains unclear. Mice, continuously exposed to arsenic for 35 days, showed decreased sperm counts and motility [91,92]. Similarly, in rats, exposure to sodium arsenite suppressed spermatogenesis and testosterone release, inhibited testicular enzyme function, and reduced the weight of the male sex organs [90,93]. Arsenic exposure decreased epididymidal sperm count and testicular 17β-HSD and 3β-HSD enzyme activities,

reduced GSH levels, and increased protein carbonyl content in mouse testes. These oxidative stress-induced changes were prevented by ascorbic acid [91]. Waalkes et al. [94] showed that repeated arsenic exposure induced proliferative pre-neoplastic lesions of the testis. Testis proliferative lesions have also been found to be the outcome of estrogen treatment. In addition, estrogen also disrupts spermatogenesis and inhibits androgen production in association with suppressed gonadotrophin secretion from the pituitary [95,96]. Later, Jana et al. [87] investigated the mechanism of the arsenic-induced estrogenic mode of action on the hypothalamo-pituitary-testicular axis and concluded that the adverse effect of arsenite on male reproductive functions occurs via oxidative stress.

8.4.5 Arsenic Carcinogenicity

Existing literature and experimental evidence suggest that arsenic is a potent carcinogen. However, unsolved questions still remain regarding the permissible dose and exposure time that lead to variation in results of different types of cancer [2,15,97–101]. The literature provides sufficient evidence to classify arsenic as a human carcinogen [102]. Its role in laboratory animals is, however, not very clear, although several studies on the carcinogenicity of inorganic arsenic in hamsters were carried out and provided positive results [103–106]. In addition, DMA(V), the predominant metabolite of inorganic arsenic, has been reported as a rat bladder carcinogen [28,102]. Arsenic possesses tumor-promoting properties as is evidenced from its efficacy in activating transcription factors and changing gene expression for cell growth, proliferation, and malignant transformation; the exact molecular mechanism of its action in carcinogenicity, however, needs more study. Recent reports suggest that transcription factors (such as AP-1 and NF-κB) are activated via the arsenic-induced MAPK signaling pathway. These factors, in turn, can alter the gene expression responsible for arsenic-associated carcinogenicity [107]. Also, focal adhesion kinase, Src, Rho, Grb2, EGFR, ERK, cadherins, etc. are reported to be activated by arsenic exposure and remain involved in various cellular functions including carcinogenesis and tumor cell necrosis [28]. In addition, DMA(V) and TMAO(V) could generate oxidative stress and enhance the level of the oxidative DNA damage marker 8-hydroxydeoxyguanosine. As an outcome, cell proliferation is stimulated and carcinogenicity is induced [108,109]. Literature also suggests that arsenic could provoke bladder epithelial cell proliferation, up-regulate c-fos, c-jun, and EGR-1 proto-oncogenes expression, and collectively contribute to bladder cancer [110]. Smoking is another factor that potentiates the adverse effect of arsenic on bladder and lung cancer. These two factors, in combination, play a negative role in healthy subjects and possibly cause DNA damage [111,112]. Similarly, enhanced risk of arsenic-induced skin cancer can be thought of as a synergistic effect of its combination with sunlight. This additive effect can block DNA repair, stimulate angiogenesis, and alter DNA methylation patterns, which might deregulate cell cycle control and block physiological apoptosis [28]. Summarizing, we can say that the carcinogenic action of arsenic in various possible modes is due to enhanced oxidative stress, direct genotoxicity, altered DNA repair, and altered growth factor expressions.

8.5 Mechanism for the Toxic Effects of Arsenic

8.5.1 Oxidative Stress

For many reasons oxidative stress is considered to play a major role in arsenic-induced toxicity. Arsenic usually creates oxidative stress both *in vitro* and *in vivo* via the induction of heat shock or stress proteins [113,114]. GSH depletion could also alter the redox status and create a stressful and toxic situation in the cell [102]. This adverse situation normally arises when excess ROS are generated and react with cellular constituents. In fact, Liu et al. [115] reported that ROS could be detected in human–hamster hybrid cells within 5 min after exposure to arsenite. More evidence in this regard came from a number of experiments in which the generation of $O_2^{\bullet-}$ and H_2O_2 after arsenic exposure was shown in some cell lines such as human vascular smooth muscle cells [116], human–hamster hybrid cells [115], and vascular endothelial cells [32]; whereas induction of only H_2O_2 was detected in HEL30 [117], NB4 [118], and CHOK1 [119] cells. In addition, generation of $^{\bullet}OH$ has been detected in rat striatum [120]. Arsenic-induced free radical formation was first demonstrated by Yamanaka and colleagues [121,122]. They were able to detect a DMA peroxyl radical *in vitro*. Further, the group reported oxidative damage in experimental mouse lungs after exposure of the mice to DMA(V). Studies suggest that oxygen in molecular form reacts with dimethylarsine, forming dimethylarsinic radical and superoxide anion. The second molecule of oxygen then combines and results in a dimethylarsinic peroxyl radical. These arsenic radicals are reported to be detrimental to cells [122]. Arsenic-induced free radical generation in mouse livers has also been reported [2]. Some other groups reported that the primary mechanism of arsenical-induced cell injury, cell death, and cancer involves the inhibition of mitochondrial respiration supported by NAD-linked substrates, the cofactor lipoic acid being used for the pyruvate dehydrogenase complex [123,124]. Specifically, inorganic arsenicals induce oxidative stress by inhibiting mitochondrial respiration and enhanced ROS generation that in turn might cause DNA mutations and ultimately contribute to the development of cancer [123].

The oxidant's metabolizing enzymes, catalase (CAT) and superoxide dismutase (SOD), have been reported to reduce arsenite-induced micronuclei in CHO cells [125]. Besides, antioxidants (vitamin E, methylamine, benzyl alcohol, etc.) could protect human fibroblasts from arsenite toxicity [102,126]. Trivalent organic arsenicals have been reported to inhibit GSH and thioredoxin reductase and thus could decrease the ability of cells to protect against oxidant-induced toxicity [127,128]. Thioredoxin has also been reported to affect gene expression because of its role in the regulation of the binding of transcription factors to DNA [129,130]. DNA damaging ROS are generated from iron released from ferritin *in vitro*, which is significantly enhanced by DMA(III) and DMA(V) in presence of ascorbic acid [131]. In this pathway, free iron plays a central role in generating harmful oxygen species by enhancing the conversion of highly reactive $^{\bullet}OH$ radical from $O_2^{\bullet-}$ and H_2O_2 via the Haber–Weiss reaction [102]. Only DMA(III), however, remains active for iron release in the absence of ascorbic acid. Here, it should be noted that arsenate, arsenite, MMA(V), and MMA(III) remain inactive in iron release from ferritin.

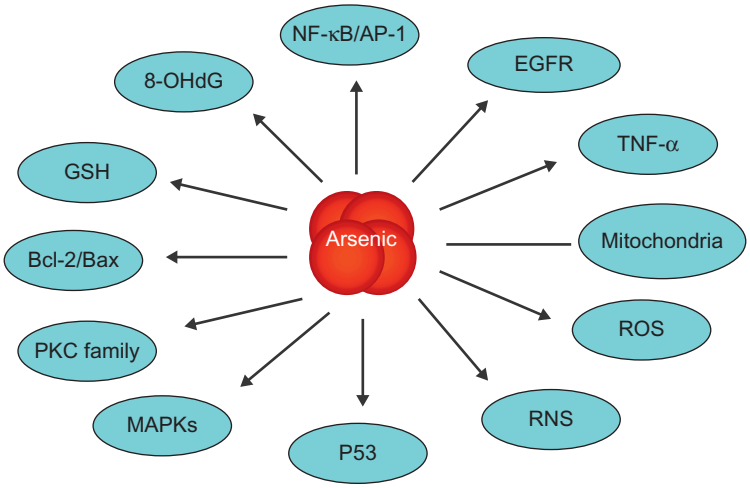

FIGURE 8–2 Different targets and signaling pathways of arsenic toxicity.

8.5.2 Signaling Mechanism

The mechanisms underlying arsenic-induced organ pathophysiology have been studied for a long period of time and some excellent recent reviews and reports have revealed the probable signal transduction pathways involved in this toxic response. Once arsenic enters the human body it can alter signal transduction pathways, which leads to either activation or inhibition of different transcription factors and regulatory proteins that bind to DNA and regulate gene transcription (Figure 8–2) [1,132–135]. Recently, Ghosh et al. [136] showed the involvement of MAPKs in As intoxicated hearts and cardiomyocytes. Data suggest a marked increase in protein content of phosphorylated p38 MAPK and p-JNK in the heart tissue as well as in cardiomyocytes without any change in total protein content of these kinases. In contrast, a less marked increment of pERK was only noted in both the heart tissue and myocytes. Liu et al. [115] also reported that arsenic exposure *in vitro* strongly activated JNK and p38 but to a lesser extent ERK. Das et al. [137] investigated the involvement of p38 and JNK MAPKs in As-induced apoptosis in hepatocytes. When hepatocytes were pretreated with SB203580 and SP600125 separately, the effect of As on cell viability and caspase-3 activation then being determined, it was shown that JNK inhibition significantly increased cell viability and reduced caspase-3 activation in $NaAsO_2$-exposed hepatocytes, whereas p38 inhibition had no effect on these events, indicating that the p38–MAPK pathway is not involved in the prevention of As-induced apoptosis. Das et al. also determined the role of major PKC isoforms in As-induced hepatic pathophysiology and observed that arsenic exposure significantly increased the expression of PKCδ both *in vivo* and *in vitro*, whereas the expression of the other two major isoforms of PKC (PKCα and PKCζ) remained unchanged [137]. All these activation processes appeared to involve generation of oxidative stress, as the free radical scavenger N-acetylcysteine inhibited the activation of the kinase pathways [1,138]. In different cell

lines (such as BEAS-2B cells, HaCaT cells, mouse epidermal JB6 cells, PC12 cells, Rat1 cells, and UROtsa cells), arsenic exposure activates the MAPK signaling pathway through the EGFR/MEK, EGFR/Ras/MEK, or Src/EGFR cascade [2]. It has been reported that arsenic-induced activation of the ERK and p38 pathway is mediated by Ras/Raf/MEK pathways and the JNK pathway is modulated by Rac, Rho, and MEKK3–4 [139]. At the same time in various cell types, EGFR-independent and MAPK-dependent activation of transcription factors by arsenic exposure has been described [2,125,134,140,141].

Arsenic exposure may cause type II diabetes. Recently Singh et al. [28] reviewed the effect of arsenic in diabetes mellitus. Prolonged exposure to it could decrease PPAR-γ expression and might reduce the sensitivity of insulin responsible for the induction of type II diabetes [142]. Replacement of a phosphate group from adenosine triphosphate (ATP) by arsenites slows down glucose metabolism, interrupts energy production, and interferes with ATP-dependent insulin secretion [143]. Because of its high affinity for sulfhydryl groups, arsenite forms covalent bonds with this group in insulin, with its receptors, as well as with the other molecules and enzymes involved in glucose metabolism [143]. In addition, a significant decrease in glucose-6-phosphatase activity due to chronic exposure to arsenic may cause hypoglycemia in both liver and kidneys [144]. Inorganic arsenic could increase oxidative stress and in turn leads to overexpression of various stress mediators such as NF-κB, JNK/SAPK, and hexosamine, causing insulin resistance and dysfunction of beta cells of the islets of Langerhans [145]. Experimental data suggest that trivalent arsenicals (like inorganic arsenic [iAs(III)], dimethylarsinous acid [DMA(III)], and monomethylarsonous acid [MMA(III)]) efficiently suppress Akt/protein kinase B phosphorylation by inhibiting 3-phosphoinositide-dependent kinase-I (PDK-1) activity and could cause significant inhibition of insulin-dependent glucose uptake. These sequences of steps result in hyperglycemia [28,146]. In summary, the results of all these studies suggest that the induction of arsenic-associated diabetes and its modulation is the outcome of decreased PPAR-γ expression, interference with ATP-dependent insulin secretion, altered glucocorticoid receptor mediated transcription, and PDK-1 inhibition.

8.5.3 Role of Transcription Factor

MAPKs are generally considered to be the mediators of apoptotic cell death under various pathophysiological conditions [147]. It has been reported that intracellular oxidative stress stimuli can activate both NF-κB and MAP kinase modules [148]. Further, signal transduction via the MAPK pathway also activates the transcription factor activator protein-1 (AP-1) [1,132,134]. Literature suggests that arsenite activates JNK and p38 in HeLa cells following the stimulation of AP-1 transcriptional activity. This process leads to increased expression of the proto-oncogenes c-Jun and c-Fos [149].

Luster and Simeonova [150] correlated the activation of the MAPK pathway with hyperproliferation of bladder epithelium and suggest that the increased activation of AP-1 followed by the expression of AP-1-related genes plays some role in cell proliferation. Simeonova et al. [151] showed that the pathway for this activation probably involves c-Src and epidermal growth factor receptor (EGFR) signaling cascades.

Barchowsky et al. [152] showed that c-Src is activated at low arsenite concentrations (<5 μM) and is also sufficient to induce cell proliferation and to increase H_2O_2 and superoxide accumulation, H_2O_2-dependent tyrosine phosphorylation, and NF-κB-dependent transcription. The protein tyrosine phosphatases (PTPs) are known to act as negative regulators of EGFR. These molecules contain a preserved thiol group, which is oxidized by ROS and ultimately results in their inactivation. This process in turn leads to the transactivation of EGFRs without EGF binding [1,134]. No direct evidence has been found so far describing the interaction of arsenic with EGFR. But some reports proposed that arsenic could interact directly with the -SH group of EGFR or indirectly via ROS and this interaction might cause structural changes or dimerization [2].

Another report suggests that arsenic increases levels of EGFR ligand and heparin-binding EGF, and activates EGFR phosphorylation in human bronchial epithelial cells (downstream of EGFR effects include increased pERK and cell cycle promoter cyclin D1 levels) [153]. In addition, phosphorylated EGFR levels were found to be higher in those specimens (in human lung tumor biopsies) obtained from subjects with elevated toe nail arsenic content compared with subjects with lower exposures [1]. Arsenic also activates p53 in a dose-, cell type-, oxidative state-, and chemical species-specific manner; e.g., arsenite (As^{3+}) has the greatest ability to activate p53, although MMA shows no effect [154]. The role of p53 is considered to be important as its activation initiates apoptosis through DNA damage [2,155–157].

The transcription factor nuclear factor erythroid-2-related factor 2 (Nrf2) is also affected by arsenic exposure. Nrf2 tightly regulates the transcriptional machinery and gene expression, and controls the cellular defense system in response to exogenous oxidative insult. In normal its physiological state, Nrf2 remains bound to Keap1 as an inactive complex. Upon a stimulus via ROS, Keap1 renders Nrf2 free for its translocation to the nucleus by some chemical or electrophilic modification. After translocation to nuclease, Nrf2 activates the antioxidant-responsive element as well as the electrophilic-responsive element and stimulates the expression of relevant transcriptional proteins [2]. Hughes et al. [1] reported that tert-butylhydroquinone (tBHQ) and sulforaphane (SF) can protect a cell from trivalent arsenic-induced toxicity by activation of Nrf2 *in vitro*. With a knockout animal model it was shown that Nrf2 null mice are more prone to arsenic-induced hepatic and bladder toxicity compared to Nrf2 homozygous mice [158]. Wang et al. [159] also suggested that Nrf2 activation by arsenite or MMA(III) is different from that by tBHQ or SF.

8.6 Preventing Arsenic-Induced Toxic Effects by Antioxidants

Among the most important factors governing the toxic effects of arsenic are the induction of ROS and depletion of antioxidant defenses in a system. It is, therefore, evident that some device that can increase the antioxidant capacity of the cells can be a therapeutic strategy in arsenic poisoning and this could be accomplished by either reducing the possibility of the metal interacting with critical biomolecules of the cells or by supplementation with antioxidant molecules from appropriate external sources. Some of the important antioxidant

molecules that have been explored for their role in reversing arsenic-induced oxidative stress and related organ dysfunctions have been discussed in brief.

8.6.1 Role of Taurine

Taurine (2-aminoethanesulfonic acid) is a derivative of the sulfur-containing amino acid cysteine and is present in many tissues of mammals in high concentrations. It has been reported that the conditionally essential amino acid taurine accounts for 25 to 50% of the amino acid pool in myocardium, possesses antioxidant properties, and has the ability to reduce arsenic-induced oxidative burden of cardiac damage [136]. It is neither metabolized nor incorporated into cellular proteins, suggesting that it may play an important role in cellular cytosolic functioning [136].

8.6.1.1 Taurine in Prevention of Arsenic-Induced Cardiac Disorder

Arsenic exposure reduced viability of cardiomyocytes, increased ROS production and intracellular calcium release, and induced apoptosis by mitochondrial-dependent caspase-3 activation and poly-ADP ribose polymerase (PARP) cleavage. In arsenic-induced cardiomyocytes death, the transcription factor NF-κB plays an important role and it has been observed that all these changes are associated with increased IKK and NF-κB (p65) phosphorylation. Results by Ghosh et al. [136] suggest that arsenic induces a marked increase in the activity of p38 and JNK MAPKs, but not ERK to that extent in heart tissue as well as in cardiomyocytes. Arsenic administration also down-regulated the anti-apoptotic (Bcl-2, Bcl-xL) and up-regulated the pro-apoptotic (Bax, Bad) Bcl-2 family proteins in cardiomyocytes. Taurine treatment, on the other hand, mitigated all these apoptotic events and maintained the balance, suggesting its protective role in arsenic-induced cardiomyocyte apoptosis is mediated via the attenuation of p38 and JNK MAPK signaling pathways. Also, arsenic administration altered a number of cardiac oxidative stress biomarkers and other apoptotic indices *in vivo* and taurine supplementation reduced the activation of this pathway and blocked the apoptotic signaling cascade. Results suggest that taurine supplementation prevented this arsenic-induced myocardial pathophysiology, attenuated NF-κB activation via IKK, p38, and JNK MAPK signaling pathways, and represents a promising approach for the protection of heart tissue against As-induced cardiovascular burden [136].

8.6.1.2 Taurine against As-Induced Hepatic and Testicular Apoptosis

The hepatotoxic effect of arsenic has been investigated extensively but its exact mechanism is still not clear. The main cause for arsenic-induced hepatic disorder is oxidative stress and the exhaustion of GSH in the liver [160–162]. Arsenic exposure significantly increased the level of all the markers related to hepatic oxidative stress. Arsenic administration also caused mitochondrial injury and disturbed the balance of pro-apoptotic and anti-apoptotic members of the Bcl-2 family proteins, which leads to increased level of Apaf-1, activation of caspase 9/3, cleavage of PARP protein, and ultimately apoptosis. Investigating the detailed cell signaling pathway reveals that arsenic exposure markedly increased JNK and p38 phosphorylation. It also up-regulated PKCδ and pre-exposure with its specific inhibitor (rottlerin) blocked the

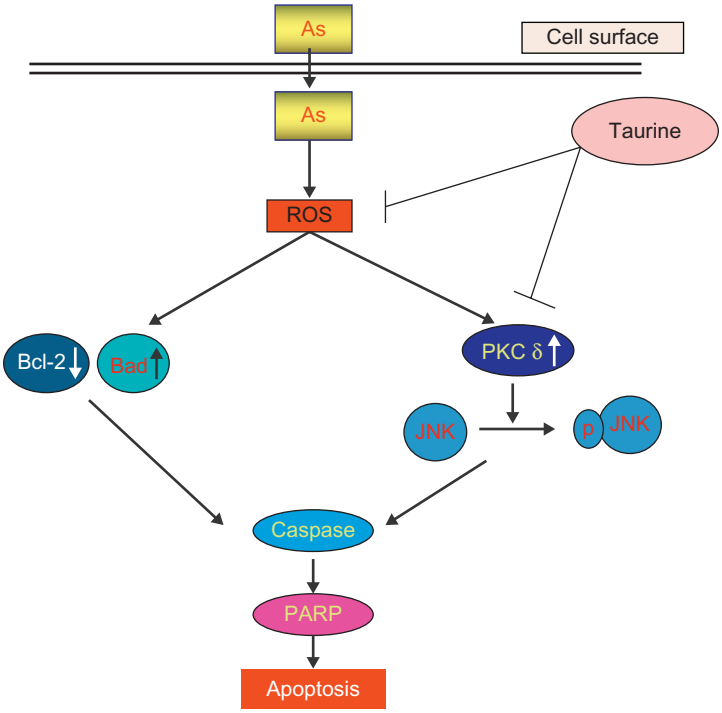

FIGURE 8–3 Schematic diagram showing the protective action of taurine against As-induced hepatotoxicity [137,160].

activation of JNK suggesting that PKCδ is upstream for As-induced JNK activation and mitochondrial-dependent apoptosis [137,160]. On the other hand, taurine treatment both pre- and post-arsenic exposure and/or incubation of the hepatocytes with taurine was found to be very effective in mitigating As-induced oxidative stress and apoptosis [137,160]. Combining all, the results suggest that taurine treatment confers its hepatic protection via blocking the activation of PKCδ and JNK, as well as the generation and accumulation of ROS (Figure 8–3).

Many reports from the literature suggest that arsenic also has a severe toxic effect on testicular tissue of experimental animals [87,163,164]. Das et al. [163] showed that arsenic exposure causes testicular apoptosis through the mitochondrial-dependent pathway via mitogen-activated protein kinases (MAPKs) and Akt as well as NF-κB (p65) in testicular tissue. As administration significantly decreased testicular steroidogenic enzymes, Δ^5-3β-HSD and 17β-HSD activities reduced the plasma testosterone level, testicular sperm count, and sperm motility [87,163]. In addition, As exposure increased the oxidative stress, ROS production, and serum TNF-α level, and decreased the activities of the antioxidant enzymes in the testicular tissue [165]. However, taurine supplementation was found to be effective in counteracting As-induced oxidative stress through its antioxidative properties and amelioration of apoptosis in testicular tissue by down-regulating the activation of phospho-ERK1/2, phospho-p38, NF-κB and other mitochondrial-dependent signaling molecules, as well as by up-regulating the expression of Bcl-2 and other molecules of the phospho-Akt signaling pathways.

8.6.1.3 Taurine against As-Induced Oxidative Cerebral and Renal Disorders

In addition to heart, liver, and testis, arsenic intoxication has also been detected in the kidneys and brain [166,167]. Increased productions of ROS and RNS as well as enhanced lipid peroxidation and decreased intracellular antioxidant functions have been reported in the kidney tissue of As-exposed experimental animals. Intracellular mechanisms suggest that oxidative stress-mediated renal dysfunction is associated with mitogen-activated protein kinases (MAPKs) and NF-κB, which ultimately induce mitochondria-dependent apoptotic cell death [166]. In the brain, both chronic and acute As toxicity have also been found to be mediated via oxidative stress, leading to enhanced ROS formation, lipid peroxidation, etc. and decreased activities of the antioxidant and membrane-bound enzyme acetylcholine esterase and levels of reduced glutathione [167]. Treatment with taurine prior to arsenic administration, however, effectively ameliorated As-induced oxidative renal and neuronal dysfunctions and apoptotic cell death [167]. These results suggest that taurine possesses the ability to ameliorate arsenic-induced oxidative insult and renal damage, probably because of its antioxidant power and functioning via MAPKs/NF-κB and mitochondria-dependent pathways. This unique molecule could, therefore, be considered as a beneficial agent in renal and cerebral oxidative stress [160].

8.6.2 Role of N-Acetyl Cysteine

N-Acetyl cysteine (NAC), the thiol-based antioxidant and derivative of cysteine and precursor of reduced glutathione, plays an important role in the protection of cellular constituents against oxidative damage and in the detoxification of many electrophiles. The protective mechanism of NAC originates from its ability to stimulate and sustain the intracellular levels of reduced glutathione levels, thereby maintaining intracellular GSH levels and detoxifying ROS [168]. The other advantage of NAC is that it possesses metal chelating properties. Recent studies by Modi et al. [169] suggested that co-supplementation of NAC along with zinc reduces the arsenic-induced oxidative burden in rat liver and kidneys. Administration of NAC and succimer in combination, post-arsenic exposure, led to a significant removal of arsenic from soft organs [170]. More recently, protective effects of N-acetylcysteine against arsenic-induced oxidative stress and reprotoxicity have been reported [168]. Moreover, Flora [2] also presented evidence of a novel therapeutic combination of NAC with meso-2,3-dimercaptosuccinic acid (DMSA), which provides greater effectiveness during chelation of arsenic in rats by co-administration.

8.6.3 Role of Melatonin

Melatonin or N-acetyl-5-methoxytryptamine, the main secretory hormonal product of the pineal gland, participates in many physiological functions, such as control of reproductive functions, modulation of immune system activity, limitation of tumorogenesis, and effective inhibition of oxidative stress due to its efficacy as a free radical scavenger and indirect antioxidant [171–174]. Due to its small size and lipophilicity, melatonin can cross biological membranes easily and reach all the compartments of the cellular system. Melatonin acts as an antagonist for a number of endogenous and exogenous free radicals during various cellular processes. Moreover, it has been shown to be an efficient protector of DNA, protein, and lipids in cellular membranes [171,175]. It can stimulate GPx in the brain and provide an indirect

protection against free radical attack. Melatonin has been shown to prevent the induction of free radical damage in various ways, such as that caused by ingestion of toxins, ionizing radiation, ischemia, reperfusion, and excessive exercise [144]. Mahabady et al. [171] recently showed that melatonin and vitamin E protect against sodium arsenite-induced skeletal malformations in rats and they suggested that melatonin has more prophylactic effect than vitamin E on the incidence of sodium arsenite-induced cleft palate and spina bifida, but this difference was not significant. Pal and Chatterjee [144] investigated the protective role of melatonin against arsenic-induced metabolic toxicity in Wistar rats and suggested that melatonin opposes the carbohydrate depleting effect of arsenic in liver. This carbohydrate depletion may in turn contribute to the accumulation of glycolytic intermediates in liver, and thus help in increasing glucose 6-phosphatase activity to provide more glucose to blood to replenish the loss of blood glucose due to arsenic treatment. Combining all these facts, melatonin is found to reduce the arsenic-induced hypoglycemic condition with associated glycosuria and increased glycogenolytic and decreased glycolytic activities of the liver. Accordingly, it may be concluded that melatonin can serve as a prophylactic agent against arsenic-induced metabolic toxicity [144].

8.6.4 Role of α-Lipoic Acid

α-Lipoic acid (LA) is a low molecular weight dithiol antioxidant and is an important co-factor in several multienzyme complexes in the mitochondria [176]. LA, and its reduced form dihydrolipoic acid (DHLA), has two free sulfhydryl groups and the two forms LA/DHLA possess a high antioxidant potential capable of quenching reactive oxygen and nitrogen species such as hydroxyl radicals, peroxyl radicals, superoxide, and hypochlorous acid [177]. In addition, LA also has metal chelating activity. Hatch et al. showed that lipoic acid acts as an antidote in arsenic-induced toxicity [178] and as it is effective in a micromolar range (millimolar NAC is needed for a similar effect), it has an advantage over NAC in protecting against GSH loss [179]. Its coadministration with DMSA has been shown to be of significant value in experimental lead intoxication and could also be expected to have the same possible beneficial effect in arsenic poisoning [170]. Samuel et al. [180] reported that lipoic acid treatment mitigates arsenic-induced protein oxidative damage of rats, because of its antioxidant nature consisting of free radical scavenging and metal chelating properties. A closer look at all these reports suggests that the protective effects of LA against arsenic-induced and oxidative stress-mediated toxicity could be attributed to its ability to be reduced by NADH to dihydrolipoic acid (DHLA), which, being a stronger antioxidant than LA, could scavenge excess oxidants and recycle other antioxidants such as vitamin E, vitamin C, and glutathione; it could form chelate with arsenic, thus preventing free radical generation and diminishing oxidant attacks on biomacromolecules. Also, LA could promote antioxidant defense by elevating the levels of antioxidant molecules (like GSH) via the induction of phase II enzymes.

8.6.5 Role of Silymarin and Quercetin

Silymarin and quercetin are polyphenolic antioxidant flavonoids which possess anticancer and cytoprotective effects and are widely available in vegetable sources [181]. Bioflavonoids,

ingested through a well-balanced diet, could function with diversified pharmacological properties and prevent a wide variety of human illnesses [182]. Bongiovanni et al. [183] investigated the effects of the plant flavonoids silymarin and quercetin on arsenite-induced oxidative stress in CHO-K1 cells and reported the biomedical potential of these flavonoids in arsenic-induced cytotoxicity in the CHO-K1 cell line. Soria et al. [182] showed the differential effects of these flavonoids on arsenite-induced cytotoxicity in two human breast adenocarcinoma cell lines. Mishra and Flora [184] showed the efficacy of quercetin and a thiol chelating agent, monoisoamyl 2,3-dimercaptosuccinic acid (MiADMSA), either individually or in combination against arsenic-induced oxidative stress and mobilization of metal in mice. They suggested that combination therapy with an antioxidant and a thiol chelator might be a better treatment option than monotherapy to treat chronic cases of arsenic poisoning. Ghosh et al. [185] demonstrated that a combination of QC and DMSA in a nanocapsulated drug delivery system provided better therapeutic efficacy than its bulk form and it protected liver from arsenic-induced fibrosis, safeguarded cells from ROS-induced damage, restored mitochondrial integrity, and down-regulated ROS-induced signaling pathways that led the cells toward p53-dependent apoptosis [186].

8.6.6 Herbal Antioxidant

Arjunolic acid (AA: 2,3,23-trihydroxyolean-12-en-28-oic acid, Figure 8–4A), a natural chiral triterpenoid saponin (isolated from the bark of *Terminalia arjuna*), is well known for various biological functions, including cytoprotective [187], anti-fungal [188], anti-bacterial [189], anti-diabetic [190], and insect growth inhibitor activity [191]. Several researchers demonstrated the role of arjunolic acid against arsenic-induced organ pathophysiology [192,193]. Sodium arsenite administration at a dose of 10 mg/kg body weight for 2 days significantly induced severe oxidative stress, altered the prooxidant–antioxidant balance in the hepatic tissue as evidenced by significant reduction in the antioxidant enzymes' activities, and depleted cellular reduced glutathione and total thiols level. In addition, the same exposure enhanced leakage of hepatic serum marker enzymes (alanine transaminase and alkaline phosphatase) indicating significant hepatic damage. It also enhanced levels of lipid peroxidation and protein carbonylation, which are considered to be the two important parameters of oxidative stress-induced organ pathophysiology. As-induced cell death is primarily necrotic in nature as confirmed by DNA fragmentation analysis. Arjunolic acid supplementation at a dose of 20 mg/kg body weight for 4 days prior to arsenic exposure prevented all the alterations and protected the cells from necrotic death. Histological findings also confirmed the disruption of liver architecture around the central vein associated with extensive centrilobular necrosis and bile duct proliferation. Arjunolic acid pretreatment prevented such changes and maintained the normalcy of the organ. Arjunolic acid treatment prevented arsenic-induced ROS production. This protective action arises because arjunolic acid itself has a radical scavenging property as was evidenced from its superoxide, hydroxyl, and nitric oxide radical quenching ability in a cell-free system [187,194]. In addition, arjunolic acid also contains one carboxylic hydrogen atom, which can easily be abstracted by any free radical (Figure 8–4B), thus making arjunolic acid a potent radical scavenger and antioxidant. Therefore, it is likely that part of the beneficial effects

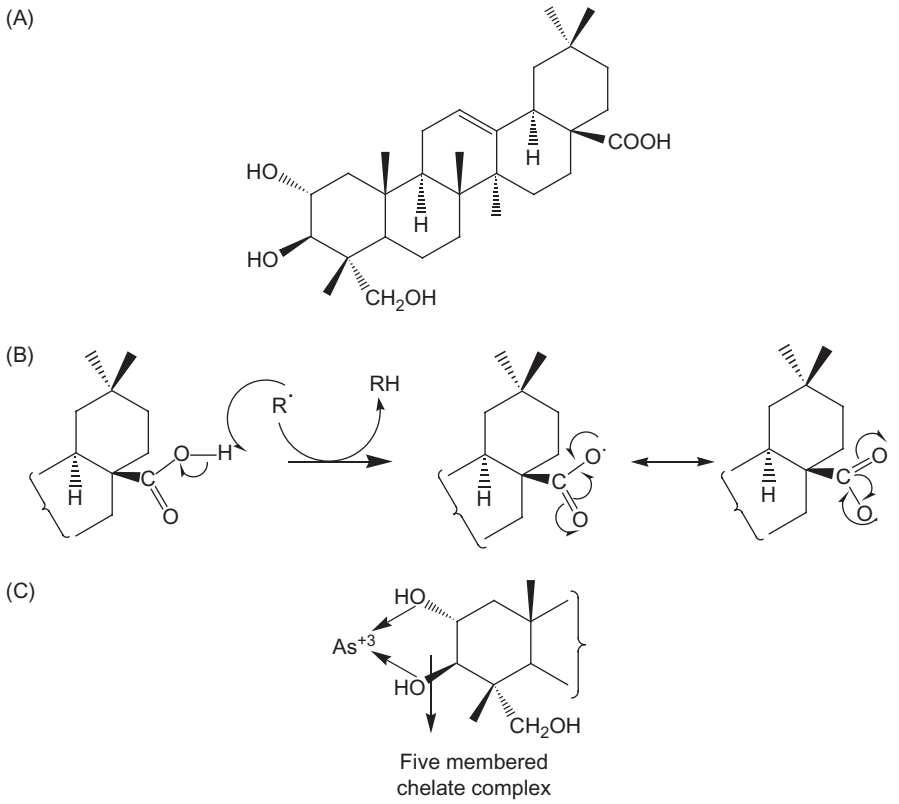

FIGURE 8–4 (A): Structure of arjunolic acid. (B): Possible mechanism of free radical scavenging activity by arjunolic acid. (C): Possible site of chelation of arjunolic acid with arsenic [187,193].

of arjunolic acid result from the detoxification of ROS produced during arsenic intoxication. On the other hand, arjunolic acid also contains polyhydroxyl groups, two of which are vicinal; these two hydroxyl groups may form a five-membered chelate complex with arsenic (Figure 8–4C) and consequently prevent arsenic from exhibiting its toxicity [187,194]. Arjunolic acid can, therefore, be used as a reliable chelator against arsenic-induced toxicity.

In addition to the liver, similar results on the beneficial role of arjunolic acid have been reported for arsenic-induced organ dysfunction in the kidneys, brain, testes, and heart tissue [136,137,163,195].

8.7 Conclusions and Future Directions

Arsenic (As)-induced organ dysfunction and apoptosis have received considerable attention in the past, which continues up to the present time. New relevant information on arsenic-induced apoptosis has been provided recently. Collective data suggest that arsenic, after entering a cell, primarily disturbs the balance between prooxidants and endogenous

antioxidants, which in turn triggers a number of pathways and ultimately determines the fate of the cell either to survive or to undergo apoptosis [123]. Although the mechanism of arsenic-induced toxicity is still not clear, findings from mechanistic studies indicate that ROS produced by arsenic play the most crucial role in exerting its toxic effects in various organ pathophysiologies. Mitochondria are considered to be one of the major targets for the generation of reactive species. The downstream cascade including mitochondrial depolarization and caspase-3 activation could be influenced by ROS produced earlier in the system. Inorganic arsenic and its methylated derivatives also play a key role in disturbing intracellular calcium while inducing apoptosis [196]. The unique molecule 8-OHdG is considered to be an excellent marker of arsenic-induced oxidative stress damage on DNA as its increased levels have been reported in arsenic-exposed animals [123]. The contribution of MAPK pathways to cell growth regulation and death during arsenic exposure has also been reported. In fact, data suggest that some important cellular functions such as proliferation, differentiation, and apoptosis might be controlled by various arsenic-induced signaling pathways. Based on the published reports, the following points can be presented as key features in arsenic-induced cellular responses leading to apoptosis. These are: generation of ROS and RNS; increased levels of 8-OHdG; intracellular GSH depletion, Ca disturbances, and PKC activation; Bax/Bak-dependent signaling; and activation of c-Jun–N-terminal kinase (JNK) as well as transcription factors, AP-1/NF-κB and DNA strand breaks [123]. Future studies are needed for a better insight into dissecting the exact mechanisms of action as well as identifying the target genes and modern techniques like microarray-based gene expression analysis and others that would be very helpful in this particular direction of research [2]. From the above discussion, it is evident that the role of oxidative stress and associated biochemical events in apoptosis deserves special attention.

It is also known that various antioxidants could protect cells from oxidative injury. Since arsenic causes toxicity by damaging the body's oxidative defense mechanism, supplementation of suitable antioxidants via chelation therapy with a proper chelator (like a thiol chelator) can be used as a possible solution. A lipophilic thiol chelator (like MiADMSA) may be logically preferred for this purpose. Here, it should be kept in mind that this approach would play a major role for the identification of a safe, suitable, and practical effective treatment for arsenic poisoning in the future. Some groups believe that chelator therapy alone may not be beneficial for better clinical recoveries and a combination therapy will be more effective [2,170]. In support of this opinion it can be said that ROS and RNS play the major role in arsenic poisoning via the direct binding with SH groups. Combination therapy using antioxidants and a thiol-chelating agent could, therefore, be a major future strategy for the treatment of chronic arsenic poisoning.

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9

Arsenic-Induced Mutagenesis and Carcinogenesis: A Possible Mechanism

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CHAPTER OUTLINE

9.1 Arsenic, a Potent Mutagen and Carcinogen

Several international agencies and government organizations recognize arsenic as one of most toxic environmental pollutants. The database of the Integrated Risk Assessment System (IRIS) of the Environmental Protection Agency (EPA) suggests that chronic and oral exposure of arsenic may result in hyperpigmentation, keratosis, and finally cancers of several organs in the body [1]. The EPA and the disease registry division pointed out from the epidemiological data that arsenic manifests carcinogenic growth in the liver, lungs, bladder, skin, and several other organs in affected individuals [2]. In line with this finding, the American Cancer Society (ACS) indicated that chronic environmental or occupational arsenic exposure may initiate free radical-associated genotoxicity, oxidative tissue degeneration, and cancerous growth [3]. The Agency for Toxic Substances and Disease Registry (ATSDR) of the Department of Health and Human Services (DHHS) has categorized inorganic arsenic as a potent human carcinogen [4]. Recently, environmental arsenic exposure has been a great concern in a number of countries. The International Agency for Research on Cancer (IARC) also suggests the potent carcinogenic

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nature of inorganic arsenic due to the consumption of arsenic-contaminated drinking water by a large population in certain areas worldwide [5,6].

The International Groundwater Resources Assessment Centre (IGRAC) suggests that exposure to inorganic arsenic (iAs), a potent human carcinogen and toxicant, occurs mainly via drinking water and industrial emissions. Long-term exposure to arsenic may result in cancerous growth in several organs [7]. The World Health Organization (WHO) has stated that arsenic is widely distributed throughout Earth's crust, and mixes with groundwaters, which are consumed by a vast population in several countries [8]. In addition to arsenic, other metals are recognized to exert a toxic impact on different metabolic processes. The European Confederation of Iron and Steel Industries and the International Council of Mining and Metals suggested that environmental and/or occupational exposure to arsenic and some other metals interferes with several genetic events, and manifests tissue/organ carcinogenesis in humans [9,10].

Moreover, the epidemiological data highlight that the adverse effects of arsenic in reproductive, pregnancy, and gestational outcomes of women are also evident with long-term health anomalies in their children [11]. Animal models to study iAs-induced cancer in skin, a major site for effects in humans, have recently been suggested by a few investigators [12–14] and this is basically in agreement with the report of the National Research Council (NRC) [15]. Gestational arsenic exposure in rodents is known to be associated with tumors of the liver, ovary, lung, and with adrenal and significant pre-neoplastic lesions in the uterus and oviduct of the offspring [16,17].

9.2 Epidemiological Perspectives of Arsenic-Induced Human Cancers

Arsenic, a human carcinogen, potentially affects ≈160 million people worldwide via exposure to it through drinking contaminated water, industrial processes, and the generation of power from coal. It is also widely used in agriculture and was formerly used extensively in medicine [18]. Exposure to arsenic may also occur through the ingestion of foodstuffs containing inorganic and organic arsenicals. It is widely established that the liver and lungs are the main target organs for arsenic-related carcinogenesis [19,20]. Arsenic induces tumors in several organs, especially the lung, which may exhibit features like squamous cell-type specificity and high tumor incidence even among non-smokers [21].

A study conducted in Michigan, USA, revealed an association between arsenic levels and several diseases outcomes, which include different forms of cancer, diseases of the circulatory and respiratory system, diabetes mellitus, and kidney and liver diseases. Skin or bladder cancers have been widely reported among arsenic-exposed persons [22–24]. Lung cancer is observed in smelter workers [25], while males are considerably more susceptible than females, and low body weight, presumably a result of poor nutrition, is predisposing [26].

A strong association between chronic arsenic exposure and various adverse health effects, including cardiovascular diseases, neurological defects, and cancers is now widely known [15,24,27,28]. Though the final disease outcomes are well defined, the processes involved in arsenic carcinogenesis remain an enigma. A variety of mechanisms, both genotoxic and

non-genotoxic, have been proposed to explain the carcinogenicity of arsenic at the cellular and molecular levels [29,30]. During the last decade, a number of mechanisms have been proposed for arsenic-induced carcinogenesis but the elucidation of a definite mechanism is still awaited. To protect the population from long-term arsenic exposure, the EPA lowered the maximum concentration limit (MCL) for arsenic from 50 to 10 µg/L. The acceptable level for maximum arsenic concentrations in safe drinking water was also reduced to 10 µg/L [31].

9.3 Arsenic-Associated Metabolism and Carcinogenesis in Animal Models

Arsenic metabolism is diversified in primates, rodents, and humans. Certain primates do not methylate inorganic arsenic [32]. Sometimes, a dissimilar metabolic profile of iAs is shown in different variants of even the same animal species. The polymorphic nature of the methyltransferase gene in primates [33] and humans [34] is one of the determining factors of the differential metabolism of inorganic arsenicals (Figure 9–1). This indicates that the same pattern of arsenic exposure may have varied cytotoxic effects in sex- and age-matched persons with similar socioeconomic/demographic and nutritional profiles, only because of their interindividual genetic variations [35] (Figure 9–2). Inorganic arsenic and its several methylated compounds are described as cytotoxic and genotoxic to different degrees [36]. Among those compounds, in terms of severity, dimethylarsinic acid (DMA) has been shown to exhibit a wide range of cytotoxicity, mitotic arrest, and tetraploid induction [37]. Thus, DMA has been used in a number of animal models to study arsenic-induced tumorigenesis and carcinogenesis. Arsenical compounds show less efficacy towards mutagenicity in animal experiments alone [38,39]. Monomethylarsonic acid (MMA) has been detected in the urine in human cases of carcinogenesis [40]. Arsenical metabolites produced during arsenic metabolism are shown in Figure 9–1.

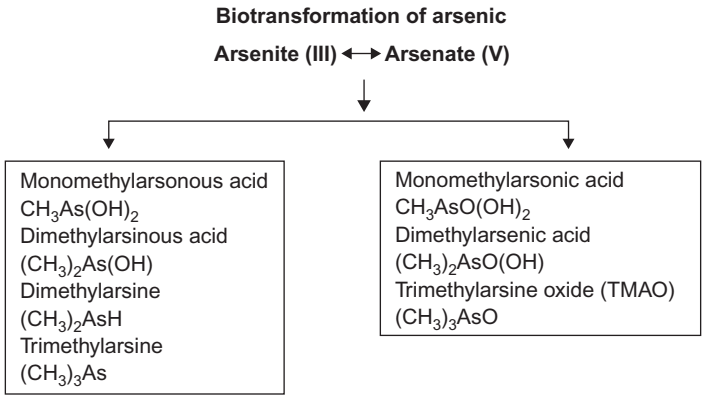

FIGURE 9–1 Predominant arsenical species found in rats and humans. Inorganic arsenic is metabolized differentially in rats and humans. The metalloid arsenic is dominant in 3+ and 5+ forms in organic and inorganic forms. Oxidation state is one of the determinants of the toxic potential of As in biological systems.

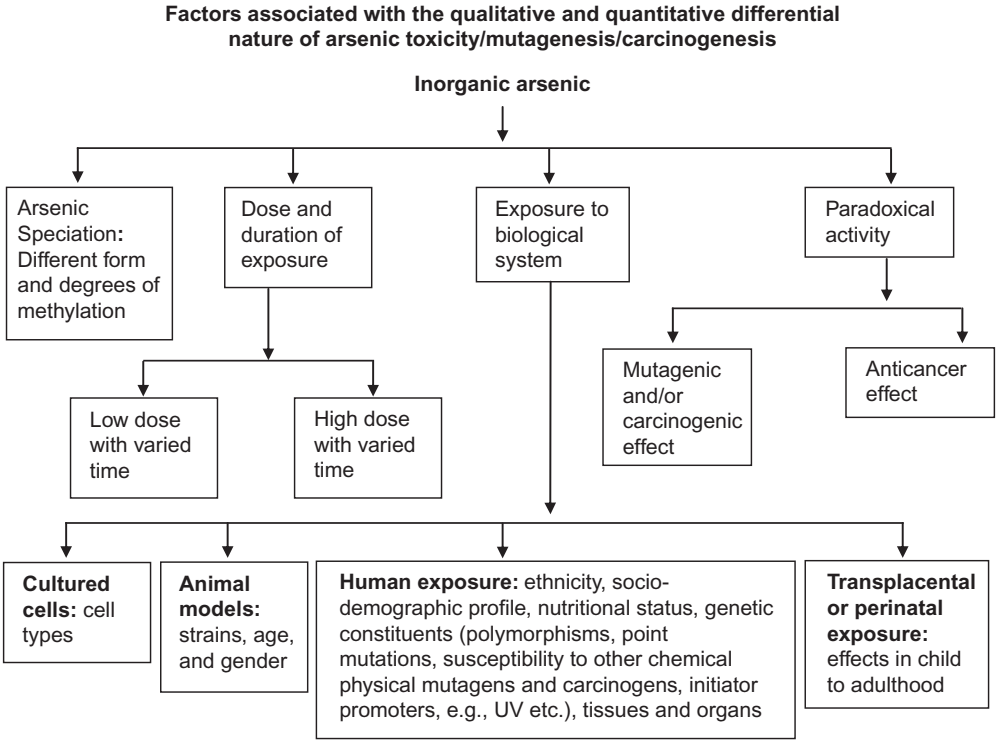

FIGURE 9–2 Outline of the differential toxicity manifestations of arsenical compounds. A large number of metabolic pathways are affected by arsenic. Almost all important metabolic regulations and rate limiting steps are influenced by arsenic. Various factors related to arsenic exposure are the determinants of this differential manifestation.

9.3.1 Arsenic Metabolism and Carcinogenesis

A differential degree of arsenic toxicity is manifested by organic and inorganic arsenicals of different valency states. DMA has been shown, with promotional activity in a rat liver carcinogenesis model, to increase oxidative DNA damage (8-hydroxy-2′-deoxyguanosine (8-OHdG)). It induces placental glutathione S-transferase (GST-ρ isoforms +) and increases the activity of ornithine decarboxylase (ODC), a rate limiting enzyme of polyamine biosynthesis. These markers are indicative of cell proliferation [41–43]. GST-ρ is regarded as a reliable tumor marker for experimental rat hepatocarcinogenesis. GST-ρ overexpression in several hepatic foci in experimental animals suggests that the essential enhancer element GPE1 is the responsible agent in the *GST-ρ* gene. Further binding of nuclear factor-erythroid 2-related factor 2 (Nrf2/MafK) heterodimer on the GPE1 element activates gene expression in preneoplastic lesions and in hepatomas. Arsenic-induced and redox-regulated activation of Nrf2 has also been reported [44].

The enzyme ODC catalyzes the formation of putrescine. It is the rate limiting step of polyamine synthesis [45]. Polyamines have been associated with cell growth and cancer. Specific oncogenes and tumor-suppressor genes regulate polyamine metabolism. Inhibition of polyamine synthesis is shown in implementing chemopreventive strategies against different types of cancers [46]. It may also be exploited against arsenic-associated carcinogenesis. All methylated metabolites of inorganic arsenicals like MMA, DMA, and trimethylarsine oxide (TMAO, which are predominant in rats) are able to induce hydroxyl-radical productions and oxidative stress-related tissue degeneration. Oral or intratracheal DMA ingestion was not able to promote lung carcinogenesis [47,48]. But the iAs with the concomitant exposure of UV light was able to promote skin tumor [49,50]. The co-promotional activity of arsenic may also be applicable in humans. Methylated species of arsenic result in increased efficiency in terms of tumorigenesis in comparison to iAs. Studies suggest that exposure to DMA may promote tumors in the urinary, bladder, liver, skin, and kidney along with other potent organic carcinogens or UV irradiation [51–55]. DMA has been shown to have a variety of carcinogenic effects in F344 male rats [56,57]. DMA in relation to long-term exposure may act as a primary carcinogen (rather than a co-carcinogen) in rats, inducing carcinoma and papilloma in the urinary bladder [58,59], and lymphoma and sarcoma in mice [60]. The inner lining of the epithelium of several organs like intestines, lungs, breasts, and skin may generate adenocarcinoma, squamous cell carcinoma, and anaplastic carcinoma. Sarcoma develops in the connective tissue such as tendons, nerve tissues, and blood vessels. Examples include alveolar soft part sarcoma and fibrosarcoma, while the lymphoma develops in lymphatic organs and vessels in the body. This suggests that a certain level of arsenic metabolites may act as an independent carcinogen.

Discrepancies arise in the case of humans where exposure to methylated arsenic species is unlikely since arsenic exposure takes place mostly through drinking water, which largely contains iAs. Thus, it is likely that the pattern of toxicity, tumerogensis, and carcinogenesis in humans may not be similar to that of an experimental animal model (Figure 9–2). Making things more complex, iAs predominantly converts to organic arsenic in certain types of cells. The qualitative and quantitative features of arsenic methylation are not ubiquitous. Although it is generally accepted that methylation is associated with arsenic detoxification, it has been recently demonstrated that methylated arsenicals have a deleterious impact on biological systems [61]. For example, human epidermal keratinocytes respond differentially against different inorganic and methylated arsenicals of varied valency state [61]. It is demonstrated that arsenite and UV irradiation together increase the incidence of carcinomas whereas only UV irradiation of cells produce papillomas in mouse skin. This increase may be due to the inhibition of DNA repair and/or distortion of tumor suppression gene *p53* functions and up-regulations of cyclin D1 action [62–64].

Chronic exposure to drinking water mixed with DMA in a male rat led to a dose-related development of tumors in the bladder, but not in other organs [65]. Administration of DMA to F344 rats and 36C3F1 mice of both sexes for 2 years led to no tumor formation in mice but rats developed urinary bladder tumors. Female rats were more sensitive to these effects. On the other hand, MMA exposure to the same strains of animals resulted in no tumor formation [65].

These observations suggest the more toxic nature of DMA over MMA, which is also diverse in different animal models.

9.3.2 Arsenic Metabolism and Free Radical Influences

The genetic basis of arsenic-induced mutagenesis and tumorigenesis may be attributed to direct free radical attack on DNA strands resulting in damage. Carcinogenic effects of DMAs are the result of metabolism of DMAs(V) to dimethyl arsinous acid DMAs(III) which induces oxidative stress in the cells followed by cytotoxicity [66]. Impairment of catalase and superoxide dismutase (SOD)-associated DNA breakage and apoptotic cell death were noticed in intestinal epithelial cells of rats chronically exposed to iAs [67]. Hydrogen peroxide (H_2O_2)-associated free radicals are implicated in the high rate of DNA fragmentation in rodent lymphocyte and liver tissues of catalase knockout mice. Electron spin resonance with the spin-trap agent detects the generation of the hydroxyl radical (·OH) via MMA(III) when H_2O_2 is present and it might thus be participating in the DNA damage mechanism [68]. These findings clearly indicate the importance of catalase in cellular protection from reactive oxygen species (ROS)-related damage (Figure 9–3).

Chronic arsenic exposure in rats also caused significant single-strand DNA damage in neuronal cell and lymphocytes as depicted by single cell comet assay. It is accelerated by an

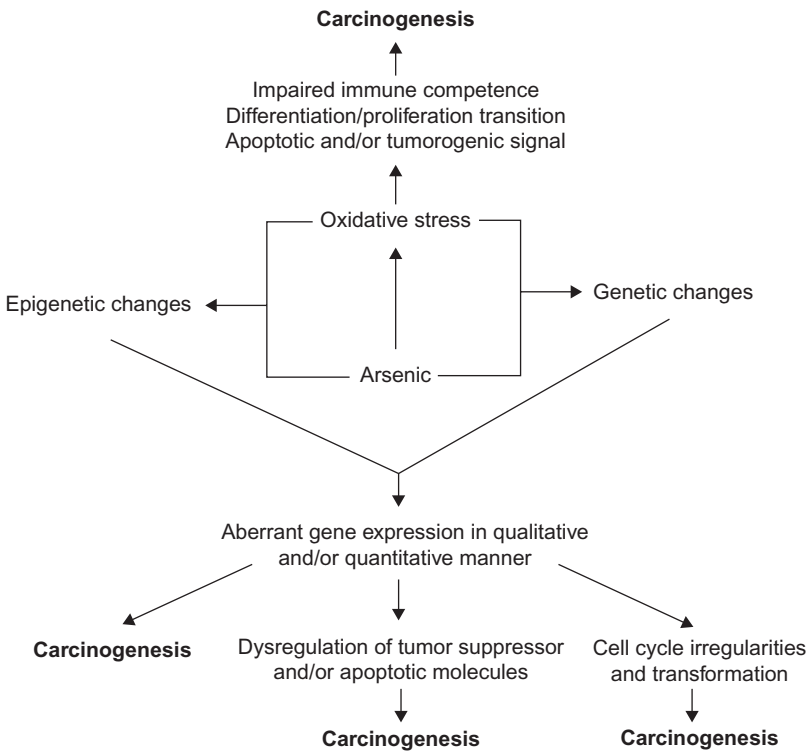

FIGURE 9–3 Interactive roles of genetic and epigenetic changes by arsenic alone or after initiating oxidative responses may influence several important modes of gene expression and signal transduction processes at nuclear and/or cellular levels. These mechanisms finally lead to carcinogenesis.

increased production of ROS [69]. These results are of significance in suggesting that chronic arsenic exposure in humans may result in a significant DNA degradation pattern in a mixed white blood cell (WBC) population with significant decrease of several serum antioxidant components including uric acid [70]. Chronic arsenic exposure also leads to ROS-mediated, mitochondrial-driven, and caspase-dependent apoptosis in hepatic cells with a significant increase in glutathione disulfide (GSSG) levels and a decrease in glutathione reductase activity [71]. This suggests that oxidative stress-related mitochondrial instability is an important factor for arsenic-related apoptotic tissue degeneration. Arsenic-induced increase in 8-OHdG, a modified DNA base as reported in several studies, suggests that oxidative DNA damage is a major cause of arsenic-related mutagenesis [72]. MMA has also been reported to induce the preneoplastic lesions in rats via the production of ROS [73].

Arsenic-associated lung damage and carcinogenesis are predominant in arsenic-exposed individuals. A study describing synergism between tobacco smoke and arsenic exposures has demonstrated more pronounced cytotoxic effects, emphysema-like lesions, and inflammatory events in mice [74]. Arsenic and chromium in drinking water promote tumorigenesis in a mouse colitis-associated colorectal cancer model via a ROS-mediated Wnt/β-catenin signaling pathway. Arsenic exposure increased nicotinaminde adenine dinucleotide phosphate-reduced, NADPH oxidase1 (NOX1) and the level of 8-OHdG suggesting ROS-related DNA damage [75]. The depletion of antioxidant enzymes, such as SOD and catalase, has been shown and linked to arsenic-related DNA and tissue damage. In addition, *in vitro* supplementation of SOD and catalase inhibited β-catenin expression and activity [75]. The study suggests that exposure to As(III) may promote colorectal cancer and tumorigenesis partially through the ROS-mediated β-catenin signaling pathway [75]. The β-catenin acts as an intracellular signaling molecule that acts through the Wnt pathway. It regulates the coordination of cell–cell adhesion and gene transcription. The study further revealed that mutations and/or overexpressions of β-catenin may result in multiple types of tumor and cancerous growth in different organs [76].

Arsenic significantly reduces mRNA expression of superoxide dismutase 2 (*SOD2*) genes possibly by decreasing the nullifications of superoxide anion radicals, leading to increased oxidative stress [77]. Dimethylarsinous acid (DMA(III)) has been reported for its urothelial toxicity and regenerative hyperplasia in arsenite methyltransferase (As3mt) knockout C57BL/6 mice [78]. DMA(V), on the other hand, does not induce hyperplasia in mice, suggesting urinary concentration of DMA(III) does not reach cytotoxic levels in DMA(V)-treated mice [79]. This further supports the important role of methyltransferase gene function in arsenic metabolism and tumorigenesis. The discovery of arsenite (+3 oxidation state) methyltransferase (As3MT) to be the enzyme responsible for arsenic methylation, genetic polymorphism, and the alternative splicing in relation to this gene regulation also contributes to the differential arsenic metabolism and toxicity [80]. Nevertheless, studies report inorganic arsenic to be carcinogenic in humans and mice. Adult offspring mice from an inorganic arsenic-exposed mother developed tumors of the lung, liver, adrenal, uterus, and ovary [81]. Male offspring had unusual testicular lesions, including rete testis (tubular meshwork in the hilum of the testicle that carries sperm), carcinomas, adenomas, and interstitial cell tumors. Overall, maternal

consumption of MMA3+ during pregnancy in CD1 mice produced similar proliferative lesions in adult offspring arising from gestationally applied inorganic arsenic [81]. Chronic exposure to arsenite in rats resulted in a high rate of DNA fragmentation with postmitotic apoptotic cell death and a significant increase in free radical-associated oxidative stress and DNA strand breakage. A study relating to arsenic therapeutics suggested that DNA and tissue were protected following supplementation of vitamin B12 and folic acid [82]. This study demonstrated that antioxidative protection and the restoration of the methyl pool were helpful in protecting DNA from arsenic-induced damage. Arsenic(III) is also known to cause developmental toxicity in human embryonic stem cell-derived embryoid bodies and in *in vivo* mouse models [83]. Developmental cytotoxicities have been observed in human embryoid bodies (EBs). *In vivo* studies suggested that arsenic exposure may lead to a significant down-regulation of gene expression in all the three germ layers, which could be correlated with high mortality and visceral and skeletal defects in pups [83]. Overall, free radicals are extremely deleterious to biomolecules. Various arsenicals exert differential potency to generate free radicals. As explained, damage to the DNA base may result in aberrant gene expression and cancer.

9.3.3 Arsenic May Influence Drug Metabolizing Enzymes

Regulation of drug metabolizing enzymes is of significance as they modify several endogenous and exogenous xenobiotic compounds. Biotransformation/activation or excretion of any compound predicts its ability to be either a toxic or a non-toxic initiator or promoter (if found carcinogenic or co-carcinogenic) in nature. In this regard, phase I and phase II drug metabolizing enzymes are of relevant concern. An arsenic-induced significant increase in phase I drug metabolizing enzyme cytochrome P450 reductase (Cyt-P450) expression has been reported [84]. Arsenic may significantly impair the metabolism of different endogenous and exogenous compounds in rat by interfering with several drug-metabolizing enzymes like Cyt-P450 reductase and Cyt b5 groups, uridine diphosphate glucuronosyltransferase (UGT), aniline p-hydroxylase (APH), and aminopyrine N-demethylase (ANDM). As a result possible bioactivation and/or excretion of several compounds may play a role in the carcinogenesis process [85].

Arsenic(III) can differentially modulate the catalytic efficacy of isoforms *Cyp1a1* and *Cyp1a2* (cytochrome P450 genes). These gene expressions are regulated by the modulation of aryl hydrocarbon receptor (AhR) sensitivity, which may also be influenced by As(III) compounds [86]. The altered P450 may regulate the metabolism of several exogenous or endogenous compounds by possible bioactivation and/or excretion [86]. Recent reports suggest that oncogenic transformations are a crucial factor in converting certain types of renal stem cells into those with cancerous phenotypic characteristic following chronic arsenite exposure [87]. Colony formation, cellular proliferation, and invasiveness are found to be associated with the altered matrix metalloprotease (MMP) activities. These results indicate multipotent stem cells being one of the targets for arsenic, which might lead to renal carcinogenesis [87]. Chronic arsenic exposure initiates an inflammatory response in the liver and also down-regulates the nuclear retinoid xenobiotic receptor α (RXRα), which is a heterodimeric pregnane xenobiotic receptor (PXR) and mediates the inactivation of the drug metabolizing enzyme *CYP3A* gene.

As a result, the lower expression in mRNA and corresponding amount of protein may impair the metabolism of several endobiotics and xenobiotics [88]. The alteration of expressions and activities of drug metabolizing enzymes by arsenic may influence the metabolism of endogenous or exogenously consumed substances. These features may influence the regular pattern of bioactivation, biotransformation, and/or excretion of several molecules. Bioactivation may sometimes result in procarcinogenic activation to form carcinogen.

9.3.4 Gut Microflora Influences Arsenic Toxicity and Tumorigenesis/Carcinogenesis

Gut microflora are known to be affected by arsenic metabolism when As is ingested through drinking water. Metagenomics studies of the gut microbiome in arsenic-intoxicated C57BL/6 mice and arsenic metabolites in their urine suggest that the gut microorganisms significantly interfere with intestinal arsenic metabolism. This is reflected in the urinary excretion of methylated and thiolated arsenical metabolites of those mice [89]. This suggests that infectious diseases and environmental exposure may be a new risk factor for individual susceptibility to carcinogenesis from environmental chemicals [89]. Under aerobic or anaerobic conditions, cecum tissue homogenates produce a certain amount of iAs MMA or DMA. These data suggest that cecum microflora utilize a high capacity methylation system that might contribute significantly to methylation-associated tumorigenesis and carcinogenesis of iAs in intact animals [90]. It is noticed that the contents of the rat small intestine or cecum reduce sodium arsenate to arsenite. In the pre-reduced media containing thioglycollate or cysteine in the absence of intestinal bacteria, sodium arsenate was reduced to arsenite. The rate of reduction of arsenate by the gut flora was increased by the presence of hydrogen sulfide or bile acids [91]. Besides the formation of monomethylarsonic acid (MMA(V)), highly toxic monomethylarsonous acid (MMA(III)) is also produced. This is an important finding of microbial thiolation leading to formation of monomethylmonothioarsonic acid (MMMTA(V)) [92].

Methylenetetrahydrofolate reductase (Mthfr) is an important enzyme that plays a key role in folate metabolism and methylation reactions. Transgenic mouse *Mthfr*+ gene loci have been reported to promote more pronounced urinary excretion of arsenic than do the Mthfr− loci, suggesting the role of folate in arsenic metabolism [93]. In contrast, the Mthfr(−/−) mouse excretes significantly lower amounts of pentavalent arsenic when fed on a folate-deficient diet compared to a control diet. This suggests that active Mthfr and folate are important not only for arsenic detoxification, but also for its increased urinary elimination [93]. Adequate folate pool and S-adenosyl methionine (SAM) are very important determinants for the arsenic methylation process [94]. Supplementation of vitamin B12/folate-related restoration of the cellular methyl pool was effective in protecting hepatic tissue/DNA from arsenic-related toxicity [82]. Chronic arsenic exposure has been demonstrated to significantly decrease SAM and phosphatidylcholine (PC) with a concomitant increase in choline. This suggests that choline has a role as a methyl donor in arsenic-intoxicated conditions [95]. Hepatic impairment due to the combined effect of arsenic and other environmental factors may come into play in the case of abnormal metabolism of micronutrients like folate, SAM, and choline [95]. Inorganic

arsenic causes the depletion of S-adenosylmethionine, the main cellular methyl donor, and represses the expression of the DNA methyltransferase genes *DNMT1* and *DNMT3A*, possibly as a consequence of these two complementary mechanisms. Long-term exposure to arsenic results in DNA hypomethylation leading to aberrant gene expression and carcinogenesis [96] (Figure 9–3). Intestinal epithelial cells are the first pass site of any ingested substance. Hence, the metabolism of ingested substances by intestinal indigenous metabolic machinery or by the gut microflora gives rise to additional factors in the manifestation of toxicity of arsenical compounds ingested.

9.3.5 Arsenic-Induced Carcinogenesis in Animal Models: Role of Signal Transduction

Tumorigenesis and carcinogenesis are multifactorial and multistep processes. They deal with the transformations of normal cells into malignant cells. Transformation may occur due to the induction of multiple cancer-specific heritable phenotypes triggered by genetic (natural mutational or DNA strand breakages and other damage) or epigenetic (non-inheritable DNA-associated modifications) events [97]. The outcome of the interactive nature of genetic and epigenetic factors on exposure to environmental and endogenous components may determine the extent of manifestations of cytotoxicity and tissue carcinogenesis [98]. For example, the toxicity outcome may alter the intracellular redox state. Imbalance in the cellular redox state and related complications may arise from impaired toxicant excretion and/or DNA damage/modifications and/or disrupted signal transduction processes [99]. One or more of these factors may result in tumorigenesis and/or carcinogenesis. The arsenic-associated animal tumorigenesis/carcinogenesis process may be mechanistically implicated via genetic, epigenetic, and signal transduction processes. There are numerous reports available in the literature suggesting that inorganic arsenic (iAs) can induce human carcinogenesis; a limited data supports the carcinogenic manifestation in animal experimental models [100]. However, mostly negative results for carcinogenicity were reported in mice, rat, hamster, rabbit, and certain other animals [101–104]; frequent tumorigenesis and distinct manifestations resembling carcinogenic features were demonstrated in arsenic-intoxicated rodent models. These investigations mostly confirmed co-mutagenic/co-carcinogenic effects rather than carcinogenicity of inorganic arsenical compounds. In most of these cases, arsenic exposure was associated with cell proliferation, differentiation, and signal transduction-related carcinogenesis, which were mostly demonstrated using cultured cell-based assay.

9.3.6 Arsenic Induces Cellular Transformation Signaling

Chronic arsenic exposure of rat-cultured liver epithelial cells induced malignant transformation with an increased expression of c-Myc and H-Ras products. The regulator gene *c-Myc* codes for a transcription factor. The mutated Myc is found in various cancers [105]. The arsenical compound phenylarsine oxide (PAO) has been shown to bind at a specific location in H-Ras and suggests mechanistically how this binding influences the pattern of H-Ras

activation in cells [106]. Such findings help in the elucidation of the involvement of the Ras ligand in arsenic-associated carcinogenesis. Ras protein is a small GTPase family member from the Ras superfamily and is involved in intracellular signal transduction in cell growth and differentiation. A mutation in *ras* genes and auto-activated/generated Ras protein signaling leads to cancer. Out of three types of human *ras* genes (*H-Ras, N-Ras,* and *K-Ras* with subtypes generated from alternative splicing [107–109]), H-ras has been mostly shown to be involved in arsenic-associated carcinogenesis in animal models. Arsenic exposure in v-Ha-ras transgenic mice (an animal model for skin cancer) caused an increase in the number of papillomas as well as overexpression of growth factors, suggesting that they participate in arsenic-induced skin papilloma development [110].

Alteration in Ras expression has been reported with a concomitant suppression of *MAPK/ERK* gene expression in perinatally arsenic-exposed mice, indicating the relation between Ras ligand signaling and the activities of different kinases [111]. Gestational arsenic exposure induces oxidative stress (as observed by the induction of the stress inducible gene heme oxygenase-1, *HO-1*) and overexpression of certain retrotransposon genes resulting in tumor formation in the offspring of certain (C3H) *Ha-Ras* mutant mice [112]. The results of this study demonstrated that gestational arsenic exposure particularly increased hepatic tumors with a C61A *Ha-Ras* mutation [112]. Though H-Ras has been linked increasingly with arsenic-associated cancers, some evidence also suggests the involvement of K-Ras in arsenic-related carcinogenesis in human-cultured prostate cells. The inorganic arsenic-induced transformation in these cells is associated with genomic DNA hypomethylation and K-Ras overexpression. Notwithstanding, this overexpression occurred without mutations and through a mechanism other than promoter region hypomethylation [113]. The mutated cells become very sensitive and susceptible to the promoter compounds, resulting in proliferation, which is noticed in several arsenic-associated animal carcinogenesis models. Transgene (mutated) mRNA expression with increased aberrant H-ras protein levels is associated with the DNA hypomethylation of specific *Msp*1-*Hpa*II sites within the transgene [114]. MspI and HpaII are the restriction enzymes which recognize the same DNA sequence with differential sensitivities to methylated DNA sites. This serves as the locus-specific discrimination between methylated and unmethylated DNA sequences. Thus, the epigenetic modification is involved in the arsenic-induced aberrant signaling process and carcinogenesis mechanism (Figure 9–3) [115,116].

The possible involvement of several factors, i.e., H-ras, K-ras, or β-catenin in arsenic-induced cancers has been described in the previous section. Aurora A, the mitotic kinase, has been designated as an evolutionary conserved oncoprotein and is frequently overexpressed in various human cancers. Reports reveal that Aurora A overexpression associates with Ha-ras codon-12 mutation and blackfoot disease in arsenic-affected populations manifesting with bladder cancer. This report explains the causative factors of aberrant H-Ras expression and cancer occurrence [117]. β-Catenin, a subunit of the cadherin protein family, serves as an intracellular signaling molecule, which acts via the Wnt signaling pathway [118]. It regulates the coordination of cell–cell adhesion and gene transcription. The report reveals that mutations and/or overexpressions of β-catenin may result in multiple types of tumor and cancerous growth in different organs [118,119]. The repression in Wnt/β-catenin signaling at the time

of early embryogenesis results in a significant decrease in the expression of several transcription factors. Arsenic exposure to P19 mouse embryonic stem cells significantly increased the expression of a transcription factor Nanog, which maintains the pluripotency of stem cells [76]. It directs a particular cell type to proceed towards the proliferation state. The decrease in Wnt/β-catenin signaling by arsenic can perturb the embryonic differentiation process. This suppression of differentiation may promote cellular proliferation and towards transformation [76]. A recent report suggests that transcriptional coregulator Mastermind-like 1 (MAML1) proteins act with p53, nuclear factor κβ (NF-κβ), and β-catenin and functions in various signaling pathways. Most of these pathways are associated with the process of cell proliferation. This result is confirmed from the finding that overexpression of MAML1 increased the proliferation of human embryonic kidney (HEK) 293 cells, while MAML1 down-regulation by siRNA decreased cell proliferation [120]. In several animal experimental studies, the impairment of the differentiation/proliferation balance has been regarded as the basis of cellular tumorigenesis/carcinogenesis. The signaling machinery for cell division/differentiation is somehow compromised by arsenic, and results in cancer (Figure 9–3).

9.3.7 Arsenic and Epigenetic Modification

At the protein level, tumor necrosis factor p53 has been found to be involved (6%) in transitional cell carcinoma (TCC). A major fraction of lesions in TCC and papillomas were noticed to be involved with decreased numbers of p27kip.1 protein molecules. Cyclin D1 and cycloxygenase 2 (Cox-2) overexpression were also involved with a major number of lesions, i.e., papillary or nodular (PN) hyperplasia and different types of TCCs [21]. p27kip.1 is a cell cycle regulatory protein and interacts with the cyclin-CDK class of molecules. It may act via transforming growth factor β (TGF-β) induction and cell cycle arrest at the G1 phase [121]. CDK-dependent phosphorylation initiates the degeneration of this molecule, which sensitizes cells to proceed through the proliferation stage [121]. Overexpression of Cox-2 may lead to several inflammatory responses and may also increase matrix metaloprotease MMP-2 and MMP-9 expression after chronic arsenic exposure in rat kidney stem cells. This expression was shown to transform these cells into non-adherent spheroid cell bodies with cancerous properties [87]. Arsenic, especially DMA(V), has been shown to induce the expression of cyclin D1 and COX-2 mRNA in the bladder epithelium of rats [122]. In addition to this, there was an increase in TGF-β1 and a decrease in tumor necrosis factor-alpha (TNF-α) levels in the urine of these treated rats. All of these findings have been correlated with a significant increase in urothelial cellular proliferation, suggesting that chronic inflammation and proliferation might be the basis of the arsenic toxicity [122]. Some investigations also suggest arsenic to be the promoter, or an initiator or an inducer of different degrees of carcinogenecity via involvement in Cox-2 regulation [123–125].

Genetic/epigenetic or signal transduction alone may not be able to result in carcinogenesis. In fact, in several cases it has an overlapping or shared activity. Epigenetic changes may influence the signal transduction-associated regulation of different gene expression modalities in a qualitative and/or quantitative manner. This series of changes may result in cellular transformation via impaired differentiation/proliferation balance. Apart from the regulations

master gene p53, the impaired expressions of other genes and altered methylation (DNA) in tumor related several gene loci have been demonstrated in arsenic-induced carcinogenesis. Epigenetic modifications such as DNA methylation and histone modifications were screened in relation to the tumor promotion in C57B1/6 mice [126]. In addition, other tumor-related genes, namely *P16* (*INK4a*), *RASSF1A*, *H-Ras*, and *ER-α*, were also tested [126]. A *de novo* promoter hypermethylation of important tumor suppressor genes such as *P16INK4a* and *RASSF1A* results in the inactivation of these genes, promoting several types of organ cancer [127]. Long-term exposure to arsenic has been reported to decrease the expression of *P16*(*INK4a*) with an increased level of dimethylated histone H3 lysine-9 (H3K9). Influence in the promoter region by histone modifications results in the transcriptional suppression of certain genes. An increase was noticed in the recruitment of H3K9 histone methyltransferase G9a to the promoter after arsenic exposure [126]. The lysine 9 methylation of histone H3 is an epigenetic event that determines the inductance of pluripotent stem cells. H3K9 methyltransferase and its corresponding demethylase act reciprocally by regulating H3K9 methylation status. This histone methylation is related to the transcriptional repression of several important genes [128,129].

Estrogen receptor α (ERα) is a marker determinant of response of breast cancers. About 30% of breast cancers, however, are hormone independent because of lack of ERα expression [130]. It is reported that arsenic trioxide (2 mg/kg b.w. to mice) induces partial demethylation of the ERα promoter, which is protected by the universal methyl donor SAM. It reduces the degree of arsenic trioxide-induced re-expression of ERα and demethylation [130]. This suggests the importance of the cellular methyl pool and restoration of DNA methylation. Thus, arsenic trioxide exhibits the induction of re-expression ERα in ER-negative breast cancer cells through demethylation of the ERα promoter [130]. The INK4a/ARF locus produces (transcribes from an **a**lternate **r**eading **f**rame) ARF tumor suppressor protein in response to continuous mitogenic stimuli, which may be generated by MYC and Ras ligand [131,132]. As described in this section, it may be concluded that epigenetic modifications are as important as genetic impairment; however, sometimes epigenetic dysregulations are more relevant in cellular transformation mechanisms.

9.3.8 Arsenic and Tumor/Heat Shock-Associated Proteins

Heat shock protein 70 (HSP70) has been implicated in the cellular stress initiation process and is associated with cellular transformation [133]. Abnormal methylation in the pertinent gene promoter and/or in the functional gene region has been implicated as an epigenetic phenomenon that regulates gene functions. This may result in cellular toxicity and even carcinogenesis. Perinatal arsenic exposure results in increased methylation in the transcribed region of the *HSP70* gene in apolipoprotein E (ApoE) knockout mice. And this altered induction of Hsp70 can cause hepatic stress and its degeneration [134]. ApoE is a class of apolipoproteins, which is primarily produced by the liver and macrophages. In the absence of this factor, such as in knockout mice [134], cholesterol metabolism is impaired, which might be an added factor for hepatic stress [135,136]. The HSP70 family is induced by a wide range of intrinsic or extrinsic

stresses and keeps proteins in a properly folded state [137]. They also protect nascent translating proteins, promote the cellular or organellar transport of proteins, and serve a general housekeeping role in maintaining protein homeostasis. Several human tumors overexpress the HSP70 family members, which result in a poor prognosis for the disease outcome [137]. Tumor suppressor p53 protein and the related gene regulation preserve the genetic stability by preventing genome-wide mutations [138]. In relation to p53 regulation, several indications demonstrate the involvement of p53 aberrant expression by iAs, which is related to both rodent and human carcinogenesis. The ubiquitin-conjugating enzymes (Ube2d) promote p53 ubiquitination and proteasomal degradation. A recent report suggests that inorganic arsenic may induce p53-dependent apoptotic pathways through down-regulation of gene expression of the Ube2d family in rat proximal tubular cells [17,50,139,140]. p53 has been shown to play a very important role in regulating the cellular proliferation/differentiation balance which is differentially maintained in different cultured cells [141]. Arsenic severely impairs this equilibrium [141].

Arsenite and UV radiation together have been reported to increase the incidence of carcinomas whereas UV cell irradiation alone produces papillomas in mouse skin [50,142]. These benign epithelial tumors grow externally. On the other hand, malignant skin tumors or cancers derived from epidermal cells are non-melanomas, while melanomas are derived from melanocytes. The non-melanomas include squamous cell carcinoma and basal cell carcinoma derived from all layers of the epidermis and the basal layer of the epidermis, respectively [143]. The increase in severity of tumorigenesis by arsenic has been demonstrated from a mechanistic point of view. It may be due to the inhibition of DNA repair or distortion of p53 functions and up-regulation of cyclin D1 activity [50]. The *p53* gene is mutated early in skin carcinogenesis and the resulting mutations are often present on the sites of dipyrimidine [144]. In general, the carcinogenic effects are not implicated by arsenical compounds alone [145]. Arsenite prevents terminal differentiation and helps pre-adipocytes to remain in an undifferentiated state by blocking the up-regulation of c/EBPα and p21. This process helps cells to move through a stage of cellular proliferation [146]. HSP70 is a general protector against cellular stress and p53 is the master regulator against tumor promotion. As described, arsenic can impair the functions of both molecules, which are lethal to living systems (Figure 9–4).

9.3.9 Arsenic and Apoptotic Signaling via Transcription Regulation

Premature ubiquitinization of proteins is noted with low doses of arsenite, which increases the cellular senescence of protein molecules [147]. Ubiquitin-like protein, which is an oncogenic product, may block arsenic-induced ubiquitination. Arsenic associated with modulation of the glucocorticoid receptor blocks this receptor's role in tumor suppression, which may be partially related to skin cancer [148]. The experimental results suggested that DMA-induced rat urinary bladder carcinogenesis might be caused by ROS generation, which might act as the initiator for further progression of the disease. This has also been shown in arsenic-exposed humans. In humans, different inorganic and organic arsenicals have been shown with varied ability in ROS production and p53 expression [58,59,149,150]. The comutagenic activity of iAs with TPA, discussed earlier, results in more invasive cellular carcinoma. The studies reveal that

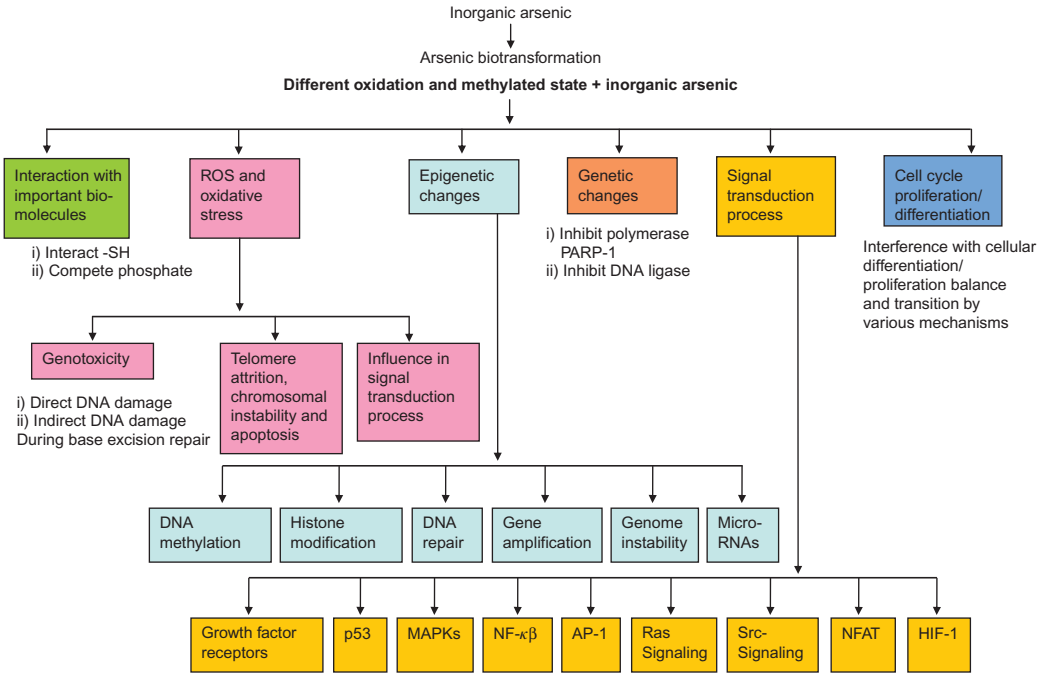

FIGURE 9–4 Description of different regulatory steps and main participants in the arsenic-related mutagenesis and carcinogenesis processes.

impaired stem cell (SC) signaling and tumor growth are also interrelated [151]. Developmental arsenic exposure affects the stem cells and it is assumed that arsenic-related carcinogenesis correlates with a few stem cell-related diseases. Arsenic impacts human SC population dynamics *in vitro* by blocking exit into differentiation pathways thereby creating further critical objectives transformation [152]. These findings may be corroborated by earlier results on the role of arsenical compounds to promote cellular proliferation after blocking of the potential for differentiation [124,125]. Pancreatic tissue degeneration and a decrease in the number of cells in islets were noticed in male rats following exposure to iAs. These alterations can be attributed to oxidative stress and inflammatory responses with high-level release of TNF-α and interleukin-6 (IL-6) [153].

The nature of arsenical compounds and the dosage are reported to have differential effects in relation to steroid receptor binding. The methylated arsenicals were found to be more potent than iAs in disrupting steroid receptor binding to DNA response elements, thus impairing the hormone-associated gene expression pattern [154]. Testicular apoptosis is initiated by arsenic in the rat by the activation of caspase-3 and degenerative signaling from Bcl-2/Bad with an increase of cytosolic cytochrome c. This suggests the mitochondrial involvement in this apoptotic process is accomplished through degeneration via the ROS-regulated pathway. Arsenite is found to induce mitogen-activated protein kinases (MAPKs), protein kinase B (AKT), and NF-κβ in testicular tissue with an increase in inflammatory response [155]. The

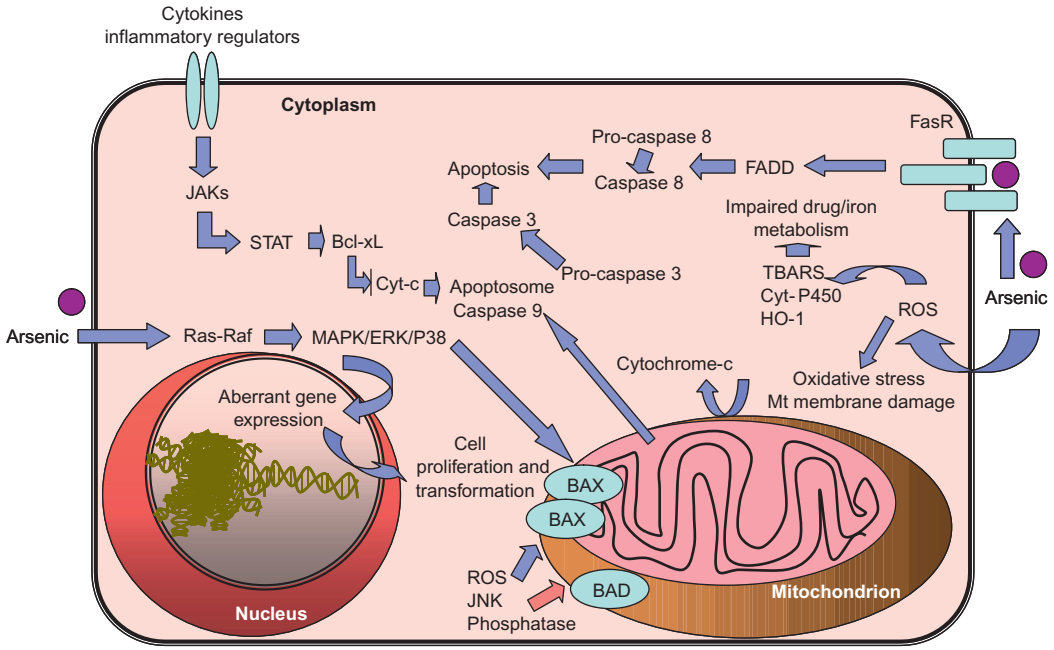

FIGURE 9–5 Arsenic may induce free radical production and cellular and organelle membrane damage. Mitochondrial membrane damage results in the release of cytochrome-c. This initiates free radical cascades and activates several pro-caspases to caspases. An apoptotic pathway is introduced under the regulation of several others proteins, namely Bcl-2, BAD, and Bcl-xL. The cumulative effects of apoptotic signals and differentiation/proliferation balance regulate cellular transformation ability. These are the important determinants of the processes of tumorigenesis and carcinogenesis.

carcinogenic potential of arsenic may be attributed to the activation of redox-sensitive transcription factors and other signaling pathways involving NF-κβ, activator protein-1, and p53. Impairment of cellular thiols is shown to induce oxidative stress-related injuries [156] and result in impaired redox regulation of not only transcriptional but also other factors.

Bcl-2-associated death promoter (*BAD*) is a pro-apoptotic gene family that initiates apoptosis. The Bax/BaK complex may also initiate apoptosis by promoting the cytoplasmic release of cytochrome c after forming mitochondrial membrane pores. Cellular redox imbalance may destabilize the mitochondrial membrane and cause release of cytochrome c (Figure 9–5). Such release is inhibited by the anti-apoptotic Bcl-2 and Bcl-xL complex. This process promotes inactivation of the cytoplasmic caspase pathway initiated by cytochrome c [157,158]. Chronic arsenic exposure increases ROS and nitric oxide (NO) (which is regarded as a strong signaling molecule) in neuronal cells of rodents [159]. The calcium influx-associated disruption of mitochondrial membrane potential has also been seen [159]. Mitochondrial degeneration, capase-3 activation, and bax/bcl2 signaling have been reported to be associated with neuronal apoptosis [159]. The reversal of some stepwise processes such as mitochondrial oxidative stress, and alteration of cytochrome c, caspase-3, and bax/bcl 2 ratio by the thiol

chelator monoisoamyl dimercaptosuccinic acid (MiADMSA), strongly support that mitochondrial instability is the prime cause of arsenic-induced apoptotic cell death [160].

Angiotensin II receptor signaling has been implicated in arsenic-induced liver toxicity. The apoptotic and cancerous death of hepatocytes has been shown to regulate the nitric oxide synthase (NOS) and Cox-2 related pathway. As explained earlier, NF-κβ, inflammatory modulator TNF-α, and caspase-3 have been shown to be involved in arsenic toxicity and the carcinogenesis mechanism (Figure 9–3) [161]. Besides direct NF-κβ signaling, transcriptional regulation of several important genes by TF is involved in cellular responses to stimuli such as stress, cytokines, and free radicals [162]. Mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinases (ERKs) are groups of cell surface receptor proteins, which, upon activation, control transcriptional regulations to produce cell division promoting proteins. Phosphorylation-based activation of these kinases is also associated with the earlier discussed Ras–RAF pathways [163,164]. Arsenic-induced impairment of cellular *cyclin D1* gene expression is shown to be mediated via miR-2909. Induction of splice switching of the tumor suppressor *CYLD* (cylindromatosis) gene as well as modulation of specificity protein 1 (*SP1*) has also been linked to this process. Thus, the important epigenomic event may play a role in arsenic-related carcinogenesis processes [165]. CYLD is a de-ubiquitinating enzyme acting via NF-κβ, c-Jun N-terminal kinase (JNK) activity, TNF receptor-associated factor 2 (TRAF2), and processes such as ubiquitination. Current data suggest that CYLD limits inflammation and tumorigenesis by regulating ubiquitination *in vivo* [166]. Impairment of immune functions by cellular and humoral levels of arsenic is a potential risk factor, which augments infections and chronic diseases, including various cancers [167].

Recently, it has been demonstrated that a signaling pathway, Salvador/Warts/Hippo (SWH), may explain the regulation of animal organ size, which is controlled by cell proliferation and apoptosis balance. Protein kinase Hpo (phosphorylates Wts and LATS1/2 in mammals) and its mutation is shown to be involved in tissue overgrowth. In other words, less phosphorylation of LAST1/2 results in impaired gene expression signaling and induction of tissue overgrowth. These kinases are the regulator of cell cycle progression and development. Important proteins like Sav and MOB activate Wts as tumor suppressors [168,169]. The report suggests that Hippo signaling plays a crucial role in organ formation during the embryonic development and its malfunctioning contributes to the pathogenesis of epithelial neoplasms. Arsenic treatment enhanced phosphorylation-dependent activation of LATS1 kinase and other Hippo signaling regulatory proteins Sav1 and MOB1. Arsenic-induced Phospho-LATS kinase inactivates the transcriptional co-activator Yap, which is usually inactivated without arsenic. Yap is indirectly related to the transcriptional regulations of several target genes. It is also important in regulating tight and adherence junctions through its binding to α-catenin. Arsenic-induced alterations of these pathways result in neoplastic lesions in mouse epidermis [170]. Arsenic-induced up-regulations of angiotensin II type I receptor (AT1R) subtypes may be caused by the ROS-mediated phosphorylations/activation of c-Jun N-terminal kinases (JNK), activated protein 1 (AP-1), and the JNK signaling pathway in the mouse aortic endothelial cell line. This step results in an aberrant signaling pathway and disease pathogenesis [171]. Arsenic trioxide can induce apoptotic morphogenesis, caspase-3 and TGF-β1 protein

expression, Bax/Bcl-2 ratio, and ERK1/2 phosphorylation in guinea pigs. This suggests that TGF-β is functionally linked to ERK1/2 and its aberrant signaling is responsible for arsenic trioxide-induced cellular apoptosis [172]. The pigment epithelium-derived factor (PEDF) plays a role in sodium arsenite-induced cell apoptosis of liver and brain in rat and the results also demonstrate that the ratio of Bax/Bcl-2 might be important in the action of PEDF. The ratio of Bax/Bcl-2 in the higher dose of the arsenic group may suggest the possible induction of a cellular apoptosis pathway [173]. Arsenic trioxide (As_2O_3)-induced apoptosis in head and neck squamous carcinoma cells has also been reported [174]. The mechanism suggests that Bax was up-regulated without a change in Bcl-2 with an ultimate consequence of Bax/Bcl-2 increase and apoptotic death signal (Figure 9–5). The results also showed that the apoptosis of PCI-1 cells by As_2O_3 was induced by the activation of the caspase-3 via cytochrome c, caspase-9, and the Apaf-1 complex [174].

Combinatorial interactions of certain transcription factors regulate the expressions of some important genes. Hepatocyte nuclear factor 1α and 4α (HNF1α and HNF4α) are key members of a transcriptional network essential for normal liver architecture [175]. Changes in HNF1α and HNF4α expression are clearly associated with the development of liver malignancies and diabetes in humans. Inorganic arsenic has been shown to down-regulate both HNF1α and HNF4α in human hepatocarcinoma (HepG2) cells and in liver of golden Syrian hamster cells. This process has been linked to arsenic-associated liver cancer [175]. HNF1α and HNF4α control pancreatic β-cell function and growth, and mutation (in this gene) may result in certain forms of diabetes [176]. Thus, it is important to address how these genetic programs control cellular functions and disease processes. Epistasis analysis with transcriptomes of single- and double-mutant islets revealed that Hnf1α and Hnf4α regulate common targets synergistically, suggesting that the mechanisms for synergistic regulation are gene specific. Hnf1α and Hnf4α control a common islet-cell regulatory program that is defective in human monogenic diabetes [176]. It is further demonstrated that the transition program between epithelial-to-mesenchymal cells lineages and reverse is controlled by HNF4α, in cooperation with its target HNF1α. This process determines the possibility of the cell lineage to move towards a terminal differentiated state or a regular step of its development, tissue repair, and tumor progression [177].

Imbalance in the cellular redox state and its associated complications has been closely associated with the regulation of nuclear factor-erythroid 2-related factor 2 (Nrf2). It is a basic–leucine zipper transcription factor that activates the antioxidant responsive element and the electrophilic responsive element. This process up-regulates the expression of a variety of downstream genes [99,178]. Exposure to a low dose of arsenic trioxide caused transformation of BALB/c 3T3 cells and, in a xenograft mouse model via induction of polycomb (PcG) proteins, BMI1 and SUZ12 functions. This protein function is related to histone H3K27 methylation (similar to H3K9 methylation as discussed earlier) after arsenic exposure. In BMI1- or SUZ12-knockdown BALB/c 3T3 cells, the expression of p16 (INK4a) and p19 (ARF) is restored compared to corresponding wild-type cells. This suggests the involvement of p16 (INK4a) and p19 (ARF) proteins in the carcinogenesis process, as mentioned in the previous section [179].

Arrigo [180] first reported that exposure to arsenic in *Drosophila* cells led to a complete abolition of methylation of histones H3 and H4 [180]. Little is known about histone

phosphorylation and arsenic-induced carcinogenesis. Histone phosphorylation may also contribute to arsenic-induced carcinogenesis. The best-studied histone phosphorylation event is that of H_2AX, a form of H_2A that represents up to 25% of the total H_2A pool in mammals. Evidence suggests that arsenic trioxide induces apoptosis by the up-regulation of phosphorylated H_2AX [181]. Studies have suggested that H3 phosphorylation induced by arsenic exposure might be responsible for the up-regulation of the oncogenes c-*fos* and c-*jun* [182] and induction of a protoapoptotic factor, caspase-10. Interestingly, As(III) exposure has also been shown to induce elevated histone acetylation, which was reportedly responsible for the up-regulation of genes involved in apoptosis or the response to cell stress after exposure to arsenic [182]. As(III) has been shown to inhibit histone deacetylase *HDAC* genes that correlate with increased global histone acetylation [183]. A small class of non-protein-coding RNAs, called microRNAs (miRNAs), participate in diverse biological regulatory events and are transcribed mainly from non-protein-coding regions of the genome [184]. These have been found to be involved in arsenic-associated carcinogenesis.

In an earlier section, ligand activated Ras signaling was described during arsenic carcinogenesis in rodent models. It is shown that the *RAS* oncogene is regulated by the let–7 miRNA family [185]. Arsenic exposure has been shown to alter methylation levels of both global DNA and gene promoters. Besides this, it influences histone acetylation, methylation, phosphorylation, and miRNA expression [186]. Thus, a comprehensive epigenomic approach may elucidate the mechanisms of arsenic-induced carcinogenesis. Such an approach would also facilitate our search for the biomarkers of arsenic exposure and early effects, associated diseases and disease progression, and factors that confer susceptibility. This comprehensive discussion on the transcriptional regulation of several important genes, including cell cycle regulators, growth factors or oncogenic materials or histone-modifying enzyme genes, is highly relevant in the mechanism of arsenic-related carcinogenesis (Figure 9–6).

9.4 Human Arsenic Carcinogenesis

9.4.1 Genetic/Epigenetic Changes in Arsenic-Associated Human Carcinogenesis

The possible mechanistic layout was described in an earlier section to explain the carcinogenesis processes in animal models. One or more of these pathways may also be implicated in arsenic-associated tumorigenesis and/or carcinogenesis in humans. The data from several *in vitro* studies further add important information to the toxicity processes. The metabolic pattern, however, varies significantly between laboratory-exposed rodent models and environmentally-exposed human individuals. This is not only because of the differences in arsenic exposure pattern, but is also due to a major variability in gene mutations, single nucleotide polymorphisms (SNPs), genetic polymorphism, and other genetic and epigenetic events that exist in rodents and humans. It indicates that arsenic is highly mutagenic and carcinogenic to humans, but is not as potent to laboratory animal models. Several *in vitro* studies further help in delineating the mutagenesis/carcinogenesis pathway, but their definite applicability

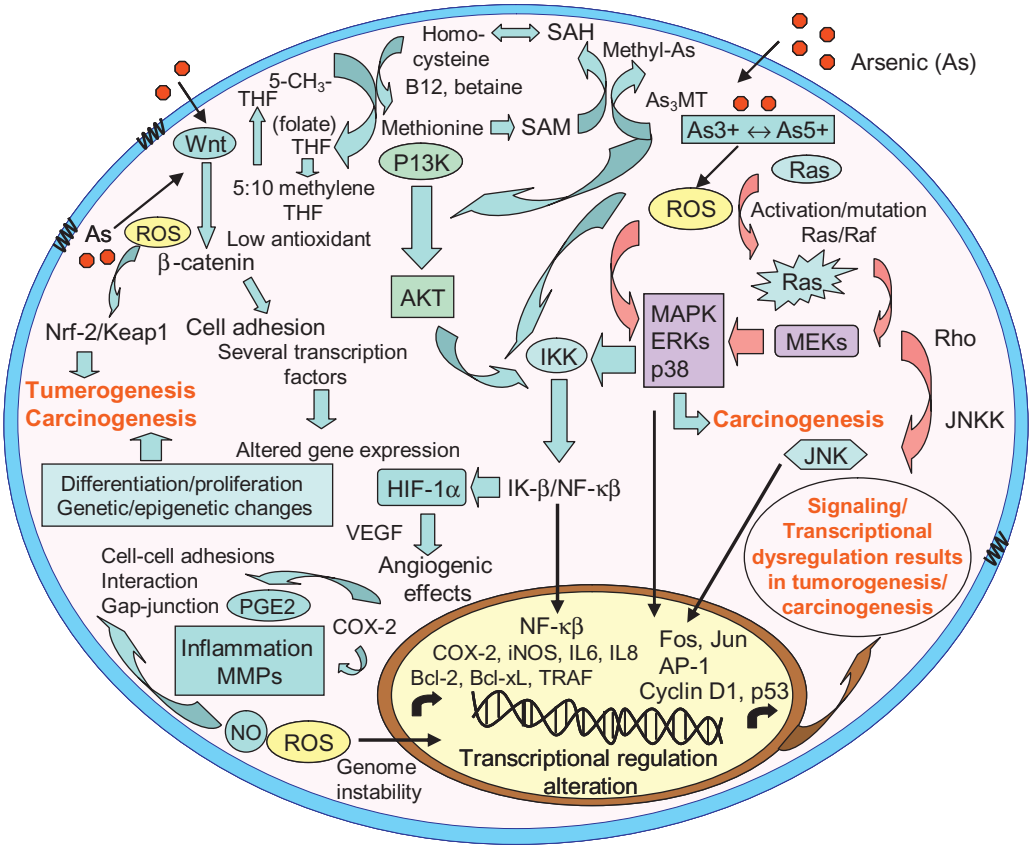

FIGURE 9–6 Arsenic can induce genetic and epigenetic changes. Alteration in gene expression regulation and impaired signal transduction at the cytosolic and the nuclear level result in differentiation/proliferation imbalance and cellular transformation. Different growth factors and oncogenic mutation/activation are the key players in the activation of several mitogen-driven kinases. These in turn modulate the function of transcription factors. Reactive oxygen species are one of the potent inducers for all these phenomena.

in whole animal models or in humans is largely inconclusive. A suitable experimental model is still a matter of debate between investigators; it is equally difficult to characterize mechanisms of arsenic-induced oncogenesis/carcinogenesis based on *in vitro* studies. However, rodent models where a xenotoxic substance causes the induction of tumors can be invaluable because they are clearly pertinent to a carcinogenic process. To explain the mechanisms behind arsenic-associated human carcinogenesis, the epidemiological data may be of immense assistance. It is agreed that there is a limitation in animal experiments due to their differential sensitivities in toxicological pathogenesis processes. With this background, the best possible and most reasonable integrative features of different mechanistic pathways resolved from animal experiments, cell culture studies, and human reports and the overlapping regions

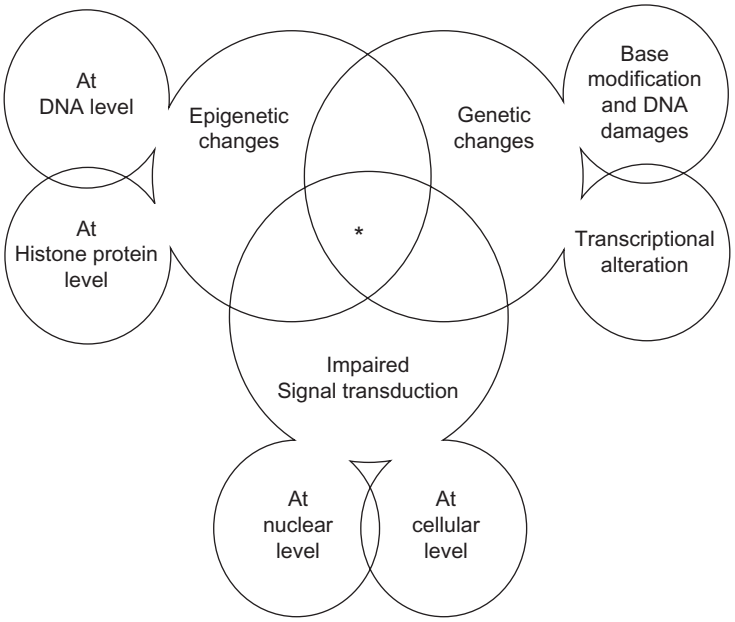

FIGURE 9–7 Different important overlapping pathways of arsenic-associated toxicity mechanism that may finally result in mutagenesis and/or carcinogenesis. In general, the fewer the number of factors involved, the less chance of disease occurrence/severity. The overlapping region denotes the interactive nature of several pathways, which usually manifest adverse cumulative impact. The central overlapping region (*) may be represented as the most severe disease status. The important unique molecules/steps at the overlapping regions might have been a good therapeutic target.

of these pathways should be taken into account in defining the arsenic-associated human carcinogenesis process (Figure 9–7).

9.4.2 Arsenic Affects Cultured Human Cells and Environmentally Exposed Human Populations

Inorganic arsenic is undoubtedly carcinogenic in humans. In the preceding section the mechanism for arsenic-associated carcinogenesis at the molecular level was discussed. The results are mostly analyzed from human-cultured cell experiments or from environmentally exposed human sample data. Arsenic can induce genotoxicities by introducing large deletion mutations in human/hamster hybrid cells and at the thymidine kinase (TK) locus of mouse lymphoma cells [187]. Genotoxic damage has also been implicated by chromosomal abnormalities, sister chromatid exchange, micronuclei induction, and abnormal DNA synthesis. DNA/protein crosslinking has also been demonstrated to occur in human cells exposed to arsenic [187]. A micronucleus is an extranuclear body containing some DNA damage and random mutations in a daughter cell [188]. DNA/protein crosslinks occur when various exogenous or endogenous agents react with two different positions in the DNA. DNA replication is blocked by crosslinks, which causes replication arrest and cell death if the crosslink is not repaired [189]. As described

in the previous section, arsenic is highly capable of influencing these molecular events. TK is a phosphotransferase (a kinase): 2′-deoxythymidine kinase, ATP-thymidine 5′-phosphotransferase. TKs have a central role in the synthesis of DNA and thereby in cell division, as they participate in the reaction chain to incorporate deoxythymidine in DNA [190].

Low dose and long-term exposure (≤0.1 μM for 8 weeks) to arsenite in human osteosarcoma cells cause transformation and mutations in another important enzyme hypoxanthine phosphoribosyltransferase (HPRT) gene locus [191]. Its product is hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and the gene is located on the X chromosome. HGPRT catalyzes conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. This reaction transfers the 5-phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate (PRPP) to the purine [192]. Arsenic binds with sulfhydryl groups of DNA repair enzymes and initiates genotoxic and carcinogenic effects. This toxic metalloid also impairs DNA ligase and poly (ADP-ribose) polymerase (PARP) activities and results in mutations in the presence of UV irradiation [191]. PARP is a family of nucleoproteins that repair single-strand DNA breaks (SSB) and control programmed cell death. PARP is activated in response to physical or chemical stress-induced DNA SSB. It repairs SSB after binding to the DNA, and begins the synthesis of a poly (ADP-ribose) chain (PAR). It acts as a signal for the other DNA-repairing enzymes such as DNA polymerase beta (polβ), DNA ligase III (LigIII), and some important scaffolding. The PAR chains are degraded via poly (ADP-ribose) glycohydrolase (PARG) after the repair process [193]. As mentioned, arsenic-associated DNA strand breakage is also repaired by these enzyme systems.

The reports from an arsenic-exposed Bangladesh population suggest that the DNA repair gene *XPD* (also known as *ERCC2*) is associated with premalignant skin lesions and hyperkeratosis. The interindividual variability in arsenical skin lesions was due to different SNPs in the *XPD* gene. XPD is a nucleotide excision repair (NER) enzyme [194], which also participates in the basal level of transcription by interacting with transcription factor TFIIH [195,196]. The important SNP in the *XPD* gene is present on codon 751 in exon 23 (A→C, Lys→Gln), which has been shown to be associated with the risk of skin cancer. Arsenic impairs DNA repair by inhibiting DNA ligase 1 [197], a required enzyme for NER mechanism. In addition, arsenic also generates oxidative DNA damage, which can be corrected by NER. This may indicate that the XPD genotype is a potential modifier of arsenic effects [198]. In brief, various genes related to DNA and nucleic acid metabolism are the prime target in arsenic-associated human carcinogenesis. Impairment of the role of some transcription factors is also a notable concern in this regard (Figure 9–6).

9.4.3 Arsenic Induces Telomere Instability

The telomeric regions of the chromosome and the telomerase enzyme have been considered as among the significant determinants of genome stability. The condensed nucleoprotein structures at chromosome termini are known as the telomere. It is composed of simple, repetitive G/C-rich DNA complexes with different histone proteins [199,200]. The investigators showed that arsenic inhibits telomerase transcription and results in the chromosomal

end lesions in human cells *in vitro*. Accordingly, arsenic may interfere with chromosome stability, which leads to clastogenicity in human cells. Telomeres play a critical role in maintaining chromosome stability by protecting chromosome ends from degradation and fusion [201]. The human telomeric DNA sequence is (TTAGGG)n and the terminal restriction fragment (TRF) contains the (TTAGGG)n sequence [200]. The DNA synthesis machinery is incapable of replicating three primed termini of the chromosome replication; thus there is a gradual loss of telomeric sequence under normal conditions during cell division. The eventual consequences of this process are senescence or apoptosis. Telomerase, a special ribonucleoprotein reverse transcriptase, is important in maintaining telomere length. It is necessary for the sustained growth of most human tumors and plays an important role in tumorigenesis [199,202]. The expression of human telomerase reverse transcriptase (hTERT) is the rate-limiting step for telomerase activity. Telomerase activity has been detected in different types of tumor cells [201–203].

The telomerase RNA template (TR) interacts with telomerase reverse transcriptase and this process regulates telomere elongation during cell division. It is reported that telomere shortening, chromosome end-to-end fusion, and reactivation of telomerase occur excessively, which promotes carcinogenesis in p53 mutant status [204,205]. Human cells are more sensitive to arsenite than are the cells of rodent origin [206], and one possible reason for this difference could be attributed to the short telomeres found in human cells [207]. Differences between mouse and human telomeres restrict the laboratory mouse as a reliable model to critically test the role of telomere dysfunction and telomerase in human malignancy. However, telomerase knockout mice show progressive telomere shortening and chromosomal instability with increasing generations. There are defects in highly proliferative tissues, affecting fetal development, growth, immune function, and carcinogenesis [208,209]. ROS are found to damage telomeres and lead to genomic instability [210]. Arsenic-induced ROS production is very much evident in all types of experimental model and in environmentally exposed human individuals. Sustained damage to telomeres is regarded as promoting the aging process. Arsenic-associated ROS production and telomere instability may mimic senescence-related features. The arsenic-associated carcinogenesis mechanism is summarized in the Figure 9–4.

9.4.4 Arsenic-Associated DNA Damage and Genome Dysfunction in Humans

Environmental conditions and addiction habits have been linked with the severity of arsenic pathogenesis. It has been reported that tobacco use may increase the risk of bladder cancer and its pathogenesis in arsenic-exposed individuals in comparison to their non-smoker controls [211]. Studies suggest that peripheral blood cells may be affected by long-term arsenic exposure. Water, blood, and urinary As have been positively correlated with global peripheral blood mononuclear cell (PBMC) DNA methylation [212]. This suggests that such epigenetic events and the gene function efficacy may be impaired by arsenic exposure in mixed white blood cell (WBC) preparations. In an epidemiological study involving a large number of arsenic-exposed populations from West Bengal in India, it has been reported that long-term

consumption of arsenic-enriched water caused skin lesions and other dermatological and multi-organ health problems resulting in cancer and death. In this study, an association between arsenicism and exonic single nucleotide polymorphisms (SNPs) in NAcht Leucine-rich repeat Protein 2, (*NALP2* gene, also known as *NLRP2* and *PAN1*), an important component of the inflammasome complex, was suggested [213]. Among several important SNPs found in the *NALP2* gene, the A1052E polymorphism is the most significant. These findings suggest that the *NALP2* A1052E SNP plays an important role in the development of arsenic-induced skin lesions and chromosomal damage [213]. NALP2 is a cytosolic, 121 kDa member of the NLRP family of proteins. It is expressed in macrophages and exhibits divergent effects on inflammation. Through the involvement of NF-κβ, it suppresses TNF-α production in response to lipopolysaccharide (LPS), and via procaspase-1 it promotes proIL-1β cleavage and release. Human NALP2 is 1062 amino acids in length [214,215]. An association has been shown between arsenic exposures levels being measured in toenails, and blood DNA methylation in Alu and Long interspersed nucleotide element-1 (LINE-1) repetitive elements in elderly men environmentally exposed to low levels of arsenic [216]. This study also revealed that higher arsenic contents are associated with increasing Alu and decreasing LINE-1 DNA methylation. The link between plasma folate, cobalamin (vitamin B12), and arsenic-associated methylation of these DNA regions suggests that the metabolism of the methyl group is an important factor [216]. The study also revealed that women had lower levels of LINE-1 methylation than did men [217]. Different arsenic species have been shown to induce strand breakages differentially. A discussion of DNA damage events which are associated with arsenic-induced carcinogenesis is given in the following paragraph.

9.4.5 Arsenic and Epigenetic DNA Modification

As(III) induces DNA strand breaks in human fetal lung fibroblast (2BS) cells [217], while DMA(V) induces DNA strand breaks in human alveolar epithelial type II (L-132) cells [218]. As(III) induces single-strand DNA breaks, DNA/protein adducts, and sister chromatid exchanges in human fibroblast cell lines [219]. Formation of apurinic/apyrimidinic sites has been shown in the human alveolar epithelial cell line L-132 as caused by DMA [220]. As(III) has been shown to increase 8-OHdG levels in human breast cancer (MCF-7 adenocarcinoma epithelial cells) [221]. Increases in 8-OHdG levels through $(CH_3)_2AsOO$ DMA(V) have also been demonstrated [55,222,223]. Both As(III) and As(V) may result in the alteration of methylation in the p53 promoter of the A549 cell line [224]. As(III) led to a marked increase in the activity in chromosome H3K9 dimethylation and decreased H3K27 trimethylation (gene silencing) events. It also increased the H3K4 trimethylation (gene-activating) process, and histone methyltransferase G9a protein levels in the human A549 cell line [225]. Changes to histone H3 acetylation and DNA promoter methylation, and decreases in expression of *DBC1*, *FAM83A*, *ZSCAN12*, and *C1QTNF6* genes in human non-tumorigenic cell lines have been observed after arsenic exposure [226]. Arsenic(III)-associated global hypomethylation has been shown in different cell lines of human sources like prostate epithelial cells RWPE-1 and keratinocytes HaCaT [186,227]. Hypomethylation of specific gene locus has also been

noticed by several investigators. As(III) and MMA(III) influence *DBC1*, *FAM83A*, *ZSCAN12*, and *C1QTNF6* genes in some cultured cells [97,226]. As(III) may influence *DAPK*, *P16*, and *P53* genes in uroepithelial SV-HUC-1 cells, myeloma cell line U266, and lung adenocarcinoma A549 cells, respectively [228].

A recent study found the occurrence of 22 CpG sites with increased methylation in WBC DNA collected in arsenic-exposed populations [229]. Some genes annotated by these 22 CpG sites are known to be involved in As-associated diseases [230]. A significant increase in 8-OHdG levels in the As(III)-exposed mouse liver was confirmed in a study that also showed the presence of severe oxidative DNA damage. These results demonstrate that arsenite is mutagenic *in vivo* and suggest that arsenite induces G:C to T:A transversions through oxidative stress induced by 8-OHdG formation [231]. The alteration in regulations of various miRNAs has been noted after iAs treatment in Jurkat cells. Several miRNAs show specific dysregulation, and particularly a panel of eight miRNAs is demonstrated to be involved in arsenic-response pathways. This identification is invaluable and can serve as a potential biomarker of arsenic toxicity with useful diagnostic value [232]. Differential dysregulation of important genes provides variability in different humans. Overall, these findings provide evidence that interindividual differences in arsenic metabolism may be an important risk factor for arsenic-related lung cancer, and may play a role in cancer risks among people exposed to relatively low arsenic levels [233].

Melanocytes are more resistant to arsenite and UVR in the stimulation of superoxide production than are keratinocytes. However, the same concentration of arsenite may promote UVR-induced DNA damage in both types of cell by inhibiting poly(ADP-ribose)polymerase. These findings suggest that arsenic, on chronic exposure, could promote retention of unrepaired DNA damage in melanocytes, acting as a co-carcinogen in melanoma [234]. Few earlier studies have demonstrated that hypermethylation of the promoter region of *p53* and *p16* genes is occurring in persons exposed to arsenic [235]. Until now no genomic hotspot has been decisively identified that is frequently hypermethylated or hypomethylated in persons chronically exposed to environmental arsenic [235]. FBJ murine osteosarcoma viral oncogene homolog B is known as FosB protein. *Fosb*, an important oncogene, has been shown to undergo alteration in DNA methylation via gestational arsenic exposure [236]. It was shown that Fosb expression significantly increased corresponding to the DNA methylation of the gene in the arsenic-exposed group. These findings suggest that DNA methylation status can be used to identify tumors resulting from gestational arsenic exposure [236].

Arsenic species patterns in urine are associated with risk of cancer and cardiovascular diseases. The organic anion transporter coded by the gene *SLCO1B1* may transport arsenic species depending on this genetic status (presence/absence or variants) [237]. Hypermethylation was found in the promoters of both *DAPK* and *p16* genes in individuals with arsenic-induced skin lesions in comparison to the individuals without skin lesions (positive controls) [238]. A significant decrease was noted in the expression of death-associated protein kinase and in the gene expression of p16 in induced cases compared to controls [238]. The lowest expression was observed in cancerous tissues. The hypermethylation of the corresponding promoter of specific genes was also associated with higher risk of developing arsenic-induced skin lesions,

peripheral neuropathy, and ocular and respiratory diseases [238]. Not only arsenic, but nickel, cadmium, and chromium (VI) are known to alter the DNA methylation profile by inducing both hyper- and hypomethylation events. Recent developments in finding a suitable therapy are based on the cancer methylome [239]. Differential DNA methylation was noted in the umbilical cord blood of infants exposed to low levels of arsenic *in utero*. It was also suggested that *in utero* exposure to low levels of arsenic may affect the epigenome [11,240]. Chronic exposure to arsenic is known to generate reactive oxygen species, which may ultimately lead to the induction of certain proinflammatory cytokines. Polymorphism of inflammatory genes and arsenic methylation capacity are associated with urothelial carcinoma [241]. Here, specific polymorphism in TNF-α -308 G/A, IL-6 -174 G/C, IL-8 -251 T/A, and urinary arsenic profiles were found to be related to urothelial carcinoma (UC) risk. The study evaluated 300 pathologically confirmed cases of UC and 594 cancer-free controls. A significant decrease in UC risk was found in subjects who carried the IL-8 -251 T/T genotype [241]. This study strongly suggests that not only metabolism associated genes, but immune system associated genes too showed impaired methylation with cancer manifestations. Arsenic-contaminated drinking water and the risk of various human malignancies were verified and the study revealed that the *FHIT* and *WWOX* genes are active in common fragile sites FRA3B and FRA16D, respectively [242]. Reduced expression of FHIT or WWOX is known to be an early indicator of carcinogen-induced cancers. An interesting study recently suggested that arsenite decreased the expressions of ATR, WWOX, and FHIT via *ERK1/2* activation in SV-HUC-1 cells. These findings also confirmed that dysregulations of these markers might also be contributing factors to arsenite-induced carcinogenesis [242]. As a whole, a large number of genetic materials may be interfered with by arsenic at their regulatory steps. Several of these interferences may result in obvious onset of tumorigenesis and/or carcinogenesis (Figure 9–6).

9.4.6 Arsenic-Associated Signal Transduction Processes and Human Carcinogenesis

Arsenic-associated mutagenesis/carcinogenesis processes may be associated with the impairment of signal transduction processes. In animal models several of these pathways have been explored. Most of these pathways are also applicable to human-associated carcinogenesis to different degrees. The interacting molecules may have a varied extent of association in terms of quality and quantity.

The regulation of p53 tumor suppressor gene product is very important in cellular transformation. Normal p53 protein is involved in cell cycle regulation, in maintenance of genomic stability, and in controlled cell apoptosis. Studies revealed involvement of arsenic-associated *p53* mutation in the manifestations of bladder and skin tumors, and the induction of carcinogenesis through inhibition of DNA repair. All these processes significantly resulted in oxidative damage to DNA [243]. It is reported that the bladder tumors from people chronically exposed to arsenic showed mutations in the *TP53* gene at codon 175 and transitions at points 9 and 10 [244]. iAs(III) treatment (10 μM) has been reported to induce an accumulation of p53 protein in different cell lines having a wild or mutated gene, with a greater effect in cells having

normal *TP53* than in those with mutated *TP53* [245]. The p53 protein is a transcription factor that accumulates in response to DNA damage and other stress stimuli. This increases the expression of cyclin-dependent kinase inhibitor p21 (CKI p21) protein, which is able to silence cyclin-dependent kinases (CDKs) essential for S-phase entry (via cyclins E and A) leading to the deactivation of cell cycle progression [246]. The p21 protein has been characterized as a cyclin kinase inhibitor that induces growth arrest by preventing phosphorylation of retinoblastoma pRb in G1/S transition. pRb regulates G1/S transition depending on its phosphorylation status, which is modified by CDKs/cyclin complexes [245,247].

Arsenite binds with the sulfhydryl (SH) group of enzymes, leading their inhibition [248]. Arsenite induces production of reactive oxygen species, which affects multiple targets in the cell such as inhibition of IκB kinase (IKK)1 by arsenite [249]. This results in the suppression of NF-κB transcription factor activation followed by a marked change in the anti-apoptotic functions of the cell [250]. Simultaneously, oxidative stress induces kinase pathways (MKK6-MAPK p38, MKK4/7- JNK, and MEK-ERK1/2), up-regulates the AP-1 (Jun-Fos, Jun- ATF2) transcription factors, and induces AP-1-dependent gene expression and the regulation of the STAT family [251,252]. In general, different cancerous growths result from the involvement of high levels of cytokines and growth factors (i.e., TNF-α, transforming growth factor-β, and TRAIL) supporting their autonomous growth [253]. Moreover, the important transcriptional regulator NF-κB pathway and two other signaling pathways, Ras-Raf-MEK-ERK and PI3K-AKT, are shown to be critically involved in the regulation of cell survival including the scenario of arsenic-associated carcinogenesis [250,254,255].

Inorganic arsenic transforms the RWPE-1 human prostate epithelial line to CAsE-PE cells, and a derivative normal stem cell (SC) line, WPE-stem, to the arsenic-cancer SC (As-CSC) line. miRNAs are non-coding but exert negative control of expression by degradation or translational repression of target miRNAs. Aberrant miRNA expression is important in carcinogenesis [256]. In both transformants, decreased miRNAs targeting KRAS and RAS superfamily members impair signal transduction processes. Reduced miR-134, miR-373, miR-155, miR-138, miR-205, miR-181d, miR-181c, and let-7 in CAsE-PE cells was correlated with increased target of *RAS* oncogenes, RAN, RAB27A, RAB22A mRNAs, and KRAS protein. Reduced miR-143, miR-34c-5p, and miR-205 in As-CSC was correlated with increased target RAN miRNA, and KRAS, NRAS, and RRAS proteins. The RAS/ERK and PI3K/PTEN/AKT pathways control cell survival, differentiation, and proliferation, and when dysregulated promote a cancer phenotype. iAs transformation increased expression of activated ERK kinase in both transformants and altered components of the PI3K/PTEN/AKT pathway, including decreased PTEN, and increased Bcl-2, Bcl-xL, and vascular endothelial growth factor (VEGF) in the absence of AKT activation. Thus, dysregulated miRNA expression may be linked to RAS activation in both transformants [256,257].

As mentioned earlier, IκBs are a family of related proteins that have an N-terminal regulatory domain, followed by six or more ankyrin repeats and a PEST domain near their C terminus. Although the IκB family consists of IκBα, IκBβ, IκBε, and Bcl-3, the best-studied and major IκB protein is IκBα. Activation of NF-κB is initiated by the signal-induced degradation of IκB proteins. This occurs primarily via activation of a kinase called the IκB kinase (IKK). IKK is

composed of a heterodimer of the catalytic IKKα and IKKβ subunits and a "master" regulatory protein termed the NF-κB essential modulator IKKγ [258]. The regulation of IκB protein is very important because arsenic-associated tumorigenesis and carcinogenesis have been shown to be influenced by NF-κB signaling [258]. The activator protein 1 (AP-1) is a transcription factor that is a heterodimeric protein composed of proteins belonging to the c-Fos, c-Jun, ATF, and JDP families, which are also shown to participate in arsenic-related cancerous growth. It regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, metal-associated carcinogenesis including arsenic, and bacterial and viral infections. AP-1 in turn controls a number of cellular processes, including differentiation, proliferation, and apoptosis [259]. Arsenic has been shown to promote tumor formation by stimulating AP-1 activity and inhibiting a JNK phosphatase [251]. The signal transducer and activator of transcription 3, also known as STAT3, is a transcription factor protein, which in humans is encoded by the *STAT3* gene, which has been shown to influence oncogenic activation upon arsenical threat [260,261]. In response to cytokines and growth factors, STAT family members are phosphorylated by receptor-associated kinases and then form homo- or heterodimers that translocate to the cell nucleus, where they act as transcription activators [260]. Cytokine mediation in STAT regulation in arsenic carcinogenesis is evident [260].

A very recently characterized gene, *mdig* (mineral dust-induced gene), is a newly identified oncogene linked to occupational lung diseases and lung cancer. It is unclear whether *mdig* is also involved in arsenic-induced malignant transformation of lung cells. By using human bronchial epithelial cells and human lung cancer cell lines, researchers demonstrated that arsenic is able to induce expression of *mdig* [261]. This group further demonstrated that *mdig* induction by arsenic was partially dependent on the JNK and STAT3 signaling pathways. Disruption of JNK or STAT3 by their corresponding siRNAs or some chemical inhibitors diminished arsenic-induced accumulation of *mdig* mRNA and protein. The group also showed that microRNA-21 (miR-21) and Akt were downstream effectors of JNK and STAT3 signaling pathways in arsenic-induced mdig expression. Collectively, these data suggest that *mdig* may play an important role in the pathogenesis of arsenic-induced lung cancer. Further, JNK and STAT3 signaling pathways are essential in mediating arsenic-induced *mdig* expression [261]. There is also evidence that miR-21 is involved in arsenite-induced malignant transformation and associated signaling pathways. During arsenite-induced transformation of human embryo lung fibroblast (HELF) cells, miR-21 is up-regulated, and the ERK/NF-κB signaling pathway is activated [262]. Moreover, superoxide dismutase (SOD, a scavenger of superoxide) and catalase (a scavenger of hydroperoxides) block the arsenite-induced effects in HELF cells and mouse embryonic fibroblasts. Overexpression of miR-21 and the activation of ERK and NF-κB promote the malignancy of HELF cells exposed to arsenite. But knockdown of miR-21 and a feedback blockage of ERK and NF-κB activation decrease anchorage-independent growth of arsenite-transformed cells. Thus, the transformation of HELF cells by chronic arsenic exposure is mediated by increased miR-21 expression. This overexpression is mediated by the ROS activation of the ERK/NF-κB pathway [21,262]. MicroRNA-21 is also known as hsa-mir-21 or miRNA21, a mammalian microRNA, which is encoded by the *MIR21* gene. MIRNA21 was one of the first mammalian microRNAs identified. The mature miR-21 sequence is strongly

conserved throughout evolution. The human microRNA-21 gene is located on the plus strand of chromosome 17q23.2 (55273409–55273480) within a coding gene *TMEM49* (also called vacuole membrane protein) [263].

Arsenic, due to its ability to regulate genes that link cell cycle control with apoptosis, has been widely recognized to play a crucial role in oncogenomics. miR-2909 regulates *CCND1* (*cyclin D1*) gene expression within these cells by inducing the splice-switching of tumor suppressor *CYLD* (cylindromatosis) gene as well as by modulation of specificity protein 1 (SP1) activity through the repression of Kruppel-like factor4 (KLF4) expression at the translational level [264].

Inorganic arsenic is a well-known human skin carcinogen. Chronic arsenic exposure results in various types of human skin lesions, including squamous cell carcinoma (SCC). To investigate whether mutant stem cells participate in arsenic-associated carcinogenesis, HaCaT cell lines were repeatedly exposed to an environmentally relevant level of arsenic (0.05 ppm) *in vitro* for 18 weeks. Following sodium arsenite administration, cell cycle, colony-forming efficiency (CFE), cell tumorigenicity, and expression of CD44v6, NF-κB, and p53 were analyzed. The expression level of cleaved caspase-3, which is a molecular indicator of cell apoptosis, was remarkably elevated after long-term arsenic exposure [265]. An arsenic-induced tumorigenesis and carcinogenesis mechanism is summarized in Figure 9–4.

Investigators also observed that chronic exposure of HaCaT cells to a low level of arsenic led to a cancer stem-like phenotype. Further, arsenic-treated HaCaT cells also became tumorigenic in nude mice and their growth cycle was predominantly in the G2/M and S phases. Arsenic was capable of increasing cell proliferation and the sprouting of the cancer stem-like phenotype. Immunohistochemical analysis demonstrated that CD44v6 expression was upregulated in HaCaT cells exposed to a low level of arsenic. Likewise, the expressions of activating transcription factor NF-κB and p53 genes in the arsenic-treated HaCaT cells were also significantly higher than in non-treated cells. Higher expressions of CD44v6, NF-κB, and p53 were also observed in tumor tissues isolated from Balb/c nude mice. Arsenic promotes malignant transformation in human skin lesions through an NF-κB signaling pathway-stimulated expression of CD44v6 [266]. The chronic arsenic-exposed breast epithelial (CABE) cells showed increases in secreted matrix metalloproteinase (MMP) activity, colony formation, invasion, and proliferation rate, indicating an acquired cancer cell phenotype. These CABE cells presented basal-like breast cancer characteristics, including ER-α, HER-2, and progesterone receptor negativity, and overexpression of K5 and p63. Putative CD44(+)/CD24(−/low) breast SCs were increased to 80% over the control in CABE cells [267]. However, the role of epithelial to mesenchymal transition (EMT) in arsenic carcinogenesis is not well understood, although previous studies have shown that short-term exposure of endothelial cells to arsenic stimulates angiogenesis. It still remains to be determined whether cells that were malignantly transformed by long-term arsenic exposure have a pro-angiogenic effect. It was found that the conditioned medium from arsenic-transformed cells strongly stimulated tube formation by human umbilical vein endothelial cells (HUVECs). Moreover, enhanced angiogenesis was detected in mouse xenograft tumor tissues resulting from inoculation of arsenic-transformed cells. Mechanistic studies revealed that β-catenin was activated in arsenic-transformed cells,

up-regulating its target gene expression, including angiogenic-stimulating vascular endothelial growth factor (VEGF). Reverse transcriptase-PCR analysis revealed that the mRNA levels of canonical Wnt ligands were not increased in arsenic-transformed cells. These findings suggest that EMT in arsenic-transformed cells promotes angiogenesis through activating the β-catenin-VEGF pathway [268].

The Nrf2–Keap1 signaling pathway is a protective mechanism promoting cell survival. Activation of the Nrf2 pathway by natural compounds has been proven to be an effective strategy for chemoprevention. Interestingly, a cancer-promoting function of Nrf2 has recently been observed in many types of tumors due to deregulation of the Nrf2–Keap1 axis, which leads to constitutive activation of Nrf2 [269]. Recently, a novel mechanism of Nrf2 activation by arsenic has been postulated that is distinct from that of chemopreventive compounds. Arsenic deregulates the autophagic pathway through blockage of autophagic flux, resulting in accumulation of autophagosomes and sequestration of p62, Keap1, and LC3. Thus, arsenic activates Nrf2 through a non-canonical mechanism (p62 dependent), leading to a chronic, sustained activation of Nrf2. In contrast, activation of Nrf2 by sulforaphane (SF) and tert-butylhydroquinone (tBHQ) depends upon Keap1–C151 and not p62 (the canonical mechanism). More importantly, SF and tBHQ do not have any effect on autophagy. In fact, SF and tBHQ alleviate arsenic-mediated deregulation of autophagy. Collectively, these findings provide evidence that arsenic causes prolonged activation of Nrf2 through autophagy dysfunction, possibly providing a scenario similar to that of constitutive activation of Nrf2 found in certain human cancers. This may represent a previously unrecognized mechanism underlying arsenic toxicity and carcinogenicity in humans [270].

The major signaling pathways altered include NRF2–mediated stress response, interferon, p53, cell cycle regulation, and lipid peroxidation. These results showed a similar process qualitatively and quantitatively for all three cell types from human sources, urothelial (1T1), keratinocyte (HEK001), and bronchial epithelial (HBE) cells, and support a mode of action involving cytotoxicity and regenerative proliferation [271]. TGF-β is a multifunctional peptide related to arsenic-associated carcinogenesis and controls proliferation, differentiation, and other functions in many cell types [272]. Another important protein TNF-related apoptosis-inducing ligand (TRAIL) has been shown to induce apoptosis and is linked to arsenic-related apoptotic tissue death [273]. Arsenic is known to influence mitochondrial permeability, transition pore opening, and loss of the mitochondrial trans-membrane potential, which down-regulates the Bcl-2 protein and activates caspase-3 activity (Figure 9–5). The disruption of mitosis was shown to be due to interference with tubulin polymerization and disruption of mitotic spindles, inactivation of centrosomes, formation of aberrant spindles, and blockage of chromosome segregation [274–277]. Another study assessed the role of Akt in the cell death induced by As_2O_3. As_2O_3 caused dose-dependent apoptotic cell death [278]. As_2O_3 activated caspase-3 and -9 and PARP cleavage in a dose-dependent manner. Altered mitochondrial membrane potential and an increased protein level of Bax indicated involvement of mitochondria [278] (Figure 9–5). As_2O_3 decreased the levels of p-Akt (Ser473), p-Akt (Thr308), and p-GSK-3β (Ser9), suggesting that As_2O_3 inactivated Akt kinase. These results demonstrated that inhibition of PI3K/Akt signaling was involved in As_2O_3-induced apoptosis of gastric cancer SGC-7901 cells [278]. The arsenic-associated carcinogenesis mechanism is summarized in Figure 9–4.

Arsenic induces the expression of angiogenesis-related factors through PI3K and MAPK pathways in SV-HUC-1 human uroepithelial cells [279]. Several important angiogenesis related-factors, including cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF), and hypoxia-inducible factor-1α (HIF-1α) were up-regulated and PI3K/AKT and MAPK signal pathways were activated in the human uroepithelial cell line (SV-HUC-1) [279]. In conclusion, arsenite-induced COX-2, VEGF, and HIF-1α expressions, mediated partially by ROS, were regulated by MAPK and PI3K/AKT signaling pathways in human uroepithelial cells [279,280]. This study also evaluated the changes in the expression and release of cytokines in Caco-2 cells, widely used as an intestinal epithelial model. Differentiated cells were exposed to 1 μM of As(III), 0.1 μM of monomethylarsonous acid (MMA(III)), and 1 μM of dimethylarsinous acid (DMA(III)) for 2, 4, 6, and 24 h. Additionally, the effect of arsenic co-exposure with lipopolysaccharide (LPS, 10 ng/mL) was evaluated [281]. The results of arsenic exposure of human carcinoma Caco-2 cells, widely used as an intestinal epithelial model, showed that arsenic increases the expression and release of the pro-inflammatory cytokines TNF-α, IL-6, and IL-8 [281]. Arsenite also caused malignant transformation of cells. Another study has also revealed the molecular mechanisms underlying inflammation during neoplastic transformation induced in HBE cells. The results showed that, on acute or chronic exposure to arsenite, HBE cells overexpressed the pro-inflammatory cytokines IL-6, IL-8, and IL-1β. The data also indicated that HIF-2α was involved in arsenite-induced inflammation. Moreover, IL-6 and IL-8 were essential for the malignant progression of arsenite-transformed HBE cells. The results provided a link between the inflammatory response and the acquisition of a malignant transformed phenotype by cells chronically exposed to arsenite [282]. A further study aimed to evaluate global miRNA and mRNA expression changes induced by a metal mixture containing $NaAsO_2$, $CdCl_2$, $Pb(C_2H_3O_2)_2{\cdot}3H_2O$ and to predict possible metal-associated disease development under these conditions [283]. Results showed that this metal mixture led to an miRNA expression profile that may be responsible for the mRNA expression changes observed under experimental conditions. The proteins coded by these mRNAs are shown to be involved in metal-associated responses to apoptotic cell death, cellular proliferation, and cancer [283]. Different species of inorganic and organic arsenical compounds are shown to induce epigenetic and genetic events in human cultured cells, which may be implicated in the carcinogenesis processes (Figure 9–6). As(III) can also alter the expression of has-miR-210, -22, -34a, -221, and -222 in human lymphoblastoid cells [284]. As(III) exposure also resulted in the reduction of miR-200 in immortalized p53-knocked down human bronchial epithelial cells (HBECs) [285].

The mir group (miRNA) of sequences is microRNAs, which are also known as hsa-mir. This mammalian microRNA is encoded by the respective *MIR* gene. MIRN21 was one of the first mammalian microRNAs identified. A recent interesting study suggested that As(III) induces MAPK activation, phosphorylation of ATF-2 and c-Jun, and elevated IL-8 release in the human BEAS 2B cell line [286]. Induction of p53-independent expression of GADD45 protein (a G2/M cell cycle checkpoint protein) by As(III) has been shown in the human BEAS 2B line [287]. Stabilization of GADD45 alpha mRNA As(III) through nucleoli is demonstrated in the human BEAS 2B line [288]. As(III) significantly enhanced centrosome amplification in p53-compromised cells. Resistance to arsenite-induced G2/M cell cycle arrest and arsenite-induced apoptosis in p53-compromised cells has been observed [289]. A higher reduction in

cell viability in BEAS-2B cells (with functional p53) vs p53 compromised cells (H1355 or p53 inhibited BEAS-2B are noticed, after a low dose (0.1–5 μM) arsenic exposure. There was a significant reduction in arsenic-induced apoptosis in p53 compromised cells. A reduction in Gadd45a expressions and increased centrosomal abnormalities were observed in arsenite-treated p53 compromised cells [289]. As(III) (≤0.5 μM) increased cell viability and resulted in the down-regulation of APE1 and Polβ mRNA (above 1 μM) in GM847-immortalized human lung fibroblast [290]. As(III) also produces a series of molecular and signaling phenomena. It increased plating efficiency (cell growth advantage), micronuclei incidence (marker of chromosomal instability), gene amplification (PALA resistance), invasive capabilities, anchorage-independent growth (oncogenic transformation), loss of β4 integrin expression, up-regulation of phosphorylation of Rb and ERK, and decreased expression of p53 protein in h-TERT-immortalized human small airway epithelial cells [291].

9.5 Conclusions and Future Directions

The inevitable scarcity of ground water and the unavoidable situation of increased use of deeper earth water is gradually increasing the possibility of unaffected areas and their associated populations becoming exposed to arsenic in the future. Inorganic arsenic and its organic species exert their toxicities in different ways at the cellular and molecular levels. These include modulation of several genetic and epigenetic factors, which are directly or indirectly associated with the cellular transformation process. Arsenicals are highly potent in initiating oxidative stress. Reactive oxygen species or nitrogen species can modulate cellular signaling pathways at nuclear and cellular levels. The consequence is an impaired differentiation/proliferation balance, resulting in cellular transformation. This chapter discusses several pathways explored by a number of researchers and scientists using cell culture or animal models or human epidemiological data. Nonetheless, it cannot be concluded that all possible mechanisms are occurring at the same time in the human carcinogenesis process in a particular location and with specific nutritional and environmental conditions. Rather, it is reasonable to believe that few or even one of those mechanisms are sufficient to initiate carcinogenesis pathways. But it is unequivocal that the greater the number of pathways involved, the greater the chances of disease pathogenesis with higher degrees of severity within a shorter period. Better investigation and a comprehensive understanding the disease mechanism will enable us to gain a better handle on a reliable therapeutic approach. It would of course be extremely beneficial for the millions of affected people around the world.

Significant new and emerging research constitute the present and future directions in the determination of the mechanisms of arsenic toxicity. Mechanistically, elucidation of the overlapping zone of the epigenetic, genetic, and cellular signal transduction pathways will pave the way in the search for important molecules that unequivocally result in arsenic-related carcinogenesis (Figure 9–7). Discovery of the molecules participating in tumorigenesis/carcinogenesis mechanisms will enable scientists to focus on obvious therapeutic targets. Recent advancements in neutragenomic and neutraceutical studies also might be a great help to counteract

arsenic toxicity at the therapeutic and/or prevention level(s). Future studies on sociodemographic profiles, nutritional status, and, above all, genetic susceptibility/predisposition, will also help to evaluate the possible progression of the toxicity mechanism in affected individuals. All the above would consolidate a better therapeutic approach.

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Arsenic Through the Gastrointestinal Tract

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CHAPTER OUTLINE

10.1 General Aspects

Arsenic (As) is widely distributed throughout Earth's crust where it can be found in several different chemical species, inorganic and organic, with a significant and variable degree of toxicity. Inorganic arsenic (iAs) is one of the most toxic species found in water and food, the main sources of As exposure for the general population [1]. The World Health Organization (WHO) estimates that >200 million persons worldwide might be chronically exposed to As in drinking water at concentrations above the WHO safety standard of 10 μg/L [2]. This estimation of the affected population would be increased if the intake of As through food were considered.

The term iAs includes both tri- (As^{III}) and pentavalent (As^{V}) oxidation states, As^{V} representing the main arsenical species in waters. The biotransformation of iAs occurs mainly in the liver and implies successive reduction and oxidative methylation steps producing different species with an increasing degree of methylation (mono-, di-, trimethylarsenic) and valency (trivalent or pentavalent), two critical features largely determining the toxicity of As [3]. Inorganic species are more toxic than methylated ones and trivalent species are more toxic than pentavalent ones. The biomethylation process was considered a detoxification process; however, there is still an open discussion ongoing on this matter because it has been

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demonstrated that trivalent mono- and dimethylated compounds [4] and thiolated intermediary metabolites have higher toxicity than iAs [5–8]. *In vitro* studies have also evidenced the existence of a presystemic biomethylation of iAs by human intestinal epithelial cells [9] and human gut microbiota [10].

Adverse health effects have been widely described after iAs intake in chronic and acute studies, with the conclusion that As is a highly potent toxicant and carcinogen [11], even at low doses. iAs is classified by the International Agency for Research on Cancer (IARC) as a carcinogen to humans (Group I) associated also with increasing prevalence of skin lesions, cardiovascular and cerebrovascular pathologies, type 2 diabetes, respiratory illnesses, and neurobehavioral and developmental disorders [1].

10.2 Arsenic in Foods: Household Processing and Toxicological Risk

Food and dietary habits constitute the most important source of As exposure for persons with limited exposure via drinking water [1]. Particular attention needs to be focused on infants during early stages of life. Moreover, persons suffering specific metabolic diseases that could affect As accumulation and metabolism, causing a potential major susceptibility to toxicity, deserve special consideration.

Studies conducted in different countries to evaluate total dietary As have shown that in areas where there is no endemic As contamination, intakes between 8 and 345 µg/day can be reached [12]. Fish and derived foods constitute the group of major contributors to As intake. In these foods As contents are usually greater than 1 mg/g, exceeding 10 mg/g in flatfish and crustaceans [13]. Arsenobetaine represents between 50 and 80% of total As content, although other species such as DMA (dimethylarsinic acid), MMA (monomethylarsonic acid), TMAO (trimethylarsine oxide), AC (arsenocholine), TMA+ (tetramethylarsonium ion) [13], and arsenosugars [14] have been detected. The iAs normally has been found in concentrations up to 0.1 mg/g [15], although unusual high levels of iAs have been described in crustaceans and bivalves (0.001–4.5 mg/kg) [16]. From a toxicological point of view, edible algae have received special attention because As content can reach concentrations between <0.04 and 141 mg/kg [17–19]. The iAs represents the major arsenical species in the alga *Hizikia fusiforme* [20], whereas dimethylated arsenosugars are the main species in the remaining seaweed [21].

With respect to terrestrial animal foods (milk, liver, and derived products), As^{III} and As^{V} contents have been described below 0.05 mg/g [22,23]. The presence of DMA and AB found in chicken meat [24] could be a consequence of the biotransformation of As^{V} [25]. Otherwise, in land-based plant foods, the concentration of As is generally lower than 0.1 mg/kg [26,27]; however, this content can be higher in areas with water and soil As contamination (e.g., *Ipomoea aquatica*, 0.367 mg/kg wet weight) [28]. Importantly, the iAs content in rice—the staple diet for a large proportion of the world population—and rice products varies between 0.02 and 0.56 mg/kg, accounting for 11 to 100% of the total As content [26,29]. It is alarming that As concentrations up to 1.8 mg/kg have been reported in rice samples or rice products [30,31].

Moreover, the presence of MMA (0.015 mg/kg) and DMA (0.486–0.539 mg/kg) has also been reported [32,33].

In the estimation of the toxicological risk, exposure assessment must take into account the frequency of consumption as well as the amount of the toxicant and its chemical form in the considered food. Since diet is the exposure path for humans, assessing the extent of exposure should also consider the influence of cooking on the concentration of the toxicant and its oral bioavailability. Most existing studies of As in the literature do not consider the effects of cooking or bioavailability, but are limited to a determination of the concentrations of the toxicant in the raw products.

Several studies have showed that contaminated waters used for cooking purposes increase the toxicological risk associated with food consumption [34–36]. While the use of water with the least amount of As is the best choice [37] this is not always possible in endemic areas of As contamination. However, there can be recommended cooking procedures that will reduce the amount of As absorbed by the food during cooking. For example, the influence that several different cooking processes used by Asian consumers may have on the retention of As in rice has been studied. If rice is boiled with an excess of water, which later on is discarded, the values for As retention (43%) are lower than those calculated when rice is cooked with a limited amount of water (72–99%) [38]. Moreover, the use of parboiled rice did not imply large variations in the concentration of the toxicant retained by the rice [39]. Similarly, around 90% of total As can be removed from the edible alga *Hizikia fusiforme* by household processes such as soaking and boiling [40]. Besides, after cooking vegetables in water containing inorganic, mono- and dimethylated pentavalent As species there have been reported changes (reduction and thiolation) in these species [41]. The production of TMA^+ has also been detected during baking, frying or grilling of seafood products, possibly because heating facilitates decarboxylation of arsenobetaine to TMA^+ with higher toxic potential [42,43].

10.3 Role of the Gut in Arsenic Toxicity: Bioavailability and Intestinal Health

Several approaches have been used to estimate relative As bioaccessibility (maximum fraction of the toxic solubilized in the gastrointestinal tract and available for absorption) and bioavailability (fraction of the toxicant that becomes distributed through blood circulation to target tissues and organs).

The limited existing data from human studies concerning As absorption indicate a high uptake rate (>50%) for the different As species calculated from the rate of urinary excretion [44]. However, this approach can underestimate As absorption because of the bioaccumulation of As in nails [45], hair, and skin [27], and in different organs. The *in vivo* measurement constitutes the only tool to estimate bioavailability, but due to the ethical issues affecting toxicants, animal models and *in vitro* approaches have also been developed as a useful alternative for screening and relative estimation of As bioavailability. Several different animal models (primates, pigs, dogs, rabbits, and rodents) have been used [46–50], but results are not

extrapolable to humans. In this sense, there can be identified marked differences for methylation capability with respect to humans; for example, the marmoset monkey and chimpanzee have been shown not to methylate iAs [51], and dog or rat hepatocytes have a higher methylation capability than those in humans [52]. *In vitro* methods have also been developed as an alternative to the use of experimental animals [53,54]. In this sense, it is worth noting the inclusion of gut microorganisms in the *in vitro* system [10] because the human gut microbiota (fecal inocula) possess a substantial metabolic potential for As [10,55].

Most current research concerning As bioavailability has been focused on contaminated soils [56]. However, few studies have considered food matrices such as algae, rice, and seafood. Total diet studies conducted in countries without As contamination in water show a wide variability in intake of As, with values ranging from traces up to 345 mg/day for the Japanese population [57], for example. Recently, iAs consumption through staple foods such as rice has emerged as a worldwide health concern, especially for groups of populations with a high consumption frequency of this cereal and derived products (infants, coeliacs, vegans, different ethnic groups) [58,59]. These studies demonstrated increased (by 14.2%) urinary As concentrations in children and infants consuming rice derivatives-based foods, which resulted in higher than maximum exposures predicted for adults drinking contaminated waters. In the case of a staple food such as rice, the juvenile swine model revealed large differences in As bioavailability (33 vs. 100%) depending on the DMA or As^V [60] concentration in the considered sample.

Arsenic intestinal absorption constitutes a complex process that implies several routes and underlying molecular mechanisms, depending on the chemical form in which the toxicant is presented. Overall, a more effective absorption for trivalent species, has been described despite their greater degree of methylation, than their pentavalent counterparts. Absorption rates have been calculated for pentavalent methylated species between 17% for MMA^V and 33–50% DMA^V [60]. Using the rat intestine model it was shown that DMA^V is absorbed at a rate directly proportional to their concentration over a 100-fold range [61]. Data concerning absorption rates for trivalent methylated species have only been obtained with intestinal epithelial cells showing increasing absorption rates according to the degree of methylation (DMA^{III}>MMA^{III}>As^{III}), contrary to the pentavalent ones (As^V>MMA^V>DMA^V) [62]. From a molecular point of view, As^V, As^{III}, and MMA^{III} showed mixed components of trans- and paracellular transport (Figure 10–1). Otherwise, DMA^{III} is mostly transported transcellularly involving energy-dependent processes.

The mucus layer represents a barrier to the entry of these highly toxic species to the bloodstream. It is possible that a thinner mucus layer by different factors such as antibiotic treatment [63] or pathological conditions such as inflammatory bowel disease [64] could negatively affect trivalent As absorption and enterocytes uptake, and therefore worsen the toxicological effects. Moreover, it would also be necessary to evaluate the effect of As on the amount and composition of glycoproteins present in the mucus, as there are studies demonstrating the ability to dissolve mucus by compounds interacting with -SH groups [65]. Moreover, the intestinal oxidative stress is associated with loss of mucosal redox balance [66], altering its protective effect.

Due to their high reactivity, trivalent As forms are highly unstable in aqueous solutions and oxidize rapidly resulting in DMA^V and MMA^V. Thus, it is considered that their presence

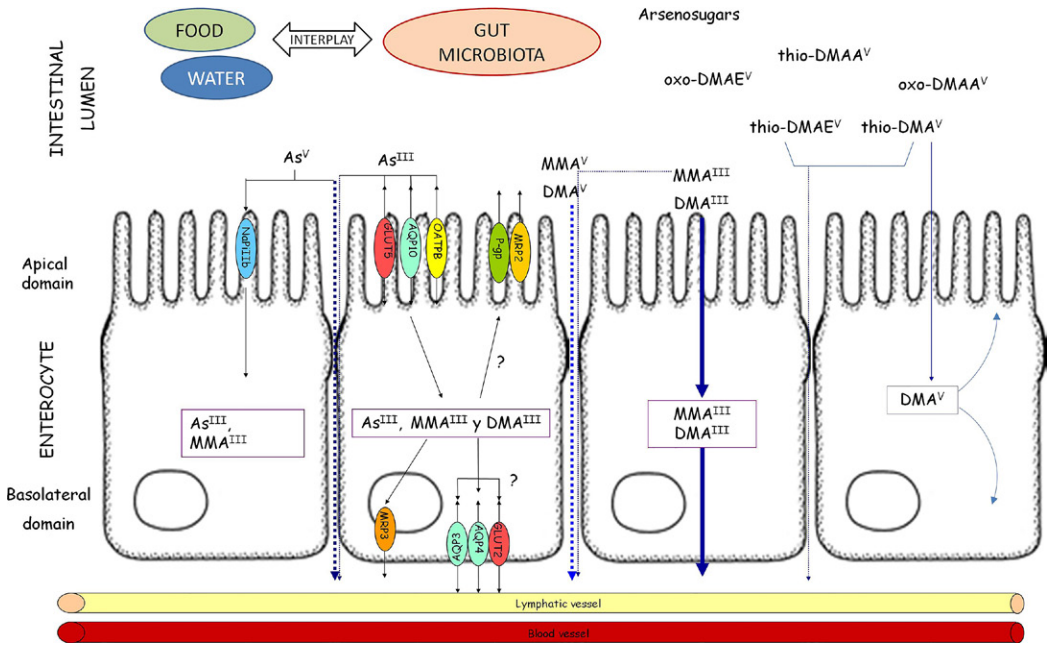

FIGURE 10–1 Schematic representation of routes for As transport across the intestinal epithelium. Arsenic intestinal uptake occurs through the paracellular and transcellular pathway. Paracellular route is relevant for intestinal absorption of pentavalent arsenic species, while for the trivalent ones, transcellular path is involved. As^{III} comes into intestinal (Caco-2) cells by various carriers, including organic anion-transporting polypeptide B (OATPB), glucose transporter 5 (GLUT5), and aquaporin 10 (AQP10). As^V enterocyte uptake is made through the sodium-dependent phosphate transporter NaPiIIb. Inside the cell iAs is metabolized to AsIII and MMA^{III}, which reach the bloodstream or are eliminated into the intestinal lumen by the same proportion. Thiolated metabolytes are absorbed in a lesser extent than iAs and only thio-DMA^V is observed inside the cell. Exposure to iAs modifies the mRNA expression of apical domain transporters (GLUT5, AQP10, OATPB and NaPiIIb) as well AQP3, AQP4 and GLUT2, transporters located on the basolateral membrane, potentially facilitating iAs intestinal uptake.

after food intake is minimal. Only Yahtvakilla et al. [67] have shown MMA^{III} in carrot samples grown in soil contaminated with As. On the other hand, after the progress made in recent years regarding the metabolism of iAs in intestinal epithelium and gut microbiota, it can be concluded that trivalent forms may be present in the intestinal lumen or produced within enterocytes and later reach the bloodstream and/or exert a toxic effect at intestinal level. In addition, As triglutathione and methyl As diglutathione have been evidenced to be present in bile from rats administered with iAs [68,69], and therefore could reach the intestinal lumen by the common hepatic duct, entering the enterohepatic circulation. More recently, the formation of monomethyl monothio arsonic acid ($MMMTA^V$) and dimethyl monothio arsinic acid ($DMMTA^V$) in rats after orally administered As^{III} has been suggested to be dependent on enterohepatic circulation, possibly with the involvement of the intestinal microbiota [70].

Over the last few years, thiolated arsenical species have gained in importance. They are shown to be metabolites of As, but their toxicokinetic and toxicodynamic behavior is still

unknown. Some of these As metabolites are directly present in food, such as thio-DMA^V in rice samples [71] or may occur via biotransformation of arsenosugars by gut microbiota [10,72,73], intestinal epithelial cells [74], and/or due to enterohepatic circulation [70]. More than 20 structures of arsenosugars have been described in foods, varying the R group attached to the aglycone [75]. Accessing new technologies of analytical chemistry will offer as yet unknown As species, increasing the complexity related to As exposure risk assessment. Currently, studies of *in vitro* bioavailability of arsenosugars (DMA^V-sugar-glycerol and DMA^V-sugar-sulfate) have indicated low absorption percentages (1.7–2.8%) [74]. Hata et al. observed a 30% excretion of the ingested As, mainly as arsenosugar AsSug328, through wakame seaweed in human volunteers [76]. These data differ from those previously presented by Francesconi et al. [77] in a volunteer that ingested the same synthesized arsenosugar, in which 80% of the administered As was excreted. *In vitro* and *in vivo* assays differ considerably, highlighting the need for further studies related to the metabolism and absorption of arsenosugars and improving *in vitro* systems to assess As bioavailability.

Due to the metabolic activity of the gut microbiota, ingested arsenosugars can be rapidly converted to sulfur analogs [72]. The bioavailability of some of these metabolites is variable depending on the chemical form, with similar values to As^{III} for thio-DMA^V and thio-$DMAE^V$, whereas oxo-$DMAA^V$, thio-$DMAA^V$, and oxo-$DMAE^V$ passed the *in vitro* intestinal barrier in small amounts [74]. The most toxic compound, thio-DMA^V, crosses the intestinal barrier by passive diffusion and facilitated transport. However, *in vitro* assays have evidenced that As absorption processes can be influenced by significant interactions between As and food components exhibiting biological activity such as antioxidants [78] or chelating properties (water spinach) [79]. These effects are supported by spinach water-mediated reduction of As concentration in internal organs of rats [80]. Similarly, there was a reduced As-induced toxicity when animals were fed with curcumin [81]. The effect of biologically active compounds such as vitamin E, vitamin C, or selenium against As toxicity has been evidenced in *in vitro* and *in vivo* models [82–85], and although this was not statistically proven when evaluated through human trials [86], epidemiological studies indicate a protective effect of these compounds against As adverse effects [87,88]. Other factors affecting gut physiology such as the low pH, reduced peristaltism, or favored bile acids production could positively increase As absorption [89,90].

10.3.1 Gastrointestinal Health

The gastrointestinal tract is the largest organ in contact with exogenous compounds and constitutes the first physiological barrier to exogenous toxicants. The intestinal epithelium displays a complex architecture where epithelial, goblet, and immune cells are involved. Moreover, the intestine supplies the body with nutrients and other metabolites, but also with signals that stem from the intestinal mucosa and immune system, forming the so-called gut–liver axis. Notably, the liver is a central organ for the turnover and transformation of As and metabolites that pass through it from the intestine. In this context, the development and function of the host cells responsible for the barrier function of the intestinal surface (e.g., M cells, Paneth cells, goblet cells, and columnar epithelial cells) are strictly regulated through both

positive and negative stimulation by the luminal microbiota. Signaling by damage-associated molecular patterns (DAMPs) and commensal bacteria-derived microbe-associated molecular patterns (MAMPs) mainly through Toll-like receptors is involved in maintaining the integrity of the intestinal epithelium. Moreover, gut-associated immune cells also play critical roles in regulating both the mucosal barrier and the relative composition of the luminal microbiota. Imbalance in these components or disruption of mucosal homeostasis causes intestinal inflammation and loss of physiological functionality of the intestinal epithelium.

In vitro studies indicated that As exposure results in cytotoxic effects on intestinal epithelial (Caco-2) cells through several different underlying mechanisms similar to those identified in As-targeted tissues: oxidative stress, lipoperoxidation, and alteration in cellular antioxidant compounds and enzymes [91–93] (Figure 10–2). As a consequence of alterations in the intracellular redox status, As^{III} causes disorganization and redistribution of cytosolic actin [94,95], a protein involved in membrane vesicle and apoptotic bodies arrangement, marginalization

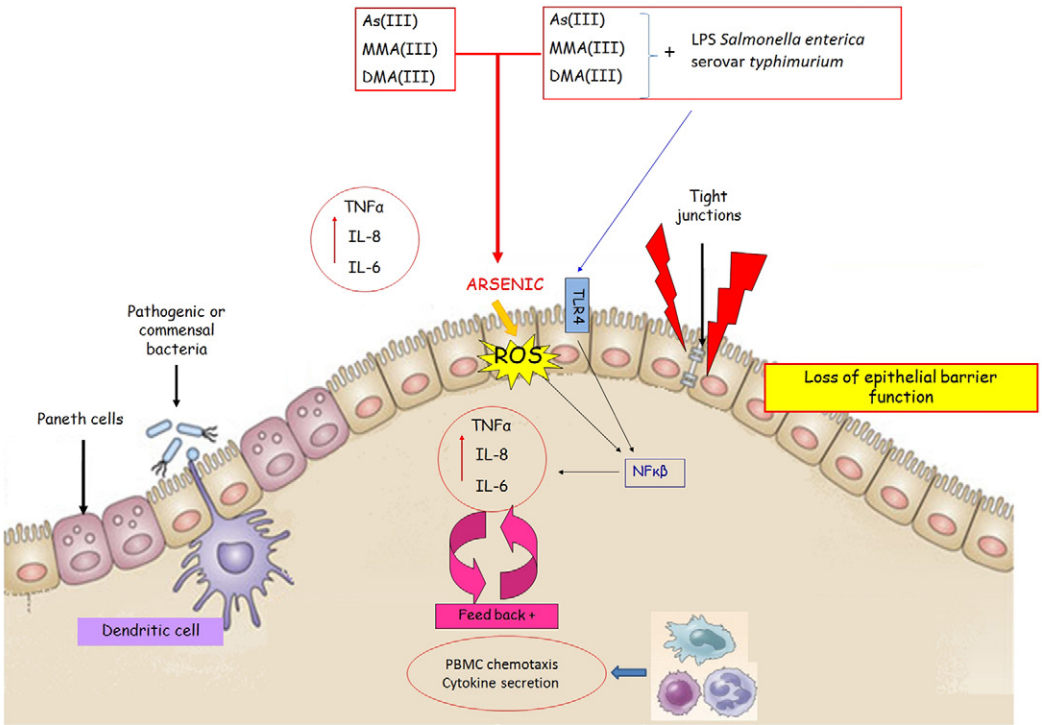

FIGURE 10–2 Hypothetic representation of As-induced immunotoxicity at intestinal level. The presence of trivalent arsenical species (especially As^{III} and DMA^{III}), with or without lipopolysaccharide (LPS) derived from *Salmonella enterica* serovar *typhimurium*, elicits oxidative stress and inflammatory cytokine secretion. This leads to an increase into cytokine levels in the interstitial space and the apical medium. This could attract peripheral blood mononuclear cells (PBMCs), intraepithelial lymphocytes, and dendritic cells, leading to an intestinal inflammatory reaction. All of these cells in response to inflammatory stimulus can generate more cytokines and positive feedback that maintain the inflammatory condition. It is not known whether damage to the tight junctions could affect epithelial barrier function and increase basal lamina contact with luminal antigenic substances.

of nuclear chromatin, and the maintenance of cell structure and signaling. This effect could be the result of activation of multiple cellular signaling pathways caused by oxidative stress [96–99]. *In situ* studies with rodents also evidenced a decreased activity of enzymes present in the plasmatic membrane of intestinal cells as well as injuries to cell membrane after As^{III} exposure [100]. Similarly to some other tissues, intestinal epithelial cells appear more susceptible to trivalent As species than their pentavalent counterparts. As^{III} and DMA^{III} induce inflammatory responses associated with increased interleukin (IL)-8 and tumor necrosis factor (TNF) α production by intestinal epithelial cells has also been reported [101]. Moreover, the coexposure of As and lipopolysaccharide (LPS) from pathogenic *Salmonella enterica* exacerbates the inflammatory response [101]. Otherwise, the intestine does not constitute an organ where a significant amount of As is accumulated in comparison to kidneys, lungs, or liver. All the aforementioned events contribute to alterations in intestinal architecture impairing gut integrity, thereby favoring local and/or systemic inflammatory reactions.

Although As is classified as carcinogenic and the drinking of water and the eating of foods are the main sources of exposure to humans, only a relatively few studies have described carcinogenic effects in the gut [102,103]. This association has shown gender (male)-specific differences in the various processes related to As toxicity. However, clinical signs of gastrointestinal irritation have been reported in cases of short-term, high-dose exposures to iAs and DMA^{V} [104,105]. Data from animal models demonstrated more severe symptoms with acute inflammation and hemorrhage of the small intestine in rhesus monkeys chronically exposed to high doses of As trioxide [106]. Also, long-term exposure of rodents to MMA^{V} led to a thickened wall, edema, and hemorrhagic, and necrotic, ulcerated or perforated mucosa in the large intestine [107]. These observations were accompanied by a significant increase in the incidence of squamous metaplasia of the epithelial columnar absorptive cells in the colon and rectum. As a function of the dose of As administered, there have been signs of stomach and intestinal inflammation (hemorrhage in stomach and intestine, rose-red inflammation, severe enteritis) in rats [108] and intestinal dysfunction related to wall movement and disrupted absorption capabilities in mice [109]. Taken together these studies demonstrate the marked impact of As on intestinal barrier, thereby potentially causing significant alterations in nutrient absorption processes and inflammatory and immune signals that can finally affect liver function.

However, not only do high-dose exposure levels of As cause intestinal physiological dysfunction. In a recent prospective population-based cohort study [110], an increased risk of diarrheal diseases has been observed in relation to prenatal low–moderate As exposure. These gastrointestinal diseases constitute the most important cause of illness and the second leading cause of death among children under 2 years [111]. For example, in Bangladesh, one of the most affected areas by As contamination in ground water, children have been identified as a sensitive population group to chronic As toxicity due to drinking As-contaminated water [112,113], which causes significant morbidity in children resulting in skin lesions, lung disease, and defects in intellectual function.

The human intestine harbors a complex, diverse, and vast microbial community, in which the genome (microbiome) plays a profound role in metabolic, immune, and cognitive development, and epithelial homeostasis. However, colonization of the intestine is a dynamic

process, which depends on both environmental and genetic factors that can be affected by As exposure. Data from rodent models clearly demonstrated that As exposure not only alters the gut microbiome at the abundance level, but also has deleterious effects on its function. These alterations have been suggested as a potential aggravating factor of human diseases [114,115]. Removal of toxic metals and toxins using probiotics (live microorganisms that when administered in an adequate dose have beneficial effects on host health) could be an inexpensive, new, promising method on top of conventional methods to eliminate, inactivate, or reduce bioavailability of As in foods [116]. However, it is important to consider that some microbes can favor As biotransformation, sometimes increasing its bioaccessibility or toxicity [117]; however, only inferential studies have addressed these aspects.

10.4 Arsenic-Induced Metabolic/Immune Toxicity

Arsenic toxicity is complex and multifaceted, not only in terms of As being a well-established agent causing skin, lung, and bladder cancers and cardiovascular diseases, but also in relation to the adversely effects on the metabolic and immune functions where its specific effects are particularly poorly understood.

As needs to cross from the intestinal lumen into the lamina propria where lymphocytes reside in order to affect their function and activity. In contrast to other tissues, intestinal mucosa has a continuous low-grade inflammation where regulatory systems participate to maintain a proper immune homeostasis and a healthy gut, but also inducing a protective immune response against pathogens and exogenous toxicants. In this sense, microbial colonization of the intestine in early life is particularly important as it is the main environmental stimulus for immune system maturation. This is a dynamic process, which depends on both environmental and genetic factors. The abnormal host–microbe interplay is largely genetically determined, which should also be considered because of As-mediated toxicity. In addition, recent animal studies demonstrated the influence of high concentrations (10 μg/mL) of As in the drinking water on gut microbiota [118]. The effects appear not only related to abundance level, but also to function, with several different microbiota-related metabolites being perturbed in multiple biological matrices.

In vitro and *in vivo* animal and human epidemiological data clearly demonstrate As immunomodulatory effects by increased apoptotic rates in immunocompetent cells, including macrophages and neutrophils [119–121]. Immune deficiencies are particularly relevant at early stages of life because of their contribution to adequate antigen tolerance or to increasing overreactions and disease risk. In this sense, the scarce existing data concerning the physiological consequences of gestational exposure to As reveal the marked negative impact of the metalloid on both branches of immune system in early stages of life, which correlate to increased fetal loss and infant mortality [122,123]. Reports on immune-related effects of prenatal exposure in newborns also showed a negative correlation between maternal urinary As concentrations and child thymic index and breast milk trophic factors such as IL-7 and lactoferrin, but a positive correlation between maternal urinary As and infant morbidity (fever, diarrhea, and respiratory infections) [124]. Recently, epigenetic modifications in microRNAs associated with

innate and adaptive immune signaling in newborn cord blood as a consequence of prenatal As exposure, have also been reported [125]. Follow-up studies indicate that these findings appear to be associated with elevated oxidative stress and inflammation in chronically-exposed adults [126,127]. Overall, the existing data increase the evidence that As-associated adverse immune-related outcomes from early life will have long-term negative consequences into adulthood.

Reduced immune surveillance caused by As could potentially play a significant role not only in carcinogenesis, but also in aggravating the severity of inflammatory and metabolic diseases such as type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) [128,129]. T2D accounts for more than 90–95% of all diabetes with unknown specific etiology, although genetic factors, aging, and obesity are important risk factors [130]. In areas with endemic As contamination such as Bangladesh a positive association of modest levels of As in drinking water ($\leq$1.7 μg/L) with increased risk of T2D has been made [131]. Being overweight or smoking are aggravating factors of the risk. However, this relation between long-term iAs exposure and risk of T2D did not show up so clearly from a more extended meta-analysis of articles published in English or Chinese (1990–2013) [132]. Several different mechanisms for As-induced T2D have also been proposed including phosphate substitution by As^{V} in the formation of adenosine triphosphate (ATP), As^{III} high affinity for sulfhydryl groups in the molecules of insulin, insulin receptors, glucose transporters (GLUTs), and enzymes involved in glucose metabolism, stimulation of glucose transport, induction of oxidative stress, and interference in signal transduction or gene expression [133]. Although the molecular mechanism remains elusive, a common conclusion from the existing literature is that insulin resistance and β-cell dysfunction can be induced by chronic As exposure.

The liver is a central organ for the transformation of nutrients and metabolites from the intestine, and long-term As immune suppression can impair the ability of liver to detoxify exogenous toxicants. The prevalence of NAFLD among Asian Indians has been reported as comparable to that in Western populations (15–30%), and its more severe steatosis with inflammation (NASH) may be present in approximately 20% of these patients [134]. Notably, about 85% of patients with NASH exhibit hallmarks of the metabolic syndrome, mainly insulin resistance, T2D, and hyperlipidemia. The interaction and effects of As exposure in NAFLD animal models have been poorly studied and the scarce existing data show an increased transport rather than biotransformation of iAs [129]. This effect may have significant implications for the overall toxicity associated with As in patients suffering from NAFLD/NASH. The exact mechanisms responsible for the pathological progression from hepatic steatosis to NASH are not entirely understood, but there are several factors involved such as mitochondrial dysfunction, oxidative stress, and pro-inflammatory cytokine production, similar to the effects resulting from As exposure.

The aryl hydrocarbon receptor (AhR) is a member of the basic-helix-loop-helix (bHLH)/Per-ARNT-Sim family of transcription proteins, the activated nuclear complex of which is known as xenobiotic responsive element regulating phase I and II enzymes [135]. Previous studies report that As^{III} exerts modulatory effects on the expression of several phase I and II AhR-regulated genes in *in vitro* systems [136]. This observation is supported by a few existing *in vivo* studies, which showed a differential modulation of AhR-regulated enzymes by As^{III} in

different organs such as kidneys, lungs, and heart of C57BL/6 mice [137]. Humans are exposed to several polyphenols, for example quercetin, resveratrol, and curcumin [138], through the diet and nutritional supplements. These compounds have been shown to activate the AhR in spite of the fact that they bind to the receptor with low affinity, differentially modulating inflammatory responses of human keratinocytes by interfering also with other activation of transcription factors such as NF-κB and the EGFR–ERK pathway [139].

Arsenic can also induce bone-marrow toxicity [140]. Mesenchymal stem cells (MSCs) are non-hematopoietic components of bone marrow involved in immune-suppressive, immune-modulatory, and cytoprotective responses. It has been shown that alterations in cellular homeostasis, such as increased oxidative stress, increases MSC senescence, poor differentiation, and cell death. These effects have been associated with the As-induced down-regulation of the peroxisome proliferator-activated receptor (PPAR)-γ by using C3H 10T1/2 cells [141]. The PPARs belong to the nuclear hormone receptor superfamily, the dysregulation of which has an important role in immune disease development.

Combining the data stated above, it is easy to conclude that there is a deleterious immunosuppressive effect due to As exposure. However, studies can also be found showing beneficial As-mediated effects derived from its synergistic action with molecules such as all-trans-retinoic acid inhibiting breast cancer and prostate cell lines [142]. Moreover, As-induced antitumor activity has also been reported in Beige/Nude/X-Linked immune-deficient mice associated with activation of PPAR-γ by the agonist RWJ-241947 (MCC-555) [143] and As reduction of lung allergic inflammatory responses in a murine model of asthma by a direct inhibitory effect on the production of eotaxin by pulmonary cells [144].

All the aforementioned aspects clearly show complex and multifaceted As-mediated immunoregulatory activity that appears conditioned, positive or negatively, by certain other environmental factors and physiological conditions, as well as As concentration. The related effects are unavoidable and might impair metabolic processes masking As toxicity in exposed populations.

10.5 Conclusions and Future Perspectives

Arsenic is widely distributed in Earth's crust and is able to penetrate to all levels of the food chain. Eating food and drinking water are the major sources for As exposure either in developing and developed countries with estimated daily intakes around 300 μg/day. Despite the progress made in As remediation from drinking water in recent years, there are currently many geographic areas with levels of As above the values recommended by the WHO. Minimizing As exposure is the best solution to reduce health problems resulting from its ingestion, but in several areas it is economically or technically not feasible to decrease As in water to levels lower than 10 μg/L. Moreover, because of the lack of knowledge and/or evidence on which the maximum dose of iAs is safe for humans, it is vital to address all the variables that could impact As toxicity, including food interactions, gut microbiota, and previous diseases.

Bioaccumulation and/or transformation of the several different As species are of particular toxicological interest because these processes can promote reduction or worsening of As

toxicity. The toxicological risk evaluation concerning As exposure from food must take into account not only the concentration of the toxicant, but also interaction with other food components and its resulting bioavailability. These processes can limit the dose of the toxicant that reaches target tissues as well as its toxicity.

Moreover, several different scientific studies have revealed different physiological alterations affecting health status derived from As exposure even to low–moderate levels. These alterations are of special importance because they affect nutrient absorption processes and immunological function, thereby affecting adequate growth and development during early stages of life. In this sense, particular attention should be paid to rice-derived products widely used for infant nutrition and replacement of wheat and other cereals for susceptible groups of the population such as celiac patients. Therefore, the risk derived from As intake should be evaluated independently in specific population groups with increased consumption of products with high content of As and/or increased susceptibility to its effects.

Although there have been major advances over the last few years concerning As effects on human health, only a comparatively small number of studies have evaluated the influence and potential contribution of this toxicant to diseases still unrelated to As exposure such as pandemic liver-related metabolic disorders in Western populations.

Finally, despite evidence that populations from non-As endemic areas are exposed to As through food, we should not forget that currently the biggest problem of As contamination continues in As endemic areas such as India, Bangladesh, Argentina, and Taiwan. Thus, it should be a priority to develop available and open technology to reduce the levels of As in drinking water in these areas, to develop economically accessible food strategies to reduce As exposure, and to implement citizenship education policies for diminishing As intake by processing or food-chelating techniques. These last issues can offer alternatives with a broad economic and social impact in areas of chronic exposure to As, until the objective of safe food and drinking water is achieved.

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11

Cutaneous Toxicology of Arsenic

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CHAPTER OUTLINE

11.1 Introduction

Arsenic is a well-known toxin and human carcinogen known to impact cellular processes in numerous organ systems in a time- and dose-dependent manner [1–5]. However, its manifestations typically first appear in the skin. These early cutaneous manifestations act as a surrogate marker for internal diseases including hypertension, ischemic heart disease, atherosclerosis [3], peripheral vascular disease, diabetes mellitus, and peripheral neuropathy [1–3,5]. Arsenic is a metalloid found in great abundance within Earth's crust. In nature, inorganic arsenic is often complexed with the mineral pyrite and easily dissociates to enter ground water with subtle changes in soil pH, temperature, oxidation state, and solution composition [3]. Human activities have also contributed to rising groundwater arsenic concentrations. Contamination has been found to occur as a consequence of mining minerals such as silver, gold, tin, copper, lead, tungsten, zinc, and cobalt [6–9]. The rainwater-induced leaching of arsenic from chromate copper arsenate (CCA)-treated wood is an additional possible source of groundwater arsenic. Though CCA-treated wood was phased out of residential use in 2004, it remains very popular among commercial builders given its service life of up to 40 years [10]. Chronic arsenic

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ingestion is the most common cause of arsenicosis, which may occur through environmental, occupational, and accidental exposure [4,5]. Ingestion of contaminated foods such as fish, cereal, algae [4], rice [11], and nutritional supplements [12] have also been associated with its toxicity.

Historically, arsenic was used as a medicine for various maladies, particularly those of the skin. It was used in ancient Greece to treat diseases such as eczema, furuncles, leprosy, lichen rubor, lupus, molluscum contagiosum, pemphigus, psoriasis, syphilis, urticaria, and warts [13]. Compounds known as Fowler's solution (liquor potassii arsenites), arsenic bromidum, arsenic iodidum, Pearson's solution (liquor sodii arseniatis), arsenicum hydrogenisatum, Donovan's solution (liquor arseni et hydrargyri iodide), and Asiatic pills (arsenic with opium or pepper) were historically used to treat disease [8,13]. Asiatic pills were one of the most commonly prescribed arsenicals and are still available for purchase in the Far East with possible continued use in traditional Chinese medicine [6,7]. Arsenic was first listed in the *United States Pharmacopoeia* in 1850 [14] and the Western world continued using Fowler's solution to treat asthma as late as the 1960s [6,7]. Interestingly, arsenic compounds are still used today in the treatment of tropical diseases such as African trypanosomiasis [14] and hematologic malignancies including promyelocytic leukemia [6,7]. Arsenic's ability to cause disease was documented as early as the late 19th century when it was noted to cause palmoplantar keratoses, papulosquamous eruptions, and pruritus [8,13].

11.2 Epidemiology

Abundant epidemiological data from countries such as Taiwan, Bangladesh, Chile, India, and Argentina have helped to show that arsenic is a serious and widespread threat to public health [11]. Current estimates suggest that as many as 45 million individuals in Bangladesh alone are at risk of arsenic exposure exceeding a level of 50 μg/L [15]. The World Health Organization (WHO) recommends a maximum allowable concentration of 10 μg/L of arsenic in drinking water, which suggests that arsenicosis in Bangladesh may be an even greater health concern than already known [16]. It is estimated that 200 million people worldwide are at risk of toxic exposure [11]. Evidence suggests a lifetime dose of 0.5 to 1 gram is capable of promoting arsenical keratoses and skin cancers [8,9]. Estimates from endemic areas of China have shown that approximately 10,000 individuals are currently suffering from arsenicosis [17]. In the United States, an estimated 1.5 million individuals were exposed to arsenic in the workplace in 1973 [18]. A retrospective analysis conducted by Focazio et al. predicted that approximately 11% of all United States public water systems had arsenic concentrations exceeding 10 μg/L with the greatest exposures occurring in the Southwest, Midwest, and Northeast [19]. The largest groundwater burden was also found to occur in water sources serving populations of less than 1000 people [19]. Arsenic's ubiquitous nature and toxic potential have led the United States Agency for Toxic Substances and Disease Registry (ATSDR) to name arsenic as the number one threat to public health. In addition, the International Agency for Research on Cancer (IARC) categorized this metalloid as a class I human carcinogen [20]. Also, other factors such as environmental UV exposure, immunosuppression, and certain dietary factors may augment

various cutaneous manifestations in chronically arsenic-exposed human populations [21]. In this regard, *in utero* exposure to arsenic results in impaired newborn and childhood immunity as assessed in chronically exposed human populations of Bangladesh [22,23].

11.3 Clinical Manifestations

Although arsenic impacts physiological cellular processes in numerous organ systems, the outcomes of its toxicity are usually first seen in the skin. Acute exposure, the significantly rarer entity, typically occurs as a result of medicinal, accidental, homicidal, or suicidal attempts. Skin manifestations of acute toxicity include flushing, erythema, facial edema, acrodynia, urticaria, alopecia, loss of nails, and Mees lines visible on nails approximately 8 weeks after exposure [6–9,24].

Chronic skin manifestations include hyperpigmentation with superimposed guttate hypopigmentation described as "raindrops on a dusty road," punctate hyperkeratoses on the palms and soles [25,26], diffuse alopecia, blackfoot vascular occlusive disease, Bowen's disease (BD, also called squamous cell carcinoma *in situ*), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) [5–9,12,24,27]. Blackfoot disease typically affects the lower extremities and often results in gangrene if left untreated [28]. An association between arsenic exposure and Merkel cell carcinoma, a neoplasm with substantially less common occurrence, has also been identified [4]. The latency period for the development of arsenical skin disease ranges from 30 to 50 years [6]. The development of skin cancer in sun-protected areas is especially concerning for the existence of chronic arsenic exposure. These cutaneous manifestations serve as a surrogate marker for associated diseases such as hypertension, ischemic heart disease, atherosclerosis [3], peripheral vascular disease, diabetes mellitus, peripheral neuropathy [2], bone marrow hypoplasia, and hepatic fibrosis [1,3,5–9,24].

Arsenical keratoses are precancerous lesions that begin as yellow pinpoint hyperkeratotic papules ranging in size from 0 to 2 mm, most commonly on pressure points of the palms and soles such as the lateral borders of the hands [12]. They eventually progress to larger corn-like papules and may eventually coalesce to form plaques [6–9,24]. These lesions are more commonly reported in patients with chronic medicinal exposure rather than that from the workplace [12].

Arsenical BD lesions typically occur in multiples on affected individuals and appear as small skin-colored to pink papules covered by a hyperkeratotic crust or horny layer. Removal of this crust often reveals an erythematous, papillomatous, oozing base [6–9,24]. In contrast to arsenical keratoses, BD lesions continually increase in size and may eventually form nodules. These lesions progress to invasive SCC in approximately 5 to 20% of cases [6–9,24].

Arsenic-induced SCC is more aggressive than its chronic UV-induced counterpart. In fact, 33% of untreated arsenical SCCs have been found to demonstrate metastatic behavior. [6–9,24,29]. These lesions may arise *de novo*, from existing benign lesions (arsenical keratosis), or may be the result of BD progression. It is believed that about half of all arsenic-induced SCCs ariose *de novo* [12]. Arsenical keratoses at risk for progression to SCC commonly present with pain, bleeding, fissuring, and eventually ulceration [28]. Early-life exposure to arsenic, multiple lesions, and acral sites are risk factors for the development of SCC [12]. Although not proven

experimentally, it is believed that pressure points on the palms and soles more commonly undergo malignant transformation due to ongoing irritation and trauma in these areas [28].

11.4 Histopathology

Arsenical keratoses display mild to moderate keratinocyte atypia, severe hyperkeratosis, and scattered parakeratosis. Adnexal structures are typically spared and basophilic degeneration of dermal connective tissue may or may not occur [30]. There is no reliable method to distinguish arsenical keratoses from actinic keratoses. However, some cases of arsenical keratoses demonstrate unique vacuolization of epithelial cells, keratin horn formation, absent solar elastosis, and a chronic dermal lymphocytic infiltrate [28]. Arsenic-induced BD, SCC, and BCC demonstrate the same histopathologic features as found in their non-arsenic-associated counterparts [6,8]. BD lesions exhibit full-thickness epidermal atypia over a broad zone with prominent pleomorphism and frequent mitoses observed in keratinocytes [30]. Well-differentiated SCCs exhibit histological features of keratoses with the addition of glassy, brightly eosinophilic keratinocytes forming detached aggregations and downward proliferating lobules. These cells display varying degrees of pleomorphism, mitoses, and inflammatory infiltrate. Keratinocytes aggregate at varying levels of the dermis and often exhibit prominent nucleoli. Poorly differentiated SCCs lack overt keratinization, may display spindle cell morphology, and more commonly exhibit perineural invasion. BCCs all display aggregations of basaloid keratinocytes within a variably fibromyxoid stroma. The cells typically exhibit large nuclei with scant cytoplasm. Lesions may demonstrate a focal connection to the epidermis, indistinct cellular borders, and retraction of the fibromyxoid stroma, which creates characteristic clefts around islands of tumor cells [30].

It is important to note that the clinical features of cutaneous arsenicosis may be similar to those of other diseases. Inherited skin diseases such as punctate palmoplantar keratoderma or Darier disease may appear clinically similar to chronic arsenicosis. However, individuals with heritable diseases typically present earlier in life and report a positive family history of the disease. Unlike arsenical keratoses, the lesions of palmoplantar keratoderma typically demonstrate crater-like pits following removal of keratin plugs. Common conditions such as corns and verruca vulgaris may be mistaken for arsenical keratoses. However, verruca exhibits characteristic thrombosed capillaries following removal of the hyperkeratotic wart surface. Corns occur in lower numbers than arsenical keratoses and rarely present on the hands [28,30].

11.5 Molecular Pathogenesis

11.5.1 Oxidative Stress

A large body of evidence has described arsenic's ability to cause oxidative and nitrosative stress by producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) respectively [3,31–33]. The indicators of arsenic-induced oxidative stress include high levels

of 8-hydroxydeoxyguanosine, lipid peroxides, glutathione, heme oxygenase-1, A170, and peroxiredoxin 1 [3,34]. In this regard, mitochondria-mediated production of hydroxyl and hydrogen peroxide plays a key role in the reduction of intracellular thiols, particularly glutathione [31,32]. Arsenic increases the expression and activity of NAD(P)H oxidase (NADPHO), an enzyme that produces the superoxide anion [3,35] through up-regulation of its subunit p22phox [36], the translocation of NADPHO-activating Rac1, and the activation of NADPHO-enhancing CDC42 [3].

Chronic arsenic exposure also depletes stores of the antioxidant nitric oxide (NO) through its reaction with the superoxide anion and as a consequence of NO synthase (NOS) uncoupling. Arsenic has been found to uncouple the NOS enzyme by reducing levels of its cofactor, tetrahydrobiopterin (BH4). Unchanged concentrations of L-arginine in the setting of decreased BH4 shift NOS's production from NO to superoxide instead [3]. Arsenic has also been found to decrease NO and increase intracellular superoxide levels by increasing the abundance of Big endothelin 1 (Big ET1), a biological precursor to endothelin 1 (ET1). ET1 increases oxidative stress by both simulating NAPHO and uncoupling NOS [37].

11.5.2 Genotoxicity

Arsenic destabilizes the genome by disrupting chromosomes, damaging DNA strands, and weakening cellular DNA repair mechanisms [4,5,38]. A higher incidence of chromosomal aberrations and inappropriate sister chromatid exchanges in the DNA of individuals subjected to chronic arsenic exposure has been identified [38]. Tri- and pentavalent arsenic species readily accept methyl groups from S-adenosylmethionine and subsequently methylate DNA strands in an unregulated manner [4]. Arsenic's predilection for sulfhydryl (SH) groups enables the metalloid to bind SH moieties on various DNA repair enzymes such as I–III DNA polymerase β, O6-methyl-guanine-DNA methyltransferase, poly ADP ribose polymerase, and excision repair cross-complementing rodent repair, complementation group 2 (ERCC2), thus rendering them inactive [5]. Consequently, DNA repair processes such as nucleotide excision repair, base excision repair, mismatch repair [37], and transcriptional proofreading [39] are negatively impacted. Arsenic acts synergistically with other carcinogens to enhance mutations and destabilize the genome with its combination of direct genotoxicity and hampered DNA repair [4,5]. These effects may ultimately be important in unraveling the molecular mechanism of arsenic's carcinogenicity.

11.5.3 Disrupted Signal Transduction Pathways

Arsenic promotes many of its deleterious effects by aberrantly activating various signal transduction pathways. This is predominantly accomplished through As binding directly and/or by ROS-mediated depletion of SH moieties [3,40]. Recent studies show that arsenic promotes growth and proliferation by altering the canonical Hippo signaling pathway (as shown in Figure 11–1) via up-regulating STE20-like kinase 1/2, Salvador homolog 1, large tumor suppressor kinase 1/2, and Mps one binder kinase activator-like 1A [41]. Independent of canonical Hippo signaling, it also promotes epidermal inflammation by promoting phosphorylation of

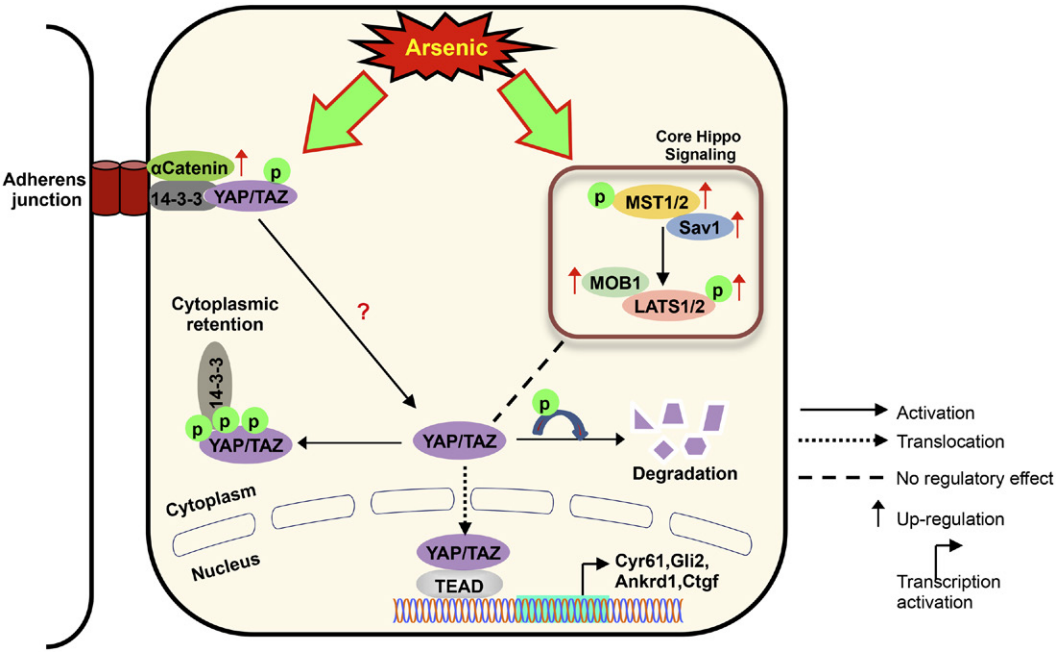

FIGURE 11–1 Flow diagram showing arsenic-mediated modulation of Hippo signaling and Yap activity in murine skin. Exposure to arsenic leads to up-regulation of core components of the Hippo signaling pathway. It is known that Mst1/2 kinases and Sav1 form a complex to phosphorylate and activate LATS1/2. LATS1/2 kinases in turn phosphorylate and inhibit the transcription co-activators YAP and TAZ, the two major downstream effectors of the Hippo signal transduction pathway. When dephosphorylated, YAP/TAZ translocate into the nucleus and interact with TEAD, other transcription factors, and genes that promote cell proliferation and inhibit apoptosis. Arsenic modulates α-catenin expression in the murine epidermis; α-catenin, together with p-YAP, is known to be a component of adherens junctions. Arsenic also activates Yap independent of its upstream regulation. Upon migration to the nucleus, Yap induces transcription of its target genes including *Cry61, Gli2, Ankrd1*, and *Ctgf*. YAP/TAZ is retained in the cytoplasm by sequestration via the 14-3-3 protein. Free and phosphorylated-Yap is targeted for β TRCP-dependent proteasomal degradation. Red arrows indicate upregulation by arsenic.

Yes-associated protein (Yap), a Hippo signaling protein that regulates tight junctions of epithelium when phosphorylated [41].

Although the Nrf2–Keap1 pathway (Nuclear factor 2–Kelch-like ECH associated protein 1) has traditionally been known for its role in promoting an antioxidant stress response, it has recently been implicated in arsenic-induced carcinogenesis. Nrf2 is a transcription factor that is constitutively inhibited by Keap1 until cell stressors inactivate Keap1. If this occurs, Nrf2 is allowed to accumulate, translocate to the nucleus, and activate the transcription of antioxidant machinery such as heme oxygenase-1 (HO-1) and thioredoxin reductase-1 (TrxR1) [42–44]. However, Nrf2 is constitutively activated in some cancer cells and may, in fact, play a role in chemoresistance. Evidence suggests that deactivation of the aberrantly overactivated Nrf2–Keap1 pathway in some cancer cells actually promotes sensitivity to chemotherapy [45].

Arsenic has also been found to promote cell proliferation and growth by stimulating constitutive activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor that has been implicated in chronic inflammation and various cancers [46]. This is accomplished, at least in part, through direct binding of arsenic with a free SH moiety on inhibitory κBα kinase (IκK), which renders it active. Activated IκK then phosphorylates inhibitory κBα (IκBα), targeting it for ubiquitin-dependent proteasomal degradation. Since IκBα is normally responsible for inhibition of NF-κB, this leads to constitutive activation of NF-κB and resultant cell survival [47–49]. Arsenic also acts to promote cell survival by hypermethylating and subsequently silencing the promoter of the pro-apoptotic death-associated protein kinase (*DAPK*) and *p16* genes [2]. It decreases expression of cell cycle control molecules such as cyclin-dependent kinase inhibitor 1B (p27Kip1), an inhibitor of the cyclin D1/CDK4 complex, resulting in uncontrolled cell proliferation [50]. Arsenic also up-regulates *cyclin D1* by altering its epigenetic modification through microRNA 2909 [51].

The metalloid also activates oncogenes such as the mineral dust-induced gene (*MDIG*), a gene that has been linked to lung [52], breast, colon, and gastric cancers, esophageal squamous cell, hepatocellular, and renal cell carcinomas, as well as some forms of lymphoma. Enhancement in the JNK and STAT3 pathways is required for this *MDIG* activation [53]. It promotes epithelial to mesenchymal transition (EMT), invasion, and migration by disrupting the Akt signaling pathway [54] and up-regulating the β-catenin–vascular endothelial growth factor pathway [55].

It is well established that BCCs are driven by the aberrant activation of the Sonic hedgehog signaling pathway (Shh) [56]. Arsenic has been shown to activate this signaling pathway by decreasing the stability of the repressor form of GLI3, one of the transcription factors that ultimately regulates Shh activity. High levels of arsenic exposure were associated with high levels of Shh activity in a cohort of bladder cancer patients [57]. It is possible that this pathway is likewise up-regulated in skin cancers, although it is yet to be demonstrated experimentally (see Figure 11–2). In addition, arsenicals have been found to antagonize the Shh pathway largely by targeting Gli transcriptional effectors in cancer cells in the short-term exposure protocols. In this regard, arsenic was shown to block Shh-induced ciliary accumulation of Gli2, the primary activator of Shh-dependent transcription [58,59]. These differences may be dependent on various contexts such as cell type (normal versus cancer cell), total dose, and duration of exposure. However, involvement of other mechanisms that may also play important roles in the antitumor activities of arsenicals cannot be ruled out at this stage [33].

11.5.4 Immune Dysfunction and Inflammatory Responses

Arsenic impairs innate and cell-mediated immune function largely by activating the unfolded protein response (UPR) homeostatic mechanism [60]. Dysregulation of the UPR has been implicated in carcinogenesis [61] as well as immune dysfunction [60]. It is activated in response to a large number of unfolded proteins present in the endoplasmic reticulum (ER) lumen. Three distinctive pathways, named after their unfolded protein sensors, work to activate the UPR (see Figure 11–3). Inositol-requiring enzyme-1 (IRE1), protein kinase RNA-like

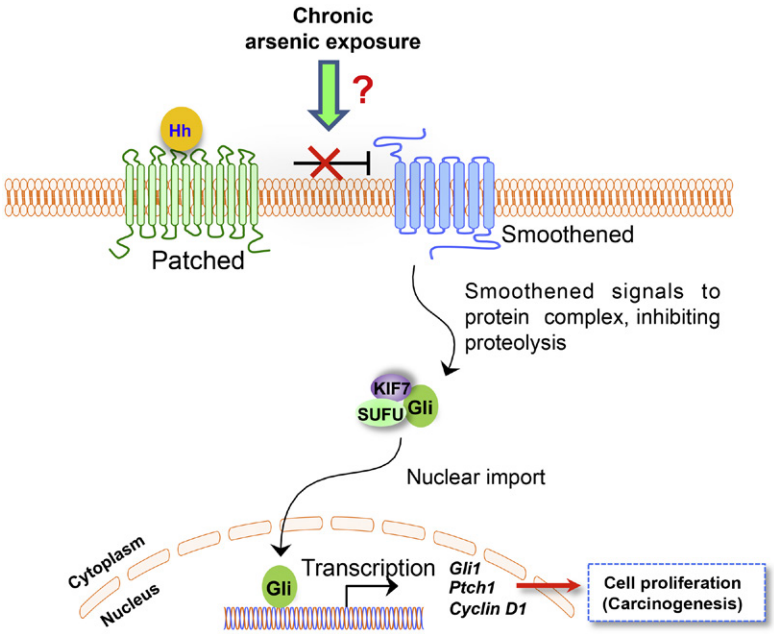

FIGURE 11–2 Arsenic-induced activation of the Hh signaling pathway. Binding of Hh ligands to PTCH de-represses SMO thereby allowing its signal for the transcriptional activation of Gli1/2. Generally, it is known that activation and nuclear translocation of Gli1/2 involves dissociation of Gli1/2 from its endogenous inhibitor SuFu. Kif7 cooperates with SuFu in catalyzing dissociation of the Sufu–Gli complex. Chronic arsenic exposure at low levels favors the nuclear translocation of Glis by an unknown mechanism. Nuclear import of Glis induces transcription of its target genes including *Gli1, Ptch1*, and *cyclin D1* that ultimately results in uncontrolled proliferation of cells.

endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) act as unfolded protein sensors. IRE1 and PERK activate signaling cascades that inhibit translation while ATF6 promotes the transcription of additional protein folding chaperones [62]. Evidence suggests that chronic arsenic exposure activates all three signaling pathways of the UPR by up-regulating IRE1, PERK, and ATF6 [25]. Arsenic has been found to disrupt macrophage functions such as bacterial engulfment, cytokine release, and bacterial digestion through activation of PERK signaling. Reactive oxygen species have been causally implicated in UPR activation because N-acetylcysteine (NAC) was found to block the detrimental effects observed in macrophages [60].

Arsenic has also been found to exacerbate lipopolysaccharide-induced inflammation, mediated by macrophages and monocytes, by ROS stimulation of the Src kinase pathway. Src kinase is a non-receptor tyrosine kinase that has been implicated in the progression of numerous solid tumors. This increases the abundance of p38 kinase, which causes monocytic-lineage cells to mount an exaggerated and damaging immune response resulting in overproduction of inflammatory cytokines [63]. Arsenic has also been found to damage cell-mediated immunity. One study found that arsenic exposure enhanced HIV's ability to infect human

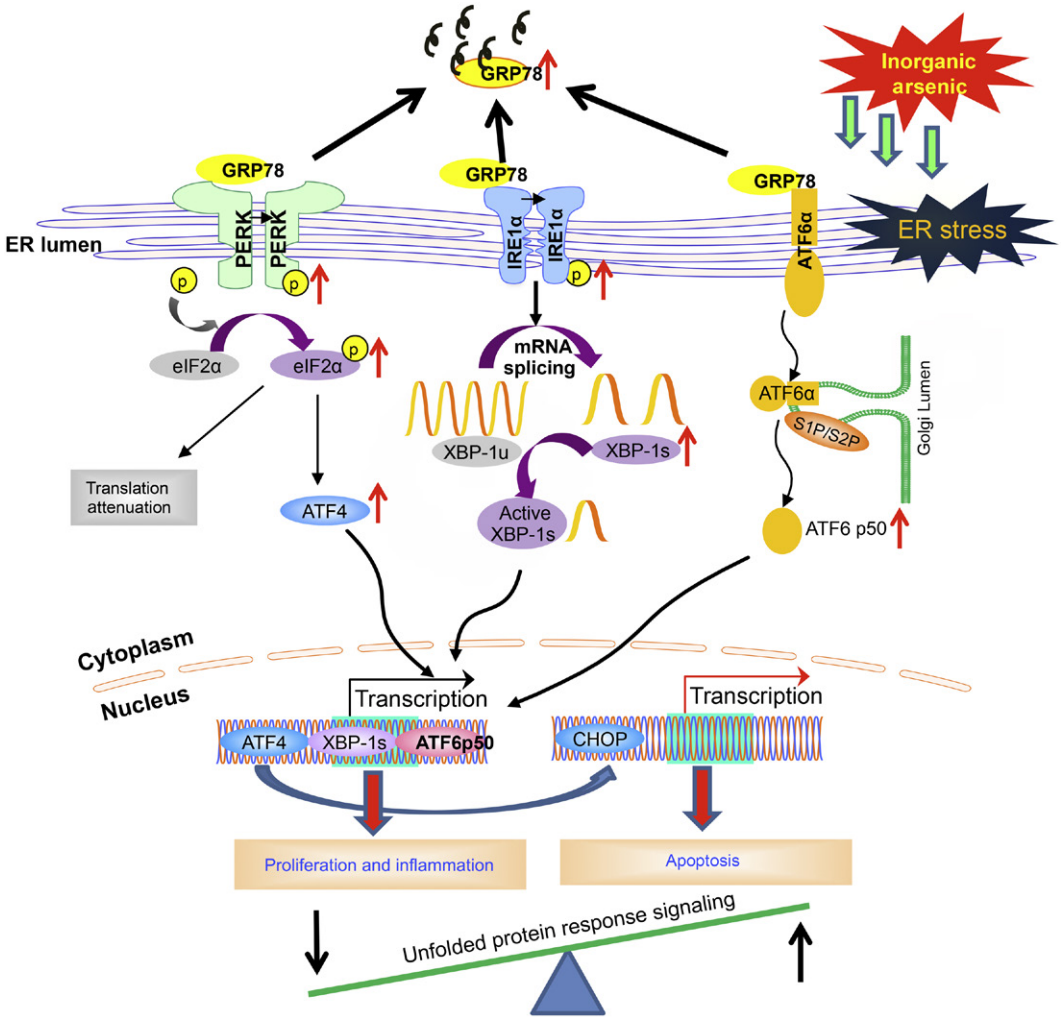

FIGURE 11–3 Flow diagram showing modulation of UPR signaling by arsenic. Under normal conditions in cells, the ER chaperone GRP78 is known to bind with the domains of the ER stress sensor proteins PERK, IRE1α and ATF6α, which is important for sustaining these proteins in an inactive form. Under the conditions of ER stress such as chronic arsenic exposure, GRP78 favorably binds to misfolded proteins and leads to activation of these sensors. Release of GRP78 from ER sensor proteins results in the activation of PERK, through PERK homodimerization and trans-auto-phosphorylation. Phosphorylated PERK then phosphorylates the translation initiation factor eIF2 and reduces the overall frequency of mRNA translation initiation. However, designated mRNAs, such as ATF4 mRNA, are preferentially translated in the presence of p-eIF2. ATF4 activates the transcription of UPR target genes. The release of GRP78 also allows IRE1α dimerization and activates its protein kinase activity through autophosphorylation. IRE1α then removes a 26-base intron from XBP-1u mRNA (u-unspliced). The spliced XBP1s (s-spliced) mRNA, which is a potent transcription factor that translocates into the nucleus and activates expression of UPR target genes. Release of GRP78 from ATF6α allows ATF6α migration to the Golgi apparatus, where it is cleaved by the proteases S1P and S2P to the ATF6 p50 fragment; following translocation to the nucleus it become transcriptionally active. Abbreviations: S1P, site-1 protease; S2P, site-2 protease; XBP-1, X-box-binding protein 1; ATF4, activation transcription factor 4. Red arrows indicate proteins upregulated by arsenic.

dendritic cells by dampening post-viral-entry cellular defenses [64]. Another investigation identified a disproportionate number of apoptotic $CD4^+$ lymphocytes in arsenical BD lesions. Additionally, the peripheral blood of individuals with these lesions demonstrated fewer $CD4^+$ T lymphocytes versus controls [65].

Arsenic promotes inflammation by activating the p38 MAPK pathway. This occurs through oxidation of an SH group on MAPK phosphatase 1, a protein tyrosine phosphatase responsible for the inhibition of an upstream MAPK effector, epidermal growth factor receptor (EGFR). Thus, oxidation of this SH group transiently inactivates the MAPK phosphatase 1 inhibitor, allowing increased pro-inflammatory MAPK signaling [3]. Furthermore, up-regulation of MAPK signaling has been causally associated with increased signaling through activated protein 1 (AP-1), a transcription factor that is known to regulate responses to cytokines, growth factors, stress, infection, and apoptosis [3,5,66]. In summary, arsenic disrupts signal transduction and effectively promotes carcinogenesis by activating oncogenes, inhibiting tumor suppressors, and up-regulating pathways that may invoke cutaneous inflammatory signaling [2,3,41,46].

11.6 Treatment

Acute arsenic toxicity is best treated by chelation therapy with agents such as dimercaprol (used most commonly), dimercaptosuccinic acid, and dimercaptopropane sulfonic acid [6,8]. There are no standard guidelines for the treatment of chronic arsenicosis or for the screening of underlying disease. Some authors recommend that patients with arsenicosis undergo a detailed history and physical as well as total skin examination every 6 months [12]. The history should focus on an individual's occupation, living conditions, environmental exposure, and medicinal exposure spanning the previous 10 to 40 years. A biopsy is indicated for any lesions that are changing, erythematous, or ulcerated to rule out malignancy. Laboratory tests such as a complete blood count, renal function tests, and liver function tests have been proposed. Hair and nail sampling may be indicated to quantify arsenic exposure [28]. Annual chest radiographs, nerve conduction studies, and other indicated clinical tests have been proposed [28] given the common coexistence of other underlying diseases such as ischemic heart disease, atherosclerosis [3], peripheral vascular disease, diabetes mellitus, peripheral neuropathy [2], and Raynaud's phenomenon [1,3,5] in the setting of arsenical skin disease. Though the treatment of arsenical keratoses is not required, these lesions are often treated to reduce patient discomfort caused by the lesions [28]. The most common treatment options include surgical excision, curettage, cryosurgery [6,8], and carbon dioxide laser ablation [6]. Although topical chemotherapy with 5-fluorouracil has also been employed, this option is less effective for the treatment of arsenical keratoses as opposed to actinic keratoses [6]. Topical keratolytics, photodynamic therapy, oral retinoids [67,68], and topical 5% imiquimod [69] have been used with some success, though these treatments are anecdotal or based upon small-scale investigations [6,8]. Evolving evidence describing the molecular pathogenesis of these cutaneous lesions may be helpful in developing novel therapeutic interventions for skin diseases and disorders among arsenic-exposed human populations.

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12

Arsenic-Induced Liver Injury

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CHAPTER OUTLINE

12.1 Introduction

Arsenic is a toxic metalloid that constitutes 0.0001% of Earth's crust. Evidence of therapeutic use of this metalloid is available from the ancient Greeks and Romans. Historically, arsenic was also used as human poison. It is believed that Napoleon died due to arsenic poisoning. Human exposure to arsenic is inevitable. Exposure can occur via three principal routes, that is, through the inhalation of air, through the ingestion of arsenic-contaminated food and water, and via dermal absorption. Worldwide, the degree of non-occupational exposure to arsenic varies greatly, being dependent on local geochemistry and the level and proximity of anthropogenic activity.

Exposure to inorganic arsenic through drinking water is a major public health problem in both developing and developed countries [1,2]. In the United States of America, over 13 million people live in areas where arsenic concentration in the drinking water exceeds the US Environmental Protection Agency's safe cut-off value of 10 μg/L [3]. But the greatest catastrophe in the history of arsenic poisoning of a population is attributed to the densely populated deltas of the great rivers flowing through South and South-East Asia, where more than 100 million people are being exposed to unsafe levels of arsenic by drinking contaminated

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groundwater [2]. In parts of West Bengal, India, and Bangladesh, arsenic levels in drinking water have been reported to be as high as 1.5 to 3.4 mg/L [4–7]. Over the last three to four decades, consumption of arsenic-contaminated drinking water in public supply systems has posed a serious health hazard among inhabitants of at least nine districts in West Bengal and Bangladesh [8–11].

Arsenic causes a spectrum of adverse responses in many species. Acute exposure to inorganic arsenic in humans results in cardiac failure, peripheral neuropathy, anemia, leukopenia, and death, while chronic arsenic exposure can cause a range of cancers as well as liver injury, neuropathy, cardiovascular lesions, and other diseases. The toxicity of inorganic arsenic usually occurs after a considerable latent period. It is responsible for the genesis of a wide range of health problems involving most of the systems of the human body and is also implicated as an etiological agent of cancers of the different organs of the body. Emerging evidence indicates that ingested arsenic favors the development of hepatic disorders. The magnitude of the public health problem posed by chronic arsenic toxicity is fairly large with newer affected areas being identified in West Bengal and some other states in India and Bangladesh day by day, and there is no treatment for established chronic arsenic toxicity with proven efficacy. This makes it essential to improve our understanding of the pathophysiology of arsenic toxicity, which is difficult to discern in humans and has not been well studied in experimental animals. The present chapter deals with understanding arsenic-induced liver injury and its possible pathways, in the context of current available knowledge.

12.2 Source of Exposure

Environmental arsenic originates from both geochemical and anthropogenic activities. The anthropogenic sources of arsenic are soil, water, mining, metallurgical activities, and use of herbicides and pesticides. The sources of arsenic exposure vary in different parts of the world, i.e., from burning of arsenic-rich coal in China and mining activities particularly in Malaysia and Japan, to the ingestion of arsenic-contaminated drinking water in many countries around the world. Due to geological factors, some coals contain high concentrations of arsenic [12]. People of Guizhou province in southwest China are exposed to arsenic due to burning coals that have a high concentration of arsenic. In some areas of Guizhou province, coals contain 100–9000 ppm arsenic [12–14]. In these areas, chronic arsenic intoxication occurs due to burning of high arsenic containing coal in unventilated stoves, which is a routine practice in the drying of various foods. Arsenic, which is a constituent of some ores, is a common occupational exposure and frequently results in widespread environmental contamination. Other sources of contamination are the manufacture and use of arsenical fertilizers, pesticides, wood preservatives, and insecticides. Non-occupational exposure occurs primarily through the ingestion of food and water, with the inhalation pathway playing only a minor role. Food is more commonly the main contributor to total intake but in areas where drinking waters contain relatively high levels of arsenic, such water may be the most important source of arsenic intake. In Latin America, South Asia, Taiwan, and Japan, arsenic levels in drinking water have been reported as high as 1.5–3.4 mg/L [4,6,7]. Groundwater arsenic contamination in

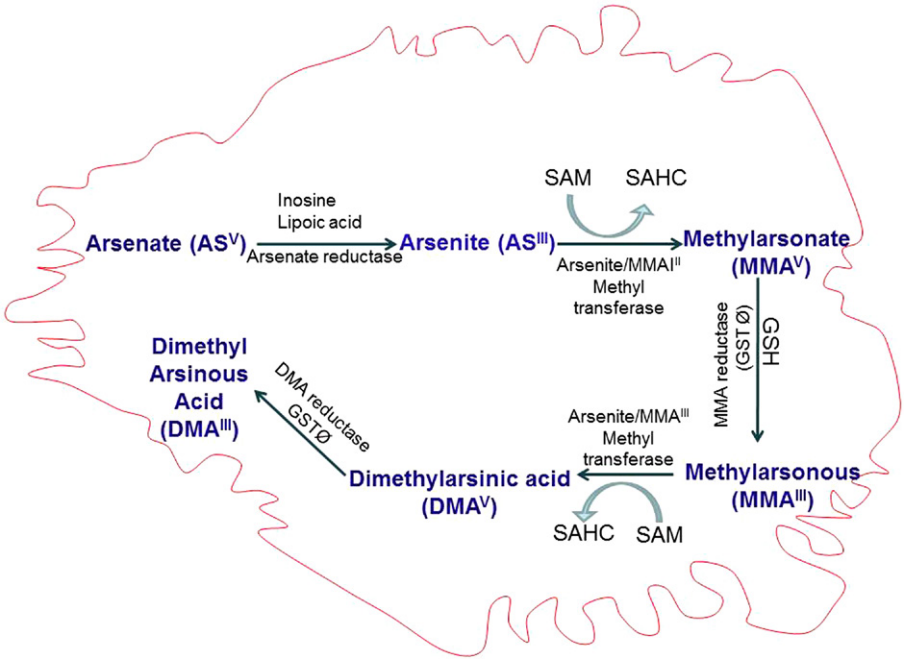

FIGURE 12–1 Pathways of inorganic arsenic metabolism within hepatocytes of the liver.

Bangladesh and the West Bengal Delta of India has received the greatest international attention due to the large number of people potentially exposed and the high prevalence of arsenic-induced diseases. Residential areas of Behala, Kolkata, also reported groundwater arsenic contamination due to industrial pollution [15].

12.3 Biotransformation and Elimination of Arsenic

Following absorption, arsenic is distributed widely in the body, with high concentrations appearing in the skin, liver, kidney, lung, and spleen. Liver is the primary organ for biotransformation of toxic inorganic arsenic into less toxic metabolites for elimination from the body. In most mammals, inorganic arsenic is metabolized to methylarsonic acid (MMA) and dimethylarsenic acid (DMA) by stepwise transfer of the methyl group from S-adenosylmethionine. It is known that after ingestion, the pentavalent form of inorganic arsenic (As^{V+}) is reduced to trivalent inorganic arsenic (AS^{III+}) and subsequently methylated in the liver to the less toxic readily excreted metabolites MMA and DMA (Figure 12–1). The methylated metabolites have a shorter half-life *in vivo* than inorganic arsenic and are less reactive with tissue components, less toxic, and more rapidly eliminated from the body through urine than inorganic arsenic [16,17]. In general, little is known about factors influencing arsenic toxicity and metabolism in humans. Arsenic methylation patterning is influenced by age as well as exposure level. Urinary DMA

content in urine is lower in children compared to adults, indicating that children retain more arsenic in their tissues and they are more sensitive to arsenic exposure. The toxicity of arsenicals is highly dependent on the chemical forms. Trivalent inorganic arsenic (As^{III}) is the main form of arsenic interacting with tissues [16,17]. It is observed that a lower capacity to methylate arsenic is associated with higher tissue concentrations [18], and possibly also higher risk for toxic effects. Thus, the methylation of arsenic may be considered a detoxification mechanism.

The mechanism involved in arsenic metabolism is not well understood; it has been shown that glutathione (GSH) plays an important role in both reduction and methylation of arsenate [19]. Experimental studies have shown that GSH elevation is a natural reaction to arsenic insult, presumably as a protective mechanism [20], and that GSH depletion prior to treatment with arsenic leads to interference with arsenic metabolism, including inhibition of methylation in the liver and decrease in the elimination rate of arsenic metabolites [21]. Since GSH is involved in arsenic metabolism, it is possible that the individual variation in methylation capacity relates to glutathione *S*-transferase M1 and glutathione *S*-transferase T1 genotypes. Reduced levels of glutathione *S*-transferase in those with null genotypes may increase susceptibility by decreasing methylation efficiency, leading to exposure of the liver to more toxic forms of arsenic. Arsenic has been shown to produce toxic effects common to oxidative stress [22–24] and these effects are thought to be mediated by the binding of arsenic metabolites (e.g., As^{III}) to critical sulfhydryl groups within proteins [25]. Glutathione *S*-transferase T1 deficiency could therefore directly exacerbate the oxidant effects of arsenic exposure.

Inorganic arsenic and its metabolites are rapidly cleared from hepatocytes through bile and ultimately eliminated from the body through feces. The biliary transport of arsenic is dependent on the arsenic–GSH complex formation that transports it out of the hepatocytes into bile. High expression of multidrug resistance-associated protein 2 (MRP2) is observed in the liver during arsenic exposure. This MRP2 actively involves the biliary transport of arsenic [26]. Any defect of arsenic–GSH complex formation, lack of intracellular GSH store, or defect of MRP2 protein formation leads to accumulation of arsenic within the hepatocytes.

12.4 History of Arsenic-Related Liver Diseases

The liver is vulnerable to prolonged exposure to small amounts of arsenic; however, the precise degree of sensitivity to arsenic in any one person cannot be predicted. Chronic arsenic exposure has been typically associated with dermatological disorders. The relationship between arsenic exposure and liver disease is not clear. However, sporadic reports of liver disorders are available in the literature. One of the earliest descriptions of arsenic-induced liver disease dates back to the late 18th century, when Bang [27] first reported development of ascites due to prolonged use of liquid arsenic in therapeutic doses. Subsequently, Sir Jonathan Hutchinson [28] described a young man with psoriasis who developed ascites after treatment with Fowler's solution (arsenic trioxide). Early investigators also described an outbreak of liver disease in beer drinkers in the north of England, where arsenopyrite had been used to produce beer from starch [29,30]. The disease was directly related to the amount of arsenic in the beer. In 1921 Stockman [31] reported the development of ascites and liver cirrhosis in a patient who had

consumed arsenic in a bromide mixture. In 1936 Cannon [32] described a young woman with an enlarged liver who had ingested fruits and vegetables sprayed with an arsenical pesticide. In 1945 Maccallum described liver damage due to organic arsenicals used therapeutically [33]. Thus, the hepatotoxic effects of arsenic when used as a therapeutic agent have long been recognized. Scanty reports of liver diseases related to chronic exposure to arsenic-contaminated water are available in the literature. Worldwide, environmental exposures remain a significant problem. Chronic liver diseases, which can be ascribed to arsenic toxicity, include steatosis, cirrhosis, hepatoportal sclerosis, angiosarcoma, and perhaps hepatocellular carcinoma [34].

12.5 Hepatoportal Sclerosis

Chronic arsenic exposure may be associated with hepatoportal sclerosis (also known as non-cirrhotic portal hypertension), which is characterized by portal hypertension without evidence of liver cirrhosis. Portal hypertension is a clinical syndrome defined by a portal venous pressure gradient between the portal vein and inferior vena cava exceeding 5 mmHg [35]. It is a disorder of unknown etiology. The disease has been reported from different parts of the world and predominantly from the developing countries. According to the consensus statement of the Asia Pacific Association for the Study of the Liver (APASL) on NCPF, the disease accounts for approximately 10–30% of all cases of variceal bleed in several parts of the world including India [36]. Clinically, this disease is characterized by features of portal hypertension, and moderate to massive splenomegaly, with or without hypersplenism with preserved liver functions and patent hepatic and portal veins [37]. Histologically, it is characterized by thickening and sclerosis of the wall of large portal vein branches. Increased perivascular fibrosis is seen in the portal tracts, which may show sclerosis of portal vein branches. The portal vein lumen is reduced and organized thrombi with recanalization may be seen. Several reports have documented the involvement of chronic arsenic exposure in the development of this disease. Zeegen and coworkers [38] described a series of 44 patients with portal hypertension, four of whom had a history of arsenic exposure. Viallet et al. [39] described the case of a patient who had consumed arsenic for the treatment of psoriasis for 12 years before development of portal hypertension in the absence of liver cirrhosis. Morris et al. [40] described two cases of non-cirrhotic portal hypertension due to treatment of Fowler's solution for 3 and 22 years respectively. Wedged hepatic vein pressures were normal, while intrasplenic pressure was high in these two patients. This indicated obstruction to portal flow in the portal tracts. Morris et al. [40] also suggested that arsenic caused damage of the intrahepatic portal veins. Huet et al. [41] reported the development of non-cirrhotic portal hypertension after intake of arsenicals. In addition, a case of massive variceal bleeding secondary to presinusoidal portal hypertension due to arsenic poisoning was reported from North America [42]. Nevens et al. [43] also described non-cirrhotic portal hypertension development in eight patients who had been treated with Fowler's solution for psoriasis. Total arsenic consumption was varied from 4 to 16 g, with an interval between treatment and onset of symptoms of 2 to 16 years. From India, Dutta et al. [44] first reported nine patients with hepatoportal sclerosis who had consumed high levels of arsenic from contaminated drinking water, adulterated

opium, and indigenous medicine. Estimated hepatic blood flow of those patients with hepatoportal sclerosis due to consumption of high amounts of arsenic was within the normal range and there was no correlation between hepatic arsenic and the hemodynamic findings. Hepatoportal sclerosis was also reported from patients consuming high levels of arsenic-contaminated drinking water [10,45]. Santra et al. reported the presence of esophageal varices only in few cases in their series [45]. Thus, the majority of the patients had no definite evidence of portal hypertension though portal fibrosis was observed in liver biopsy in most of the cases. Hepatoportal sclerosis is often a result of damage to the local vasculature [43,46].

12.6 Arsenic-Related Hepatic Fibrosis and Cirrhosis

The hepatotoxic action of arsenic, when used as a therapeutic agent, has long been recognized. Development of ascites has been reported in patients taking liquor arsenic in therapeutic doses for prolonged periods [27–28]. Cases of cirrhosis of the liver resulting from continued use of Fowler's solution containing potassium arsenite have also been described [47]. Early investigators also described a few cases of chronic liver disease due to drinking arsenic-contaminated beer [30,31]. Baldridge [48] described cirrhosis in patients being treated with arsenic for syphilis. Luchtrath [49] reported that cirrhosis among German vintners had decreased since the banning of arsenical pesticides. However, data on liver involvement following chronic exposure to arsenic-contaminated water are scanty. Guha Mazumder et al. highlighted that non-cirrhotic portal fibrosis occurred in chronic arsenicosis due to drinking arsenic-contaminated water [10,50]. Subsequently, Santra et al. reported the largest series showing significant involvement of the liver in the form of non-cirrhotic portal fibrosis among people drinking arsenic-contaminated water [45]. In hospitalized arsenicosis patients who had been exposed to arsenic-contaminated water for up to 15 years and had hepatomegaly, from West Bengal, India, liver fibrosis is very common (Figure 12–2). A few of these patients

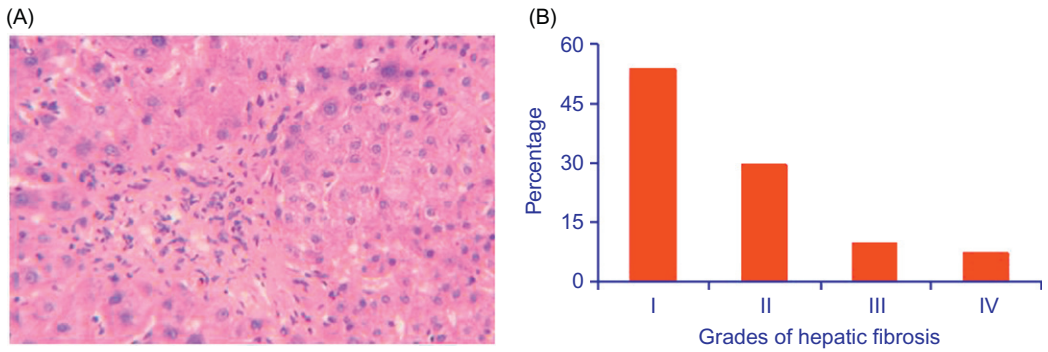

FIGURE 12–2 (A) Representative liver histology of a patient who had hepatomegaly due to drinking high arsenic-contaminated water for a long period. Evidence of expansion of the portal zone is also observed in the section. (H & E staining; original magnification ×20.) (B) Grades of liver fibrosis in patients suffering from chronic arsenicosis due to drinking high levels of arsenic-contaminated water. Most of the patients had mild fibrosis (grades I and II) and few had moderate to severe fibrosis (grades III and IV). The data are based on 69 hospitalized arsenicosis patients [45].

had liver cirrhosis. The portal fibrosis was characterized by expansion of portal zones of varying degrees (Figure 12–2). Fibrous extension from portal tracts into liver lobules producing septa was observed in some cases. The fibrosis in the liver was mild in most cases, moderate to severe fibrosis being observed in few cases (Figure 12–2). At some regions the expanded portal zone contained a leash of vessels replacing the portal vein branches. The features of periportal fibrosis and multiple vascular channels in expanded portal zones reported by Santra et al. [45] are similar to those observed in non-cirrhotic portal fibrosis/idiopathic portal hypertension. These multiple portal venous vascular channels are most likely the end results of recanalization of thrombosed intrahepatic portal veins and have been observed in patients who develop non-cirrhotic portal hypertension [51]. This neovascularization appears to be an attempt to re-establish portal venous blood flow in previously injured or obstructed vessels.

Chronic arsenic exposure has been associated with peripheral vascular diseases. One such disorder, known as "blackfoot disease," seems to result from an early destruction of vascular endothelial cells [52]. In an editorial review, Boyer [53] raised an important issue—whether non-cirrhotic portal hypertension results from primary injury to the endothelial cells within the portal vein radical. Long-term follow-up of patients with arsenic-induced hepatic fibrosis is helpful to define the relationship between chronic arsenic toxicity and development of non-cirrhotic portal hypertension.

Most of the authors described liver profiles to be normal or near normal, but Santra et al. described elevated levels of serum ALT and AST in a significant number of cases with hepatomegaly [45], and arsenic in the liver tissues was detected in 23 of 29 samples tested. The maximum arsenic content in the liver tissue was found to be 6 mg/kg. However, the arsenic content of the liver tissue did not correlate with the degree of hepatic fibrosis or with the arsenic content of the water consumed. While the nature of the toxicological mechanisms involved in hepatic damage due to inorganic arsenic is not clear, some reports have demonstrated that arsenic can accumulate in the liver [10,44,45]. Figueroa and colleagues examined mummies hundreds of years old that were found in region II of Chile, an area that has had arsenic levels in drinking water. Kidney, liver, nail, and lung tissues had some of the highest concentrations of total arsenic followed by skin, intestine, hair, and ribs, in that order [54]. High arsenic content in the liver was also demonstrated in patients suffering from non-cirrhotic portal fibrosis associated with chronic arsenic toxicity [10,44,45].

12.7 Epidemiological Study to Assess Arsenic-Related Liver Dysfunction

A few epidemiological studies related to liver dysfunctions due to chronic arsenic exposure are available. High prevalence of hepatomegaly was reported among inhabitants of six villages of three arsenic-affected districts in West Bengal [55]. Subsequently, Guha Mazumder et al. [10] also reported high prevalence of hepatomegaly among subjects in a hamlet drinking high arsenic-contaminated water (>50 μg/L). Ma et al. reported occurrence of hepatomegaly and abnormal liver functions in a study population of 1447 cases of chronic arsenicosis due to drinking arsenic-contaminated water in Inner Mongolia, People's Republic of China [56].

Reports of liver dysfunction in chronic arsenicosis are also available from Bangladesh [57]. However, most of the reports described above have some limitations in selecting the subjects from the viewpoint of an epidemiological study.

Population-based studies of liver injuries related to chronic arsenic exposure are necessary for accurate information on the risk of liver diseases. One such example is a population-based study reported from Kolkata. This study was designed to assess the prevalence of liver involvement and various health effects associated with chronic arsenic exposure through drinking high arsenic-contaminated water. This cross-sectional epidemiological study was conducted in one of the most affected districts of West Bengal [6]. This district was found to be most suitable for the epidemiological study because of heterogeneity in arsenic exposure. The drinking-water arsenic content in this district ranged from non-detectable to 3400 µg/L. In this study two particular areas of the district were selected. The first one had had high levels of arsenic detected in a number of shallow tubewells, but not all. The other area selected was from another part of the district where people used shallow tubewell water that did not contain increased levels of arsenic. A total of 7683 participants were included in this study. Convenience sampling was adopted in both high and low exposure zones. Enlargement of the liver was observed in 2.99% of participants drinking water containing arsenic at <0.05 mg/L (low exposure group), and while in 10.21% of participants who were drinking water containing arsenic at >0.05 mg/L (high exposure group). Hepatomegaly in this population was found to have a linear relationship proportional to increasing exposure of arsenic in drinking water in both the sexes ($p < 0.001$). The prevalence was greater among males than females. The prevalence of hepatomegaly among females was 3.46% in the lower exposure category (<50 µg/L) while its incidence was 17.83% in the highest exposure category (≥800 µg/L). A stronger trend appeared also in males; the prevalence increased from 2.62% in the lowest exposure category to 28.81% in the highest exposure category.

This epidemiological study is thought to be the first large-scale population study assessing the prevalence of hepatic enlargement due to chronic arsenic ingestion in an arsenic-affected area in a structured population survey. A major advantage of this study was the participation of subjects from all the different age groups of the community, and thus the data generated from this study truly reflect the actual prevalence of hepatic enlargement due to arsenic exposure.

12.8 Animal Studies for Understanding the Pathogenesis

The pathogenesis of liver injury in arsenicosis is not clear; however, some experimental studies on animals are available. Based on various experimental studies, it is now clear that arsenic induces liver injury from an early phase of exposure followed by a phase of adaptation, and if the exposure is continued long term, there is evidence of chronic liver injury. Evidence of oxidative stress in the liver following arsenic exposure is available. Cellular events at an early phase of high arsenic exposure that initiates liver injury in mice have been described [58]. The pathological lesions in liver include fatty degeneration and to a lesser extent necrosis and

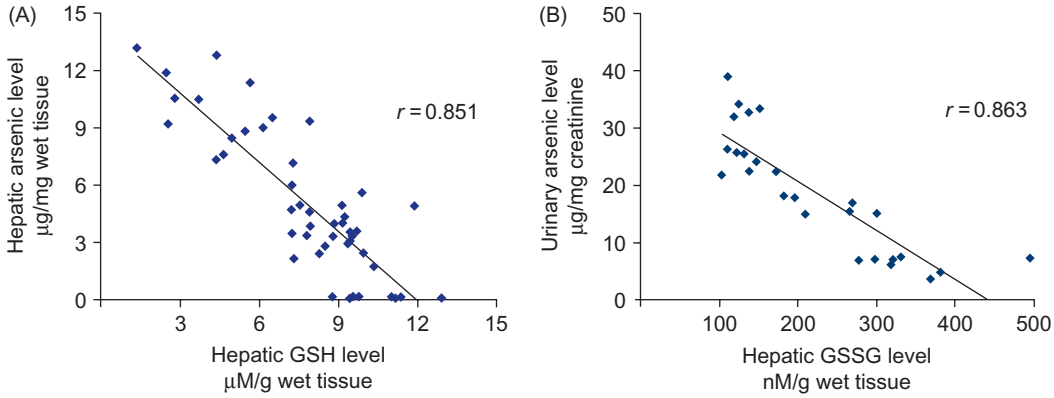

FIGURE 12–3 Correlation between oxidative stress with arsenic content in liver and urinary excretion. (A) Data showing a negative correlation between hepatic GSH level and arsenic deposition in the liver in control and arsenic-exposed mice ($r = 0.851$; $p < 0.001$). (B) Decrease in urinary excretion of arsenic with increase in GSSG levels ($r = 0.863$; $p < 0.001$).

inflammation due to high-dose arsenic exposure. Hepatic steatosis is macrovesicular in nature and apoptosis is the predominant mode of hepatocyte death due to arsenic exposure. Further, apoptosis of the hepatocytes in an early phase of arsenic exposure is dose dependent. Urinary arsenic is generally used as a marker of current arsenic exposure. Animal studies showed increased deposition of arsenic in the liver despite an increase in urinary arsenic excretion, thus indicating the inability of the liver to eliminate the increased arsenic that the animals were exposed to. The deposition of arsenic in the liver was found to be related to the increasing doses of arsenic exposure. Deposition of arsenic in the liver inversely correlated with depletion of hepatic GSH content (Figure 12–3). Moreover, the urinary arsenic elimination was also found to be inversely correlated with the accumulation of GSSG in liver, a marker of oxidative stress, as a result of arsenic exposure (Figure 12–3).

Many endogenous or xenobiotic lipophilic substances are eliminated from the cells by the sequence of oxidation and conjugation to an anionic group (glutathione, glucuronate, or sulfate) and transport across the plasma membrane. The latter step is mediated by integral membrane glycoprotein belonging to the superfamily of ATP-binding cassette (ABC) transporters, most commonly known as the multidrug resistance-associated proteins (MRPs). MRP-2, a subfamily of MRPs, also contributes to the control of the intracellular glutathione disulfide (GSSG) levels. Although this protein has a low affinity towards GSSG, it can play an essential role in response to oxidative stress. The elimination of arsenic is one of the important criteria of adaptation, and this elimination depends on the function of MRP-2. Ghatak et al. had studied the role of MRP-2 protein as it has been well documented that arsenite appears to be a stronger inducer of MRP-2. Interestingly, Ghatak et al. [59] have found that the expression of MRP-2 is directly related to the level of cellular GSH.

One potential central mechanism of hepatic injury at the early phase of arsenic exposure is the development of oxidative stress due to generation of reactive oxygen species (ROS) from

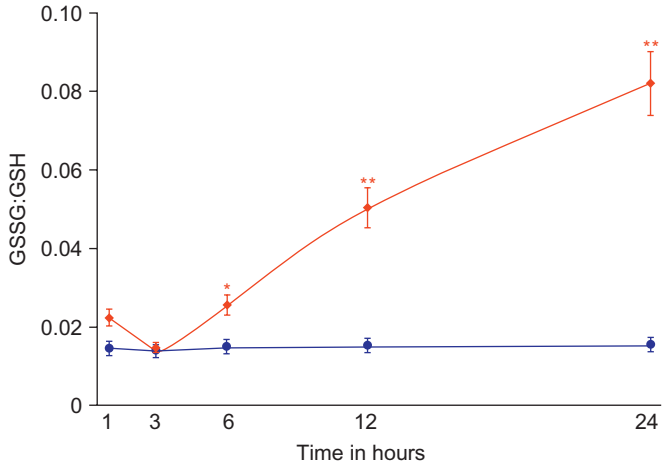

FIGURE 12–4 Effect of arsenic exposure on hepatic oxidative stress. Mice were treated with 6 μg/g body weight p.o. of arsenic and sacrificed at different time intervals. The ratio of GSSG:GSH was plotted graphically using varied hours of arsenic exposure. Results were expressed as mean ± SD of 10 mice per group (*$p < 0.05$; **$p < 0.001$). The red trace indicates the arsenic-treated groups while the blue trace indicates control.

mitochondrial as well as from non-mitochondrial pathways [58,60]. The ratio of GSSG to GSH is often regarded as the marker of oxidative stress. Animal studies indicated that from an early phase of arsenic exposure, there is evidence of increased GSSG to GSH ratio in the liver, suggesting development of oxidative stress in the liver of arsenic-exposed animals (Figure 12–4). Hepatocytes are vulnerable to increased oxidative stress due to arsenic exposure. Generation of oxidative stress in the liver and its impact on cellular integrity and function are critical early events in arsenic-induced liver injury. It has been observed that high levels of reduced glutathione (GSH) are associated with cellular resistance to arsenic [61–63] and decreasing intracellular GSH concentration causes increased sensitivity to arsenic toxicity [62]. Thus, the hepatotoxic effects of arsenic are attributed to the development of oxidative stress and the consequent depletion of GSH from the liver. GSH has been suggested to be important for arsenic methylation as well as transport [64,65] and thus helps in the removal of arsenic from the body. Depletion of hepatic GSH facilitates accumulation of arsenic in the liver and thus causes oxidative stress particularly at higher dose. However, at comparatively low dose of arsenic exposure, evidence of oxidative stress, especially in the mitochondrial fraction, is not significantly increased because at lower dose there is an adaptive response to re-establish the normal cellular redox status [58].

Arsenic exposure in animal models showed increased oxidative stress in the liver mitochondria, which causes mitochondrial functional alteration. A potentially deleterious effect of ROS production in mitochondria is facilitation of Ca^{2+}-dependent mitochondrial permeability transition (MPT), which plays a key role in certain modes of cell death. Arsenic causes progressive induction of MPT pore opening of the liver mitochondria in an early phase of exposure (Figure 12–5) and decreased fatty acid oxidation leading to accumulation of triglycerides within the

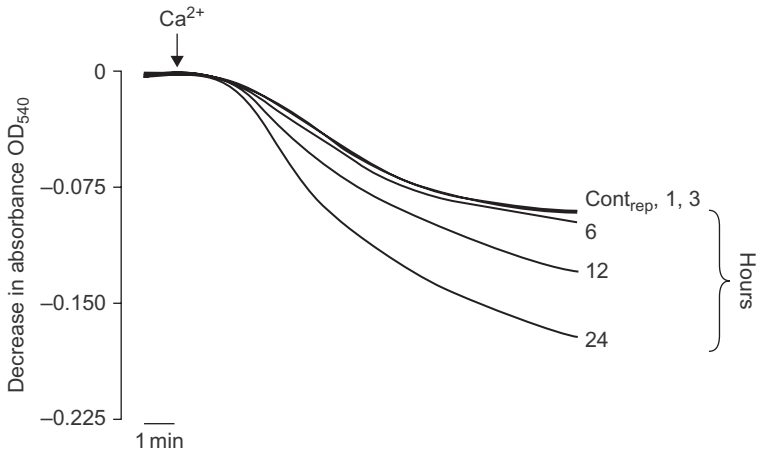

FIGURE 12–5 Effect of arsenic on the functional activity of mitochondria. Arsenic-treated mice were scarified and the mitochondria were isolated. 1 mg/mL of mitochondria suspension was prepared and loaded with Ca^{2+} (20 nmol/mg protein) and the mitochondrial swelling was monitored by the O.D. at 540 nm.

hepatocytes. At present, although the detailed mechanisms regulating MPT are still largely elusive, it is known that the oxidative stress markedly sensitizes mitochondria toward MPT induction [66,67]. It is well known that onset of MPT always leads to a loss of mitochondrial membrane potential (MMP), though not all MMP losses are caused by MPT [66]. The onset of MPT results in alterations in the equilibration of ions between the mitochondrial matrix and the inner membrane space, leading to the loss of MMP [68]. Mitochondrial GSH plays an essential role in maintaining healthy mitochondria, and its depletion may be a key event in sensitization of hepatocytes to oxidant injury. Santra et al. [58] reported altered MPT associated with translocation of cytochrome c in the cytosol and activation of caspase 3 and caspase 9 activities in the early event of arsenic exposure in mice. Translocation of cytochrome c into cytosol is a primary event that leads to the formation of apoptosomes and activation of the caspase cascade. Higher doses of arsenic cause an increased DNA fragmentation, suggesting increased apoptosis. Moreover, a significant increase in caspase 3 and caspase 9 activities in arsenic-exposed mice suggests that apoptosis is caspase dependent [58]. Caspase, which is cysteine protease, cleaves different intracellular target molecules, resulting in cell shrinkage, chromatic condensation, and DNA fragmentation [69]. Caspase 3 is activated via either an extrinsic pathway (death receptor mediated) or an intrinsic pathway (mitochondria-dependent pathway [70]. The activation of caspase 9 in arsenic-induced mice strongly suggests the involvement of a mitochondrial pathway [58].

Treatment with an antioxidant such as N-acetyl cysteine (NAC) in animal models could replete cellular stores of the tripeptide GSH and is an effective intervention against oxidative stress developed due to arsenic exposure. One explanation of the observed hepatoprotection provided by NAC could be that the liver of NAC pretreated animals effectively detoxifies or removes arsenic via a GSH-dependent pathway. This is probably due to an enhanced ability to maintain GSH homeostasis during exposure to toxic electrophiles generated by arsenic as well as its rapid elimination/excretion from the body.

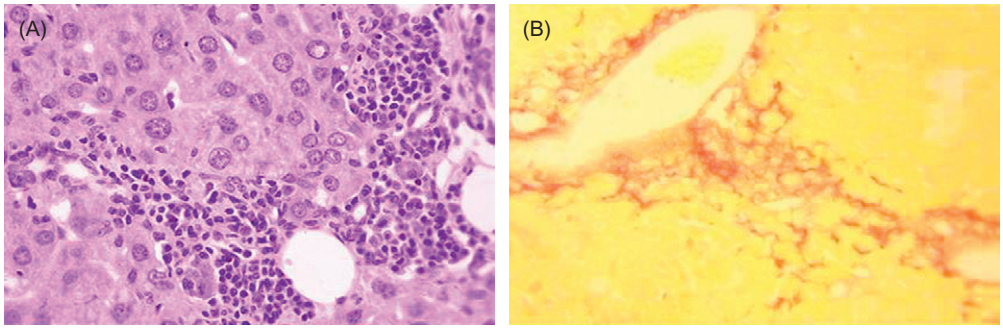

FIGURE 12–6 Liver histology of arsenic-exposed mice. (A) Hematoxylin and eosin staining of arsenic-exposed mice for 12 months (magnification: ×40). The infiltrations of inflammatory cells are shown in the section. (B) Histology with Sirius red collagen staining of liver section of arsenic-exposed mice for 12 months (magnification: ×20). There is evidence of marked portal fibrosis and pericellular fibrosis accumulated near the portal tract.

Evidence for adaptation against oxidative assault in the liver of experimental models due to short-term arsenic exposure is available in the literature [71]. Arsenic exposure for short periods initiates activation of a liver antioxidant defense system by increasing hepatic GSH content to counteract and protect the hepatocytes against free radical-mediated oxidative damage. This antioxidant defense system becomes less effective subsequently, due to its overreactivity and this might initiate hepatic injury.

The histological abnormalities observed in the liver of the experiment model in long-term arsenic exposure include hepatocellular degeneration with focal mononuclear cell collection, fatty degeneration (macrovesicular steatosis), Kupffer cell proliferation, and hepatic fibrosis (Figure 12–6). The hepatic fibrosis is not associated with nodular regeneration.

Exposure to arsenic-contaminated drinking water for a prolonged period is associated with pathological changes in the liver as evidenced by clinical and experimental studies. Providing arsenic-contaminated water to mice for 15 months and sacrificing those mice at different intervals revealed hepatocellular steatosis associated with significant reduction in hepatic antioxidant defense system at 9 months and evidence of hepatic fibrosis associated with further impairment of the antioxidant defense system at 12 months [72]. Oxidative stress is thought to underlie several chronic liver diseases that are associated with fibrosis. Oxidative stress is a complex process that can result in the peroxidative damage of the major cellular components including amino acids, proteins, lipids, carbohydrate, and nucleic acids [73]. Damage of the membrane lipids and associated alteration of the bulk properties of the membranes are considered to be the primary basis of chemical-induced hepatocellular injury. The increased lipid peroxidation and plasma membrane damage as evidenced by progressive reduction of Na/K ATPase activity in mice drinking arsenic-contaminated water parallel GSH depletion of the liver. Peroxidation of the lipids in the cell membrane, damage to DNA and macromolecules, recruitment of inflammatory cells, and activation of hepatic stellate cells can occur as a result of oxidative stress in sequence or in parallel and may contribute to the

progression of liver injury that may proceed the development of hepatic fibrosis. It is known that the lipid peroxidation products in the liver parenchymal cells are needed to initiate the fibrogenic process. Increased hepatic fibrogenesis with minimal hepatocellular injury due to high arsenic exposure in animal models was also observed by Sarin et al. [74]. In this model, hepatocellular necrosis and inflammation were either insignificant or mild. There was neither any feature of non-cirrhotic portal hypertension nor significant splenomegaly observed in this animal model. Das et al. showed elevated tissue necrosis factor-α (TNF-α) levels in the liver of mice exposed to arsenic for prolonged periods [75]. TNF-α plays an important role in activation of stellate cells [76], the predominant cells involved in the production of type I collagen.

The mechanism underlying the arsenic-induced oxidative stress response is not clearly understood. One potential candidate for the generation of ROS in chronic arsenic exposure may be NADPH oxidase (NOX), which is a potential non-mitochondrial source of ROS. Progressive increase of 4-HNE adduct, a marker of oxidative stress in the tissue, in the liver associated with increased NOX activity parallel to the duration of arsenic exposure was reported [77]. The role of NOX in arsenic-induced liver injury and subsequent development of fibrosis is suggested, since it is well established that ROS activate hepatic stellate cells (HSCs) and stimulate fibrogenesis [78]. Arsenic-induced oxidative stress in liver may up-regulate CD14 expression and thus increase the susceptibility of Kupffer cells to arsenic or its metabolites. It seems likely that the Kupffer cells, in turn, up-regulate TNF-α production, which plays an important role in the activation of HSCs, the predominant cells involved in the production of type I collagen [76,79,80]. Increased hepatic TNF-α production associated with up-regulation of CD-14 and TLR-4 mRNA transcript was reported after long-term arsenic exposure to mice [77]. However, the mechanism of Kupffer cell activation by arsenic remains unknown. Kupffer cell activation seems to be mediated by CD14 and TLR-4 [81].

In experimental studies, several lines of evidence suggest that arsenic activates HSCs as documented by immunohistochemical staining as well as mRNA levels for α-smooth muscle actin (α-SMA), a hallmark for stellate cell activation as demonstrated in the liver of mice exposed to arsenic for 1 year [77]. Further, the growth factors transforming growth factor-β (TGF-β) and platelet-derived growth factor receptor β (PDGF-Rβ) were reported to be increased in the liver tissues after arsenic exposure. Both proteins are important for the induction of HSC activation and proliferation and the development of fibrosis [82].

Evidence for progressive development of hepatic fibrosis after long-term exposure to high levels of arsenic comes from experimental works [72,77] as documented by increased accumulation of collagen in the liver associated with increased hepatic levels of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) mRNA, matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9). Heightened expression of MMPs probably contributes to the pathogenesis of arsenic-induced liver injury, as several studies also report that MMP-2 and MMP-9 were up-regulated in the fibrotic liver [83]. The normal extracellular matrix (ECM) is essential for maintaining the homeostasis of all resident liver cells. Therefore, it is likely that degradation of the ECM by MMPs alters cell matrix as well as cell–cell interactions and enhances hepatocyte susceptibility to necrosis and/or apoptosis caused by prolonged arsenic exposure. The ECM also serves as a binding reservoir for several key cytokines such as TNF-α

and growth factors such as TGF-β and PDGF [84,85]. MMPs release soluble bioactive factors through ECM degradation and regulate macrophage chemoattractants and leukocyte infiltration during injury [86]. Collectively, these data strongly suggest that arsenic-mediated liver injury is associated with HSC activation and hepatic fibrogenesis.

In vitro experiments using co-culture models of HSCs with hepatocytes provided evidence of the involvement of oxidative stress in the hepatocytes due to arsenic treatment and were responsible for activation of HSCs that may transdifferentiate into myofibroblasts [77]. This model provided several lines of evidence that prove the role of oxidative stress in the hepatocytes in arsenic toxicity. First, the authors identified excessive ROS generation in both hepatocytes as well as HSCs. ROS are known to play a key role in HSC activation [87]. Second, they detected H_2O_2 and thiobarbituric acid reactive substance (TBARS) accumulation, derived from hepatocytes, in the incubating medium of HSCs. Third, they found are higher levels of α-SMA, PDGF-Rβ, and Pro-(α) collagen I expression in HSCs. H_2O_2 derived from hepatocytes may induce collagen transcription in HSCs [87]. Further, pretreatment of hepatocytes with the antioxidant NAC abrogates arsenic-induced generation of ROS in the cells, H_2O_2 and TBARS accumulation in the medium, and expression of α-SMA along with decreased expression of PDGF-Rβ and type I collagen. This study clearly supports a role of oxidative stress in the hepatocytes due to arsenic exposure in the activation of HSCs.

The results reported by Ghatak et al. from India differ from another study from the USA that used a comparatively low to moderate exposure to arsenic (250 ppb in drinking water) for a shorter duration (5 weeks) [88]. Such exposure caused increased sinusoidal endothelial cell (SEC) capillarization, vascularization of the peribiliary vascular plexus, and constriction of hepatic arterioles in mice. However, this lower level of arsenic exposure, equivalent to a human dose of 32 μg/day for 3.75 years, did not cause arsenic deposition in the liver or result in portal fibrosis, an important feature in liver histological lesion in chronic arsenic-induced hepatotoxicity of humans [10,40–45]. The rationale for the relatively high dose of arsenic used in the study of Ghatak et al. [77] comes from epidemiological studies of arsenicosis in this region of the Indian subcontinent [89]. These studies suggest that clinical manifestations of arsenic-induced liver injury develop only after a long latency with the intake of large volumes of arsenic-contaminated water, usually since childhood [10,44,45,89]. The dose used by Ghatak et al. [77] in their study was simulated to be equivalent to a human exposure of 6 mg of arsenic/day for more than 20 years, a time over which liver toxicity might be expected to develop. However, the precise relationship between duration of arsenic exposure and the onset of liver lesions is uncertain and remains to be clarified.

Non-cirrhotic portal hypertension associated with portal fibrosis has also been a major consequence of chronic arsenic toxicity found in human clinic-pathological studies from India and the West [10,41–45,89]. The pathophysiological mechanisms that contribute to angiogenesis and vascular remodeling in response to arsenic exposure are not very clear. Liver SECs are unique endothelial cells in respect of their architecture and function. In response to oxidizing agents [90], SECs differentiate into regular endothelium in a process termed capillarization. The hallmarks of capillarization are SEC defenestration, development of laminin-rich basement membrane, and junctional expression of platelet endothelial cell adhesion molecules (PECAMs).

Capillarization precedes the development of many liver diseases, which include portal hypertension and liver fibrosis. Interestingly, low levels of arsenic exposure cause SEC injury that includes SEC defenestration, capillarization, and decreased liver clearance in experimental animal models [88]. Arsenic stimulates capillarization of SECs by disrupting the SEC signaling that maintains fenestrations and suppresses cell spreading, which may be the initial events in the pathogenic changes in the liver. Progressive SEC capillarization is mediated through activation of p47phox containing NADPH oxidase [91]. However, these animal models did not develop features of portal fibrosis, which is an important feature of arsenic-related hepatotoxicity in humans.

Activation of hepatic fibrogenesis is therefore a likely consequence of this arsenic-induced liver injury. Sustained arsenic-induced oxidative stress in the liver leads to increased apoptosis of hepatocytes and liberation of some mediators, which contribute to the activation of HSCs, the predominant fibrogenic cell type producing collagen type I in the liver [80,92]. HSCs also produce tissue inhibitor of matrix metalloproteinases (TIMP), which inhibits collagen-degrading MMPs and shifts the balance between ECM synthesis and degradation towards fibrogenesis [79,93]. A high fat diet also enhances chronic arsenic-induced hepatic fibrogenesis [94].

12.9 Arsenic and Liver Cancer

Arsenic and arsenic-containing compounds are human carcinogens. Two types of liver cancer have been associated with chronic arsenic exposure: hepatocellular carcinoma (HCC) and hepatic angiosarcoma (HAS). HCC has been identified as a tumor type associated with arsenic exposure in humans [46,95–97]. HCC, non-alcoholic liver cirrhosis, and ascites are leading causes of mortality in the arsenic endemic area of Guizhou, China [97,98]. HAS is a malignant neoplasm of the liver constituting only 2% of all primary tumors in Western countries. A causal association between ingested inorganic arsenic and HAS is supported by a series of case reports of arsenic-poisoned winegrowers in Germany [99] and several studies in southwestern Taiwan where drinking-water has a naturally arsenic content [100]. HAS is often associated with exposure to vinyl chloride. Roth [101] reported three cases of HAS in a series of 27 autopsies among arsenic-poisoned German vintners. A study in Chile found that among a group of 16 male cancer patients exposed to high arsenic levels through the water supply (200–2000 μg/L), 15 had skin carcinomas and only one had an HAS in addition to chronic arsenical dermatosis [102]. Falk et al. [103] identified 168 cases of HAS in the USA during the period 1964–1974; seven of these cases had used Fowler's solution for 6 to 17 years. Case reports of liver angiocarcinoma associated with medicinal ingestion of arsenic are also available [104–107]. Several epidemiological studies based on data from an area of southwestern Taiwan, having high levels of inorganic arsenic in the artesian well water supply, have found elevated rates of liver cancer death [107,108]. Childhood cancer was studied by Liaw et al. [109] in northern Chile, where the arsenic content in drinking water was as high as 860 μg/L. No increases were detected for all cancers combined. However, childhood liver cancer mortality under age 20, which is normally extremely rare, was markedly increased for those who were young children when they would have experienced high arsenic concentrations in water.

Liver is a target organ for arsenic-induced carcinogenesis. Liu and Waalkes [98] reviewed the mechanisms of arsenic-related liver cancer and the authors described the potential mechanisms of arsenic-induced hepatic carcinogenesis. The potential mechanisms include oxidative DNA damage, impaired DNA damage repair, acquired apoptotic tolerance, hyperproliferation, altered DNA methylation, and aberrant estrogen signaling. Some of these mechanisms may be liver specific.

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13

Arsenic and Respiratory Disease

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CHAPTER OUTLINE

13.1 Introduction

The lung is frequently the target of airborne and systemic environmental exposures. An adult at rest breathes in more than 7000 liters of air daily into a series of branching airways, which become shorter, narrower, and more numerous with every division. Gas exchange occurs across the extremely thin blood–gas interface of the alveoli and pulmonary capillary network [1]. This makes the lung uniquely exposed to airborne toxins. In addition, the lung receives the entire cardiac output from the right side of the heart, placing it at risk of systemic toxins. As a result, the lung is highly susceptible to the development of respiratory disease caused by exposure to environmental toxins.

Arsenic is the only environmental agent that has been linked to both malignant and non-malignant respiratory disease following ingestion, rather than inhalation, making arsenic a unique toxicant to the respiratory system. Chronic arsenic exposure via drinking water has been shown to increase the risk of developing lung cancer along with other malignant diseases [2–5]. The increased risk of developing lung cancer is similar whether the arsenic has been ingested or inhaled [6]. Chronic arsenic exposure has been associated with a variety of respiratory symptoms such as chronic cough and shortness of breath, as well as the development of non-malignant respiratory diseases such as bronchiectasis and chronic obstructive pulmonary disease (COPD). The lung is highly sensitive to environmental toxins during stages of development both *in utero* and in early postnatal life, and the risks of developing non-malignant respiratory disease appear highest when the exposure occurs in early life [7,8].

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13.1.1 Lung Development

Lung development begins within 3 weeks of fertilization and continues after birth. The lung is highly susceptible to environmental insults during both the *in utero* and postnatal stages of development. Within the first weeks of gestation the lung bud (ventral bud of primitive foregut) forms into trachea and primary and secondary bronchi [1]. This is followed by differentiation of the airway epithelial lining and formation of cartilage, bronchial smooth muscle, and pulmonary blood vessels by week 17. Respiratory bronchioles, alveolar ducts, and primitive alveoli develop by week 27. Further development and differentiation of the alveolar cell types occurs between weeks 28 and 36. The final alveolar stage of development occurs from week 36 of gestation to early childhood and involves the septation and multiplication of alveoli and growth of the airways [1]. The switch at birth from the fluid-filled intrauterine environment to breathing air is dramatic and requires absorption of the fetal lung lining within the first few breaths. The lungs increase in volume from approximately 80 mL at birth to 6000 mL in adulthood, and therefore the lung remains susceptible to external insults throughout postnatal life [9].

13.2 Chronic Arsenic Exposure and Respiratory Health

13.2.1 Chronic Arsenic Exposure and Respiratory Symptoms

A high prevalence of respiratory symptoms has been reported among adults chronically exposed to arsenic [10]. The prevalence of cough, shortness of breath, and chest sounds correlated positively with arsenic concentrations in drinking water, and were most pronounced in individuals with skin lesions in West Bengal, India [11]. In the same population, arsenic-exposed men with skin lesions had a higher prevalence of respiratory symptoms compared with arsenic-exposed men without skin lesions, in particular morning cough (odds ratio (OR) = 2.8, 95% confidence interval (CI) 1.2, 6.6) and shortness of breath (OR = 3.8, 95% CI 0.7, 20.6) [12]. In arsenic-exposed men in Pakistan, a positive correlation between arsenic in drinking water and arsenic in scalp hair was found, but was stronger in those with respiratory disease symptoms (coughing, chest sounds, and shortness of breath) ($r = 0.785$, $p < 0.001$) than in those without respiratory disease symptoms ($r = 0.511$, $p < 0.001$) and the severity of respiratory symptoms was increased in those men with skin lesions [13]. Data from the Health Effects of Arsenic Exposure Longitudinal Study (HEALS), a large prospective longitudinal cohort study in Bangladesh, found a positive dose–response relationship between arsenic exposure and respiratory symptoms (chronic cough, breathing problems, or blood in sputum) [14] and the odds of having dyspnea in the previous 6 months was 1.32 times greater (95% CI 1.15, 1.52) in adults exposed to arsenic at high concentrations (>50 μg/L) compared with those exposed to arsenic at low concentrations (<7 μg/L, $p < 0.01$) [15].

13.2.2 Chronic Arsenic Exposure and Lung Function

Chronic arsenic exposure throughout life has also been linked to impaired lung function. There have been a small number of studies that have examined lung function in arsenic-exposed

populations of South Asia. These studies have reported low forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) among individuals with skins lesions or exposed to high levels of arsenic (>250 μg/L); however, the type of lung function impairment varies among studies, with some studies recording predominantly obstructive lung disease [16], and others predominantly restrictive or mixed obstructive/restrictive lung disease [12,17]. The degree of lung function impairment is clinically significant, with 256.2 mL lower FEV_1 (95% CI 113.9, 398.4) and 287.8 mL lower FVC (95% CI 134.9, 440.8) in arsenic-exposed men with skin lesions in West Bengal [12]. The HEALS study published the largest lung function study from their population-based cohort in 2013. They observed significant associations between both water and urinary arsenic levels and decreases in FEV_1 and FVC [18]. These data indicate that arsenic exposure can lead to both restrictive (lower FVC) and obstructive (lower FEV_1) patterns of lung function impairment and the balance may shift depending on a multitude of confounding factors, such as arsenic dose, speciation, timing, susceptibility, and additional insults. Arsenic exposure may alter lung function by impairing lung development or promoting lung remodeling. As lifetime exposure to arsenic is difficult to accurately quantify, it is unclear whether the reduction in lung function is due to recent or past exposure to arsenic.

13.2.3 Chronic Arsenic Exposure and Immunosuppression

Arsenic has been found to be immunosuppressive in humans [19–21]. A microarray study in the USA showed that arsenic exposure altered the expression of genes involved in defense responses, immune function, cell growth, apoptosis, cell cycle regulation, and T-cell receptor signaling in lymphocytes [22]. Arsenic exposure has also been shown to modify the expression of genes, cytokines, and growth factors involved in inflammation in lymphocytes [21], alter T-cell proliferation [19], and impair T-cell function [20]. In children in Mexico exposed to arsenic in drinking water, urinary arsenic levels were associated with reduced lymphocyte proliferation and interleukin-2 secretion [23]. Arsenic exposure in adults in West Bengal was associated with loss of cell adhesion capacity and impaired phagocytic capacity of macrophages [24]. A study examining humoral immune responses following chronic arsenic exposure found that patients with arsenicosis had elevated serum levels of immunoglobulins (IgG, IgE, and IgA) and subjects with arsenic-associated respiratory complications (chest sounds, asthma, bronchitis, and cough) had higher mean IgE levels than exposed subjects without respiratory complications [25]. An inverse association was also found between urinary arsenic concentration and serum Clara cell protein 16 in arsenic-exposed subjects with skin lesions, indicating potential damage to respiratory Clara cells [26]. Therefore, arsenic-induced immunosuppression in adults and children may contribute to increased susceptibility to respiratory infections and the development of chronic respiratory disease.

13.2.4 Chronic Arsenic Exposure and Non-Malignant Respiratory Disease

There is strong evidence that chronic exposure to arsenic increases the susceptibility to developing respiratory disease. The prevalence of chronic bronchitis and chronic cough was found to

be significantly higher in arsenic-exposed residents of Bangladesh compared with unexposed residents and increased with increasing concentrations of arsenic [27,28]. A study of 108 arsenic-exposed subjects in West Bengal found an increase in bronchiectasis in patients with arsenic-related skin lesions (adjusted OR = 10, 95% CI 2.7, 37) compared to exposed subjects without skin lesions [29]. In Taiwan, the risks of developing bronchitis were higher in men (standardized mortality ratio (SMR) = 1.48, 95% CI 1.25, 1.73) and women (SMR = 1.53, 95% CI 1.3, 1.80) when arsenic-associated blackfoot disease was prevalent following the consumption of well water containing 780 μg/L arsenic [30]. After the peak arsenic exposure event occurred in Antofagasta, Chile (1958), mortality rate ratios for tuberculosis in males started increasing reaching a rate ratio of 2.1 (95% CI 1.7, 2.6) 20 years after the peak exposure event [31]. While the SMR for COPD over all ages was not significantly increased in Antofagasta, there was a very high relative risk for COPD deaths for men and women aged between 30 and 39 years. The individuals who died of COPD in this age group would have been children during the peak exposure event and this thus highlights the significance of early life arsenic exposure on the development of respiratory disease [3].

13.3 Early Life Arsenic Exposure and Lung Health

13.3.1 Early Life Arsenic Exposure, Birth Outcomes, and Growth

Exposure to arsenic during pregnancy has been shown to increase the risks of spontaneous abortions (OR range 1.14–6.07, 95% CI range 1.04, 24.0) [32–36], still births (OR range 1.23–2.5, 95% CI range 0.87, 4.9) [33,37], neonatal death (OR range 1.8–2.81, 95% CI range 0.9, 10.8) [32,33,35,38], and post-neonatal death (relative risk (RR) range 1.17–1.26, 95% CI range 1.03, 1.32) [32,34,36]. Exposure to arsenic during pregnancy is also associated with decreased infant birth weight [39,40]. The mechanism for this is not understood but may be a result of arsenic-induced oxidative stress resulting in placental insufficiency or disruption of the endocrine control of glucose homeostasis and cellular growth [41,42]. Fetal growth restriction is associated with impaired lung function and greater respiratory morbidity in early childhood [43–46] and adulthood [47,48]. This is significant as children who are small for gestational age are more likely to be hospitalized for respiratory tract infections [49,50]. This effect on somatic growth is just one of the potential mechanisms that may explain the link between *in utero* arsenic exposure and the development of chronic lung disease. However, as discussed below, there is also evidence that arsenic can have a direct effect on the lung over and above an effect that can be explained by impaired somatic growth.

13.3.2 Early Life Arsenic Exposure and Immune Development

Arsenic exposure during pregnancy has been linked with altered immune development and increased susceptibility to respiratory infections in infancy [36,51]. Arsenic exposure during pregnancy can increase oxidative stress and inflammation in the placenta, reduce placental T cells and alter the expression of cord blood cytokines (IL-1β, IL-8, IFN-γ, TNF-α) [51]. Infants exposed to arsenic *in utero* had evidence of impaired thymic development and higher levels of

fever, diarrhea, and acute respiratory infections [52]. In a community-based prospective study of 1552 live-born infants in Bangladesh, exposure to arsenic at concentrations above 250 μg/L *in utero* increased the risk of developing lower respiratory tract infections by 69% (RR = 1.69, 95% CI 1.36, 2.09), severe lower respiratory tract infections by 54% (RR = 1.54, 95% CI 1.21, 1.97), and diarrhea by 20% (RR = 1.20, 95% CI 1.01, 1.43) compared to those exposed to arsenic at concentrations below 39 μg/L [7]. The New Hampshire Birth Cohort Study in the USA also reported increased upper and lower respiratory tract infections, as well as increased respiratory symptoms in 4-month-old infants exposed to relatively low level (0.45–58.3 μg/L) arsenic *in utero* [53]. Recurrent lower respiratory tract infections in early childhood and impaired pulmonary immune defenses are important risk factors for respiratory morbidity and mortality in childhood, and the development of chronic respiratory diseases in adulthood [46,54].

13.3.3 Early Life Arsenic Exposure and Non-Malignant Respiratory Disease in Children

The unique arsenic exposure event in Antofagasta, Chile, revealed the significance of early life arsenic exposure in the development of respiratory morbidity and mortality. In the 1950s the establishment of a new drinking water source resulted in the residents of Antofagasta consuming water containing high concentrations of arsenic (90–860 μg/L) for more than 20 years [55]. Two years after the peak arsenic exposure in Antofagasta, children presented with arsenicosis associated with respiratory disease, including diffuse and segmentary bronchiectasis [56]. Autopsies performed on five children with evidence of arsenic poisoning who died between 1968 and 1969 in Antofagasta revealed lung abnormalities in four of the five children including evidence of pulmonary interstitial fibrosis and mild bronchiectasis [57]. In a study of school-aged children in Antofagasta, 38.8% of children with abnormal skin pigmentation had chronic cough and 15.9% had bronchopulmonary disease ($n = 144$), compared with 3.1% and 6.2% of children with normal skin ($n = 36$), respectively [58]. The presence of cough and dyspnea correlated with the mean drinking water arsenic concentrations in 398 children [59] and after the installation of a water treatment plant, the prevalence of cough and dyspnea dropped from 38 to 7% [58]. Following a series of postmortem examinations, Zaldivar [60] described the pathology of those who died from chronic arsenic poisoning in Antofagasta, which included a combination of cardiovascular, pulmonary, digestive, and neurological abnormalities. Among the children who were examined, the prevalence of bronchiectasis and bronchopneumonia was 23 and 3.4 times higher in those with chronic arsenic dermatosis than the estimated prevalence in the general population of Chilean children [60]. It was postulated that the combination of malnutrition and dietary arsenic exposure was resulting in the high prevalence of bronchiectasis and bronchopneumonia in the children of Antofagasta, and arsenic was acting as an immunosuppressant in infants and children. In addition, children in Bangladesh exposed to high (>500 μg/L) arsenic *in utero* and early life had a marked increase in chronic respiratory symptoms such as wheezing and shortness of breath when walking or climbing [61]. These studies clearly demonstrated the importance of ingested inorganic arsenic in determining respiratory health early in life.

13.3.4 Early Life Arsenic Exposure and Non-Malignant Respiratory Disease in Adults

It was not until a study by Smith and colleagues that the long-term impacts of early life arsenic exposure, and particularly *in utero* exposure, were realized [8]. They found that the SMRs for lung cancer and bronchiectasis in those born just prior to the peak exposure event in Antofagasta (1950–1957) and exposed to arsenic in early childhood were 7.0 (95% CI 5.4, 8.9) and 12.4 (95% CI 3.3, 31.7), respectively. Those born during the peak exposure period (1958–1970), who were exposed to arsenic *in utero* and early childhood, had corresponding SMRs of 6.1 (95% CI 3.5, 9.9) for lung cancer and 46.2 (95% CI 21.1, 87.7) for bronchiectasis [8] (Figure 13–1). This groundbreaking study was the first to show that *in utero* and early life exposure to arsenic had pronounced effects on the lungs and the long-term risk of developing non-malignant lung disease, which is consistent with the literature on cigarette smoke [62]. In a subsequent study Dauphiné and colleagues [63] showed that a small group of residents of Antofagasta, exposed to >800 µg/L arsenic before the age of 10 years ($n = 32$), had long-term deficits in lung function as adults, with 11.5% lower FEV_1 ($p = 0.004$), 12.2% lower FVC ($p = 0.04$), and increased breathlessness (OR = 5.94, 95% CI 1.36, 26.0) compared to unexposed adults. Surprisingly, the magnitude of lung function deficits as a result of exposure to arsenic in early life was equivalent to the effects of smoking cigarettes throughout adulthood. These studies provide clear associations between early life exposure to arsenic and long-term impairment in respiratory health, but do not provide crucial mechanistic data to explain these associations.

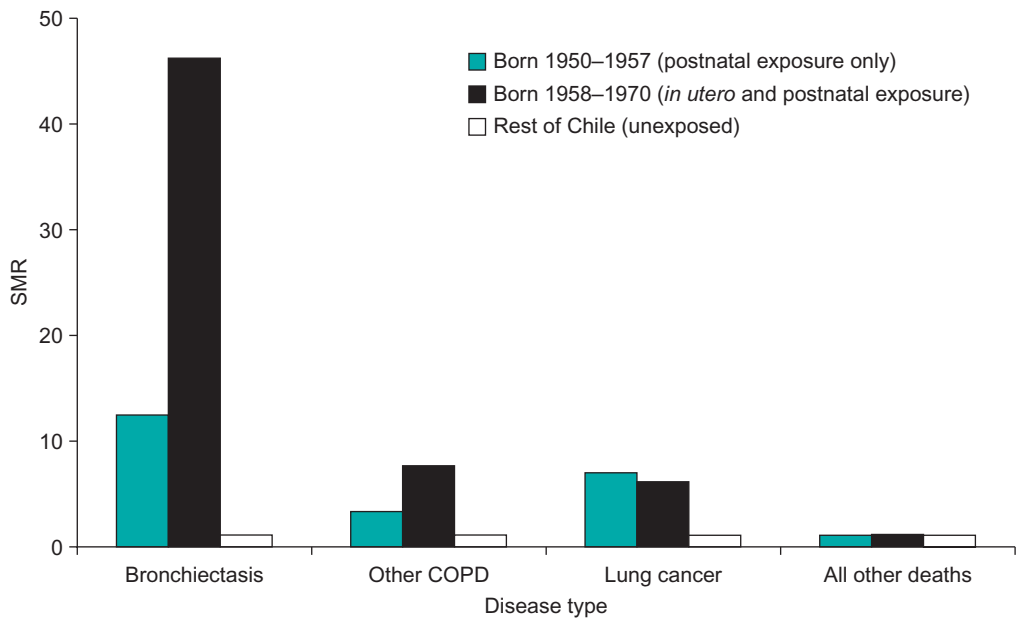

FIGURE 13–1 Increased mortality from bronchiectasis, COPD, and lung cancer following exposure to arsenic in early life. *Reproduced with permission from Smith et al.* [8].

13.4 Mechanistic Data on Arsenic Exposure and the Lung

Despite many epidemiological studies reporting links between chronic arsenic exposure and non-malignant lung disease, these investigations are constrained by a range of limitations including low sample sizes, unreliable measures of arsenic exposure (when mean/median levels in a community are used), heterogeneity of drinking water sources, migration, uncertainty of response latency, varied dose–response relationships, and population/genetic differences [64]. Mechanistic studies are therefore essential to show causality and unravel the mechanisms by which the ingestion of arsenic leads to respiratory disease [65].

13.4.1 Mechanistic Data on Early Life Arsenic Exposure

Experimental animal models have been critical to our understanding of the impact of arsenic on the lung. Some of the earliest animal studies using arsenic exposure were able to demonstrate that arsenic can pass through the placenta, the fetus thereby being exposed to the toxicant [66–68]. Additional studies have shown that methylated arsenic products (DMA and MMA) are present in fetal blood, lung, and liver tissue following *in utero* arsenic exposure [69]. As described earlier, arsenic exposure *in utero* is associated with impaired somatic growth in humans [40], which may have downstream effects on respiratory morbidity and mortality. Importantly, a recent mouse study was able to replicate this finding whereby mice exposed to arsenic *in utero* had significant deficits in growth in early life compared with unexposed offspring [70]. These observations highlight the validity of mouse models for exploring the mechanisms of arsenic-induced lung disease.

13.4.2 Mechanistic Data for Arsenic Exposure and Immune Function

Various studies have investigated the effect that arsenic has on the immune system. Mouse models of *in vivo* arsenic exposure have shown that arsenic alters the expression of key innate immunity genes in the lungs [71,72]. Exposing mice to arsenic suppresses antibody formation, inhibiting T-cell proliferation and macrophage activity, and altering cytokine expression [73–78]. Inhalation of arsenic trioxide in mice increased mortality from a pulmonary bacterial infection and decreased bactericidal activity [79]. Zebrafish exposed to arsenic at concentrations of 2 and 10 μg/L had drastically reduced ability to clear viral and bacterial infections [80]. Exposure to drinking water containing 100 μg/L arsenic in adult mice compromised the lung's immune response to influenza (H1N1) infection, resulting in greater viral load and higher mortality [81]. *In utero* and postnatal exposure to 100 μg/L arsenic in mice increased viral titer and exacerbated the inflammatory response to early life influenza A infection [82].

In human cell cultures, exposing macrophages to arsenic results in loss of adhesion, altered morphology, and impaired function [83]. Recent studies have found that arsenic increases ubiquitination and degradation of cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels in the gills of killifish [84,85] and human airway epithelial cells [86].

The CFTR protein, a chloride channel in the apical membrane of airway epithelial cells, regulates the volume of airway surface liquid (ASL) by secreting chloride into the periciliary space, which drives the secretion of sodium and fluid into the ASL. When the CFTR protein is degraded or defective, as in cystic fibrosis lung disease, excess sodium is transported back into the cell and the ASL becomes dehydrated. Dehydrated ASL impairs the mucociliary clearance of pathogens and increases susceptibility to chronic respiratory infections [87]. Therefore along with impairing the morphology and function of immune cells, arsenic exposure can alter the distribution of cell types in the airway epithelium resulting in impaired mucociliary clearance, a significant component of innate immunity.

13.4.3 Mechanistic Data for Arsenic Exposure and Lung Structure and Function

Exposure to arsenic *in utero* has been shown to have profound effects on the lungs of experimental animals. Arsenic was shown to be a complete transplacental carcinogen in a series of studies involving exposure of mice to arsenic in their drinking water at concentrations of 0, 42.5, and 85 mg/L between days 8 and 18 of gestation [85,86]. There were dose-dependent increases in hepatocellular carcinomas, liver, and adrenal tumors in male offspring, and ovarian tumors and lung carcinomas in female offspring. This was the first study to show that ingestion of arsenic in drinking water during gestation could induce tumors in rodents [88,89]. *In utero* exposure to arsenic has also been shown to reduce lung size and alter gene expression in the lungs at birth—particularly genes involved in lung development and cell trafficking [90].

In utero and early postnatal arsenic exposure has been shown to irreversibly alter lung structure and lung function in mice [91]. Mice exposed to arsenic ($\leq$100 μg/L) *in utero* and early postnatal life via drinking water had hyper-responsive airways, increased airway smooth muscle, and altered airway matrix proteins compared to controls. These changes were not reversible and were only observed in mice exposed to arsenic in adulthood [91]. A study of *in utero* exposure to arsenic on three strains of mice found that the response to arsenic in the lungs of mice is genetically determined and the strain most susceptible to oxidative stress, the C57BL/6 strain, was also the most susceptible to arsenic exposure [92]. *In utero* exposure to arsenic impaired somatic growth, lung volume, and parenchymal lung mechanics in C57BL/6 mice, and altered the expression of genes that function in mucociliary clearance, innate immunity, and lung growth in the offspring [92,93]. Arsenic induced mucous cell metaplasia and increased expression of a calcium-activated chloride channel (CLCA3) in the offspring, known to regulate mucous production and secretion [92].

Arsenic exposure and infection with influenza in early life resulted in long-term deficits in lung mechanics, airway hyper-responsiveness, and increased airway smooth muscle and numbers of mucus producing cells [82]. These data demonstrate how exposure to arsenic in early life can alter the response to influenza infection resulting in both acute and long-term effects on respiratory health. Altered lung growth, lung structure, and increased mucous secretion may together impair the ability of the airways to clear pathogens, increasing the risk of chronic localized infections and chronic lung diseases, such as bronchiectasis.

13.5 Conclusions

Further investigation into the effects of arsenic exposure on lung structure and function will be critical to understanding arsenic-induced lung disease. The epidemiological data discussed suggests that exposure to arsenic, particularly *in utero* and early life, can result in long-term impairments to lung function and increased risk of developing non-malignant lung disease such as bronchiectasis. It is clear that arsenic has the potential to modulate lung growth and development and alter immune function. Impaired development of the lung may also alter the susceptibility and response to additional respiratory insults and pathogens. The development of chronic respiratory symptoms and progressive respiratory diseases in arsenic-exposed populations can have debilitating effects on the quality of life for individuals and the overall productivity and wellness of communities.

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14 Arsenical Kidney Toxicity

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CHAPTER OUTLINE

14.1 Introduction

Arsenic, which is atomic number 33 in the Periodic Table of Elements, may exist in the −3, +3, and +5 oxidation states, all of which have been shown to produce cellular toxicity to varying degrees. Humans may be exposed to arsenicals from air, food, and water under a variety of environmental, occupational, and medical therapy conditions [1,2]. Arsenic is metabolized into several methylated species *in vivo*, which also vary in their toxic potential. There is genetic variation in the enzymes responsible for the methylation of arsenicals *in vivo* [2]. Urinary excretion is the major route for elimination of arsenicals from the body and the kidney concentrates these during this process. Hence, this physiological concentration process likely plays a major role in making the kidney a major target organ for arsenical toxicity. The present chapter will focus on the renal handling of the various arsenical species, target kidney cell populations, and the known mechanisms of arsenical cellular toxicity. A general summary of reported clinical manifestations of toxicity produced by exposure to arsenicals is presented in Table 14–1.

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Table 14–1 Reported Clinical Manifestations of Arsenical Nephrotoxicity from Acute or Chronic Arsenical Exposures

Acute Renal Failure	Arsenical	Reference #	Measured Endpoint(s)
	Arsine gas	[1–4]	Increased BUN, hemoglobinuria
Chronic renal damage			
	Arsenate, arsenite	[1,5,6]	Proteinuria, glucosuria
Renal cancer			
	Arsenate, Arsenite	[7]	Cancer cells

14.2 Clinical Manifestations of Arsenical Toxicity in Humans

14.2.1 Epidemiological Studies

In recent years, there have been a number of studies of human populations exposed to arsenic in drinking water in India [8], Taiwan [9], Bangladesh [10], Chile [11–13], and Argentina [14] as a result of geological contamination of water passing through arsenic-containing rock formations. The clinical manifestations of such arsenic exposures on the kidney are consistent across studies and may involve effects on the blood vasculature [5,6,10,15] leading to axotemia and/or tubular toxicity, and in some studies renal carcinogenesis. Arsenic-induced diabetes [16,17] will further exacerbate the effects of this element on the kidney. The overall conclusion to be derived is that arsenic is a broad-spectrum nephrotoxin which is also capable of playing a role in the development of renal cancer in some individuals [7]. The chapter sections that follow will attempt to provide more specific information on the mechanisms underlying these clinical outcome measures and suggest ways in which this mechanistic information may be used to develop biomarkers for early detection of arsenical-induced nephrotoxicity or carcinogenicity prior to the onset of clinical disease.

14.2.2 Mechanisms of Arsenical Toxicity to Specific Renal Cell Populations

14.2.2.1 Renal Blood Vasculature

As noted above, the kidney is a highly vascular organ, which has a number of arteries, arterioloes, capillaries, and veins lined with endothelial cells. Recent studies [18] have demonstrated that endothelial cells and renal podocytes [19] are highly sensitive to arsenicals ostensibly as a result of arsenical inhibition of cellular respiration with attendant increases in formation of reactive oxygen species (ROS). ROS are highly toxic [20,21] and the endothelial cells are most likely highly sensitive as a result of a limited capacity to produce antioxidants such as glutathione. Endothelial cell death from arsenical exposures will lead to inflammation followed

by scarring via a complex set of processes [22]. It should be noted that arsenical disruptions of cellular signaling pathways [23–28] have been demonstrated to lead to a number of effects related to altered regulation of normal cell death and replacement processes [29].

14.2.2.2 Proximal Tubules

The renal proximal tubules are a major metabolic component of the kidney, which is responsible for reabsorption of proteins, glucose, and electrolytes from the urinary filtrate [30]. Renal proximal convoluted tubule cells (PCTCs) have high energy requirements in the form of ATP in order to meet their metabolic functions for the kidney and for this reason contain many mitochondria [31] that are highly sensitive to arsenical inhibition of respiration [32]. The high metabolic activity of the renal PCTCs coupled with their high mitochondrial content combine to make these cells and this portion of the renal nephrons major targets for arsenical toxicity.

14.3 Nephrotoxic Arsenical Compounds

As noted above, arsenicals may exist in a number of oxidation states and as both inorganic and organic chemical species, which may undergo a number of metabolic interconversions *in vivo*. All of these chemical species of arsenic may exert nephrotoxic effects if given at sufficient dose levels or levels of exposure. A more detailed summary discussion of arsenical metabolism and the putative importance of methylated arsenical species in arsenic-induced toxicity and cancer has recently been presented elsewhere [2]. The following sections will focus on the nephrotoxicity of the major forms of inorganic arsenicals since these are of greatest public health interest.

14.3.1 Arsine

Arsine (AsH_3) exists as a gas at room temperature and has been known for many years as a potent hemolytic agent [1–4], which produces renal failure following acute high dose exposure. The mechanisms underlying these effects involve occlusion of the renal tubules by hemoglobin with resulting loss of urinary output. In addition, the direct action of arsine on respiration of renal tissue slices has also been reported [33] suggesting a more complex mechanism of nephrotoxicity.

14.3.2 Arsenate

Arsenate (As^{5+}) is a common form of arsenic found in water supplies, and is generally less acutely toxic than arsenite (As^{3+}), which is the other common oxidation state discussed below. It is worth noting that the relative concentrations of these two oxidation states may vary depending on local redox conditions and hence it is not uncommon for both forms to be reported as present in water systems [34]. Both forms may also undergo biological methylation reactions *in vivo* as a result of tissue methylation processes or of microbial actions by the microbiome in the gastrointestinal tract following ingestion [35].

14.3.3 Arsenite

Arsenite (As^{3+}) is another commonly encountered form of inorganic arsenic, and is more acutely toxic than arsenate [1,2] ostensibly as a result of complexation with the dihydrolipoic acid cofactor required for mitochondrial metabolism of NAD-linked substrate-supported mitochondrial respiration. Studies in recent years have demonstrated that the mechanisms underlying arsenical inhibition of mitochondrial respiration are complicated by *in vivo* interconversions between oxidation states and methylation reactions, as discussed below.

14.4 Mechanisms of Arsenical Toxicity

Arsenical exposures produce a broad spectrum of clinical renal effects that may result from either direct toxic and carcinogenic effects on specific cell types of this multifunctional organ system or related secondary effects such as diabetes that also have negative consequences for the kidney. The following sections will examine some of the known molecular mechanisms of arsenical nephrotoxicity to specific biological pathways/organelle systems and possible roles these perturbations may play in development of renal cancer and interactions with diabetes.

14.4.1 Mitochondrial Effects

14.4.1.1 Mitochondrial Respiratory Function

The inhibitory effects of arsenicals on cellular respiration have been known for many decades [1,2]. The mitochondrion plays a major role in cellular respiration, and arsenical exposures have been shown to produce concomitant alterations in mitochondrial respiration and high-amplitude swelling of renal mitochondria *in situ* [31]. Other studies [36,37] have shown that this swelling is related to inhibition of energy-linked structural transformations in this organelle, which are essential to mitochondrial respiration and may be disrupted by arsenical exposure [38]. These data suggest that arsenical inhibition of mitochondrial respiratory function is a more complex process than originally thought, as noted above, with regard to arsenicals binding to the vicinal thiols of the dihydrolipoic acid cofactor of the pyruvate dehydrogenase complex. In addition, it should be noted that inhibition of mitochondrial respiration will also produce ROS, which play a central role in a number of toxic processes [39] such as altered cell signaling pathways, apoptosis, and proteotoxicity from oxidation of labile protein functional (e.g., SH) groups. The bottom line is that these interrelated effects may act in concert to exacerbate arsenical nephrotoxicity at the level of cellular respiration.

14.4.1.2 Heme Biosynthetic Pathway

Arsenical exposure has also been demonstrated to produce a characteristic porphyrinuria with greater concentrations of uroporphyrins relative to coproporphyrins in both animals [40–42] and humans [43] exposed to inorganic arsenic in drinking water. This uroporphyrin-dominated porphyrinuria pattern has also been observed in experimental animals exposed to arsine gas via inhalation [44]. The value of this information rests with the potential of this

porphyrinuria pattern as a putative biomarker of arsenical toxicity since porphyrins are also toxic molecules capable of catalyzing formation of ROS, thus exacerbating arsenic-induced oxidative stress in target organs such as the kidney. The elimination of these toxic compounds via the urine indicates that exposure of kidney cells must occur and suggests that arsenical-induced porphyrinuria may be an exacerbating factor in mediating arsenical nephrotoxicity.

14.4.1.3 Arsenic and Renal Cancer

As noted above, some epidemiological studies of human populations exposed to arsenic in drinking water have reported mild increases in renal cancer [7]. Such findings are reasonable given the increased formation of ROS discussed above with the attendant genetic and epigenetic cellular responses known to result from increased ROS formation. The relatively modest magnitude of renal cancer outcomes is most likely related to the robust nature of renal tubule cells with regard to antioxidant defense systems such as reduced glutathione [45], and induction of glutathione synthetase [46], superoxide dismutase [47], and stress proteins [48–50] such as heme oxygenase (HSP32). The combined impact of these antioxidant defense systems would be to attenuate the carcinogenic response in the kidney unless other factors such as genetic predisposition factors and nutritional deficiencies [39,45] are also operating. The bottom line is that the multiple factors are most likely operating to delineate subpopulations at special risk for both renal toxicity and carcinogenic outcomes.

14.4.1.4 Membrane Transport Systems

In addition to porphyrinurias, arsenical exposures in humans has been shown to produce alterations in the renal handling of a number of substances including glucose [51], phosphate [52], amino acids, and low molecular weight proteins [53], which are normally reabsorbed by membrane transport proteins and/or energy-dependent pinocytosis. Arsenate, which is chemically similar to phosphate, has been shown to be actively taken up by renal tubules via a phosphate transport system [54]. The overall results of these studies indicate that arsenic may accumulate in the kidney via an active transport system and also interfere with the membrane transport of a number of other metabolically important substances by competitive inhibition. Arsenical-induced decrements in mitochondrial energy production could compromise ATP-requiring transporters of a number of metabolically important substances. In either case, the cellular membrane of renal tubule cells should also be regarded as a target organelle system for arsenical toxicity.

14.4.1.5 Altered Cellular Signaling Pathways

As noted above, arsenical-induced ROS will have an impact on normal regulation of a number of cellular processes in the kidney and other organs via alteration of cellular signaling pathways that regulate a variety of renal processes including transport of metabolic products, cell death, and replacement processes including initiation of renal cancer. Table 14–2 is intended to summarize some of the reported effects of arsenicals on a number of these cellular signaling pathways in renal cells following either *in vivo* or *in vitro* exposures.

Table 14–2 Effects of Arsenical Exposures on Cellular Signaling Pathways in the Kidney

Pathway	Exposure Route	Impact	Reference #
Apoptosis	*In vitro*	+	[19,23,25,28]
Cell replication	*In vitro*	–	[24]
Glucose transport	*In vitro*	+ or –	[55]

14.5 Biomarkers of Nephrotoxicity

The field of biomarkers has rapidly expanded over the past 30 years and biomarkers related to chemical-induced renal toxicity are particularly prominent due to the release of putative biomarker substances into the urine and the reserve capacity of the kidney, which limits the ability to detect early ongoing signs of toxicity until the kidney is approaching overt renal failure and end stage renal disease (ESRD). Epidemiological studies of human populations exposed to arsenic in drinking water have demonstrated elevated rates of renal damage [56,57] and cancer [58], so development of renal biomarkers for early detection of arsenical-induced renal damage is of particular importance. The following section is intended to review some of the more current renal biomarkers for arsenic-induced toxicity and suggests needed areas of future research.

14.5.1 Omic Biomarkers

In recent years, the need for sensitive and specific biomarkers for delineating early cellular manifestations of arsenic toxicity has encompassed the field of "omics," which includes genomics, proteomics, and metabolomics/metabonomics, which are measurable cellular responses to toxic cell injury. In the case of arsenic, these responses are on an interconnected biological continuum, and the oxidative stresses produced by arsenic exposure described above are a common generic trigger for a number of specific "omic" responses. As also noted above, the kidney is a primary target for arsenical-induced toxicity since arsenic is concentrated by this organ and urinary excretion of arsenical metabolites is the major route of elimination for this element from the body. This section will review some of the published works on arsenic-induced alterations of omic biomarkers with particular emphasis on the kidney.

14.5.2 Altered Gene Expression Patterns (Genomics)

There are a number of arsenic-induced alterations in renal gene expression patterns that have been reported *in vivo* in intact animals [41] and *in vitro* cell systems [48–50]. Generally, these genomic responses usually demonstrate both up- and down-regulation of a number of genes. The extent to which this occurs is mediated by a number of factors including dose, duration of exposure, gender, and test system being evaluated [59]. Not all up-regulated genes are actually expressed. The mitigating effects of epigenetic responses and interconnecting signaling pathways will also influence measurable outcomes.

14.5.3 Altered Protein Synthesis Patterns (Proteomics)

Proteomic responses to arsenic exposure have the advantage that one is measuring an actual expressed gene product produced in response to arsenical exposure. The question of the duration of expressed proteomic responses *in vivo* is an important issue with regard to interpretation of biological significance since the degree and nature of the response will vary with time. Given the complexity of proteomic responses to arsenical compound exposures, computerized analysis of digitized 2D gel images [59] is essential to interpretation of generated data. For example, the intensity of proteomic responses of hamster renal tubule cells following acute *in vivo* exposures to gallium arsenide or indium arsenide particles at 10 days or 30 days post-exposure (Figure 14–1) have been shown to be both time and gender dependent [59].

14.5.4 Metabolomics

As noted above, arsenical exposure produces alterations to a number of metabolic pathways including those in the kidney [31]. These include those involved in intermediary metabolism [60] and heme biosynthesis [40–43].

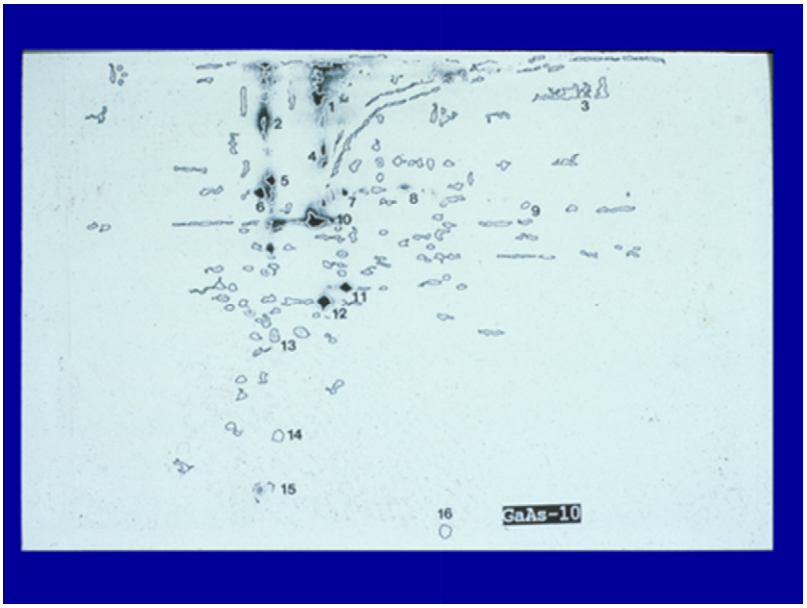

FIGURE 14–1 A standard digitized image of a 2D gel-^{35}S methioine autoradiogram of kidney tubule cells from a male hamster treated with GaAs particles on day 10 post-exposure. The image shows computer assigned numbers for each identified protein spot on the gel. The computer then ratios the density of the spots to those from control hamster kidney cells loaded with an equal amount of ^{35}S radioactivity. See [58] for details and further discussion.

14.5.5 Arsenic-Induced Posttranslational Alterations of Proteins

Arsenic-induced alterations in posttranslational modifications of histones have been reported in both humans [61] and experimental systems [62,63] exposed to inorganic arsenic. These findings have potentially important risk assessment implications for both renal toxicity and carcinogenesis in human populations exposed to arsenicals.

14.5.6 Altered Heme Biosynthesis/Porphyrinuria Patterns

Arsenic exposure also results in inhibition of enzymes of the heme biosynthetic pathway resulting in a characteristic porphyrinuria dominated by uroporphyrins with lesser amounts of coproporphyin [40–43] and induction of heme oxygenase (HSP32), which is the rate limiting enzyme in the heme degradative pathway [59]. These metabolic disturbances in this essential biological pathway have been used successfully as biomarker endpoints for studies in both experimental animals and humans exposed to arsenic in drinking water [40,43].

14.5.7 Proteinuria Patterns

Increased excretion of proteins into the urine has also been reported in humans [56,57] following chronic exposure to arsenic in drinking water. Depending on the dose level, the proteins may be either low molecular weight at lower dose levels or both low and higher molecular weight at higher dose levels [2,56,57].

14.6 Arsenical Interactions with Other Nephrotoxic Elements

It is also common for arsenic exposures to occur in combination with other nephrotoxic metals with resultant additive or synergistic increases in toxicity. The most common co-exposures occur with lead and cadmium under environmental conditions, and gallium and indium in the semiconductor industry. Arsine gas is used in the fabrication of gallium arsenide and indium arsenide chips as the source of arsenic. The following short review is intended as an overview of the published literature on these co-exposures and known mechanisms of renal cell toxicity stemming from interactions among these elements.

14.6.1 Lead

Combined exposures to lead and arsenic are common in both occupational and environmental situations [64,65]. Since both these elements are nephrotoxic, additive or synergistic manifestations of renal cell injury are frequently reported depending on dose or exposure level. Toxic effects on renal tubule cells from combined lead and arsenic exposures have been reported following both moderate and low level dosing of experimental animals *in vivo* [66,67].

14.6.2 Cadmium

Nephrotoxic effects of combined cadmium arsenic exposures have been reported in human populations [68], experimental animals [66,67], and *in vitro* exposure situations [50]. As with lead, both additive and synergistic toxicities may be observed depending on dose or exposure level [66,67].

14.6.3 Gallium

Interaction studies between gallium and arsenic stem from the use of both elements in the semiconductor industry for the production of gallium arsenide from computer chips and light emitting diodes (LEDs). Worker exposures to arsenic may occur in the grinding and polishing of GaAs wafers and maintenance of fabrication equipment [69].

14.6.4 Indium

Indium arsenide (InAs) is a similar semiconductor to GaAs whose use has expanded due to the greater electronic speeds of InAs chips over those of GaAs chips. Both GaAs and InAs are toxic to the kidney but InAs appears to exert greater toxicity on kidney cells relative to GaAs equivalent exposure conditions [59].

14.7 Summary and Future Research Needs

Based on the above short review, it is clear that each of the various forms of arsenic discussed is capable of producing renal toxicity in both humans and experimental animals either on the basis of arsenic alone or in combination with other nephrotoxic metals. The basic toxicological issues stemming from these general observations serve as the basis for future research needs including, in particular, the need for sensitive and specific biomarkers of arsenical nephrotoxicity on an individual or multi-element mixture basis particularly at low dose exposures.

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Arsenic-Induced Developmental Neurotoxicity

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CHAPTER OUTLINE

15.1 Introduction

Arsenic toxicity has become a global health problem affecting millions of people. Aside from causing gastrointestinal, respiratory, cardiovascular, genitourinary, endocrine, hematopoietic system and skin diseases [1], acute and chronic arsenic exposure is associated with neurologic consequences in adults [2]. Arsenic is a known neurotoxicant that affects the peripheral nervous system, with effects which may last for several years or even a lifetime, causing a symmetrical peripheral neuropathy characterized by sensory nerves being more sensitive than motor nerves to arsenic effects, and neurons with large axons being more affected than neurons with short axons [3–5]. Acute arsenic exposure was reported to cause central nervous system (CNS) alterations [6–9], including learning, memory, and concentration.

When exposure occurs in the early developmental stages, the CNS is more susceptible to toxic agents. Children may be particularly susceptible to neurotoxic substances as suggested by findings from studies on the effects of lead [10], methylmercury [11], and PCBs [10]. Gestation in humans and rodents is a period of high sensitivity for offspring to chemical toxicity [12]. Recently, results of some epidemiological, experimental animal, and *in vitro* studies have suggested a possible association between arsenic exposure and neurodevelopment.

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Although neurotoxic effects of arsenic in adults have been well documented [2,13], there is no review addressing the effects of arsenic on neurodevelopment. The purpose of this review is to integrate the reports on possible interactions of arsenicals with neurodevelopment and to promote interest in exploring the mechanisms of arsenic developmental neurotoxicity.

15.2 Arsenic Exposure Impairs Intellectual Function in Children

Arsenic poisoning related to occupational exposure causes CNS alterations, including impairments of recent memory, learning, and concentration [14]. When exposure occurs in the early developmental stages, the CNS is more susceptible to toxic agents. Therefore, many studies were designed to investigate the possible association between arsenic exposure and neurodevelopment of children.

Calderon et al. [15] reported a cross-sectional study in Mexico to address the neurological effects on children of chronic environmental exposure to arsenic. In this study, two populations chronically exposed to either high (41 children) or low (39 children) levels of As and Pb were analyzed using the Wechsler Intelligence Scale for Children, Revised Version, for Mexico (WISC-RM). Mean urinary arsenic (UAs) values were 62.9 ± 0.03 (μg As/g creatinine) for the exposed group and 40.2 ± 0.03 (μg As/g creatinine) for the reference group. After controlling for significant potential variables, verbal intelligence quotient (IQ) decreased with increasing concentrations of urinary arsenic (UAs). Higher levels of UAs were significantly correlated to poorer performance on WISC-RM factors examining long-term memory and linguistic abstraction [15]. Another cross-sectional study compared 49 junior school students drinking arsenic-containing well water and 60 controls in Taiwan. The former was divided into two groups, exposed to arsenical levels of 184.99 ± 225.29 μg/L (high dose group, $n = 29$) and 131.19 ± 343.70 μg/L (low dose group, $n = 20$) over periods of 11.28 ± 2.58 and 8.10 ± 6.07 years, respectively. After adjusting for education and sex, pattern memory and switching attention were found to be significantly affected by long-term cumulative exposure to arsenic [16]. A study conducted in Shanyin County, China, involved 720 children between 8 and 12 years of age. The children were exposed to arsenic at the concentrations of 190 ± 183 μg/L (high-As group), 142 ± 106 μg/L (medium-As group), and 2 ± 3 μg/L (low-As group, control). A modified Raven's test was used to determine the effects of these exposures on children's intelligence. The mean IQ scores for the high-As group (95 ± 17) ($p < 0.01$) and medium-As group (101 ± 16) ($p < 0.05$) were significantly lower than that of the control group (105 ± 15) [17]. A study among 351 children aged 5 to 15 years in West Bengal tried to address the possible impairment of children's intellectual function in relation to arsenic exposure *in utero* and during childhood. Authors found associations between arsenic and reductions in the adjusted scores of the vocabulary test, the object assembly test, and the picture completion test. However, exposure to arsenic during pregnancy or childhood was not associated with these test results. UAs, not water arsenic (WAs), were associated with small decrements in intellectual testing in school-aged children in West Bengal [14].

Wasserman et al. conducted two separate cross-sectional investigations of intellectual function, in 201 10-year-olds and 301 6-year-olds in Bangladesh, respectively [18,19]. In the 10-year-olds study, drinking water As exposure was associated with reduced intellectual function after adjustment for sociodemographic covariates and water Mn. WAs was associated with reduced intellectual function in a dose–response manner, such that children exposed to WAs levels >50 μg/L achieved significantly lower performance and full-scale scores than did children exposed to WAs levels <5.5 μg/L. The association was generally stronger for WAs than for UAs [18]. In the 6-year-olds study, with covariate adjustment, WAs remained significantly negatively associated with both performance and processing speed raw scores, but the associations were less strong than in 10-year-olds. Neither study found an association between As exposure and aspects of verbal intelligence [18,19]. It seems that children with less lengthy exposure to As may have diminished adverse consequences for intellectual function [19].

A consecutive investigation on the IQ of 408 children (two age groups: 9 and 10 years; 4 and 5 years) in Bangladesh was reported [20]. The level of UAs was associated with reduced IQ in a dose–response manner. The association between reduced IQ and UAs was stronger than that between reduced IQ and WAs. However, there was no association between verbal IQ scores and UAs of children in early childhood (aged 4 and 5 years). Based on these results, the authors concluded that UAs were negatively associated with the IQ of the children tested, and that this adverse effect of As may also gradually accumulate over time among the poor.

In their study, Rosado et al. [21] recruited 602 children 6–8 years old living within 3.5 km of a metallurgic smelter complex in Mexico. The mean level of UAs was 58.1 ± 33.2 μg/L. After adjustment for hemoglobin concentration, blood lead concentration, and sociodemographic confounders, the results showed a significant inverse association between UAs and visual-spatial abilities with figure design, the Peabody Picture Vocabulary Test, the WISC-RM Digit Span subscale, visual search, and letter sequencing tests ($p < 0.05$). Boys excreted significantly more UAs ($p < 0.05$) and were affected in different cognitive areas than were girls. Impairment of children's cognitive development caused by As is independent of any effect of lead [21]. McDermott et al. [22] conducted a retrospective cohort study, which involved 3988 mother/child pairs who had lived during pregnancy and early childhood in South Carolina, to describe the complex association between soil concentrations of As combined with Pb and the probability of intellectual disability (ID) in children. The probability ID was increased with increasing concentrations of As and Pb in the soil. For normal weight for gestational age children, when As = 22 mg/kg and Pb = 200 mg/kg, the risk for ID was 11% and when As = 22 mg/kg and Pb = 400 mg/kg, the probability of ID was 65%. The probability of ID was significantly associated with the interaction between Pb and As for normal weight for gestational age infants [22].

Wright et al. [23] conducted a small ($n = 32$) pilot study to explore the potential associations between hair metal levels and the neuropsychological function and behavior of 11–13-year-old children. The mean hair Mn and As levels were 471.5 and 17.8 ng/g. Children's general intelligence (particularly verbal IQ) scores and scores on assessment of verbal learning and memory were significantly negatively related to hair Mn and As levels. In some cases, a significant Mn-by-As interaction was found. No significant association between children's hair As level and parent or teacher ratings of children's behavior was found [23]. In another

study, Wasserman et al. [24] recruited a new sample of 299 children (8–11 years old) to examine possible interactive effects of exposure to both As and Mn on neurobehavioral function. After adjustment for each other, both As and Mn (BAs, BMn) in whole blood were found to be significantly negatively related to most WISC-IV subscale scores. With further adjustment for sociodemographic features and ferritin, association between BAs and verbal comprehension was significantly negative. UAs (per gram creatinine) was significantly negatively associated with verbal comprehension scores, even after adjustment for BMn and other contributors. Mn by As interactions were not significant in either adjusted or unadjusted models [24].

The nervous system of an embryo is vulnerable to adverse impacts from pollutants because it goes through a long developmental process. This developmental process requires precise coordination of cell growth and movement, which may be disrupted by even short-term exposures to environmental contaminants [25]. A study with 4436 pregnant women in Matlab, Bangladesh, was conducted to assess the effects of *in utero* arsenic exposure on infants' problem-solving ability and motor development. Urine specimens at 8 and 30 weeks of gestation were collected to measure arsenic concentration. Infants' problem-solving ability and motor development were assessed at 7 months of age. With covariate adjustment, there was no significant effect of urinary arsenic concentration on children's motor and PST scores and behavior ratings. It is possible that some other effects are as yet unmeasured or that effects will become apparent at a later age [26]. A hospital-based birth cohort study with 100 pregnant women in Nepal was conducted to investigate the association between *in utero* toxic (Pb and As) and essential element Zn levels and neurodevelopmental indicators after birth. Infants' neurodevelopmental indicators at 1 day after birth were tested using the Brazelton Neonatal Behavioral Assessment Scale, third edition (NBAS III). The cord blood levels of Pb and As, but not Zn, showed significant inverse association with the neurodevelopment of newborns [25].

Trying to find information on critical windows of arsenic exposure, Hamadani et al. [27] conducted a population-based longitudinal study, which recruited 1700 children at 5 years of age in rural Bangladesh. Arsenic exposure was measured by arsenic concentrations in mothers' urine collected in early (gestational week 8) and late (gestational week 30) pregnancy and in children's urine at 1.5 and 5 years. After controlling for all potential confounders and loss to follow-up, it was found that verbal IQ and full-scale IQ (FSIQ) were negatively associated with (log) UAs in girls. It was calculated that 100 μg/L UAs was associated with a decrement of 1–3 points in both verbal IQ and FSIQ in girls. However, UAs showed consistently low and non-significant associations with all IQ measures in boys [27].

To explore whether prenatal exposure to toxic heavy metals is associated with neonatal development, a stratified multi-stage cluster sampling methodology was utilized to investigate 1652 mother/infant pairs from 2008 through 2009 in Shanghai. The median concentrations of blood Pb, Hg, Cd, As, and Tl were all in the level considered safe. Increasing exposure to Cd, Hg, As, and Tl during pregnancy was associated with decreasing neonatal behavioral neurological assessment (NBNA) scores. Those exposed to medium-level As (which is considered safe) had lower NBNA scores than those exposed to low-level As, which implied that the considered safe level of As was not safe to the newborns' development [28].

Some epidemiologic studies have focused on the possible associations between impaired motor function and arsenic exposure in children. Parvez et al. [29] conducted a study to investigate the associations of drinking water arsenic and Mn with motor function in 304 children in Bangladesh, 8–11 years of age. The motor function was assessed with the Bruininks-Oseretsky test, version 2, in four subscales. After adjustment of blood Mn, lead, selenium, and other covariates, blood As was associated with decreases in total motor composite (TMC), fine manual control (FMC), and body coordination (BC). Other As exposures (WAs, UAs, and toenail As) were also inversely associated with motor function scores, particularly TMC and BC. In addition, the study found blood selenium was positively associated with TMC, FMC, and MC in the unadjusted models. Mn exposure was not significantly associated with motor function [29]. Another study involved 526 children aged 6–7 years living near a metal foundry in Torreón, Mexico. Children's behavior was assessed by parents and teachers with Conners Behavior Rating Scales. The mean behavior scores from parent and teacher ratings were within the clinically normal range ($T < 65$). There was no significant association between any measure of UAs and parent ratings of behavior. However, there existed an association between higher total UAs and urinary dimethylarsinic acid (DMA) with higher ratings on the oppositional, cognitive problems and attention deficit hyperactivity disorder (ADHD) subscales of the teacher ratings. But after adjustment for the Peabody Picture Vocabulary Test scores, the associations between total UAs and behavior became statistically non-significant, suggesting that the harmful effects of arsenic on behavior may be secondary to arsenic-induced cognitive deficits [30].

In a cross-sectional study, 130 junior high school students aged 12–14 years were recruited and examined for the motor and sensory nerve conduction velocity of peripheral nerves in their right upper and lower limbs. After adjustment for gender and height, the development of slow nerve conduction velocity of the sural sensory action potential (SAP) was found among those subjects with a cumulative arsenic dosage of >100.0 mg. Thus chronic exposure to arsenic might induce peripheral neuropathy. The slowing of the nerve conduction velocity of sural SAP might be an early marker of chronic arsenic neuropathy [31].

15.3 Developmental Neurobehavioral Toxicity in Animals

Studies in animals suggest that arsenic exposure can impair cognitive development and neurobehavioral development. Sprague-Dawley (SD) rats were exposed to arsenite (36.70 mg/L in drinking water) from gestation day 15 (GD 15) or postnatal day 1 (PND 1) until approximately 4 months old. Rat offspring exposed from GD 15 showed increased spontaneous locomotor activity and both exposed groups showed increased numbers of errors in a delayed alternation task in comparison to the control group [32]. Xi et al. [33] exposed offspring rats to sodium arsenite in drinking water from GD 6 until PND 42. Exposure to 100 mg/L arsenite induced a significant decrease in the number of incidences in tail hung, auditory startle, and visual placing in rat pups. A dose-related increase in the trained numbers of rats and latency time were

observed among As-exposed groups in the square water maze test [33]. Pregnant C57BL6/J mice consumed drinking water containing sodium arsenite from GD 4 until birthing. Arsenic produced a range of behavioral impairments in male and female offspring at each of the test ages. The most striking effects of arsenic were on the development of gait and other motor responses including acoustic startle, righting reflexes, and forelimb grip [34]. Compared to control, exposure to arsenic during gestation and lactation significantly prolonged the time of completing reflex response of surface righting, negative geotaxis, and cliff avoidance of rat offspring in a 13.6 mg/L As-exposed group [35]. Moderate perinatal arsenic (50 ppb) exposure disrupted the regulatory interactions between the hypothalamic-pituitary-adrenal axis and the serotonergic system, and increased learned helplessness and measures of immobility in a forced swim task in adult mouse offspring [36]. Perinatal arsenic exposure significantly reduced corticosterone receptor (CR) levels in the hippocampus and impaired the performance of mouse offspring in an object recognition task and in the eight-way radial arm maze [37]. However, in the gestational administration of arsenic (0, 1.5, 3.0, and 4.5 mg/kg/day/po) from GD 8 till parturition, rat offspring showed no significant changes in the day of appearance of eye opening, startle reflex, negative geotaxis, and spontaneous alteration performance in comparison to the control group [38]. The different results might be due to different exposure duration and dose.

Weaned SD rats exposed to arsenic for 3 months showed poor performance in Morris water maze and Y maze tasks [39,40]. Another study found arsenic exposure impaired the performance, in Morris water maze, of Wistar rats [41]. Twenty-one-day-old male SD rats were exposed to iAs in drinking water for 1 year. There were significant decreases in the total-distance number of movements, and movement time during the dark phase of the dark–light cycle in the group treated with 50 mg iAs/L in comparison to the control group [42]. Zebrafish embryos exposed to arsenic between 4 and 120 h post-fertilization (hpf) resulted in alterations in neural development including weak tactile responses to light (2.0–5.0 mM, 30 hpf), malformation of the spinal cord, and disordered motor axon projections (2.0 mM, 48 hpf) [43].

15.4 Arsenic Distribution After Exposure in Early Life

When exposure occurs in the early developmental stages, the CNS is more susceptible to toxic agents. Gestation in humans and rodents is a period of high sensitivity for offspring to chemical toxicity [12]. During gestation, both inorganic arsenic and its methylated metabolites (MMAs and DMAs) can readily cross the animal and human placenta and enter the fetal system. Concha et al. [44] found the concentration of arsenic in cord blood (median, 9 μg/L) was almost as high as in maternal blood (median, 11 μg/L), and these two were strongly correlated. Hall et al. [45] also found the same correlation. Thus, at least in late gestation, arsenic is easily transferred to the fetus. The main speciated arsenic in newborns and their mothers was DMA, which may indicate that DMA is the major form of arsenic transferred to the fetus [44]. Animal

studies also found arsenic can readily cross the placenta. When mice were exposed to arsenic during gestation and lactation, concentrations of iAs, MMA, and DMA in the liver and brain of newborn mice increased with the concentration of arsenic in drinking water [33,46–48]. Compared to those in the brain of mother mice, summation of arsenic species in the brain or liver of newborn mice was higher [46].

While arsenic concentrations in maternal blood and urine were high, averaging 10 and 320 μg/L respectively, the mean arsenic level in human milk was 2.3 μg/kg [49]. Fängström et al. [50] also found arsenic levels in human milk were much lower than in blood. An animal study confirmed the low level of arsenic in mothers' milk [48]. Therefore, breast-fed infants might be protected from arsenic exposure.

Animal studies found that, when exposed to arsenic in early life, arsenic can cross the immature blood–brain barrier (BBB) easily. When mice were exposed to arsenic during gestation and lactation, arsenic concentrations in the brain of newborn mice increased with the concentration of arsenic in drinking water [33,35,46–48,51]. Compared to those in the liver of newborn mice, contents of iAs in the brain were almost the same (1:1), and contents of DMA in brain were much higher (1:2). So, as an organic metabolite, DMA might accumulate preferentially in the brain [46].

The brain is particularly vulnerable during gestation and early life, and the damage induced by toxic agents is often permanent [47]. In arsenic-contaminated areas, fetuses and infants may be exposed to iAs and its metabolites via the placenta. The BBB is formed by blood vessel endothelial cells, astrocytes, pericytes, and a basement membrane [52], which can separate the brain from the circulatory system and protect the central nervous system (CNS) from potentially harmful chemicals. However, as the barrier is immature at early developmental stages, the transfer of toxic agents from blood into brain might not be limited efficiently. In addition, arsenic exposure could impair the structure and function of the BBB, and have deleterious effects such as the formation of impaired astrocytes [53], hippocampal neurons and vascular endothelial cells, irregular capillary lumina, and obvious edema around capillaries [35].

15.5 Mechanism of Developmental Neurotoxicity

15.5.1 Effects of Arsenic on Neurotransmitter Systems

Efficient communication among large numbers of brain cells (i.e., neurons) is necessary for the normal function of the nervous system. A central mechanism of neuronal communication involves the release of neurotransmitters that bind to specialized receptors on the target cell, changing its activity [54]. Any alteration in neurotransmitter systems could be reflected in the neurobehavioral deficits seen in humans and animals. Arsenic alters cholinergic, glutamatergic, and monoaminergic systems in adult rodents, with the dopaminergic system being the most affected [2]. Rodríguez et al. [2] have described the effects of arsenic on neurotransmitter systems in an excellent review, but we will not go further on this issue here.

15.5.2 Arsenic and Neurite Growth, Astrocyte, and Myelin

During prenatal development each neuron establishes neurites that develop into a unique and complex pattern of dendrites, which function as the sites of input for all connecting neurons, and an axon that carries neuronal output. The production and elaboration of dendrites and axons during fetal development is essential for neuron function and connectivity [55]. The establishment of the correct patterning of dendrite elaboration and axon outgrowth is highly regulated during fetal development [56]. Many studies indicate that developmental exposure to arsenic can alter neurite growth and complexity.

A study was designed to examine the effects of arsenic on brain explants (human fetal brain and newborn rat brain) and cells grown and maintained in a tissue culture system. Arsenic exposure resulted in changes in brain cell membrane function, reduced brain explants' growth, and caused loss of cells, inhibition of intracellular networking, and the loss of ground matrix [57]. Exposure of rats to arsenic in drinking water (3 ppm) from gestation to the age of 4 months resulted in moderate to severe alterations of thickness, organization, surrounding space, and shape of fiber tracts and axons, while cell bodies remained healthy. These anomalies were accompanied by significant decrease of all nitrergic markers in striatal tissue [58].

Hong and Bain [59] used P19 mouse embryonic stem cells to examine whether arsenic exposure could alter their differentiation into skeletal muscles and neurons. Although embryoid body formation was not altered, arsenic suppressed their differentiation into muscles and neurons. The altered differentiation was due to the repression of muscle- and neuron-specific transcription factors (Pax3, Myf5, MyoD, myogenin, neurogenin 1, neurogenin 2, and NeuroD) caused by repressed Wnt/β-catenin signaling pathways in early embryogenesis.

Frankel et al. [55] investigated the effects of sodium arsenite on the early stages of neurite production and growth. The results showed that arsenic has concentration- and time-dependent effects on initial neurite outgrowth *in vitro*. Exposure to low levels of arsenic for 5 days results in reduced neurite production, outgrowth, and complexity in newly differentiating PC12 cells. Another study found that exposure to low levels of arsenic ($\leq 5\,\mu M$) during differentiation reduced the neurite outgrowth in N2a cells in the absence of cell death [60]. Reduced neurite outgrowth induced by arsenic resulted from deficient activation of AMPK as a consequence of a lack of activation of LKB1. Oxidative stress induced by arsenic, especially excessive superoxide, played a critical role in blocking the LKB1–AMPK signaling pathway [60]. Primary cultured neurons obtained from the cerebral cortex of mouse embryos exposed to sodium arsenite (1 and 2 μM) showed significant inhibition of total neurite length and expression of GluA1, a specific subunit of AMPA receptor. Compensatory overexpression of GluA1 protected against the deleterious effects of arsenic on neurite outgrowth [61].

A study was designed to determine whether sodium arsenite (0, 1, 5, or 10 μM) affects both neurite outgrowth and/or induces apoptosis. The results showed that the area ratio of neurite to cell body in SCAT3-expressing cells was significantly reduced by 5 and 10 μM sodium arsenite. Apoptosis was significantly induced when arsenic exposure continued after the

significant effects on neurite outgrowth were found. Arsenic treatment increased the mRNA levels of the neurofilament light (NF-L) and neurofilament medium (NF-M) and decreased the mRNA levels of tau and tubulin in a dose-dependent manner [62]. Male Wistar rats exposed to single doses of arsenite (15 and 20 mg/kg) showed reduced expression of NF and fibroblast proteins in sciatic nerve. Some fibroblast protein bands had disappeared in the 20-mg/kg dose group. The analyzed NF-M and NF-L proteins decreased dose dependency over time [63]. Subchronically exposed to arsenic reduced expression of NF-L protein and increased expression of mu- and m-calpain protein in the sciatic nerve of rats, both in a dose/time pattern. Furthermore, NF-H protein was hypo-phosphorylated, while NF-L and microtubule-associated protein tau (MAP-tau) proteins were hyper-phosphorylated [64]. Arsenic treatment of differentiated NB2/d1 cells and cultured dorsal root ganglion neurons decreased NF transport into axonal neurites and increased perikaryal phospho-NF immunoreactivity. Both of these effects were mediated by increasing activity of JNK, while GSK-3β activity may selectively interfere with NF transport following arsenic exposure [65]. Dubey et al. [66] also found arsenic exposure inhibited translocation of NFs into axonal neurites in culture and increased perikaryal NF phosphoepitopes.

Astrocytes are the most numerous cell type within the CNS and perform a variety of tasks, from axon guidance and synaptic support, to the control of the BBB and blood flow [67]. Some studies showed that arsenic exposure can impair astrocytes. Exposure of primary cultured astrocytes to 0–30 μM arsenite for 24 h resulted in damage to astrocytes in a concentration-dependent manner, and inhibited glutamate metabolism reflected as lower activities and protein expression of glutamine synthetase and glutamate transporter (GLAST and GLT-1) [53]. Incubation of astrocytes for 2 h with arsenate in concentrations of up to 10 mM caused a time- and concentration-dependent loss of GSH from viable astrocytes, which was accompanied by a matching increase in the extracellular GSH content. The results suggested that arsenate exposure disturbs astrocytic GSH metabolism [68]. Primary neuron cultures were divided into four groups, which were neurons without astrocyte-conditioned medium (ACM) exposure (group I) and neurons exposed to ACM from 0, 5 or 10 μM arsenite-treated astrocytes (groups II–IV). Synaptic formation and protein expression of NR2A, NR2B, CaMKII, and AC in groups III and IV were significantly suppressed when compared to results in group II. Moreover, synaptic formation and protein expression of CaMKII and AC in group II were significantly enhanced when compared with group I. The results suggested that arsenic in astrocytes might impair synaptic formation through disturbing astrocytic effects on neuronal signal transduction [69].

Arsenic exposure also affects the myelin of nerve fibers. Adult female Wistar rats were exposed to arsenic (3 and 36 ppm) from gestation until 1, 2, 3, and 4 months of age. Arsenic intake resulted in myelin damage reflected as empty spaces in fiber tracts, and significant lower levels of dimethyl arginine in exposed animals, as compared with the controls [70]. Male SD rats weighing 40–60 g exposed to sodium arsenite through drinking water for 3 months showed abnormal structural changes in the myelin sheaths of nerve fibers and decreases in the terminals of mossy fibers [39].

15.5.3 Arsenic and Neuron Apoptosis

Studies have shown apoptosis to be involved in arsenic-induced neurotoxicity. After exposure to arsenic, human fetal and rat neonatal brain explants cells showed disturbance in lipid peroxidation, generation of nitric oxide (NO) and reactive oxygen species (ROS), apoptosis, and necrosis. The administration of vitamins C and E and DMSA partially reversed these effects [71]. Milton et al. [72] found apoptosis in a neuronal cell line peaked between 10 and 20 μM of arsenic trioxide, while higher concentrations of arsenic caused increasing cell death. Zinc significantly decreased arsenic-induced apoptosis in a dose-dependent manner at 50 and 75 μM zinc. Embryonic primary rat midbrain (GD 12) neuroepithelial cells exposed to sodium arsenite (0, 0.5, 1, 2, and 4 μM) did not show apparent apoptosis until 4 μM at 24 h [73].

Kaler et al. [74] studied the effects of arsenic exposure during the rapid brain growth period (RBGP) (postnatal days 4–11) on pyramidal neurons and granule cells of rat hippocampus. Microscopic study found decreased numbers and isolation of pyramidal neurons in superficial layers, misalignments of pyramidal cells in stratum pyramidale of CA1 and CA3 in rats exposed to 1.5 and 2.0 mg/kg sodium arsenite, and the presence of polymorphic cells in the subgranular zone of the ectal limb of the dentate gyrus. Morphometric assessments quantified and confirmed the microscopic findings. Increased apoptosis was found in the dentate gyrus of rats exposed to 2.0 mg/kg sodium arsenite. These results confirmed the vulnerability of pyramidal and granule cells of the hippocampus during RBGP [74]. Another study found arsenic exposure (5 and 10 μM) for 24 h caused obvious apoptosis in primary cultured rat cerebellar granule neurons. Neuroglobin protein and mRNA expression were significantly down-regulated shortly after arsenic exposure and then up-regulated after a longer time of exposure. Exposure to arsenic for 16 weeks induced higher neuroglobin expression in rat cerebellum [75].

Exposure to sodium arsenite (5, 10, or 15 μM) or dimethylarsinic acid (1 or 5 mM) induced apoptosis in cerebellar neurons, with the inorganic form being more toxic. Arsenite-induced cerebellar neuron apoptosis requires new gene expression and caspase activation. Sodium arsenite selectively activated p38 and JNK3 (not JNK1 or JNK2) in cerebellar neurons. Blocking the p38 or JNK signaling pathways protected cerebellar neurons against arsenite-induced apoptosis [76]. Arsenic exposure also induced cortical neuron apoptosis through activating JNK and p38 MAP Kinases [77]. Rat cerebellar granule neurons (CGNs) exposed to sodium arsenite (from 0 to 50 μM) showed obvious apoptosis. Meanwhile, arsenite treatment increased phosphorylation expression of p38 and extracellular signal-regulated kinases (ERK1/2), but not of c-Jun N-terminal kinases. Furthermore, SB203580 (an inhibitor of p38) decreased the percentage of apoptotic cells whereas U0126 (an inhibitor of ERK1/2) enhanced arsenite-stimulated toxicity. These data suggest that p38 may contribute to arsenite-induced apoptosis of rat CGNs, but that ERK1/2 is involved in cell growth and survival [78].

Arsenic-induced free radicals are known to cause cellular apoptosis through the mitochondrial-driven pathway. Dwivedi et al. [79] investigated the effect of arsenic interactions with various complexes of the electron transport chain and evaluated if there was a complex preference for arsenic that could trigger apoptosis. They found that arsenic exposure induced free radical generation in rat neuronal cells, which reduced mitochondrial potential and enzyme

activities of all the complexes of the electron transport chain. These early events along with reduced ATP levels could be correlated with the later events of cytosolic migration of cytochrome c, altered bax/bcl2 ratio, and increased caspase-3 activity. Monoisoamyl dimercaptosuccinic acid could reverse most of these arsenic-induced events except DNA damage [79]. One study investigated the toxic effects and possible mechanisms of arsenic exposure (0.5 and 5 mg/L As_2O_3 in drinking water for 6 weeks) in the cerebrum of mice. Arsenic exposure induced oxidative stress, apoptosis, and activation of caspase-3, up-regulation of Bax and Bak, and down-regulation of Mcl-1 in the cerebral cortex. Exposure to arsenic also triggered the expression of ER stress-related genes, including *GRP78*, *GRP94*, and *CHOP*. Meanwhile, increased phosphorylation of p38 and decreased phosphorylation of ERK1/2 were shown in the cerebral cortex as a result of arsenic exposure of mice. These results suggested that arsenic-induced oxidative stress causes cellular apoptosis in the cerebrum and that signaling of p38 and ERK1/2, and ER stress, may be involved in iAs-induced cerebral toxicity [80]. Recently, other research has found that iAs exposure induced apoptosis in N2a cells, increased oxidative stress damage, and induced several features of mitochondria-dependent apoptotic signals, including: mitochondrial dysfunction, the activation of PARP and caspase cascades, and the increase in caspase-3 activity. A further study found that JNK/ERK1/2, but not p38-MAPK, mediated mitochondria-dependent apoptosis in N2a cells. In addition, GRP 78/CHOP pathways were also involved in the apoptosis [81].

15.5.4 Arsenic and Methylation

There is currently an intensive focus on epigenetic regulation of phenotypes to identify chronic enhancement of disease risk resulting from arsenic exposure during discrete developmental windows [82]. Arsenic exposure can cause changes in all three epigenetic markers, namely, DNA methylation, histone modifications, and expression of non-coding RNAs [83]. After its absorption, arsenic undergoes hepatic biomethylation to form monomethylarsenoc acid (MMA) and dimethylarsenic acid (DMA). S-adenosylmethionine (SAM) is the principal methyl donor in the organism and is present in every cell participating in almost all methylation reactions, from small molecules to DNA, RNA, and proteins [70]. It has been proposed that a high demand of S-adenosylmethionine (SAM) for arsenic methylation as well as failure in the regeneration of SAM would compromise the availability of the methyl group in the organism leading to the characteristic toxic effects of arsenic, namely, nervous system damage, vascular disease, hepatic and renal damage as well as different types of cancer [84,85].

Methylation has an important role in the synthesis of myelin basic protein (MBP), an essential component that confers compactness on myelin. Arsenic intake resulted in myelin damage accompanied by significant reduced levels of dimethyl arginine in rats [70]. In another study, Wistar rats were exposed to arsenic in drinking water (3 and 36 ppm) from gestation until 4 months of age. The results showed DNA hypomethylation in the brain cortex and significant increase in the non-methylated form of PP1 gene promoter either in cortex or hippocampus. Furthermore, the rats showed progressive and dose-dependent deficits of fear memory [86].

As one of the most important methyl donors is folate, folate deficiency contributes to a variety of age-related neurological and psychological disorders. One study examined whether or not folate deprivation potentiated the impact of arsenic on neurofilament dynamics. The study found that folate deprivation potentiated the toxicity of arsenic. Supplementation with SAM attenuated the impact of folate deprivation on arsenic neurotoxicity [66]. Another study found that in adult rats with life-long exposure to arsenic through drinking water (3 ppm), there were no changes of SAM, choline, and PC concentrations found in the brain, but SAM and phosphatidylcholine were severely decreased in liver accompanied by a significant increase of choline. The results indicated that choline plays an important role as methyl donor in arsenic exposure [85].

Recently, one study applied chromatin immunoprecipitation followed by high-throughput massive parallel sequencing (ChIP-seq) to evaluate the H3K9 acetylation pattern in the offspring of arsenic-exposed and control mice. The results showed that arsenic exposure during embryonic life caused global hypo-acetylation at H3K9 and changes in functional annotation with highly significant representation of Krüppel-associated box (KRAB) transcription factors in brain samples from exposed pups. The authors also found that arsenic exposure impaired spatial and episodic memory, as well as fear conditioning performance in adult mice [82].

15.5.5 Arsenic and Nitric Oxide

In the brain, nitric oxide is involved in long-term potential (LTP) in cortex and hippocampus [87], neuronal transmission [88], and potent neuroprotectant [89], and its production depends mainly on calcium entry through NMDA receptors for glutamate [90]. Several studies have found that arsenic exposure disturbs NO production.

Female Wistar rats exposed to arsenite in drinking water (4–5 mg/kg/day) from gestation until 4 months of age showed a significantly lower response to NMDA receptor stimulation, reduction of nitric oxide synthase (NOS) activity, and decreased levels of nitrites and nitrates in striatum. These markers of NO function were accompanied by significantly higher levels of LPx and ROS production [91]. Furthermore, on exposure to a lower concentration of arsenic in drinking water (0.4 mg/kg/day) from gestation until 4 months age, rats showed brain structural changes and significant decreases of all nitrergic markers (except the expression of nNOS-mRNA). These anomalies were not accompanied by ROS and/or LPx increases at low exposure levels [58]. In another study, rats exposed to arsenic showed significantly decreased levels of NOS and acetylcholinesterase—however, accompanied by increased levels of NO generation both *in vitro* and *in vivo* [41].

15.5.6 Arsenic and Gene Expression

Arsenite, at low and subcytoxic concentrations, appears to induce oxidative stress leading to activation of early transcription factors (NF-κB and activator protein-1), whereas addition of antioxidant inhibited the activation of these factors [92]. Subchronic exposure to As increased 8-OHdG remarkably, and down-regulated expression of succinate dehydrogenase subunit A (Sdha) and SDH activity in brain tissue of mice [93].

In an attempt to investigate whether acute arsenate treatment could alter the expression of several cell cycle genes during murine neurulation, pregnant LM/Bc dams were injected

i.p. on GD 7:12 (day:hour) and 8:12 with 40 mg/kg of arsenate. Following arsenate treatment, the gene expression of Bc1-2 and p53 was significantly up-regulated at GD 9:0, compared to control. The results indicated that arsenic inhibits cell proliferation, rather than inducing apoptosis, which delayed neural tube closure [94]. In order to understand the mechanism of arsenate-induced neural tube defects (NTDs), highly sensitive Folr2 nullizygous mice were injected i.p. with sodium arsenate at the beginning of the neural tube formation process. The investigators identified several candidate genes as well as important ontology groups that may be responsible for arsenic's teratogenicity. These genes included: engrailed 1, platelet-derived growth factor receptor alpha, and ephrin A7. The gene ontology groups included: morphogenesis, oxidative phosphorylation, redox response, and regulation of IKK/NF-κB cascade [95]. One study sought to evaluate proliferation and differentiation of adult neural progenitor cells (NPC) in the dentate gyrus after 50 μg/L As exposure throughout the perinatal period of development in mice. Compared to controls, proliferation of the NPC was decreased by 13% (not significantly); however, the number of differentiated cells was significantly decreased by 41% in As-exposed mice. Brief, daily exposure to environmental enrichment significantly increased proliferation and differentiation in both control and As-exposed animals. Expression levels of 31% of neurogenesis-related genes were altered after As exposure and restored after enrichment [96].

In a further study, mice were exposed to 1 and 4 ppm As_2O_3 through drinking water for 60 days. Arsenic exposure induced learning and memory impairment and down-regulated Camk4 expression, a very important regulator in the LTD pathway [97]. Weaning rats exposed to arsenic for 3 months had impaired neurobehavioral performance, reduced expression of NR2A, a subunit of NMDA receptor [40], and altered expression of postsynaptic signaling proteins (CaMKII, PSD-95, SynGAP, and ERK1/2) in rat hippocampus [98]. Polysialylation of NCAM (PSA-NCAM) is a critical functional feature of NCAM-mediated cell interactions and functions. Maternal and early life arsenite exposure up-regulated the expression of NCAM, PSA-NCAM, and polysialyltransferases (STX and PST) in hippocampus of rat offspring on PND 21 and PND 120 [35].

Perinatal arsenic exposure increased basal plasma corticosterone levels, decreased hippocampal glucocorticoid receptor (GR) levels in the hippocampus, and impaired learning and memory performance [99]. Reduced expression of MAPK/ERK genes was found in perinatal As-exposed offspring, induced by GR deficits [99]. One study found that perinatal As-exposed offspring showed an increase in hypothalamic corticotrophin-releasing factor, altered corticosterone secretion both at baseline and in response to a stressor, decreased hippocampal 11β-HSD 1, and altered subcellular GR distribution in the hypothalamus [100].

Exposure of SN56.B5.G4 cells expo to both sodium arsenite and DMA resulted in enhanced levels of APP and sAPP in the membrane and cytosolic fractions, respectively. Exposure of primary neuronal cells overexpressing the Swedish mutation of human APP to sodium arsenite resulted in an enhanced level of membrane-bound APP, a slightly higher level of sAPPβ, and a reduced level of Aβ peptides in the culture medium. In contrast, exposure of neuronal cells to DMA considerably increased formation of Aβ and sAPPβ, accompanied by an enhanced membrane APP level. The results indicated that sodium arsenite and DMA can affect processing of APP *in vitro* [101].

15.6 Neuroprotective Agents Against Arsenic Toxicity

Plant extracts and pharmacological agents have been used to investigate their neuroprotective efficacy against arsenic-induced neurotoxicity [102]. Curcumin, a non-toxic polyphenolic non-flavanoid compound, has been found effective in the treatment of neurodegenerative disorders [103] and chemical-induced neurotoxicity including lead and cadmium [104]. The efficacy of curcumin against arsenic-induced neurotoxicity has been investigated in rats. In a comparison of rats treated with arsenic as against those co-treated with arsenic (sodium arsenite, 20 mg/kg b.w.) and curcumin (100 mg/kg b.w.) orally for 28 days, co-treatment increased the neurobehavioral performance, binding of striatal dopamine receptors, and TH expression while it decreased arsenic levels and oxidative stress in brain regions [102]. Decrease of dopamine (DA), norepinephrine (NE), epinephrine (EPN), serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in corpus striatum and hippocampus observed in arsenic rats showed a trend of recovery in rats co-treated with arsenic and curcumin. Furthermore, increased levels of NO in different brain regions in arsenic-treated rats were found decreased in rats co-treated with arsenic and curcumin [105]. Co-treatment with arsenic and curcumin increased learning and memory performance associated with increased binding of 3H-QNB and activity of acetylcholinesterase in brain as compared to arsenic-treated rats. Increase in the expression of ChAT protein, immunoreactivity of ChAT, and staining of Nissl bodies in the hippocampal region was also observed in rats co-treated with arsenic and curcumin as compared to those treated with arsenic alone [106].

Taurine, an end product of L-cysteine metabolism, is the most abundant free amino acid in many tissues. It protects many of the body's organs against toxicity and oxidative stress caused by various toxic substances [107]. Oral administration of taurine (100 mg/kg b.w. for 5 days) was found to be very effective in the prevention of As-induced oxidative impairment in the brain tissue of the experimental rats [108]. In another study, As exposure resulted in swellings, evident vacuolar degeneration in cytoplasm, karyorrhexis and karyolysis, and intensive expression of 8-nitroguanine in the brain of mice. Weak expression of 8-nitroguanine and relatively light pathological changes were observed in brain of the mice co-administered arsenic and taurine [109]. The protective effect of taurine on the disturbed biogenic amine neurotransmitter levels in the mouse brain induced by arsenic was examined in a recent study. The brain of rats exposed to As alone showed significantly decreased concentrations of NE, DA, and 5-HT, number of synaptic vesicles, and gene expressions of TH, TPH, and DBH. Administration of taurine significantly alleviated these toxic effects [110].

The neuroprotective effects of Zn, vitamin C, and vitamin E were investigated. Perinatal arsenic exposure resulted in less body weight gain, and retardation in development of morphology and sensory motor reflexes of the pups. Significant decreases in motor behavior along with significant decreases in GSH levels in the serum were observed in young adults. On the other hand, serum γ-GT and TBARS were significantly increased due to arsenic treatment. However, zinc in animals co-exposed to arsenic with zinc showed a remarkable ameliorating effect on all observed teratological and biochemical arsenic toxicities in male offspring [111]. Zinc also protected against arsenic-induced apoptosis in a neuronal cell line in a concentration-dependent manner [72].

One study was designed to investigate the protective effect of the antioxidants Zn, vitamin C, and vitamin E against toxic effects of arsenic exposure on the brain during pregnancy and lactation. Arsenic exposure enhanced alkaline phosphatase (ALP) activity, acetylcholinesterase activity, TBARS, and catalase activity. Co-exposure of arsenic and antioxidants (a mixture of Zn, vitamin C, and vitamin E) reversed the increase of ALP and TBARS [112]. Arsenic exposure caused disturbance in lipid peroxidation, and generation of nitric oxide (NO), ROS, apoptosis, and necrosis in human fetal brain explants. The administration of vitamins C, E, and DMSA showed partial reversal of the effects, indicating possible protection from arsenic toxicity [57].

Some studies found that exogenous glutathione and methionine can ameliorate the effects of arsenicals on NO metabolism in the brain and reduce arsenic burden in both blood and brain. Mice were exposed to sodium arsenite (50 mg/L in drinking water) for 4 weeks and treated with saline solution and glutathione (200, 400, or 800 mg/kg b.w.) at the 4th week. It was found that exogenous glutathione could promote both primary and secondary arsenic methylation capacity in the liver, which might facilitate excretion of arsenicals, and consequently reduce arsenic burden in both blood and brain. Administration of glutathione could significantly increase NOS activities and NO contents [113]. Exogenous methionine had almost the same effect as exogenous glutathione on arsenic burden and NO metabolism in brain of mice exposed to arsenite [114].

One study reported that arjunolic acid, a triterpenoid saponin, possessed the ability to ameliorate arsenic-induced oxidative insult in murine brain and is probably due to its antioxidant activity [115]. In another study, therapeutic efficacy of some thiol chelators was compared in terms of reducing arsenic burden, as well as recovery in the altered biochemical variables particularly suggestive of oxidative stress. Among these thiol chelators, monoisoamyl DMSA (MiADMSA) was the most effective chelator in reducing ROS and in counteracting arsenic-induced inhibition of ALAD, SOD, and GPx activity in the brain [116].

15.7 Conclusions

Epidemiologic (Table 15–1) and animal studies (Table 15–2) have demonstrated that arsenic exposure impairs neurodevelopment, and this adverse effect of arsenic may gradually accumulate over time. During gestation and lactation, both inorganic arsenic and its methylated metabolites (MMAs and DMAs) can readily cross the animal and human placenta and enter the fetal system. Animal studies found that, when exposed to arsenic in early life, arsenic can cross the immature BBB easily. Furthermore, arsenic exposure could impair the structure and function of the BBB. Our current knowledge indicates that arsenic may impair neurotransmitter systems, and impair neuronal signal transduction through impaired astrocyte and neurite growth, and myelin formation. In addition, arsenic can cause neuron apoptosis, and alter methylation status, level of nitric oxide, and expression of some important genes in the brain. Some plant extracts, vitamins, Zn, and pharmacological agents have shown their neuroprotective efficacy against arsenic-induced neurotoxicity. In the future, more studies are needed to uncover the molecular mechanisms of arsenic-induced neurodevelopmental toxicity and find more effective neuroprotective agents to counter toxicity.

Table 15–1 Neurodevelopmental Effects of Arsenic Exposure in Children

Population Studied				
Number (male; female)	**Age (years)**	**Concentration of As (mean ± SD)**	**Neurodevelopmental Effects**	**Reference**
Exposed: 41 (18; 23) Control: 39 (23;16)	7.6 ± 0.8 7.4 ± 1.0	UAs: 62.9 ± 0.03 (μg As/g Cr) UAs: 40.2 ± 0.03 (μg As/g Cr)	Decreased verbal IQ, poor performance on long-term memory and linguistic abstraction	[15]
High As: 29 (16; 13) Low As: 20 (10; 10) Control: 60 (27; 33)	13.5 ± 0.6 13.4 ± 0.5 13.4 ± 0.6	WAs: 184.99 ± 225.29 μg/L WAs: 131.19 ± 343.70 μg/L NR	Alterations in pattern memory and switching attention	[16]
351 (190; 161)	5–15	Peak lifetime water: 147 ± 322 μg/L Average lifetime water: 59 ± 133 μg/L Pregnancy water: 110 ± 243 μg/L Current child urine: 78 ± 61 μg/L	Decrements in intellectual testing: (1) vocabulary test; (2) the object assembly test; (3) the picture completion test	[14]
201 (98; 103)	10 ± 0.4	Mean (range) (μg/L) WAs: 177.8 (0.094–790)	Reduced intellectual function: lower performance and full-scale scores	[18]
301 (150; 151)	6 ± 0.18	Mean (range) (μg/L) WAs: 120.1 (0.1–864)	Reduced intellectual function: lower performance and processing speed raw scores	[19]
High As: 91 (37; 54) M-As: 243 (135; 118) H-F: 180 (92; 88) Control: 196 (112; 84)	8–12	WAs: 190 ± 183 μg/L WAs: 142 ± 106 μg/L WAs: 3 ± 3 μg/L; F: 8.3 ± 1.9 mg/L WAs: 2 ± 3 μg/L; F: 0.5 ± 0.2 mg/L	Reduced intellectual function	[17]
9–10 years: 232 4–5 years: 176	9–10 4–5	UAs mean (range) 181.9 (0.6–2336) μg/L UAs mean (range) 137 (5.0–1021) μg/L	Reduced intellectual function in 9–10 group	[20]
591 (319; 272)	6–8	UAs: 58.1 ± 33.2 μg/L BPb: 11.5 ± 6.3 μg/dL	Reduced cognitive development Different effects on boys and girls Independent of any effect of lead	[21]
299 (151; 148)	9.64 ± 0.77	BAs: 4.81 ± 3.22 μg/L UAs: 78.09 ± 72.16 μg/L BMn: 14.78 ± 3.72 μg/L	BAs negatively related to most WISC-IV subscale scores UAs negatively related to verbal comprehension scores	[24]

1652 (894; 758)	Mother's age: 27.7 ± 4.1	Median (μg/L) BAs: 0.86; BPb: 41 BHg: 1.88; BCd: 0.03	Decreased neonatal behavioral neurological assessments (DNBN) score; considered safe level of As related to lower DNBN scores	[28]
3988 (1861; 2127)	6.5–11.5	In soil: Mean (range) (mg/kg) As: 2.6 (0–42.1); Pb: 35.4 (0.9–1800)	Increased probability of intellectual disability (ID) Interaction between Pb and As on probability of ID	[22]
1700 (884; 816)	1.5 years old 5 years old	Median (10th and 90th percentiles) Maternal UAs (mg/L) GW 8: 81 (24, 380) GW 30: 84 (26, 415) Children UAs (mg/L) At 1.5 years: 34 (12, 155) At 5 years: 51 (20, 238)	Reduced verbal IQ (VIQ) and full scale IQ (FSIQ) in girls	[27]
100	Mother's age: 22.9 ± 3.7 GW: 38.9 ± 1.4	Mean (range) (μg/L) As (n = 94): 1.46 (0.51–9.58) Pb (n = 79): 31.7 (6.83–220.8) Zn (n = 94): 2286 (1299–6430)	Cord blood levels of Pb and As inversely related with neurodevelopment of newborns	[25]
1799	7 months	Maternal UAs: Median (IQR) (μg/L) GW8: 81 (37–207); GW30: 84 (42–230)	No significant effects on children's motor and PST scores and behavior ratings	[26]
117 (62; 55)	12–14	WAs (μg/L): undetectable–3590	Slow nerve conduction velocity	[31]
303 (151; 152)	9.6 ± 0.7	BAs (μg/L): 4.8 ± 3.2 WAs (μg/L): 43.3 ± 73.6 UAs (μg/L): 78.0 ± 72.1 UAs (μg/g Cr): 246.5 ± 183.9	Low levels of As in drinking water inversely associated with motor function scores; Blood selenium was positively associated with motor function scores	[29]
526 (292; 234)	6.9 ± 0.4	Median (IQR) (μg/L) Total UAs: 55.2 (39.7); InAs: 7.5 (6.6) MMA: 6.7 (5.9); DMA: 39.3 (28.5)	Moderate association between TAs and DMA in urine and teacher ratings of oppositional, cognitive problems and ADHD-like behaviors among the children	[30]

TAs, total arsenic; InAs, inorganic arsenic; UAs, urinary arsenic; WAs, water arsenic; BAs, blood arsenic; M-As, medium-arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; BMn, blood manganese; BHg, blood mercury; BPb, blood lead; Cr, creatinine; H-F, high fluoride; IQ, intelligence quotient; IQR, interquartile range; GW, gestational week.

Table 15–2 Neurobehavioral Toxicity of Arsenic Exposure in Animals

Animal	Exposure Duration	Arsenic Dose	Neurobehavioral Toxicity	Reference
Male SD rat	GD15 or PND1 to 4 months	36.70 mg/L in drinking water	Increased spontaneous locomotor activity Increased number of errors in a delayed alternation task	[32]
Wistar rat	GD 6 to PND 42	0, 10, 50, 100 mg/L $NaAsO_2$ in drinking water	100 mg/L group: Decreased number of incidences in tail hung, auditory startle and visual placing; Poor performance in water maze test	[33]
C57BL6/J mice	GD4 to birth	0, 8, 25 or 80 ppm $NaAsO_2$ in drinking water	Impairment in development of gait, acoustic startle, righting reflexes, and forelimb grip	[34]
Male SD rat	GD 1 to PND 21 or PND 120	0, 2.62, 13.6 mg/L $NaAsO_2$ in drinking water	Impaired performance in surface righting, negative geotaxis, and cliff avoidance	[35]
C57BL6/J mice	GD 1 to PND 23	50 ppb $NaAsO_2$ in drinking water	Increased learned helplessness and measures of immobility in a forced swim task in adult mouse offspring	[36]
C57BL6/J mice	GD 1 to PND 23	50 ppb $NaAsO_2$ in drinking water	Reduced level of corticosterone receptor Impaired performance in object recognition task	[37]
Wistar albino rat	GD 8 to parturition	0, 1.5, 3.0, and 4.5 mg As/kg/day/p.o.	No significant changes in neurobehavioral developmental tests	[38]
Male weaning SD rat	3 months	0, 2.62, 13.6, and 68 mg/L $NaAsO_2$ in drinking water	68 mg/L group: impaired performance in Morris water maze test	[40]
Male weaning SD rat	3 months	10 and 15 mg/kg $NaAsO_2$ in drinking water	15 mg/kg group: Impaired performance in the Morris water maze and Y maze tasks	[39]
Male Wistar rats	6 months	1 mg/kg $NaAsO_2$ or 10 mg/kg GaAs, oral	Significant deficit in hidden platform acquisition	[41]
Weaning male SD rat	1 year	0.05, 0.5, and 50 mg As/L in drinking water	Decrease in total-distance number of movements, and movement time during the dark phase	[42]

SD, Sprague-Dawley.

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16

Developmental Arsenic Exposure Impacts Fetal Programming of the Nervous System

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CHAPTER OUTLINE

16.1 Introduction

Developmental processes (i.e., gestation and early childhood) in the nervous system are vulnerable to disruption by some chemicals at doses that may not be toxic to mature systems [1,2]. The vulnerability of the brain originates from the combination of immaturity and ongoing development [3]. The fetal and infant environment is known to be critical for the development of adverse health effects later in life [4]. Fetal and early postnatal development constitutes the most vulnerable stages with regard to adverse effects of some toxicants [5]. It has been reported that one in every six children has a developmental disability and in most cases these disabilities affect the nervous system [6]. The most common neurodevelopmental disorders include learning disabilities, sensory deficits, developmental delays, and cerebral palsy [6]. Treatment of these disorders is difficult and the disabilities may be permanent [3]. There is increasing evidence for adverse effects of early-life exposure to toxic chemicals on organ function late in life [4]. Thus, it is plausible that arsenic (As) and its metabolites, which easily pass to the fetuses, may contribute to such effects. Moreover, arsenic has been recognized as a cause of neurodevelopmental disorders [7,8].

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There is a growing body of evidence that the intrauterine or early childhood exposure to arsenic also induces changes that will become apparent much later in life [5,8]. Epidemiological studies indicated that exposure to arsenic during early childhood or *in utero* was associated with an increased morbidity from nervous system disease. Several cross-sectional studies have shown the links between arsenic exposure and neurobehavioral deficits in school children [3]. For example, the incident of the Morinaga dried milk poisoning, where children eating As-contaminated milk powder revealed neurologic diseases, neurobehavioral dysfunction, and decreased cognitive skills [3]. Similar results were also obtained in children with arsenic exposure from a smelter [1]. Such evidence supports the notion that arsenic is a developmental neurotoxicant [3]. The developmental neurotoxic effects of arsenic are dependent on dose, route, and the day of gestation when exposure occurs [4]. It has been reported that a significantly lower dose of arsenic was sufficient to induce apoptosis in neuronal cells as compared to non-neuronal cells, illustrating the sensitivity of the nervous system to this metal [9]. It was reported that rats exposed to arsenic during development presented deficits in neurological function, suggesting neurodevelopmental risk with low concentrations [4]. Research has indicated that prenatal arsenic exposure increases the risk of adverse effects during early childhood [5] and produces developmental toxicity, including malformations, decreased prenatal rate of growth, and increased mortality and neural tube defects [3]. Some studies in neuronal cells exposed to arsenic showed decreased cell viability, altered cell growth, and apoptosis [10,11] reflecting mechanisms by which low doses of arsenic may lead to neurodevelopmental toxicity.

In this chapter, the accumulation of arsenic in fetal brain tissue, its effects on neurodevelopment and neurobehavior, and the mechanisms of As-induced developmental neurotoxicity are stated. Moreover, effects of developmental arsenic exposure on fetal programming of the nervous system are also discussed.

16.2 Accumulation of Arsenic in Fetal Brain Tissue

Although the placenta serves as a natural barrier between mother and fetus, it has been fully demonstrated that arsenic readily passes the placenta in humans [12–14] and in other mammals [15,16]. Concha et al. reported that the median maternal and cord blood arsenic levels in northern Argentina were 11 μg/L and 9 μg/L, respectively [13]. Hall and colleagues reported that the mean maternal and cord blood arsenic levels in Matlab, Bangladesh, were 11.9 μg/L and 15.7 μg/L, respectively [14]. These investigations suggested that the level of exposure of the fetus to arsenic is similar to that in the mother. Research by Concha et al. indicated that in contrast to the free passage of arsenic over the placenta to the fetus, the passage over the mammary gland is limited, and little arsenic is excreted in breast milk [13]. Working in Argentina, these researchers found a decrease in arsenic concentrations in the urine of the infants from about 80 μg/L during the first 2 days of life to less than 30 μg/L at 4 months of age [13]. This research has showed that the main route of arsenic into the developing organism is transplacental exposure *in utero*.

Once in the developing body, both arsenic and its methylated metabolites (MMA, DMA) cross the immature blood–brain barrier (BBB) easily and arrive at the brain, which has been

proved in animal experiments. Jin et al. treated maternal mice with arsenic-containing drinking water at concentrations of 0, 10, and 30 ppm for the entire pregnancy [17]. It was detected that the levels of total arsenic in the brains of newborn mice were <0.01 μg As/g in controls, and 0.07 μg/g in the 10 ppm and 0.22 μg/g in the 30 ppm groups, respectively. In terms of content, the level of total arsenic in the brain of newborn mice was similar to that in their own liver, and was far higher than that in the brain of their mothers. Xi et al. administered arsenic to rats from day 6 of gestation (GD 6) until postnatal day 42 (PND 42) at concentrations of 0, 10, 50, and 100 mg/L. It was shown that total arsenic content in brain tissue of pups on PND 42 was significantly increased in all three As-exposed groups compared with control [18]. A dose-related trend was found between total brain arsenic content and arsenic concentrations in drinking water. This indicated that the protection of the immature BBB against arsenic passing from blood to brain is very limited. Together with imperfect eliminating function of developing kidney, all these factors result in relatively high levels of arsenic in the brains of early-life organisms.

16.3 Effect of Arsenic on the Development of the Nervous System

The normal developmental processes of the nervous system are an outcome of extremely precise, interdependent cellular and molecular events such as protein synthesis, membrane development associated with axonal extension, dendritic outgrowth, synaptogenesis, and response to growth factors. Any type of alteration could affect the final outcome of this complex coordination [19]. It has been demonstrated that arsenic exposure during the developmental period leads to structural alterations in neuronal circuitry, and these provide the morphological evidence of structural alterations and functional deficits encountered in the later period of life.

Neurodevelopmental toxicity is shown in Table 16–1. Wlodarczyk et al. reported that after pregnant LM/Bc dams were injected intraperitoneally on GD 7 and GD 8 with 40 mg/kg of arsenate, the exencephaly was shown in 90 to 100% of the exposed fetuses [20]. Hill et al. found that, after maternal intraperitoneal exposure to sodium arsenate at 9.6 mg/kg in LM/Bc/Fnn mice on GD 7.5 and 8.5, neural tube defects were induced in all mice pups [21]. Similar results were also reported by Wlodarczyk et al. [22]. Nagaraja and Desiraju observed reduced body and brain weight in rats following arsenic administration from days 2 to 60 after birth and drew an association between decreased body and brain weight during the developmental period of the exposed animals [23]. Tyler et al. found that exposure to a low concentration of 50 ppb arsenic over the first three trimester equivalents significantly decreased the number of differentiated neural progenitor cells in adult mice. Additionally, prenatal arsenic exposure altered 31% of target neurogenesis-related genes as well, including several involved in growth and differentiation in adult animals [24]. Dhar et al. reported that exposure to arsenic in the stage of rapid brain growth in rats was associated with defects in migration, delayed maturation, and alteration in the nuclear area of the Purkinje cells of the cerebellum. Developmental arsenic exposure alters cerebellar morphology in the brain [19].

Table 16–1 Experimental Studies on Arsenic Neurodevelopmental Toxicity

First Author and Year	Species	Treatments	Reported Effects
Single-or multiple-day administration during embryogenesis			
Finnell et al. (1996) [20]	mice (LM/BC)	Arsenate, single i.p. injection, GD 7:12 (day: hour) and 8:12, 40 mg/kg	40 mg/kg: increased % malformed fetuses (90–100%), especially neural-tube defect
Hill et al. (2009) [21]	mice (LM/BC)	Sodium arsenate, single i.p. injection, GD 7.5 and 8.5, 9.6 mg/kg (fetuses assessed GD 9.0)	100% NTDs
Wlodarczyka et al. (2000) [22]	mice (STOCK-Folr2tm Fnn null)	Arsenate, single i.p. injection, 9.6 mg/kg, E 7:12 and 8:12 (assessed 3 h, 12 h, 24 h later)	Developmentally delayed embryos; most with closed neural tube only at closure site I; significantly fewer somites
Nagaraja and Desiraju (1994) [23]	Rat (Wistar)	Arsenate, 5 mg/kg/day, PND 2 to 60	Decreased body and brain weights
Tyler and Allan, (2013) [24]	mice (C57BL/6J)	Arsenate, 50 ppb, drinking water, GD 0 to PND 23–25 (fetuses assessed on GD 34, 35, 63, 70)	41% decrease in number of differentiated neural progenitor cells, 31% alteration of target neurogenesis-related genes
Short- or long-term arsenic exposure *in vitro*			
Frankel et al. (2009) [25]	PC12 cells	Arsenite, 0, 0.5, 1, 2.5, 5, 10 mM cells (assessed 48, 72, and 120 hours later)	Reduced neurite production, outgrowth and complexity in newly differentiating PC12 cells; inhibited further neurite development in more mature PC12 cells
Chattopadhyay et al. (2002) [11]	human fetal brain explants[a] rat neonatal brain explants[b]	Arsenite 0.3 mg/L, 24 h[a]; arsenite, 0.3 mg/L, drinking water, GD 0 to PND 1[b]	[a] Cytoplasm vacuoles, frothing and nuclear condensation with the appearance of intact membrane [b] Intact membrane, large vacuoles and loss of cell–cell junction
Namgung and Xia, (2000) [26]	Primary cortical neurons from newborn SD rats	Arsenite 0, 2, 5, 7, 10 μM; 12, 24, 48 h	Apoptosis; As concentration and time dependence
Sidhu et al. (2005) [27]	Primary embryonic rat midbrain (GD 12) neuroepithelial cells	Arsenite 0, 0.5, 1, 2, and 4 μM; 48 h	Cell viability reduction and cell cycle progression, As concentration and time dependence, 4 μM 24 h: apoptosis

GD: gestation day; PND: postnatal day: NTDs: neural tube defects.

A series of morphological changes were also found in developing neurons subsequent to arsenic exposure *in vitro*. Observation on human fetal brain explants exposed to arsenic showed cytoplasm vacuoles, frothing, and nuclear condensation with the appearance of intact membrane on day 12. Exposure to arsenic for 18 days resulted in signs of cellular degeneration, appearance of large vacuoles, and loss of cell–cell junction [11]. Arsenic exposure induced

reduction in the viability of embryonic primary rat midbrain neuroepithelial cells with concentration- and time-dependent effects *in vitro* [27]. Namgung and Xia [28] and Chattopadhyay et al. [10] reported that primary neonatal rat neural cells exposed to arsenic showed a reduction of neuronal viability and morphological changes. Frankel et al. reported that arsenic has concentration- and time-dependent effects on initial neurite outgrowth *in vitro*. Exposure to low micromolar levels of arsenite for 5 days resulted in reduced neurite production and outgrowth and complexity in newly differentiating PC12 cells. Furthermore, it was found that exposure of more mature PC12 cells to arsenite could inhibit further neurite development. These results indicated that exposure to arsenic disrupts early stages of neuron differentiation [25].

Neurite outgrowth is an indispensable process for proper development of the nervous system. Abnormal formation of neurites during development is correlated with developmental disabilities and impaired behavioral functions in rodents and primates, including humans. Wang et al. demonstrated that arsenic inhibits neurite outgrowth by inhibiting the LKB1–AMPK signaling pathway. Oxidative stress induced by As, especially excessive superoxide, plays a critical role in blocking the LKB1–AMPK pathway [29]. Neurite outgrowth is mainly orchestrated by three cytoskeletal components: microtubules, microfilaments, and neurofilaments. The coordinated rearrangement of cytoskeletal components, especially microtubules and microfilaments, plays a key role in the transition of neurons from an undifferentiated state to neurite-bearing morphology [30]. $NaAsO_2$ increased the mRNA levels of the light and medium subunits of neurofilaments and decreased the mRNA levels of tau and tubulin in a dose-dependent manner in SCAT3-expressing cells. The area ratio of neurite to cell body was significantly reduced by $NaAsO_2$. The changes in cytoskeletal gene expression are likely responsible for the inhibitory effects of $NaAsO_2$ on neurite outgrowth [30].

16.4 Effect of Arsenic on Neurobehavior

It was reported that arsenic exposure induced neural and behavioral changes and the alterations caused were correlated with the exposure dose, the duration of exposure, and the experimental conditions. Moreover, the animals exposed to arsenic had taken longer to acquire the learned behavior and to extinguish the operant [31]. Epidemiological studies on adverse effects of exposure to arsenic on neurobehavior are included in Table 16–2. Epidemiological studies indicated that arsenic exposure correlated with various learning and cognitive deficits in children in Bangladesh [32–35], West Bengal [45], Thailand [46], India [17], China [37,38], and Mexico [39–41] as well as in adults in Texas, USA [42] (Table 16–2). The intellectual function of 529 children aged 6–9 years in the As-exposed region of Thailand was assessed by using the Wechsler Intelligence Scale for Children (WISC). The percentage of children in the average intelligence quotient (IQ) group decreased remarkably from 56.8 to 40.0 as the arsenic level increased. Moreover, a statistically significant relationship with arsenic levels could explain 14% of variance in children's IQ was observed after adjusting for confounders [46]. Meta-analysis suggested that a 50% increase of arsenic levels in urine would be associated with a 0.4 decrease in the IQ of children aged 5–15 years [47]. Rocha-Amador et al. observed a decrease in full scale IQ in children aged 6–10 years when total arsenic levels were increased in drinking water [41].

Table 16–2 Summary of Epidemiological Studies Assessing Neurobehavioral Disorders from Arsenic Exposure

Location	Age	Sample Size	Study Design	Confounders Accounted for	Exposure Measure
Bangladesh	10 years	201	Cross-sectional	Maternal education, maternal intelligence, house type, TV access, height and head circumference	Water, Urine
India	5–15 years	351	Cross-sectional	Age, sex, maternal and paternal education, father's occupation, number of rooms in the house, type of house building material, BMI, mother's age	Water, Urine
Bangladesh	5 years	2260	Longitudinal cohort	Age, HOME, father's education, mother's BMI and IQ, assets, housing, number of children in the household, gestational age, birth length, concurrent HAZ and testers	Maternal urine at pregnancy Child urine
Bangladesh	8–11 years	299	Cross–sectional	Maternal intelligence, maternal age, school months, head circumference, plasma ferritin and blood manganese	Blood
India	5–15 years	351	Cross-sectional	Age, sex, maternal and paternal education, father's occupation, number of rooms in the house, type of house building material, BMI, and mother's age	Water, Urine
China	8–12 years	720	Cross-sectional	None	Water, Urine
China	4.5 years	106	Prospective cohort	Cord blood Pb, maternal age, height, weight, gestational weeks, maternal education, method of delivery, breastfeeding, nursery school age, tobacco exposure and family income	Placenta, maternal blood, cord blood
Mexico	6–9 years	41	Cross-sectional	Sex, age, socioeconomic status and parent's education	Urine
Mexico	6–8 years	602	Cross-sectional	Age, sex, mother's school level, Hb, Pb and for Pb × AsU interaction	Urine
Mexico	6–10 years	132	Cross-sectional	Blood Pb, socioeconomic status, mother's education, height-for-age z-score and transferrin saturation	Water, Urine
USA	40–96 years	434	Community-based participatory research	Age, sex, education, ethnicity, APOE4 genotype, residence	Water
USA	11–13 years	31	Cross-sectional	Sex, maternal education	Hair
Taiwan	13–14 years	109	Cross-sectional	Sex, education	Water

[a]Median.
[b]Interquartile range.
[c]Geometric mean.
[d]10th and 90th percentiles; AsH: arsenic in hair; AsN: arsenic in nails; AsS: arsenic in soil; AsU: arsenic in urine; AsW: arsenic in drinking water; BMI: body mass index; DMA: dimethylarsinic acid; HAZ: height-for-age z-score; Hb: hemoglobin; In-As: inorganic arsenic; IQ: intelligence quotient; MMA: monomethylarsonic acid; MQ: methodological quality; Pb: lead; T-As: total arsenic; WHZ: weight-for-height z-score; CBPR: community-based participatory research; DPAA: diphenylarsinic acid.

Type of Arsenic	Mean ± SD (range)	Psychological Test	Observed Effect	MQ	Reference
T-As	118 ± 145 µg/L water 116.6 ± 148.8 µg/L urine	WISC-II	AsW↓ Full-scale and performance IQ	High	[32]
T-As	147 ± 322 µg/L water (1–2480) 78 ± 61 µg/L urine (2–375)	WISC-III CRT SBIS	AsU↓ Vocabulary test, object assembly test and picture completion test	High	[33]
In-As T-As	84 µg/L maternal urine[a,b] (26–415)[d] 51 µg/L Child urine[a] (20 –238)[d]	WPPSI–III	AsU↓ Full-scale and verbal IQ in girls, but not in boys	High	[34]
T-As	4.81 ± 3.22 µg/L	WISC-IV	AsB↓ Full-scale score, verbal comprehension and working memory	High	[35]
T-As	147 ± 322 µg/L water (1–2480) 78 ± 61 µg/L urine (2–375)	WISC-III CRT SBIS	AsU↓ Vocabulary test, object assembly test and picture completion test	High	[36]
T-As	190 ± 183 µg/L water 73 ± 3 µg/L urine	CRT-RC2	AsU↓ Full-scale IQ	High	[37]
T-As	0.15 µg/g placenta[a] 1.80 µg/L maternal blood[a] 0.60 µg/L cord blood[a]	WPPSI-R	CdCB↓ Full-scale and performance IQ	High	[38]
T-As	62.9 ± 0.03 µg/g crea (27.5–186.2)	WISC-RM	AsU↓ Full-scale and verbal IQ	Medium	[39]
In-AMMA, DMA	58.1 ± 33.2 µg/L[c]	WISC-RM NLS CAT	AsU↓ Digit span subscale, letter sequencing, visual search	High	[40]
T-As	194 ± 1.3 µg/L water[c] 116 ± 2.2 µg/g crea[c]	WISC-RM	AsW↓ Full-scale, performance and verbal IQ AsU↓ Full-scale IQ	High	[41]
T-As	2.19–15.26 µg/L water	CBPR	AsW↓ Language, visuospatial skills, executive functioning, global cognition, processing speed, and immediate memory	High	[42]
T-As	0.018 ± 0.014 µg/g (0.001–0.055)	WASI CVLT-C WRAML	AsH↓ Full-scale and verbal IQ and memory test	Low	[43]
T-As	184.99 ± 225.29 µg/L	NES2-T	AsW↓ Pattern memory and switching attention	High	[44]

Wang et al. also obtained a similar result when arsenic levels were increased in drinking water, in 720 children aged 8–15 years in rural villages in Shanyin county, Shanxi province, China. Wasserman et al. also documented the same association with total arsenic levels in water in relation to 6- and 10-year-old children's intellectual function [32,33]. In the study of 201 children in Araihazar, Bangladesh, it was found that exposure to arsenic from drinking water was associated with reduced intellectual function in WISC III for children, in a dose–response manner after adjustment for sociodemographic covariates and water Mn. Children with water arsenic levels >50 mg/L achieved significantly lower performance and full-scale scores than did children with water arsenic levels <50 mg/L. The association was generally stronger for well water arsenic than for urinary arsenic [32]. In a later study conducted in children aged 8–11 years, a decrease in full scale IQ score, verbal comprehension and working memory was observed, associated with increased levels of total arsenic in blood [35]. Wright et al. also observed a negative effect on full-scale and verbal IQ when they studied total arsenic levels in hair among children aged 11–13 years [43]. Developmental and continuous arsenic exposure induced significant deficits in verbal IQ and long-term memory in children aged 6–9 years, as measured by the WISC [39]. A cross-sectional study was conducted for examining cognitive function in 49 adolescents exposed to high and in 60 controls exposed to low levels of arsenic in drinking water in Taiwan. The results showed that memory and switching attention were significantly affected by long-term cumulative exposure to arsenic after adjusting for education and sex [44]. Von Ehrenstein et al. conducted a cross-sectional study among 351 children age 5 to 15 years in West Bengal, India. They found associations between arsenic concentration in the children's urine and intellectual function assessed using six subtests from the WISC as well as using the Total Sentence Recall test, the Colored Progressive Matrices test, and a pegboard test. Moreover, there were also significant relations between urinary arsenic and reductions in the adjusted scores of the vocabulary test, the object assembly test, and the picture completion test [36]. Neurobehavioral outcomes are influenced by the age at examination and many other covariates, such as nutrition. The exposure to arsenic and factors influencing the susceptibility at the time of the study may be very different from that occurring prenatally or in early childhood. Therefore, longitudinal studies are warranted for evaluation of late effects of early-life exposure. No such studies have yet been reported [5]. But it was found that infants who survived the Morinaga milk arsenic poisoning incident had highly raised risks of neurological disease during adult life [48].

Transplacental and early-life exposure to inorganic arsenic affected learning and memory functions and neuromotor reflex in offspring rats [49]. After administration of 100 mg/L diphenylarsinic acid (DPAA) in drinking water for 14–21 days, young rats showed behavioral abnormalities, such as gait disturbance and deficient acclimatization to a novel condition in the open field test [50]. Markowski et al. assessed the effects of prenatal exposure to arsenite on motor and food-motivated behaviors from birth to adulthood in C57BL6/J mice. The results showed that developmental arsenic exposure can produce other behavioral impairments in children in addition to cognitive impairment [51]. Rats exposed to arsenite at concentrations of 37 mg/L drinking water from GD 15 until 4 months of age showed increased spontaneous locomotor activity and alterations in a spatial learning tasks compared to control rats [4]. Rodriguez et al. also reported that spontaneous locomotor activity and alterations in

a spatial learning task were deficits among the rats exposed to arsenic during development [52]. Developmental subchronic exposure to diphenylarsinic acid induced transient increased exploratory behavior, impaired learning behavior, and decreased cerebellar glutathione concentration in rats [53]. Exposure of young rats to 5 mg/kg arsenate resulted in increased time to acquire operant learning. At non-maternally toxic levels, arsenic given to pregnant dams via drinking water affected fetal brain development and postnatal behaviors [54]. However, the underlying mechanisms responsible for these behavioral outcomes in adulthood after developmental exposure to arsenic have not yet been elucidated [24].

16.5 Mechanisms for the Effect of Arsenic on the Nervous System During Development

The mechanisms involved in arsenic-induced developmental neurotoxicity likely include inductions of oxidative stress, apoptosis, and oxidative DNA damage, effects on neurotransmitters and the neuroendocrine and cell cycle, perturbation of one-carbon metabolism, epigenetic effects, etc. The major mechanisms are discussed as follows.

16.5.1 Induction of Oxidative Stress

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses. Oxidative stress is believed to cause a number of diseases. The oxidative stress induced by chronic exposure to arsenic is related to cytotoxic and genotoxic effects in the cells, playing a crucial role in the pathogenesis of nervous system disorders [55]. One of the principal mechanisms of arsenic toxicity is the induction of a strong oxidative stress with production of ROS in cells [56]. Several studies have shown that cell populations chronically exposed to arsenic have significant oxidative stress that, in turn, induces DNA damage [57,58], as well as lipid peroxidation and decreased glutathione levels [59,60]. There is increasing evidence that the induction of ROS plays a crucial role in arsenic neurotoxicity. Arsenic exposure was found to induce an increase in the production of ROS in human fetal brain explants [11]. Chattopadhyay et al. also reported that the human fetal brain cells that were exposed to arsenic in culture and neonatal rat brain cells derived from arsenic-treated mothers showed an increase in NO and ROS [10].

Arsenic may induce the production of ROS by impacting on antioxidant enzymes. It was reported that arsenic exposure resulted in a significant decrease in the activity levels of antioxidant enzymes such as manganese-superoxide dismutase (Mn-SOD), Cu/Zn superoxide dismutase (Cu/Zn-SOD), catalase (CAT), and glutathione peroxidase (GPx), while malondialdehyde (MDA) levels were significantly increased in the cerebral cortex, hippocampus, and cerebellum of developing rats [61]. Kadeyala et al. demonstrated that arsenic exposure significantly decreased the activities of SOD isoforms, CAT, GPx, and glutathione reductase (GR) with an increase in glutathione-S-transferase (GST), while lipid peroxidation (LPx) and arsenic levels were significantly increased in different brain regions. The induced alterations in these parameters were more pronounced in the cerebral cortex [62]. Gupta and Flora reported that

the rats exposed to arsenic showed significant increases in oxidized glutathione (GSSG) levels in the brain [63]. However, the activities of brain SOD and CAT decreased marginally on arsenic exposure [63]. Developmental subchronic exposure to DPAA also induced reduction in the concentration of cerebellar GSH, which might be a primary cause of oxidative stress [53]. Comprehensive gene expression analyses in primary cultured rat cerebellar cells exposed to arsenic revealed significant alterations in the mRNA expression of genes encoding antioxidative stress proteins (heme oxygenase 1 and heat shock protein 72) [50]. Zhang et al. demonstrated that high-level arsenic induces severe redox imbalance by decreasing the levels of GSH and increasing the levels of ROS through the oxidative stress adaptor $p66^{Shc}$ [65]. Knockdown of $p66^{shc}$ improves the developmental competence of As-exposed embryos *in vitro* by increasing the resistance to oxidative stress. It is suggested that p66Shc-linked redox imbalance contributes to As-induced developmental retardation in mouse preimplantation embryos [64]. Zhang et al. showed that p66Shc-linked redox imbalance and metabolic abnormality of amino acids promote developmental delay in arsenite-exposed mouse preimplantation embryo. An antioxidant, N-acetyl-L-cysteine, improves the development of arsenite-exposed embryos by reducing intracellular ROS and adjusting amino acid metabolism. These results support further that redox imbalance is involved in arsenite-induced embryonic toxicity [65].

16.5.2 Induction of Apoptosis

It is well known that apoptosis is a form of cell death, also known as programmed cell death. Apoptosis can be triggered by exogenous and endogenous stimuli such as oxidative stress and genotoxic chemicals. It is well known that Bcl-2 family proteins and the caspase protein family play important roles in regulating the mitochondrial (intrinsic) apoptotic pathway [66,67]. Following an intrinsic death signal, proapoptotic Bcl-2 members, such as Bax, increase, and antiapoptotic Bcl-2 members, such as Bcl-2, decrease, which subsequently increases mitochondrial permeability transitions, cytochrome c release, and caspase 3 activation. The mitochondrial apoptotic pathway indicators, including mitochondrial membrane potential, cytochrome c protein, and caspase 3 activation, were all significantly changed correlating to the apoptosis trigger. A growing body of evidence indicates that abnormal apoptosis may be involved in the neurotoxicities induced by some neurotoxicants. Experiments on human fetal brain explants on exposure to arsenic in culture showed disturbance in lipid peroxidation, ROS, and apoptosis [11]. Namgung and Xia reported that apoptosis was induced in primary cerebellar and cortical neurons which were exposed to 5, 10 and 15 μM sodium arsenite [28]. *In vitro* studies showed that arsenite-induced apoptosis in cortical neurons prepared from newborn mice [68] and C17.2 cell lines originally derived from the developing mouse cerebellum neuronal cells [69], suggesting that arsenate exhibits neurotoxicity by inducing apoptosis. Arsenite leads to an increase in intracellular Ca levels and generation of ROS, which may cause a decrease in mitochondrial transmembrane potential, release of cytochrome c, and consequent activation of caspases. A slight activation of calpain also takes place, which might favor activation of the mitochondrial pathway or might activate other pathways [69]. Arsenic exposure resulted in an increase in mRNA expression levels of caspase-3 and caspase-9 in brain

regions (cerebral cortex, hippocampus) and cerebellum of developing rats [61]. Namgung and Xia reported that arsenic-induced apoptosis in cortical neurons was mediated by c-Jun N-terminal protein kinase 3 and p38 mitogen-activated protein kinase [26]. These investigators also reported that sodium arsenite selectively activated p38 and JNK3, but not JNK1 or JNK2, to induce apoptosis in cerebellar neurons from 6 to 8-day-old rats [28]. Blocking the p38 or JNK signaling pathways using the inhibitors SB203580 or CEP-1347 protected cerebellar neurons against arsenite-induced apoptosis. These data suggest that arsenite neurotoxicity may be at least partly due to apoptosis caused by activation of p38 and JNK3 MAP kinases [28].

16.5.3 Effect of Arsenic on the Cell Cycle

Arsenic exposure disrupts cell cycle dynamics of neuroepithelial cells *in vitro* [19,27]. It was reported that arsenic interferes with cell regulatory control via the induction of p53 and Bcl-2 [20]. p53 is a critical regulator of the cell cycle and has been shown to undergo a dose- and time-dependent induction in response to arsenic [70,71]. Induction of p53 in response to As_2O_3 exposure was associated with subsequent cell cycle arrest at gap 2 phase (G2)/mitosis (M) [72–74]. It has been shown that arsenic induces p21, a critical cell cycle regulatory protein. Moreover, it was reported that the induced p21 by arsenic blocked cell cycle progression at G2/M [75] as well as G1/S (synthesis phase) [76]. Cell cycle arrest at G2/M induced by arsenic [73,77] has been associated with subsequent apoptosis, indicating the latter may occur due to As-mediated cell cycle inhibition. These studies highlight the impact of arsenic on cell cycle progression and its potential link to arsenic-associated neurodevelopmental effects. Sidhu et al. reported a dose-responsive delay in cell cycle progression in primary embryonic neuroepithelial cells exposed to arsenic [27]. They also suggested that cell cycle arrest may contribute to the neurodevelopmental toxicity induced by this metal.

16.5.4 Effect on Central Neurotransmitters/Neuroendocrine

It has been reported that the neurotransmitter disrupting effects of arsenic are likely to occur at very low concentrations and to have long-term consequences, particularly if these effects are induced early in life. Moreover, it was demonstrated that developmental exposure to arsenic alters components of the hypothalamus-pituitary-adrenal (HPA) axis including increased hypothalamic corticotrophin releasing hormone (CRH), altered corticosterone (CORT) secretion, decreased hippocampal 11b-HSD 1, and altered subcellular glucocorticoid receptor (GR) distribution in the hypothalamus [78]. Arsenic has been shown to perturb components of the HPA stress axis, such as glucocorticoid receptors, and to alter gene expression of inducible genes. Perturbation of the HPA stress axis has been implicated in both cognitive damage and the promotion of carcinogenesis [79]. Mice given water with 50 μg/L arsenic during the prenatal period showed impaired regulatory interactions between the HPA axis and the serotonergic system in the dorsal hippocampus at adult age, indicating predisposition for depressive-like behavior, which was also indicated in behavioral studies [80]. There was also aberrant expression of genes related to insulin growth factor-signaling pathways [81].

It is reported that after weaning mice were exposed to arsenic over a period of 9 weeks, dopamine was significantly decreased in the hypothalamus [82]. Huo et al. reported that immature rats exposed to realgar showed significant changes of amino acid neurotransmitters, including aspartate, glutamate, glutamine, homocysteine, serine, glycine, γ-aminobutyric acid, and taurine, in the brain. The results indicated that the neurotoxicity induced by realgar may be associated with its effects on amino acid neurotransmitters [83]. Rat pups were exposed to arsenic from the second day after birth up to 60 days of age. The results showed that glutamic acid decarboxylase (GAD) activity and gamma-aminobutyric acid (GABA) levels were reduced in the cerebellum, hypothalamus, and brainstem [84]. Chandravanshi et al. investigated the changes in brain cholinergic receptors and acetylcholinesterase activity in male rats exposed to arsenic from PD 22 to PD 59 [45]. They found the decrease in the binding of muscarinic-cholinergic receptors (CHRM2) in frontal cortex and hippocampus was associated with reduced CHRM2 mRNA levels, acetylcholinesterase (AChE) activity, and expression of choline acetyltransferase (ChAT) and protein kinase C β-1. Moreover, the cholinergic alterations and AChE modifications were found to be linked with increased arsenic levels in the frontal cortex and hippocampus [45]. Nagaraja and Desiraju reported that exposure of young rats to arsenate resulted in increased time to acquire operant learning and decreased AChE activity in the brain [23]. It was reported that arsenic induced aberrant estrogen receptor signaling in pregnant mice, which may affect early-life genetic programming [5].

16.5.5 Other Mechanisms

Epigenetic mechanisms are crucial to regulate the expression of different genes required for neuronal plasticity. Neurotoxic substances such as As, which induces cognitive deficits in exposed children before any other manifestation of toxicity, could interfere with the epigenetic modulation of neuronal gene expression required for learning and memory. There is evidence that arsenic interferes with DNA methylation [85,86] and global histone acetylation [87] at very low exposure levels. The effects of arsenic may be implicated in long-term fetal programming. Martínez et al. assessed the effects of arsenic on DNA methylation patterns in the hippocampus and frontal cortex of treated rats from gestation until 4 months of age. Their results demonstrated alterations of the methylation pattern of genes involved in neuronal plasticity in an animal model of memory deficit associated with arsenic exposure [88]. Cronican et al. exposed C57Bl6/J mice to 100 mg/L arsenic in the drinking water starting 1 week before conception until birth. They found that arsenic exposure during embryonic life caused global hypoacetylation at H3K9 in brain samples from exposed pups [89].

Neural cell adhesion molecules (NCAMs) play critical roles during the development of the nervous system. Polysialylation of NCAM (PSA-NCAM) is a critical functional feature of NCAM-mediated cell interactions and functions. Luo et al. investigated the effects of maternal and early-life arsenite exposure on NCAM and PSA-NCAM in rat offspring. The results showed that maternal arsenite exposure increased the expression of PSA-NCAM, NCAM, and polysialyltransferases in the hippocampus of rat offspring on PND 21 and PND 120, which indicated

that the increased expression of these molecules might contribute to impaired neurodevelopment following arsenite exposure [2].

16.6 Conclusions and Future Directions

Taken together, epidemiological investigations and animal experiments indicate that chronic arsenic exposure can induce developmental neurotoxicity. Although the molecular mechanisms are still poorly understood, As-induced neurodevelopmental disorders may be associated with these mechanisms as discussed above. These data suggest that developmental arsenic exposure can have long-lasting effects on the nervous system well into adolescence, consistent with the fetal basis of adult disease hypothesis [90], which postulates that many adult diseases have a fetal origin. Further studies detailing the molecular effects of arsenic on the central nervous system during development are essential to explain further the mechanism of As-induced neurodevelopmental disorders including learning and memory deficits.

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17

Health Effects of Prenatal and Early-Life Exposure to Arsenic

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CHAPTER OUTLINE

17.1 Introduction

The presence of inorganic arsenic (iAs) in drinking water presents a major threat to human health around the globe. While the World Health Organization (WHO) has recommended that the levels of inorganic arsenic (iAs) in drinking water should not exceed 10 μg/L [1], it is currently estimated that up to 100 million people worldwide are exposed to drinking water containing detrimental levels of iAs [2]. The presence of elevated levels of arsenic in drinking water predominantly stems from the use of naturally contaminated groundwater, but groundwater sources can become contaminated due to anthropogenic activities such as mining as well [3]. This contamination of groundwater sources is particularly problematic in rural areas and developing countries as these areas must often rely on ground water as the predominant source of drinking water. The detrimental impact of iAs-contaminated drinking water has been particularly evident in Southeast Asian countries such as Bangladesh and India [4,5]. Bangladesh is a severely affected country, as estimates suggest 35–77 million of the 125 million inhabitants are exposed to levels of iAs in drinking water that exceed the WHO standard [6–8]. For Bangladeshis exposed to iAs levels in their drinking water at levels that exceed 50 μg/L, there are yearly estimates of over 9100 deaths and 174,000 disability-adjusted life years (DALYs) attributed to iAs-associated disease [9]. While the problem of iAs in drinking water may be most evident in Southeast Asia, iAs levels in drinking water that exceed the

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WHO standard have been detected in numerous areas around the world including Mexico and the United States of America (USA), emphasizing the worldwide nature of the problem [10].

As discussed in more detail below, numerous adverse health effects have been associated with arsenic exposure in human populations, including increased susceptibility to infections and increased risk of developing cancers and other non-communicable diseases (NCDs) later in life. The impact of NCDs on human health is of major concern as they were among the top causes of death worldwide in 2011 and were attributable for up to 67% of premature deaths, i.e., those occurring before age 60, in developing countries [11]. The susceptibility to NCDs is known to be dependent on both genetic and environmental components, and mounting evidence suggests that for most NCDs, environmental factors generally have a greater impact than genetic factors [12]. In developed countries, environmental factors such as physical inactivity and high caloric intake are believed to contribute significantly to NCD development. However, it is becoming increasingly clear that exposure to environmental toxicants, and particularly exposures that occur during critical developmental windows, is also linked to the development of NCDs such as diabetes, obesity, cardiovascular disease, and respiratory disease later in life [13]. Of great concern is the capacity of developmental toxicant exposure to not only affect the health of individuals throughout their lifetimes but also adversely affect the health of subsequent generations. For example, *in utero* exposure to maternal cigarette smoke is associated with increased risk of asthma [14] and lung function deficits [15] in children, and this risk is further increased if both the mother and maternal grandmother smoked during pregnancy [13,16]. These results underscore the urgent need for the identification of vulnerable populations such as pregnant women and the implementation of preventive measures.

In the following sections, we provide an overview of the health impacts associated with arsenic exposure and focus on health effects associated with prenatal/early-life exposure. We detail how defined time periods of arsenic exposure in human populations and animal models have helped to increase the understanding of the diversity and latencies of the observed effects, differences between susceptible populations, and the impacts of factors such as sex and co-exposures. Lastly, we discuss the molecular mechanisms implicated in the development of these latent effects and identify needs in current research trends to help better understand these mechanisms.

17.2 Adverse Health Effects Associated with Chronic and Early-Life Arsenic Exposure

17.2.1 Health Effects Associated with Chronic Exposure to iAs

Some of the best-characterized health effects associated with arsenic exposure are those observed in chronically exposed adults. The first clinical symptoms of chronic arsenic poisoning, or arsenicosis, are often specific lesions in the skin that include areas of aberrant pigmentation (both hypopigmentation and hyperpigmentation) and hyperkeratosis [17]. More prolonged exposure periods are associated with the development of numerous cancers,

especially non-melanoma skin cancers and cancers of the lung and urinary bladder, but cancers in the liver, prostate, and kidney have also been observed [1,18]. Based on epidemiological data, it has been estimated that drinking water containing 50 µg/L of iAs has a combined cancer risk of approximately 1 in 100, which is least 100 times greater than any other drinking water contaminant with a defined maximum contaminant level (MCL) [18]. Chronic arsenic exposure is also associated with non-malignant adverse health effects, including the development of NCDs such as diabetes mellitus, cardiovascular disease, and peripheral vascular disease as well as gastrointestinal effects and neurological effects [19]. The nature and severity of clinical symptoms/diseases observed in iAs-exposed individuals are likely multi-factorial and include exposure dose and duration, the genetic background and nutritional status of the population, and co-exposure to factors such as other environmental toxicants [20]. Human population studies have indicated that both cancerous and non-cancerous health effects associated with iAs exposure can be a significant cause of mortality. In a Bangladeshi population, there was an increased risk of death due to all non-accidental causes [hazard ratio (HR) = 1.16; 95% confidence interval (CI): 1.06–1.26)] at even relatively low levels of arsenic in drinking water ranging from 10 to 49 µg/L [21]. At higher levels of exposure (50–149 µg/L), there were dose-dependent relationships between arsenic exposure and deaths from several causes including cancers (HR = 1.44; 95% CI: 1.0–1.95), cardiovascular disease (HR = 1.16; 95% CI: 0.96–1.40), and infectious diseases (HR = 1.30; 95% CI: 1.13–1.49). Another report identified an increase in hazard ratios associated with all-cause mortality and chronic disease mortality in Bangladeshi adults [22]. The investigators reported that approximately 21.4% of deaths and 23.5% of deaths associated with chronic diseases could be attributed to arsenic levels in drinking water that exceeded 10 µg/L [22]. In summary, experimental evidence in human populations underscores the numerous diseases associated with chronic arsenic exposure and that arsenic exposure can be a significant cause of death in exposed populations.

17.2.2 Health Effects Associated with iAs Exposure during Gestation and Infancy

Arsenic has been shown to readily cross the placenta in humans and experimental animals and therefore the developing fetus is not protected from arsenic exposure [23]. Pregnant women and their unborn children are particularly vulnerable to the adverse effects of arsenic exposure, which include increased risks of poor pregnancy/birth outcomes such as spontaneous abortion, preterm birth, stillbirth, and decreased birth weight [24–29]. While prenatal exposure to arsenic has been shown to be associated with some developmental abnormalities in mice including dose-dependent trends in neural tube defects and vertebral and calvarial malformations [30], data that support the association of developmental defects such as these in human populations are generally lacking [29]. In newborns from Chitwan Valley, Nepal, cord blood levels of arsenic were shown to be inversely associated with neurodevelopmental indicators [31]. Health effects in infants exposed to iAs *in utero* include reduced thymic index, higher rates of morbidity (e.g., diarrhea and pneumonia), and higher rates of mortality [32–34].

For example infants in Bangladesh born to mothers exposed to iAs levels in their drinking water that exceeded 50 μg/L had an increased risk of death, a response that showed a dose-dependent effect [26]. It is important to note that infants exposed prenatally to arsenic are likely also exposed throughout their childhood and into adulthood, so adverse health effects that are observed throughout the lifetime of an individual cannot be discerned as specifically associated with prenatal exposure or postnatal exposure. As a result there is a need for controlled toxicological experiments to clearly define the specific windows of developmental susceptibility to these exposures.

17.2.3 Health Effects of iAs Exposure Observed in Childhood

Children who are chronically exposed to iAs may exhibit skin lesions similar to those observed in chronically exposed adults [35]. However, unlike adults, epidemiological evidence in general does not support an association between chronic iAs exposure and an increase in cancer incidence and cancer mortalities in children [36]. As discussed further below, this may be due to the long latency times that are commonly observed for cancers associated with chronic and early-life exposure to arsenic. A study based in Chile did report an association between very high iAs exposure in early childhood (approximately 860 μg/L in drinking water) and an increase in mortality individuals <20 years of age from liver cancer, which is generally a rare cancer observed in children [37].

Due to the sensitivity of the developing brain to the effects of toxicants, the impact of arsenic exposure on the neurological development and intellectual capacity of children has been a major endpoint of interest. There is evidence of neurotoxic effects of arsenic associated with chronic exposure in rodents, including the impairment of spatial memory in rats [38] and gender-dependent alterations in brain neurochemical markers and spontaneous locomotor activity in mice [39]. In rats, prenatal and early postnatal life exposure to arsenic is also associated with poor reflex response and ultrastructural changes and altered expression of neuronal cell adhesion molecules in the hippocampus [40]. Aside from neurological impairments observed in prenatally exposed newborns [31], several studies have linked iAs exposure with cognitive impairments in children or adolescents [41–46]. For instance, a dose-dependent inverse relationship between iAs exposure and child intelligence in both 10-year-old [42] and 6-year-old children [41] was observed in a Bangladeshi population. A negative association between iAs exposure and motor function in children 8–10 years of age was also observed in Bangladesh [47]. Reduced intellectual function was observed in 14-year-old children who were exposed to arsenic-contaminated milk powder early in life (as reviewed in [48]).

Some sex-specific neurological effects have been observed. For instance, considering Bangladeshi children aged 1.5 and 5 years of age, the concentration of total arsenic in urine (U-tAs) was inversely associated with verbal IQ (VIQ) and full-scale IQ (FSIQ) in girls but not boys [45]. In a cohort from the smelter community of Torreón, Mexico, iAs exposure was associated with cognitive deficiencies in both girls and boys aged 6–8 years [44]. Overall, while there was a greater impact in boys compared to girls, the particular effects observed differed between boys and girls and were dependent on levels of iAs exposure as well, with some

effects only evident in lower exposure groups (U-tAs <50 μg/L). There is also evidence that adverse effects may be associated with co-exposures to other toxicants. For example, a study of 11–13-year-old children in the United States indicated that high levels of either arsenic or manganese were associated with lower indicators of intelligence, with the most impact observed when there was co-exposure to both metals [49].

17.2.4 Delayed Health Effects Associated with Chronic and Prenatal Arsenic Exposure

Studies of a Chilean population that had a defined period of high arsenic levels in drinking water have provided increased insight into the diverse effects of arsenic exposure on human health, the latent nature of these health effects, and the impact of early-life exposure on adverse health effects apparent in adulthood. These studies focused on Region II of northern Chile, which had a period of very high arsenic concentrations in municipally supplied drinking water between the years of 1958 and 1970 [50]. Almost all who live in this region rely on municipally supplied water as their source of drinking water, which is obtained from a few river sources that originate in the Andes Mountains. Prior to 1958, Antofagasta, the main city of Region II, had approximately 90 μg/L arsenic in its municipally supplied drinking water [51]. Starting in 1958, the drinking water supply of this region was supplemented with water from two additional rivers with very high arsenic content. This initiated of a 13-year period (1958–1970) in which more than 250,000 people in the city of Antofagasta were exposed to average levels of 860 μg/L arsenic in drinking water, levels that are 86 times the current recommended limit of 10 μg/L [52]. After the drinking water treatment plant was installed in 1970, the levels of arsenic in the drinking water of Antofagasta quickly declined and are less than 10 μg/L today [52,53]. Thus, this defined period of high exposure to iAs provides a rare opportunity to examine the impacts of arsenic on human health, including exposure limited to early life and childhood.

Following the high exposure period, mortality rates of various causes from arsenic-exposed Region II compared to those from unexposed regions of Chile revealed increases from numerous cancers such as those of the skin, lung, urinary bladder, and kidney [50,51,54,55]. Increased mortalities from non-cancerous causes such as pulmonary tuberculosis and acute mycocardial infarction (AMI) were also observed in Region II [56,57].

These studies not only provided insight into the diverse health effects associated with iAs exposure, but also revealed differential latencies associated with these diseases. Specifically, increases in death from most causes, including cancers and pulmonary tuberculosis, started around 1968, approximately 10 years after the start of the high exposure period [54,55,57]. The exception to this was deaths due to AMI, which had high highest rates of mortality during the high exposure period [56]. Comparisons of mortality rate ratios for urinary bladder cancer and lung cancer of the high exposure area (Region II) to the lower exposure area (Region V) between the years 1950 and 2000 demonstrated an increase in both urinary bladder cancer and lung cancer in the iAs-elevated area that began approximately 10 years after the start of the high exposure period and peaked in 1986–1997 [54]. A similar comparison based upon the 1950–2000 death records in Regions II and V showed an increase in deaths due to AMI in Region II versus

Region V during the high exposure period of 1958–1970 [56]. At that time, the AMI mortality rate ratios were 1.48 for men (95% CI: 1.37–1.59; $p < 0.001$) and 1.26 for women (95% CI: 1.14–1.40; $p < 0.001$). A comparison of the mortality data for bladder cancer, lung cancer, and AMI showed that excess deaths in Region II during the high exposure period were predominantly attributable to AMI. This was in contrast to the data representing the time period 10 years after the high exposure period where the excess AMI-associated deaths were reduced, while those associated with urinary bladder cancer and lung cancer predominated [56]. These results highlight the differences in latency periods, and that the latency periods can be very long. Long latencies were particularly observed for various cancers, as the deaths from lung, urinary bladder, and kidney cancers were high at least 25 years after the end of the high exposure period [54,55].

The data also highlight the differences in the health outcomes of arsenic exposure between men and women. For instance, the increase in mortality from lung cancer, urinary bladder cancer, and AMI combined peaked in 1991–1995, with an estimated 10.9% of all deaths in men, yet 4.0% of all deaths in women were attributable to arsenic exposure during this period [56]. The peak kidney cancer mortality rate ratio was 3.4 (95% CI: 2.2–5.1) for men in 1981–1985, which later declined to 1.6 (95% CI: 1.2–2.1) in 1996–2000 [55]. Between 1981 and 1985, the mortality rate ratios were 2.9 (95% CI: 1.8–4.7) for women and were therefore lower than those observed for men, but further increased to 4.4 (CI: 3.0–6.4) in 1991–1995 and therefore remained high longer than for men [55].

Studies of the high exposure period in Region II of Chile also highlight the delayed impact of early-life exposure to arsenic. These analyses focused on individuals born during the peak exposure period of 1958–1970 and those born just before this period, i.e., between 1950 and 1957 and were "young adults" aged 30–49 at the time of their deaths. Comparison of mortality rates of young adults in Region II to those from the rest of Chile (excluding Region II) revealed an association between arsenic exposure in early life/childhood and mortality from non-malignant lung disease (i.e., bronchiectasis) and lung cancer [58]. In this study, individuals born just prior to the high exposure period had a standardized mortality ratio (SMR) for lung cancer of 7.0 (95% CI: 5.4–8.9; $p < 0.001$) and an SMR for bronchiectasis of 12.4 (95% CI: 3.3–31.7; $p < 0.001$). For those born during the high exposure period that were likely exposed prenatally and during early childhood, the SMR for lung cancer was 6.1 (95% CI: 3.5–9.9; $p < 0.001$) and the SMR for bronchiectasis was 46.2 (95% CI: 21.1–87.7; $p < 0.001$) [58]. Other effects on the lung have also been observed, in which adults living in Antofagasta younger than 10 years of age during the high exposure period demonstrated reduced lung function nearly 40 years after the high exposure period ended compared to adults who were unexposed as children [59]. These adults with early-life exposure had 11.5% lower forced expiratory volume in 1 s (FEV_1; $p = 0.04$), 12.2% lower forced vital capacity (FVC; $p = 0.04$), and increased breathlessness (prevalence odds ratio = 5.94, 95% CI: 1.36–26.0) compared to those who did not experience the exposure. In addition, an exposure–response relationship was observed between exposure concentrations in early life and adult FEV_1 and FVC. The authors estimated that the decrease in lung function was similar to that observed for 45 pack-years of cigarette smoking [59].

Further examination of health effects in addition to those on the lung indicated that prenatal or early childhood arsenic exposure was associated with increased mortality from several

causes, including cancers of the urinary bladder (SMR = 18.1; 95% CI: 11.3, 27.4), larynx (SMR = 8.1; 95% CI: 3.5, 16.0), and liver (SMR = 2.5; 95% CI: 1.6, 3.7), as well as chronic renal disease (SMR = 2.0; 95% CI: 1.5, 2.8) [60]. Similar effects were observed with mortality rates from kidney cancer in young adults aged 30–39 who were born in two Region II cities, Antofagasta and Mejillones, during or just prior to the high exposure period compared to the rest of Chile (mortality rate ratio = 7.1; CI = 3.1–14) [55]. In addition, the highest mortality rate ratios for AMI observed in high exposure Region II versus the low exposure Region V were those observed in young adult men aged 30–49 who were born during the high exposure period (rate ratio = 3.23, 95% CI: 2.79, 3.75; $p < 0.001$). Taken together, the data from the Antofagasta region indicate that prenatal/early-life exposure to arsenic is associated with the greatest increase in mortality in adults <50 years of age compared to any other prenatal toxicant identified to date [60].

Consistent with the effects observed in Chile, exposure of Japanese infants to arsenic in milk powder for a defined period of time also indicates early-life exposure to arsenic is associated with latent impacts on human health. A mass poisoning of bottle-fed infants occurred in 1955 in Japan due to arsenic-contaminated milk powder (as reviewed in [48,61]). Okayama Prefecture was the most severely affected area, with acute poisoning cases that resulted in 24 deaths, 2005 surviving cases, and 84 suspected cases in the time period between August 1955 and April 1956 [48]. Follow-up studies of poisoning victims from Kyoto and Hiroshima Prefecture victims at age 14 demonstrated increased rates of neurological symptoms/disorders and lower intelligence quotient (IQ) including mental retardation compared to unexposed children (as reviewed in [48]). A comparison of cancer mortality in Okayama between the years of 1970 and 2006 in individuals 15–54 years of age was performed. These analyses focused on comparisons of mortality of cohorts of individuals who were less than 5 years old at the time of the poisoning and therefore potentially exposed to contaminated milk powder and that in control groups of unexposed individuals. The results revealed an increase in cancer mortality risk in the exposed group from pancreatic cancers in all age groups and leukemias in the 30–39 age group [61]. A follow-up study that focused on individuals who were ≤1 year old at the time of the poisoning and unexposed individuals born approximately 1 year later revealed evidence of increased mortality from all cancers (aged 48–53 years; MR = 1.36, 95% CI: 1.03–1.78) and liver cancer (all ages; MR = 1.72, 95% CI: 0.99–2.97) in the exposed cohort. Unlike the results observed in Chile that identified health effects associated with chronic and prenatal exposure, there was no evidence of increased risk of lung, urinary bladder, or kidney cancers. The authors suggested that early-life exposure may result in different cancers than those observed due to lifetime exposures or chronic exposures that started later in life [62].

Taken together, these studies of defined exposure periods were informative in several ways. They have indicated that both chronic and early-life arsenic exposure is associated with deaths from a wide variety of causes, and that the latency periods between exposure periods and the development of adverse health effects differ by each disease. In addition, these studies highlight the important finding that there are sex-specific trends in the outcomes between men and women. They have also indicated that early-life exposure to arsenic is sufficient to increase risk of death/premature death due to various diseases later in life. The link between arsenic exposure and increased death due to pulmonary tuberculosis highlights that arsenic may increase

the risk of death due to infectious diseases, and suggests that the full impact of arsenic on morbidity and mortality due to various causes is likely currently underestimated. In addition, these studies also indicate that there may be differences in diseases that develop in those exposed to arsenic in early life compared to those exposed in adulthood or throughout their lifetimes—a finding that requires study in animal models. There is also some indication that characteristics of exposed populations, such as genetic background and nutritional status, may influence disease development. Thus far, studies on these populations have not indicated the transgenerational potential of health risks associated with prenatal exposure, nor have molecular alterations of study subjects been studied. In the following section, we summarize research on animal models and currently exposed human populations that have provided insight into the molecular mechanisms that drive disease development associated with early-life exposure.

17.3 Mechanisms Implicated in Disease Development Associated with Prenatal Exposure

Considering the multitude of adverse health effects associated with early-life exposure to arsenic, complex biological mechanisms are likely an underlying feature. Much remains to be learned about the precise molecular mechanisms that link prenatal exposure to altered disease risk, especially those associated with latent health effects.

Arsenic-associated cancers have been the most intensely studied disease associated with arsenic exposure in which several molecular events are believed to play a role. These include the generation of reactive oxygen species (ROS)/oxidative stress, alterations in key cellular signaling cascades, enzyme inhibition, interference with DNA repair, epigenetic alterations, induction of iAs biotransformation, immunomodulatory effects, endocrine disruption, and the formation of genetic damage such as chromosomal aberrations [63,64]. No single mechanism has emerged as a key event, and it is likely that several mechanisms are involved. For example, ROS generated by arsenic exposure can cause macromolecule damage as well as activate various cell signaling pathways implicated in cancer development [65,66]. In terms of prenatal arsenic exposure, a major research focus has been to understand the molecular alterations that may lead to delayed health effects and, in particular, cancers. As discussed below, studies in animal models and examinations of human populations have identified several factors that may be involved in the development of one or more effects associated with prenatal exposure including altered gene expression/hormone signaling, epigenetic reprogramming, the emergence of cancer stem cells (CSCs), and perturbed immune function.

17.3.1 Epigenetic Reprogramming as a Potential Key Event in Latent Disease Development

It has been proposed that permanent changes regulated by the epigenome are a plausible link between prenatal toxicant exposure and disease development, particularly for NCDs that develop later in life. The epigenome, which means “above the genome,” refers to potentially

heritable biological information contained outside the DNA sequence that impacts gene expression [67–69]. The epigenome is widely accepted to include three components, namely DNA methylation, histone post-translational modifications (PTMs), and microRNAs (miRNAs), which control gene expression at either the transcriptional level (DNA methylation/histone PTMs) or at the post-transcriptional level (miRNAs) [68,69]. Histone PTMs and especially DNA methylation patterns are the most extensively studied and best-characterized components of the epigenome. In mammals, DNA is methylated almost exclusively at the cytosine residue of CpG dinucleotides to generate 5′-meCpG-3′ [70]. CpGs are clustered in two main areas of the genome: (1) ≈15% of CpGs are found in regions of euchromatin known as CpG islands and CpG island shores and (2) ≈85% of CpGs are found in repetitive, interspersed regions of DNA, e.g., transposable elements, in heterochromatin [71–73]. The association of transcriptional competency with particular epigenetic "marks," i.e., particular histone PTMs and/or DNA methylation patterns, has been established, especially in the context of gene promoters (see [74–76] for reviews). In general, there is a negative relationship between promoter CpG island/CpG island shore methylation and gene expression [77], and CpGs are heavily methylated in the transcriptionally silenced regions of heterochromatin [73]. However, it should be noted that this relationship is complex and there are numerous instances in the literature in which no relationship or positive relationships between promoter DNA methylation and transcriptional competency have been observed [77,78]. This is important to consider in the context of functional implications of arsenic-associated DNA methylation.

The integrity of the epigenome is critical to cellular homeostasis and genetic stability as it has been shown to regulate and fine-tune gene expression changes associated with virtually every cellular process, including cellular differentiation during organismal development as well as response to environmental stimuli [79–81]. The epigenome is considered an especially sensitive target of the effects of perturbations to the fetal environment as the DNA methylome, for instance, undergoes many changes during early development [67]. Changes to the epigenome that occur during early developmental periods are particularly concerning as they may be metastable and therefore persist throughout the lifetime of an individual as well as be maintained in subsequent generations [82,83]. Such "reprogramming" of the epigenome is believed to be a mechanism that supports the fetal origins of adult disease through the altered, persistent expression of key genes involved in disease development such as the overexpression of growth factors or the silencing of tumor suppressors [80,84].

The DNA methylome is the most extensively studied epigenetic component in terms of examining the effects of toxicant-induced perturbations in the fetal environment and later-life health effects. In animal models, evidence that the reprogramming of the DNA methylome may play a role in altering disease risk later in life is supported by the link between transient toxicant exposure during particular developmental periods, perturbed DNA methylation and gene expression patterns, and altered disease risk in adulthood [85]. For instance, neonatal male rats exposed to bisphenol A (BPA) or estradiol demonstrated permanent alterations in the DNA methylation patterns of several cell-signaling and apoptosis genes and an increased risk of developing prostate cancer later in life [85]. One of these modulated genes, namely phosphodiesterase type 4 variant 4 (*Pde4d4*), becomes transcriptionally silenced in the normal

prostate during aging via hypermethylation of certain CpG sites within the *Pde4d4* promoter. However, the prostate tissue of the BPA- and estradiol-exposed rats, these CpG sites within the *Pde4d4* promoter remain hypomethylated with sustained *Pde4d4* expression during aging. In addition, *in vitro* evidence revealed that these CpG sites are hypomethylated with increased *Pde4d4* expression in the prostate tumor-cell line AIT compared to the normal prostate epithelial cell line NbE-1, suggesting this gene may play an important role in latent prostate cancer development [85]. Considering early-life exposure to arsenic is also associated with altered, delayed risk of the development of NCDs such as cancers, pulmonary disease, and cardiovascular disease in humans, it is plausible that DNA methylation changes provide a link between prenatal iAs exposure and altered disease risks in adulthood [86,87]. Thus, alterations in gene expression profiles and DNA methylation patterns have been a major focus of investigations of molecular impacts associated with early-life exposure to arsenic.

17.3.1.1 Epigenetic and Genomic Alterations in Experimental Animals

The transplacental exposure models developed by the Waalkes group have been valuable tools in investigations of the molecular events that may link prenatal arsenic exposure and cancer development later in life. While experimental evidence generally does not support that iAs acts as a complete carcinogen in any organ in any experimental animal when administered in adulthood [88], iAs is a complete transplacental carcinogen in multiple organs in several strains of mice [89]. This effect was first observed in C3H mice, in which exposure of offspring born to pregnant females to sodium arsenite [$NaAs_2O_3$; iAs(III)] in drinking water [42.5 or 85 ppm arsenic during gestational days (GDs) 8–18] resulted in increased incidence and/or multiplicity of tumors in multiple organs in adult offspring [89,90]. Sex-specific effects were observed in the rodent model. Specifically, female offspring had dose-related increases in lung adenocarcinoma, benign and malignant ovarian tumors, and preneoplasias in the uterus and oviduct. In male offspring, there was a dose-dependent incidence of liver adenoma, hepatocellular carcinoma (HCC), liver adenoma/carcinoma, and adrenal cortical adenoma. A very pronounced effect was the strong dose response in HCC incidence and multiplicity in male C3H offspring, a form of cancer that is epidemiologically linked to chronic arsenic exposure in humans [37,90,91].

Prenatal exposure to a carcinogenic dose of arsenic was also found to be associated with the development of cancers, tumors, and/or proliferative lesions in some organs may have initiating effects in other organs after postnatal application of tumor promoting agents such as diethylstilbestrol (DES) and tamoxifen (TAM) in CD1 mice [92,93] and 12-O-tetradecanoyl phorbol-13-acetate (TPA) in C3H mice and Tg.AC mice [94–96]. For instance, prenatal arsenic exposure alone had no effect in Tg.AC mice, but postnatal TPA application resulted in the development of papillomas and squamous cell carcinomas (SCCs) [96]. Mice that received prenatal arsenic and postnatal TPA had increased the multiplicity of SCCs compared to TPA exposure alone, and the SCCs were much more aggressive [96]. In terms of internal cancers, postnatal TPA alone did not result in any statistically significant changes compared to controls [95]. In male offspring, arsenic exposure was associated with the development of adrenal cortical adenomas (ACAs) independently of the administration of TPA, and urinary bladder (UB) hyperplasia was observed in the arsenic + TPA group. In females, prenatal arsenic exposure

alone was associated with the development of ACAs, UB hyperplasia, uterine hyperplasia, and to a lesser extent uterine tumors and UB papillomas, with higher incidence of ACAs in the arsenic + TPA group. In both males and females, no dose response was observed [95].

The Waalkes group also developed a whole-life arsenic exposure model in CD1 mice, in which mice were exposed to 0, 12, and 24 ppm iAs(III) starting in both males and females 2 weeks before breeding, in females during pregnancy and lactation, and in the resulting offspring until 2 years of age [97]. These exposures resulted in dose-dependent increases in tumors in male and female mice in similar target sites as observed after *in utero* arsenic exposure only, except the tumors associated with whole-life exposure were more aggressive, which may reflect the cumulative effects of prenatal and postnatal exposure [97].

Importantly, the carcinogenic effects observed in these transplacentally exposed mice are comparable in many ways to those observed in human populations exposed to arsenic in early life, namely: (1) prenatal/early-life exposure is a particularly sensitive period to the effects of arsenic, (2) exposure during this period is sufficient to cause increased risk of cancer development later in life, and (3) cancers develop in multiple organs in a sex-dependent manner. These transplacental models also illustrate that prenatal exposure to arsenic can have an initiating effect as well as enhance the tumorigenic effects of other chemicals.

Molecular examinations of tumors and normal tissue from transplacentally exposed mice have supported the hypothesis that the persistent, altered expression of key genes is a plausible early event involved in cancer development after prenatal iAs exposure. Experimental evidence also suggests epigenetic reprogramming may underlie these altered gene expression profiles. For instance, compared to unexposed mice, the fetal livers of C3H male mice exposed to hepatocarcinogenic doses of arsenic *in utero* have alterations in the DNA methylation levels of GC-rich regions throughout the genome and altered expression of numerous genes, including several implicated in liver tumorigenesis and liver disease such as cytokeratins and insulin-like growth factors [89,98]. Similar gene expression profiles were observed in both HCCs and peritumor liver tissue from transplacentally exposed male C3H mice [99]. Together, these results indicate that alterations in gene expression profiles were long-lasting and their detection in prenatal and peritumorous tissue suggests they may be early events in the carcinogenic process. Of particular interest is the increased expression of estrogen receptor alpha (*Er-α*) and the estrogen-responsive gene cyclin D1 (*Cd1*) as well as the feminized expression of P450 genes in normal-appearing tissue and HCCs of prenatally exposed male mice [99,100]. Concurrent with increased *Er-α* expression, several DNA regions within the *Er-α* promoter were found to be hypomethylated in the livers of adult male mice that had developed HCCs, which may be significant as loss of promoter methylation is often associated with increased transcriptional activity [100]. Increased *Er-α* expression and hypomethylation of the *Er-α* promoter were also observed in the livers of mice chronically exposed to arsenic [101], and both ER-α and CD1 are implicated in the development of liver cancers [102,103]. Similar to findings in the liver, the aberrant expression of *Er-α* was also implicated in the development of lung cancers in prenatally exposed C3H mice. The fetal lung had aberrant levels of Er-α at both the mRNA and protein levels in addition to the modulation of several estrogen-regulated genes and genes associated with lung cancers [104]. In addition, lung adenomas and adenocarcinomas that

developed in the adult lung had intense ER-α staining, which was not observed in normal adult lung or lung adenocarcinomas associated with diethylnitrosamine (DEN) exposure. Interestingly, postnatal exposure to estrogens was shown to increase the carcinogenic response after prenatal exposure to arsenic in male and female CD1 mice, as there was high expression of ER-α protein in UB lesions and HCCs in males after postnatal DES/TAM exposure and uterine and urinary bladder tumors were present in females after DES exposure [92,93].

Together, these results are informative as they indicate that estrogen-responsive pathways may play a role in arsenic-mediated carcinogenesis, and that aberrant promoter DNA methylation of key target genes such as *Er-α* are associated with altered gene expression and cancer development associated with prenatal arsenic exposure. Given the similarities between early-life arsenic exposure and cancer development in adulthood between these animal models and human populations, it is plausible that animal models such as these provide insight into the molecular mechanisms that underlie the development of latent diseases associated with prenatal exposure in humans. However, it must be emphasized that a correlative, and not causal, relationship has been identified between altered promoter DNA methylation (e.g., *Er-α*), aberrant gene expression, and disease development in these models. Assuming that altered DNA methylation is an initial event in the alteration of the expression of a key gene such as *Er-α*, the exact mechanism by which prenatal arsenic exposure changes the DNA methylome is unknown, as well as the role that other epigenetic modifications, i.e., histone PTMs and miRNAs, may play in this process.

It is important to note that prenatal iAs exposure is also associated with accelerated/exacerbated atherosclerosis in apolipoprotein A-knockout ($ApoE^{-/-}$) mice, a mouse model often used to study atherosclerotic disease. Transplacental exposure of pregnant $ApoE^{-/-}$ females to 85 ppm sodium arsenite from GD 8 to birth (GD 20) is associated with evidence of increased atherosclerotic disease in male offspring 10 and 16 weeks after birth compared to controls [105]. In addition, prenatal exposure to 49 ppm sodium arsenite from GD 8 to birth was shown to cause changes in the mRNA and miRNA profiles in the liver of male offspring that were evident at postnatal day (PND) 1 and PND 70 [106]. Genes altered at PND 1 included those associated with gluconeogenesis/glycolysis whereas genes altered at PND 70 were associated with diverse processes such as protein export and antigen processing/presentation. These altered, sustained gene expression profiles are consistent with arsenic reprogramming gene expression signaling within the fetal liver and are of particular interest as liver stress/injury may be a contributing factor of atherosclerosis development. The authors suggest that sustained stress and pro-inflammatory response in the liver may play an important role in the development of early-onset atherosclerosis in prenatally exposed $ApoE^{-/-}$ mice, but the molecular mechanisms associated with this reprogramming have not been explored [106].

Taken together, the animal models of arsenic-induced disease support that fetal reprogramming may be a common and important link between prenatal/early-life exposure to iAs and the delayed development of both cancerous and non-cancerous NCDs.

17.3.1.2 Epigenetic and Genomic Alterations in Human Populations

Studies that examine perturbed gene expression and epigenetic profiles associated with prenatal arsenic exposure in human populations represent a growing area of interest. The first study of

gene expression changes associated with prenatal arsenic exposure examined the gene expression profiles of cord blood from newborns from the Ron Pibul District, Nakhon Sri Thammarat, Thailand [107]. This analysis identified 447 iAs-associated genes in cord blood leukocytes, of which ≈90% were up-regulated in the exposed group. These genes were enriched in functions associated with immune and inflammatory response, stress response, cell signaling, apoptosis, and cancer/tumorigenesis. In particular, this study identified the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling cascade as a key mediator of the genomic response to prenatal arsenic exposure in newborn cord blood, which is of particular interest given the role of NF-κB in inflammation-associated cancer development [108]. A set of 11 genes that exhibited a strong dose–response relationship with prenatal arsenic exposure was identified. These genes demonstrated a high level of accuracy (83%) in predicting prenatal arsenic exposure within this cohort and therefore were suggested to be potential biomarkers of prenatal exposure [107]. The effect of prenatal iAs exposure was also examined at the protein level in the cord blood plasma of Bangladeshi newborns [109]. Analysis of the association of 18 cord blood cytokines with maternal U-tAs at gestational week 30 (GW 30) revealed that the level of several pro-inflammatory cytokines, namely, interleukin 1 beta (IL-1β), interleukin 8 (IL-8), tumor necrosis factor (TNF), and interferon gamma (INF-γ), had U-shaped curves across quartiles of maternal U-tAs with significant differences between quartiles 2 and 8 for IL-8 and TNF [109]. Although these studies are limited in number, results at both the mRNA and protein level are consistent with the up-regulation of pro-inflammatory genes in arsenic-exposed newborns [107,109].

To date, studies that have examined epigenetic effects associated with prenatal arsenic exposure have primarily focused on changes in DNA methylation. Some studies have examined methylation changes at the gene level, which may be significant as they may be correlated with the aberrant expression of disease-promoting genes. Another area of interest includes examining changes in the overall methylation status of the genome as vast changes may be an important factor in disease development. For instance, DNA hypomethylation within heterochromatic regions may lead to genetic instability, which is implicated as a predisposing event in the development of some cancers [110,111].

In Thailand, examination of DNA isolated from newborn cord blood revealed a positive relationship between arsenic exposure *in utero* and promoter DNA methylation of the tumor suppressor, tumor protein 53 (*TP53*), although no relationship was identified between prenatal exposure and the methylation of Long Interspersed Element 1 (LINE-1) elements, which were used as indicators of global DNA methylation levels [112]. In iAs-exposed mother/child pairs in Bangladesh exposed to drinking water levels of <1–230 μg As/L, the relationships between iAs exposure and DNA methylation patterns of LINE-1 and the promoters of tumor suppressors *TP53* and cyclin-dependent kinase inhibitor 2A (*CDKN2A*/*p16*) were examined in maternal and umbilical cord blood leukocytes [113]. The DNA methylation trends were similar between mothers and newborns, in which a positive association was identified between maternal U-tAs and LINE-1 methylation and some CpG sites within the *p16* promoter. The greatest effects were observed in the middle tertiles of exposure, therefore revealing evidence of a nonlinear dose–response relationship and highlighting that epigenetic effects may be observed at relatively moderate levels of exposure. Changes in DNA methylation have also been observed in leukocytes derived from the cord blood of newborns in the USA exposed to relatively low

levels of iAs [114]. This genome-wide analysis of cord blood isolated from newborns from New Hampshire indicated there was no overall change in DNA methylation levels in relationship to maternal U-tAs. However, several differentially methylated areas were identified, most of which occurred in CpG islands [114]. Another genome-wide analysis of cord blood of prenatally exposed Bangladeshi newborns identified differential methylation within CpG regions of cord blood leukocytes in relation to prenatal arsenic exposure [115]. Importantly, this analysis identified a positive association of DNA methylation of CpG regions within 71 genes and levels of iAs in maternal drinking water after adjusting for the shifts in white blood cell populations that were observed in the blood of prenatally exposed newborns [115]. Another study identified sex-dependent DNA methylation profiles in prenatally exposed newborns in Bangladesh [116]. Using several different methods to analyze global DNA methylation status in newborn cord blood, data trends indicated the relationship between global DNA methylation levels and maternal U-tAs was positive for newborn males and negative for newborn females, which is interesting in light of various sex-dependent effects associated with iAs exposure [116].

Recently, we reported the first analysis of miRNA perturbations of newborns born to iAs-exposed mothers from Gómez Palacio, Mexico [117]. This analysis identified 12 miRNAs and 334 mRNAs in newborn cord blood that were associated with maternal U-tAs. Functions associated with the modulated miRNAs included diseases and cellular responses with known links to iAs exposure including cancer, diabetes mellitus, respiratory diseases, and inflammatory responses. MiRNAs act as post-transcriptional regulators of gene expression by binding to their mRNA target(s), and nine of the U-tAs-associated miRNAs were predicted to regulate the expression levels of ≈20% of the U-tAs-associated mRNAs. Major functions associated with the modulated mRNAs included an enrichment of those associated with innate or adaptive immune response signaling. These results highlight that multiple forms of epigenetic regulators may play a role in mediating the cellular response to iAs exposure.

Taken together, these results demonstrate that arsenic exposure *in utero* has been associated with both genome-wide and gene-specific changes in DNA methylation levels, including changes in genes such as *TP53* that are implicated in the development of arsenic-associated diseases. However, many of these changes have been examined in leukocyte-derived DNA and it is unknown whether these changes have functional consequences in terms of altered expression levels of these genes at the mRNA level and subsequently the protein level. It is also unknown whether these epigenetic alterations are persistent over time, how postnatal exposure to arsenic or other toxicants impacts the epigenetic changes observed in prenatally exposed individuals, or whether the relationship between these epigenetic alterations and disease susceptibility later in life.

17.3.2 Role of Cancer Stem Cells

Evidence from *in vitro* and *in vivo* studies has implicated cancer stem cells (CSCs) as potential origins of cancers associated with prenatal arsenic exposure. CSCs are believed to arise in the fetal environment from normal stem cells (SCs) or slightly differentiated SC progeny as a result of prenatal exposure [118]. These CSCs, which have altered SC functions, remain quiescent in

target organs until ultimately transforming into malignant cells [118]. The triggers that end the quiescent state to produce cancers are unknown but may include the effects of continued exposure to arsenic during the postnatal period or postnatal exposure to other chemicals as observed in the transplacental mouse exposure models described above [92–96]. Evidence that supports CSCs as potentially important factors in the development of arsenic-related cancers includes their high numbers in arsenic-transformed cell lines and malignant tumors associated with arsenic exposure [118]. For instance, the malignant transformation of the prostate epithelial cell line RWPE-1 by arsenic exposure *in vitro* results in an overabundance of CSCs compared to RWPE-1s malignantly transformed by cadmium or *N*-methyl-*N*-nitrosurea [119]. The malignant transformation of prostate stem cells (WPE-stem) by arsenic exposure *in vitro* resulted in the production of highly aggressive CSCs. Here the CSC phenotype was accompanied by the loss of the expression of normal SC markers and reduced expression of the tumor suppressor phosphatase and tensin homolog (*PTEN*), thus indicating the capacity of SCs to serve as progenitors of a CSC population [120]. An overabundance of CSCs is observed in numerous tumors associated with prenatal arsenic exposure in mice, such as the lung and liver cancers in CD1 mice after whole-life exposure to arsenic [97] and in the highly aggressive SCCs that developed in Tg.AC mice that were exposed prenatally to arsenic and postnatally to TPA [96].

Evidence for CSCs developing in the fetal environment and the potential for aberrant gene expression as a key, early event in CSC development is also observed in the fetal skin (GD 19) of Tg.AC mice prenatally exposed to arsenic. This is characterized by the increased expression of several genes such as the oncogene *v-Ha-ras*, *CD34*, a cell-surface marker for keratinocyte stem SCs and cancer skin cells, and ras-related C3 botulinum toxin substrate 1 (*Rac1*), a gene involved in keratinocyte SC renewal, compared to unexposed mice [96]. *v-Ha-ras* activation is considered a potential key, early event in Tg.AC skin cancer development, and while *v-Ha-ras* expression has been shown to be dependent on promoter DNA methylation status [121], no alteration in the DNA methylation status of the *v-Ha-ras* promoter was observed after prenatal arsenic exposure [96]. These results suggest that not all arsenic-associated aberrant gene expression observed in the fetal environment is associated with changes in promoter DNA methylation status of that gene. This finding suggests that other transcriptional regulators play a role in the control of *v-Ha-ras* expression after prenatal iAs exposure.

Similar to results observed in fetal skin, an examination of the highly aggressive SCCs that developed in Tg.AC mice after prenatal arsenic exposure and postnatal TPA exposure also revealed high expression of *v-Ha-ras* and the CSC markers *CD34* and *Rac* compared to tumors that arose after TPA treatment alone. A comparison of the tumors arising from these two treatment groups showed increased expression levels of the pro-growth gene *Cd1* and decreased expression of tumor suppressor *p16* in the aggressive SCCs in the iAs arsenic/TPA group. These data illustrate how subsequent alterations in genomic signaling may contribute to the acquisition of the malignant phenotype and potentially end the quiescence period in these prenatally exposed mice [96].

Taken together these data support that tumors associated with prenatal arsenic exposure have high numbers of CSCs and have possibly acquired aggressive characteristics through the altered expression of pro-growth genes and tumor suppressors. In human populations, it is unknown if tumors associated with prenatal arsenic exposure have an overabundance of

CSCs as observed in the aforementioned animal models. In addition, the stimuli and molecular events that end the quiescent state associated with prenatal arsenic exposure to produce malignancies have yet to be elucidated.

17.3.3 Immunomodulatory Effects

Arsenic has been shown to be a potent immunomodulatory agent *in vitro*, *in vivo*, and in human populations [122]. Analyses of perturbed mRNAs and miRNAs in the cord blood of iAs-exposed newborns indicated that the immune response is a major altered function associated with prenatal iAs exposure [107,117]. In chronically and prenatally exposed human populations, there is evidence that arsenic can cause both inflammation and immunosuppression, which is likely of major significance as each effect has been linked to adverse health outcomes.

As a potent inducer of ROS, arsenic can cause inflammation via the activation of ROS-sensitive pro-inflammatory signaling pathways such as those that involve mitogen-activated protein kinases (MAPKs) and the transcription factor NF-κB [123]. There is evidence that prenatal iAs exposure is associated with inflammation as there was increased expression of pro-inflammatory genes at the mRNA level and protein level in newborn cord blood, oxidative DNA damage in the placenta, and induction of pro-inflammatory proteins in the placenta such as IL-1β, TNF, and IFN-γ [107,109]. One of the adverse effects of a prolonged inflammatory state is the inflammation-induced generation of oxidative stress that can cause tissue/macromolecule damage, and inflammation during gestation has been associated with adverse pregnancy outcomes such as preterm birth, preeclampsia, and fetal growth restriction [124,125]. In addition to effects in early life, sustained inflammation is implicated in the development of chronic diseases that occur later in life through the activation of disease-promoting proteins such as growth factors and angiogenic factors [123,126]. This pro-inflammatory state is believed to be a major mechanism by which ROS-producing metalloids elicit carcinogenic effects [66]. Furthermore, inflammation is believed to play a role in the development of other iAs-associated diseases such as diabetes mellitus, atherosclerosis, and liver fibrosis [127–129].

Arsenic exposure has also been associated with immunosuppression, which has been demonstrated in several *in vivo* systems including zebrafish [130] and mice [131]. Arsenic decreases the functionality of several types of cells involved in the host defense system including peripheral blood mononuclear cells [132], macrophages [131], and T helper cells [133]. Arsenic exposure is associated with insufficient immune response to infection in mice and markers of immunosuppression in chronically exposed children and adults [132,134–137]. Prenatal exposure is associated with alterations of T cell populations in the cord blood of exposed newborns [115] and increased morbidity and mortality and reduced thymic index in infants [33,34,138]. The immunosuppressive effects *in utero* may not only be linked to the increased morbidity and mortality that is associated with iAs exposure in early life [33,34]; if this immunosuppressive effect is maintained, it likely also increases the susceptibility to infections and chronic diseases later in life [139,140]. Interestingly, it has been shown that the immunosuppressive effects of arsenic can lead to complex adverse health outcomes. For instance, arsenic-exposed C57BL/6J mice have been shown to exhibit a biphasic response in response to influenza A (H1N1) challenge in the lungs [134]. Whereas arsenic-exposed mice

initially have an inadequate immune response to influenza challenge compared to arsenic-unexposed mice, this response is followed by an excessive inflammatory response in the lung resulting in extensive tissue damage that is not observed in arsenic-unexposed, H1N1-challenged mice [134]. Since markers of both immunosuppression and inflammation have been observed simultaneously in arsenic-exposed populations as well [135], it is likely that these two seemingly divergent effects are both involved in the development of the diverse adverse health effects associated with prenatal arsenic exposure.

17.4 Conclusions and Future Directions

In summary, it is clear that there are diverse and delayed adverse health effects associated with arsenic exposure in early life. Defined periods of exposure in human populations have proven invaluable in understanding the diversity of the health effects involved and the different latency periods associated with these effects, and how various factors may impact the nature of the health effects that develop. Both human studies and animal models have demonstrated that the prenatal/early-life period is a particularly vulnerable time in relation to these toxic effects and that exposure during this period is sufficient to significantly alter susceptibility to disease. Due to the latent nature of many of these effects, the true health impacts of such exposure are likely vastly underestimated. These results underscore the dire need to identify susceptible populations and critical windows of susceptibility. This information will be critical to successfully implement preventive measures to protect the health of currently exposed populations and potentially the health of future generations particularly, if the health effects observed are transgenerational in nature. Therefore, there is also a need to determine the potential of altered disease risk to be heritable in the germ line of both males and females.

Lastly, while many of the current research trends have identified several molecular mechanisms that may be involved in latent disease development, much remains to be learned about these mechanisms, particularly for non-cancer diseases. For instance, while studies of prenatally exposed individuals have identified alterations in DNA methylation profiles, it is unknown what, if any, biological relevance these alterations have in terms of altered gene expression or disease development. It is also unknown which persistent molecular effects contribute to disease development, which molecular effects are disease specific, and what factors (e.g., postnatal environment) contribute to the length of latency periods. Importantly, emerging technologies, which allow for rapid and increasingly comprehensive and affordable analyses, will likely greatly increase our knowledge of the molecular events associated with early life exposure to toxicants and their relationship with human disease development.

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18

Arsenic, Kidney, and Urinary Bladder Disorders

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CHAPTER OUTLINE

18.1 Introduction

Both the incidence and prevalence of type 2 diabetes mellitus, hypertension, chronic renal failure, and cancer have risen constantly over the last three decades, and these diseases are now a growing public health problem, so the identification of risk factors associated with them is essential in order to take preventive measures.

Recently, the study of the toxic effects of arsenic (As) on the human body has become especially important. This is because, in the last 100 years, arsenic began to be used as a pesticide, a chemotherapeutic agent, and a constituent of consumer products; however, was arsenic concentration in drinking water and foods the most important concern for public health?

Arsenic is one of the most common heavy metals found, and the causal association between As and the formation of tumors in the skin, lungs, bladder, liver, and kidneys has been exhaustively described. Some epidemiological studies have shown an association between exposure to high levels of As and increased risk of cardiovascular disease, hypertension, and diabetes mellitus; however, the literature on the effects of As on the renal function of the general population is still scarce.

The aim of this chapter is to analyze the epidemiology, physiopathology, and clinical manifestations of nephrotoxicity and bladder complications associated with arsenic exposure.

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18.2 Arsenic and Renal Disease

Chronic kidney disease (CKD) is an important public health problem that is characterized by poor health outcomes and very high healthcare costs; CKD is a devastating disease that represents a high socioeconomic burden to family and society.

CKD is defined as a reduced glomerular filtration rate, increased urinary albumin excretion, or both for a period longer than 3 months [1]. The incidence and prevalence of end-stage kidney disease differ substantially across countries and regions, and prevalence is estimated to be 8–16% worldwide [2]. Complications include increased all-cause and cardiovascular mortality [3], acute kidney injury [4], cognitive decline [5], anemia [6], fractures, and other mineral disorders [7]. Identification and staging of CKD rely on measurement of glomerular filtration rate and albuminuria [8].

Worldwide, diabetes mellitus is the most common cause of CKD, but in some regions other causes, e.g., herbal and environmental toxins such as long-term exposure to heavy metals and pesticides, are more common [9–11](Table 18–1). Tobacco smoke is another risk factor for kidney disease and there is some evidence that its effects on kidney function begin in childhood [12].

The poorest populations are at the highest risk and national screening and intervention programs can prevent CKD, and where management strategies have been implemented the incidence of end-stage kidney disease has been reduced.

18.2.1 Studies in Animals

Few experimental studies have evaluated the renal effects of arsenic exposure in animal models. In dogs, low-dose administration of sodium arsenate (0.73 mg/kg) results in mild degeneration and vacuolation of the ascending thick portion of the nephron; at this dosage level there was no change in glomerular filtration rate, fractional reabsorption of sodium, potassium, and chloride. Higher doses (14.66 mg/kg) result in moderate glomerular sclerosis and severe acute tubular necrosis involving all the nephron segments [13].

In mice, arsenic exposure through drinking water (22.5 mg/L) increases urine *N*-acetyl-β-D-glucosaminidase (NAG) but not urine albumin (UAlb) excretion. In that model, mice given both arsenic in drinking water and cadmium (Cd) in food exhibit increases in urine protein and NAG excretion that are markedly higher compared with those in mice given cadmium or arsenic alone [14].

In Chinese Dragon-Li cats exposed to arsenic trioxide to induce renal injury, resveratrol was used to evaluate its effects on arsenic nephrotoxicity. Resveratrol significantly attenuates the accumulation of arsenic in renal tissues, decreases markers of oxidative stress, and shows less morphologic injury and tubular necrosis [15]. Other compounds such as naringenin [16], a naturally occurring citrus flavanone; silibinin [17], a naturally occurring plant bioflavonoid found in the milk thistle of *Silybum marianum*; *Pleurotus florida* lectin [18]; green tea extract [19]; *Curcuma aromatica* leaf extract [20]; and taurine [21], have shown promising effects protecting the kidney against arsenic nephrotoxicity (Box 18–1).

Table 18–1 Environmental Toxins Associated with Chronic Renal Disease

Environmental Toxin	Type of Renal Disease
Lead	Chronic tubulo-interstitial nephropathy
	Fanconi syndrome
	Hyperuricemia
Cadmium	Chronic tubulo-interstitial nephropathy
	Fanconi syndrome
Arsenic	Glomerulosclerosis
	Chronic tubulo-interstitial nephropathy
Aristolochic acid	Chronic tubulo-interstitial nephropathy
	Acute kidney injury
	Tubular dysfunction with unchanged glomerular filtration rate
Organochlorine pesticides	Decrease GFR
Smoke (tobacco exposure)	Proteinuria
	Decrease GFR
Unknown	Mesoamerican nephropathy

GFR: glomerular filtration rate.

BOX 18–1 NUTRITIONAL PRODUCTS THAT HAVE SHOWN PREVENTION OF ARSENIC NEPHROTOXICITY IN ANIMALS

- Selenium
- Naringenin
- Silibinin
- *Pleurotus florida* lectin
- Green tea extract
- *Curcuma aromatica*
- Taurine
- Antioxidants

18.2.2 Epidemiological Studies in Humans

Arsenic was first used in ancient Greece as a medicinal agent but renal toxicity associated with this metal was not described until recently. In 1915, Pearce and Brown [22,23] did histological analysis of kidneys from dogs with acute poisoning with arsenic compounds and they detected hemorrhages in the cortex, hyaline thrombus in the glomeruli, and acute tubular necrosis. In the 1960s it was evident that acute poisoning by arsenic was an important industrial hazard associated with hemolysis and acute renal failure [24,25].

Serial renal biopsies done in humans with acute arsenic poisoning showed acute tubular necrosis followed by rapid regeneration of tubular cells; however, biopsies done 6 months later in the same patients showed evolution to diffuse interstitial fibrosis [26]; subsequent reports suggested that acute arsenic poisoning may originate as renal cortical necrosis and chronic kidney disease [27].

Table 18–2 Summary of Epidemiological Studies on Renal Injury Mediated by Arsenic

Author	Year	Country	As Concentration (water)	As Urinary Excretion	Outcome
Lewis et al. [28]	1999	USA	14–166 μg/L	NA	Increased mortality from kidney disease
Meliker et al. [29]	2007	USA	10–100 μg/L (mean 11 μg/L)	NA	Increased mortality from kidney disease
Hsueh et al. [30]	2009	Taiwan	NA	Controls 20.7 ± 1.1 μg/g CKD 31.95 ± 2.59 μg/g	High urinary total arsenic associated positively with CKD
Chen et al. [31]	2011	Bangladesh	<7–869 μg/L	1 – >206 μg/g	Adverse effects of As exposure on higher risk for proteinuria; these effects are modifiable by changes in As exposure
Robles-Osorio et al. [33]	2012	Mexico	NA	0.56 to 89 μg/g	α1MG urinary excretion increased with the U-As concentrations
Zheng et al. [32]	2013	USA	NA	Median 12.7 μg/g	Increasing urine arsenic concentrations were cross-sectionally associated with increased albuminuria

CKD: chronic kidney disease α1MG: α1-microglobulin.

More recently, the assessment of health risks associated with exposure to low to moderately elevated levels of arsenic in drinking water has become the subject of considerable interest as some studies found a direct association between arsenic concentrations in drinking water and mortality (Table 18–2).

In a cohort-mortality study in Utah, USA, Lewis et al. [28] found an increase in the mortality ratio for renal disease (nephritis and nephrosis); this finding was significant only in men but not in women. Meliker et al. [29] found that arsenic in drinking water at levels in excess of 200–300 μg/L was associated with higher mortality rates for diabetes mellitus, cerebrovascular diseases, and kidney disease in both males and females.

There are few epidemiological studies linking chronic arsenic exposure and renal disease (Table 18–2). Hsueh et al. [30], in Taiwan, studied 125 people with GFR ≤ 60 mL/min and 229 people with normal renal function and found a weak association between urinary levels of As and decreased renal function ($r^2 = 0.04$, $p \leq .001$). They found that high urinary total arsenic or low plasma lycopene level was associated positively with CKD. The results also suggest that the capacity for arsenic methylation may be associated with CKD in individuals who ingest arsenic at low levels and lycopene at low plasma levels.

Chen et al. [31] in Bangladesh used urinary dipsticks test to detect proteinuria in 11,122 men and women, the results showing a positive association between long-term As exposure and the prevalence of proteinuria. During follow-up, increasing levels of urinary As were

associated with an increased risk of proteinuria, and decreasing levels of urinary As were related to a reduced risk of proteinuria.

Zheng et al. [32] evaluated the association between inorganic arsenic and albuminuria in American Indian adults living in rural areas of the United States and low to moderate exposure to As. This population has a high burden for diabetes (49.7%), obesity and albuminuria (30%), and after statistical adjustments for diabetes, urinary Cd excretion, hypertensive medication, and systolic blood pressure they found a higher prevalence ratio of albuminuria by urine arsenic concentration in subjects with higher As urinary excretion.

Robles-Osorio et al. [33], in central Mexico, in an open non-diabetic population found an increase in the α1-microglobulin urinary excretion, but not albuminuria associated with higher urinary arsenic excretion.

There is not enough information about the effects of arsenic exposure and renal injury in children, and in the only epidemiological study to date there was found to be no association with renal disease [34].

Some animal data has shown that combined exposure to inorganic As and Cd gives rise to more pronounced renal toxicity than exposure to each of the agents alone [14]. This finding has been corroborated in some epidemiological studies done in humans [35].

In China, Hong et al. [36] studied 245 subjects co-exposed to arsenic and cadmium, 122 in the arsenic–cadmium polluted area, 123 in the non-polluted area. Arsenic exposure biomarkers β_2-microglobulin (Uβ_2MG), albumin (UAlb), and *N*-acetyl-β_2-glucosaminidase (UNAG) in urine were used; the levels of Uβ_2MG, UAlb, and UNAG in the polluted area were significantly higher than those in the non-polluted area.

Nordberg et al. [37] also studied renal dysfunction in 619 persons residing in two metal-contaminated areas in China; measurements of UB2MG, UNAG, URBP (urinary retinol-binding protein), and UAlb were used as markers of renal dysfunction. An interaction effect between As and Cd was demonstrated at higher levels of a combined exposure to both elements as an increased prevalence of B2MG-uria, NAG-uria, and ALB-uria was found in relation to Cd combined with As.

In conclusion, the evidence linking arsenic concentration in urine or drinking water and CKD is still scarce and limited to some populations; more studies are needed in open populations to determine the effects of low to moderate arsenic levels on renal disease.

Huang et al. [38] evaluated the effect of co-exposure to environmental low-level Cd and As on urinary biomarkers and oxidative stress. Urinary excretion of NAG and the oxidative stress indices urinary malondialdehyde and 8-hydroxy-2-deoxyguanosine were positively correlated with both Cd and As in urine and these effects were more pronounced with co-exposure to Cd and As than for exposure to each metal alone.

18.2.3 Early Biomarkers of Arsenic Exposure and Nephrotoxicity

One of the major challenges in the fields of As toxicology has involved the monitoring of risk populations for early signs of exposure and toxicity even though almost no attention has been focused on the development and use of sensitive biomarkers of As nephrotoxicity.

The United States National Institutes of Health have defined the term "biomarker" as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [39].

Biomarkers are by definition objective and quantifiable characteristics of biological processes, and could only serve as true replacements for clinical relevant endpoints if we understood the normal physiology of a biological process, the pathophysiology of that process in the disease state, and effects of an intervention on these processes [39].

A useful biomarker of arsenic nephrotoxicity has to be associated with a statistically significant increase in the relative risk (odds ratio or hazard ratio) of developing CKD; also, levels of the biomarker must be significantly different in individuals who will go on to develop CKD than in those who do not [40].

Unfortunately, arsenic has not been established as an important risk factor for CKD, and there are no studies that report progression to CKD according to As urinary excretion, so it is a poor predictor of the level of risk, as it is neither sensitive nor specific for development of CKD.

The measurement of GFR, levels of proteinuria and albuminuria, NAG, β_2-microglobulin, α_1-microglobulin, and retinol-binding protein has been reported in the studies on arsenic nephrotoxicity.

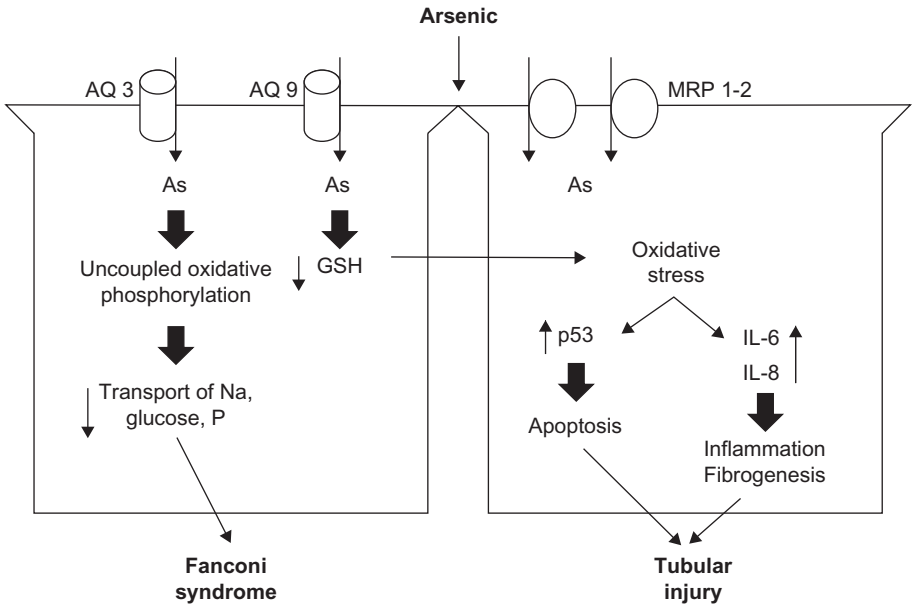
Microalbuminuria may be defined as the urinary excretion of 30–300 mg/d of albumin in a timed urine collection in adults, and macroalbuminuria as a urinary excretion higher than 300 mg/d. Albuminuria has long been recognized as a marker for renal disease and is a well-known predictor of poor renal and cardiovascular outcomes in patients with type 2 diabetes and essential hypertension [41]; albuminuria indicates glomerular and endothelial injury [42], and some studies have used this marker as clinical surrogate of kidney disease related to arsenic exposure [32]; however, there is some controversy about the role of microalbuminuria in CKD progression [43].

Urinary markers characteristic of tubular damage essentially consist of urinary enzymes or LMW plasma proteins that are normally freely filtered through the glomerulus. The impaired reabsorption of these proteins through the proximal tubule results in an increased excretion in urine and tubular or low-molecular-weight proteinuria reflecting tubular injury and tubular dysfunction. Proteins with a molecular weight lower than 40 kDa, such as β_2-microglobulin, α_1-microglobulin, and retinol-binding protein, are freely filtered through the glomerular membrane, and when tubular dysfunction is present their reabsorption in this segment is reduced [44–46].

NAG is a high-molecular-weight lysosomal enzyme found in many tissues of the body, and is not filtered through the glomerular membrane due to its high molecular weight. However, this enzyme shows high activity in renal proximal tubular cells, and leaks into the tubular fluid when proximal tubular cells are injured as in arsenic exposure. Its urine level increases and thus is used as a reflection of proximal tubular cell necrosis; however, whether this protein has a role as a useful marker of risk for progression of renal disease is still unclear [47].

18.2.4 Physiopathology

Arsenic is absorbed by the intestine and lungs (inhalation), and, to a lesser extent, through the skin. Inorganic arsenic ingested in drinking water is rapidly and almost totally (80–90%)



AQ: Aquaporin, **MRP**: Multidrug Resistant Protein, **GSH**: Glutathione

FIGURE 18–1 Mechanisms of arsenic-related nephrotoxicity.

absorbed by humans and animals [48]. Once it has been absorbed, it is transported to all tissues in the body. The intake of selenium and vitamin B decreases intestinal absorption of As in the diet. In addition, genetic variables influence arsenic methylation, so arsenic toxicity may be greater among those with poorer diets [49]. Arsenic is methylated in the liver in a glutathione (GSH)-mediated process, which decreases its toxicity and facilitates its biliary and urinary excretion [50], and then enters the intracellular medium through the aqua-glyceroporins AQ3 and AQ9. Studies in cell cultures have shown that the increase in AQ3 and AQ9 cellular expression increases intracellular accumulation of As. In the liver, AQ9 is important for biliary excretion of As [51]. Arsenic induces synthesis of metallothioneins, small, cysteine-rich, metal-binding proteins expressed in all eukaryotes and some prokaryotes; these proteins are highly inducible by heavy metals and represent a major means of metal detoxification in the body to maintain trace elements within a physiological range or to protect the body from damage by metal overload [52].

Another group of As transport proteins includes MRP-1 and -2 (ATP binding cassette-multidrug resistance protein), which were first described in the liver, where they transport As bound to GSH to the bile. The MRP-2 transporter is also located in proximal tubule cells, which favors entry of As into these cells [53]. Arsenic toxicity in PCT cells is due to GSH depletion and an increase in oxidative activity by free radicals. As also increases the expression of interleukin-6 and interleukin-8 expression, and it seems that it activates other pathways to increase the synthesis of reactive oxygen species (ROS) [54] (Figure 18–1).

Other mechanisms related to tubular toxicity are apoptosis through activation of the p53 apoptotic pathway [55] and activation of the caspase-9 and -3 signaling pathway [56].

Arsenic uncouples oxidative phosphorylation, and this graded uncoupling of oxidative metabolism causes graded reductions in the net transport of sodium, phosphate, and glucose transport; this type of tubular injury is manifested clinically as Fanconi syndrome (phosphaturia, glucosuria, and low-molecular-weight proteinuria) [57].

Widespread vascular endothelial dysfunction or chronic low-grade inflammation also may be underlying mechanisms for albuminuria. Arsenic has been associated with vascular cell adhesion molecule 1 (VCAM-1), and up-regulation in the expression of the angiotensin type I receptor [58].

18.2.5 Clinical Manifestations of Arsenic-induced Renal Disease

The literature does not currently offer sufficient information on clinical manifestations of As nephrotoxicity, but it is likely to manifest as tubular damage, such as low-molecular-weight proteinuria, aminoaciduria, glycosuria, and phosphaturia, as well as progressive deterioration of renal function.

Acute arsenic poisoning is manifested as acute tubular necrosis with oliguria and azotemia, and some patients even require advanced support with dialysis and plasma exchange. Acute tubulointerstitial nephritis has been described as a clinical manifestation of acute As poisoning [59].

18.3 Arsenic and Bladder Disease

Inorganic arsenic is a known human carcinogen, and chronic exposure leads to an increase in the incidence of skin, lung, liver, kidney, bladder, and prostate tumors.

Bladder cancer is the most common malignancy involving the urinary system and is the 10th most common cancer worldwide; the incidence increases with age, arising most commonly in the seventh decade of life and rarely occurring before the age of 50. Histologically, most cases of bladder cancer are transitional cell carcinomas (90%); 70% of these are superficial and papillary subtypes [60]. Hematuria, frequent urination, and pain during urination are the most common symptoms of bladder cancer.

Cigarette smoking is the primary risk factor for bladder cancer. Current cigarette smokers have a three-fold higher bladder cancer risk than non-smokers, and ex-smokers experience a two-fold increased risk; about half of male bladder cancer and one-fifth of female bladder cancer cases were attributable to cigarette smoking [61,62].

Epidemiologic studies based on high arsenic exposure in Taiwan [63], Argentina [64], Japan, [65] and Chile [66] suggest an increased risk of cancer of the urinary tract, and the evidence for a link between bladder cancer and exposure to arsenic in drinking water at concentrations exceeding 300–500 μg/L is strong; however, the risk with As concentrations below 200 μg/L, except for the smokers, is controversial.

Few epidemiological studies have evaluated the association between low arsenic exposure levels and bladder cancer, and more studies are needed to confirm this association. In the United States [67], a case–control study in which arsenic concentrations in drinking water ranged from 0.5 to 180 pg/L showed no overall association between arsenic exposure and cancer risk, but arsenic and cigarette smoking tended to have a synergistic effect on the risk of bladder cancer. Kurttio et al.[68] reported in a Finnish study a weak association between arsenic and bladder cancer risk in an open population exposed to very low As levels (0.5 µg/L), this study again providing evidence for synergistic effects between smoking and As. In a more recent study done in Chile, subjects were exposed to arsenic water concentrations < 200 µg/L (median = 60 µg/L); bladder cancer odds ratio for subjects in the upper tertile of monomethylarsonic (MMA) excretion compared to subjects in the lower two tertiles was 2.37 (1.01–5.57) [69].

The major metabolic pathway of inorganic arsenic in humans is its methylation in the liver. In most organisms, As is metabolized by an alternating series of reductions of pentavalent forms to trivalent forms followed by sequential oxidative methylation, and the most abundant metabolite excreted in urine of humans and rodents is dimethylarsinic acid (DMAV). The trivalent arsenicals have been shown *in vitro* to be highly reactive and considerably more cytotoxic compared with pentavalent forms, especially for the methylated arsenicals. These trivalent forms are believed to play a critical role in As-induced toxicity and carcinogenicity [70].

Arsenic methylation is an important mechanism that induces oxidative DNA damage and the acquisition of an *in vitro* cancer phenotype, as measured by invasiveness, colony formation, and secretion of matrix metalloproteinases in rat and human epithelial cell lines [71,72]. There is mounting evidence that metabolism of arsenic to methylated species plays a predominant role in its toxic and carcinogenic effects. Metabolism of arsenic is variable between species, human populations, and individuals. Polymorphisms in *AS3MT* and other genes may influence arsenic metabolism and potential susceptibility to its toxic and carcinogenic effects [73,74].

The mechanism of arsenic-induced bladder cancer remains unclear, but some proposed mechanisms are mediated through reactions with thiols in cells [75], generation of ROS [76], oxidative DNA damage, acquired tolerance to apoptosis, enhanced cell proliferation, altered DNA methylation, genomic instability, and aberrant estrogen signalling [77].

Accumulation of trivalent arsenite in bladder tissue mice exposed to 0.01% sodium arsenite in drinking water demonstrated proliferation of the bladder uroepithelium within 4 weeks after initiating treatment [78].

Arsenic is a tumor promoter that affects specific cell signal transduction pathways responsible for cell proliferation. The activation of the epidermal growth factor receptor (EGFR) extracellular signal-regulated protein kinase (ERK) pathway is important in mediating gene expression related to regulation of cellular growth. Arsenic-induced cell proliferation in the bladder epithelium is induced by activation of the MAP kinase pathway, leading to an increase in the activity of EGFR, ERK, and an increase in c-Src levels interacting with EGFR [79,80].

Arsenic induces AP-1 activation and expression of AP-1-associated genes causing activation of specific signaling pathways that lead to chronic, increased cell proliferation. This may play a non-epigenetic role in carcinogenesis by increasing the proliferation of initiated cells or increasing the mutational rate [81].

Arsenic also exerts its toxicity through mitochondrial dysfunction and generation of ROS; this mechanism plays a crucial role in the control of apoptosis, and arsenic-induced oxidative stress promotes telomere attrition, chromosome end-to-end fusions, and apoptotic cell death [82].

Angiogenesis is a key event for tumor initiation. Arsenic stimulates this process by a mechanism related to reorganization of actin filaments, activation of Cdc42, increasing the activity of the enzyme NADPH oxidase with generation of free radicals, and stimulation of cell migration [83]. Arsenic increases the expression of other enzymes and proteins such as COX-2, VEGF, and HIF-1α, which regulates the angiogenesis process; this is mediated by ROS through the MAPK and PI3K/AKT signaling pathways [84].

The inflammatory response as a pathogenic factor has gained attention since there is evidence showing that *in vitro* the exposure to arsenicals induces the production or activation of inflammatory mediators, and there is some evidence that suggests a crucial role of IL-8 in bladder cancer development [85].

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19

Developmental Arsenic Exposure: Behavioral Dysfunctions and Neurochemical Perturbations

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CHAPTER OUTLINE

19.1 Introduction

Arsenic is a naturally occurring metalloid in the environment and present in both organic and inorganic forms. Arsenic compounds typically lack odor or taste and therefore they are difficult to detect and easily consumed by people through various modes. Exposure to arsenic may occur through air, food, and drinking water. Several processes such as geothermal, alkali desorption, reductive dissolution, and sulfide oxidation are responsible for groundwater contamination by arsenic. Mining and industrial processing, and leaching of natural geological sources also contaminates drinking water with arsenic [1]. The World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA) have set the maximum contamination level for arsenic in drinking water as 10 μg/L or ppb [2,3]. Arsenic contamination in ground water is a serious concern in some parts of America, Asia, and Australia. In Asia, arsenic contamination is widely reported in Bangladesh, India, and Taiwan, Bangladesh being worst affected as more than 30% of the population has been reported to be exposed to arsenic-contaminated water [4,5].

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Arsenic compounds are also added in the preparation of several products used in our daily life, such as cosmetic products. Arsenic has some medicinal values as well when used in low doses. Its medical use has been documented from ancient times by popular philosophers and physicians such as Hippocrates and Aristotle. Arsenic was used in the preparation of Fowler's solution (1% potassium arsenite), which was used to treat diverse health problems including asthma, chorea, malaria, syphilis, and psoriasis [6,7]. In the early 19th century, Salvarsan, an arsenic-based drug, was highly popular for treating syphilis [8]. Arsenic products have been used in chemotherapeutic agents for treating various cancers such as skin cancer and acute promyelocytic leukemia [8–10].

Arsenic has also been used in the preparation of pigments used for example in candles, fabrics, toys, and wallpapers. Even though some of these uses have been discontinued due to toxic effects, arsenic is still used in the preparation of glass, pesticides, semiconductors, and wood preservatives. Industrial uses of arsenic potentially serve as a source for contaminating the soil and ecosystem [11,12].

19.2 Toxicity

Arsenic forms multiple compounds, which are highly poisonous. Arsenic has been referred as the "king of poisons" or "poison of kings" especially due to its lethal properties during the Middle Ages and the Renaissance [13]. According to a Health Agency report, exposure to inorganic arsenic has low-mild to severe route-dependent effects on various organs or biological systems [14]. Inorganic arsenic is considered to be a potential carcinogen and its effects are believed to be modulated via genotoxic mechanisms [14,15].

Arsenic affects many organs and impacts a number of functional systems in the body. Acute and chronic intoxication of arsenic is well known and extensively tested in laboratory models [16–21]. Acute arsenic exposure often causes gastrointestinal irritation, diarrhea, dehydration, and nausea. Higher doses may also result in cardiovascular, neurological, and respiratory complications and even lead to hypotension and heart failure [13,22]. Neurological effects can be undetected for some time but often exhibit a severe response leading to neuropathy [13,23]. Chronic exposure to low levels of arsenic causes mild to severe health hazards like diarrhea, skin keratosis, and cancer in skin, bladder, liver, lung, and kidneys.

19.3 Developmental Toxicity

Humans, especially children, might be exposed to arsenic via various sources including soil, water, and copper chromated arsenate (CCA)-treated woods. The Agency for Toxic Substances and Disease Registry (Atlanta, GA) have cited several incidences of arsenic exposure in children in the United States. Exposure in children is more likely to be through drinking water, hand-to-mouth activity, and playing in soil. There are incidents where children were exposed to arsenic due to CCA-coated woods in playgrounds, recreational constructions, and patio decks, and even by burning of CCA-coated plywood in poorly or non-ventilated areas. Toxicological

responses to arsenic suggest that infants and children are more vulnerable to arsenic. Considering the severity of the problem, the United Nations Children's Fund (UNICEF) and World Bank are participating in joint projects to construct wells in the affected areas, thus helping in reduction of infant and child mortalities due to chronic exposure to arsenic.

19.3.1 Effects on the Nervous System

19.3.1.1 Neurochemical Effects

It is well established that continuous exposure to arsenic may lead to detrimental effects on the nervous system [24–28]. It is also reported that the developing brain is more susceptible to the toxic effects of arsenic [29–34]. Experimental studies have been conducted to delineate the neurochemical changes in the developing brain and several mechanisms have been proposed to ascribe the behavioral and intellectual deficits induced by arsenic.

Several studies confirm that arsenic penetrates into cerebrospinal fluid, breaching the blood–brain barrier and resulting in neurological disorders, brain injury, and neurodegeneration [35–40]. Inorganic arsenic is known to induce neurotoxicity and the development of the hallmarks of neurodegenerative disorders [23,40–43]. The mechanisms behind arsenic-induced neurotoxic and neurodegenerative effects have been described in several investigations. The popular mechanisms believed to be associated with arsenic-induced neurotoxic and behavioral perturbations include the induction of oxidative stress, disruption of neurotransmitter systems, and neuronal outgrowth.

19.3.1.1.1 NEUROTRANSMITTER SYSTEMS

As arsenic easily crosses the blood–brain barrier it disrupts several biological functions, leading to neurotoxicity [44,45]. The neurotoxic effects of arsenic are primarily associated with modulations in the neurochemical changes occurring in the cholinergic, gamma-amino butyric acid (GABA)-ergic, and monoaminergic systems [26,46]. *In vitro* assays demonstrated that 0.1 μM and above concentrations of arsenite inhibited the synthesis and release of acetylcholine (ACh) in cerebral slices. Arsenite also decreased the activity of acetylcholinesterase (AChE) but potentiated the choline acetyltransferase (ChAT) activity [47]. Arsenic dose-dependently inhibited the Ach content and AChE activity in brain regions, cerebellum, cerebral cortex, hippocampus, and pons medulla, leading to cholinergic dysfunction. Rats treated with 20 mg/kg body weight of sodium arsenite daily for 28 days showed a significant decrease in AChE activity in hippocampus (46%) and frontal cortex (33%) [48].

Laboratory experiments showed that sodium arsenite modulates the levels of monoamines. Arsenic affects dopamine (DA), norepinephrine (NE), serotonin (5-HT), and monoamino oxidase [49]. Chronic (60 days) exposure to arsenic (1–4 mg/L) via drinking water decreased the levels of DA, NE, and 5-HT and this effect was severe with an increase in concentration of arsenic, showing a dose-dependent effect [50]. In another study, chronic (60 days) exposure to moderate levels of arsenic (4 ppm) in mice significantly reduced the levels of dopamine beta hydroxylase (DBH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TPH), and of NE, DA, and 5-HT [51]. These studies suggest that arsenic affects cholinergic and dopaminergic neurotransmitters.

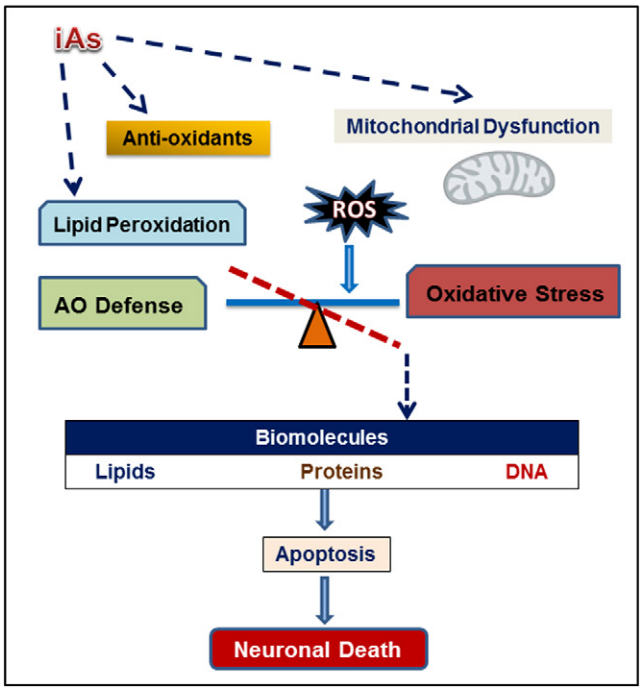

FIGURE 19–1 Arsenic causes apoptosis and neuronal death via modulating oxidative damage. Generation of ROS is induced by xenobiotic exposure. ROS are removed by anti-oxidant (AO) defense mechanisms and balanced by the biological processes of oxidation and reduction (redox). Inorganic arsenic (iAs) promotes generation of ROS and increased ROS shift the balance in the redox systems leading to the accumulation of oxidative stress. ROS interact with biomolecules such as lipids, proteins, and DNA, thereby inducing apoptosis and neuronal cell death.

19.3.1.1.2 OXIDATIVE STRESS

Inorganic arsenic is known to induce the generation of reactive oxygen species (ROS) and reactive nitrogen oxide species (RNOS) [40,52–57]. A number of studies has provided evidence for a co-relation between oxidative stress and arsenic-induced neurological disorders [58–62]. The central nervous system, particularly the brain, contains high levels of polyunsaturated lipid, which utilize large volumes of oxygen and rely on excessive aerobic respiration [63,64]. These factors make the brain sensitive to oxidative stress. Arsenic may cause morphological and structural alterations leading to neuronal death [65,66]. An imbalance between pro-oxidant and anti-oxidant systems results in the accumulation of free radicals, which induces oxidative stress followed by cellular damage and neuronal death (Figure 19–1). Cheng et al. [67] reported that arsenic trioxide induced oxidative damage and disrupted the cerebral cortex in Chinese Dragon Li cats. This group also reported that pretreatment with anti-oxidant resveratrol protected against cerebral injury by up-regulating anti-oxidant enzymes. These findings support the association of arsenic-induced oxidative stress in cerebral injury [67].

Arsenic exists in different oxidation states; however, trivalent (arsenite) and pentavalent (arsenate) states have major roles in environmental exposure [68]. Arsenite reacts with

sulfur-containing compounds and it can act as a potent inhibitor of glutathione reductase, which catalyzes the reduction of glutathione disulfide to glutathione (GSH) [69,70]. Since GSH is involved in resisting oxidative stress and plays a critical role in the maintaining redox state of cells, the inhibition of glutathione reductase by arsenic may trigger the accumulation of cellular oxidative stress.

The role of signaling pathways has been proposed in arsenic-induced oxidative stress and apoptosis. ROS potentially disrupts the activation of certain signaling pathways involving mitogen-activated protein kinases (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), Akt, mechanistic target of rapamycin (mTOR), and endoplasmic reticulum (ER). These signaling pathways regulate the events related to cell growth and differentiation. Disrupting such critical signaling pathways triggers apoptosis, thereby leading to neuronal cell death [57,71–75].

19.3.1.1.3 NEURITE OUTGROWTH

Neurons establish outgrowth by axons and dendrites, which is essential for connectivity and subsequent neuronal function. The neuronal morphology is critical for the function of the nervous system [76] and it is considered as a sensitive marker for assessing neurotoxicity. Neuronal outgrowth can be affected by manipulating the critical components of the cellular cytoskeleton such as microtubules, microfilaments, and neurofilaments light (NFs). NFs are classified as light (NF-L), medium (NF-M), and high (NF-H) depending on the subunits. Aung et al. studied the effect of arsenic on neurite outgrowth using *in vitro* assays and found that sodium arsenate alters the expression of genes associated with the regulation of cytoskeletal components, NF-L, NF-M, tau, and tubulin; however, it has no effect on NF-H and actin [77]. This group also shown that by acting on target cytoskeletal components, sodium arsenate inhibits axon formation and neurite outgrowth [77,78]. Arsenic-induced neuronal outgrowth was further evaluated by other investigators [79,80], and it was found that arsenite exposure (0.5–5 μM sodium arsenite) caused characteristic changes in the morphology of PC12 cells. It was also shown that sodium arsenite exposure caused thickening of neurites, reduced neuronal outgrowth and branching, and inhibited network complexity in PC12 cells [80].

19.3.2 Behavioral Effects

Arsenic is believed to induce neurochemical alterations in the developing central nervous system, which result in neurotoxicity and behavioral perturbations. Exposure to arsenic is known to cause impaired visual perception in Thai children aged 6–9 [81]. The concentration of arsenic was measured in 6- to 9-year-old children (total 529 children). These children were given a Motor-Free Visual Perception test and Visual Motor Integration test and their results were analyzed. A study conducted in Mexico showed a strong correlation between arsenic exposure with poor verbal intelligence and long-term memory in primary school children [82]. In another study, intellectual function in relation to arsenic exposure was evaluated in 351 children aged 5–15 years in West Bengal, India, and it was found that arsenic can induce detrimental effects on intellectual performance [2].

Animal studies also suggest that developmental exposure to inorganic arsenic causes a variety of toxicological effects including growth retardation and malformation, leading to death in hamsters, mice, rats, and rabbits [21,83]. Gestational exposure to higher doses of arsenic exhibited irresistible postnatal malfunctions in cardiac, behavioral, and cognitive functions. Gestational exposure to low levels of arsenic may fail to cause apparent toxic responses during development; however, such developmental exposure may cause behavioral perturbations in adulthood [31,84,85].

Low levels of arsenic caused mild perturbations in psychomotor activity and attentiveness; however, verbal learning and memory are severely affected by arsenic poisoning [13]. Exposure to arsenic also caused neuropathy and consequential brain damage leading to behavioral abnormalities. Impairments could occur in critical neurological functions including deficits in learning and memory [61]. Epidemiological studies also support the association of exposure to low levels of arsenic with cognitive abnormalities in children and adolescents in various parts of the world [86]. Arsenic easily crosses the placenta and may affect the embryo [45,49,87,88]. Maternal exposure to arsenic may occur when pregnant women consume arsenic-contaminated food or water. The effect of *in utero* exposure to arsenic was tested in rats. Pregnant albino rats were exposed to inorganic arsenic (0 or 4.5 or 7.5 mg/kg/day) via gavage from gestation day 8 through birth and pups were monitored for alterations in normal development and behavioral parameters such as righting reflex, neuromotor reflex, forelimb grip strength, and open field activity. This study showed that perinatal exposure to arsenic could lead to detrimental effects in the pups. Arsenic resulted in a dose-dependent increase in fetal mortality and disruptions in developmental and cognitive behavioral activity of offspring [89].

Laboratory studies showed that the neurochemical effects caused by exposure to arsenic could lead to several behavioral and cognitive deficits (Figure 19–2). Gestational exposure to arsenic significantly reduces exploratory behavior, open field activity, and locomotor activity in rats. Consistent with other behavioral observations, arsenic-treated animals showed lower grip strength [90]. Experiments were performed to determine the performance of animals in acquisition and learning using the Morris water maze and found that arsenic exposure significantly affecting the performance of the animals. Animals treated with arsenic showed a poor performance, which was improved when treated with anti-oxidant supplements and chelating agents [34,90–93]. A review of literature on rodent models suggests that regardless of dosing (moderate/high), exposure period (acute/subchronic/chronic), and timing (developmental/adult), arsenic can induce behavioral deficits in memory by causing perturbations in the hippocampus [94].

Epidemiological studies have demonstrated that moderate arsenic exposure could alter cognitive functions of learning and memory during childhood. A significant association was established between urinary arsenic levels (>50 μg/L) and poor scores on language, vocabulary, memory, intelligence, and math skills [82]. Similar results were also found in children exposed to arsenic (147 μg/L in water) throughout development and childhood [2]. An interesting observation from various epidemiological studies is the differential effects of arsenic, with young females suffering a greater impact in terms of growth development compared to males [95,96]. In contrast to arsenic exposure during development, *in utero* As exposure did

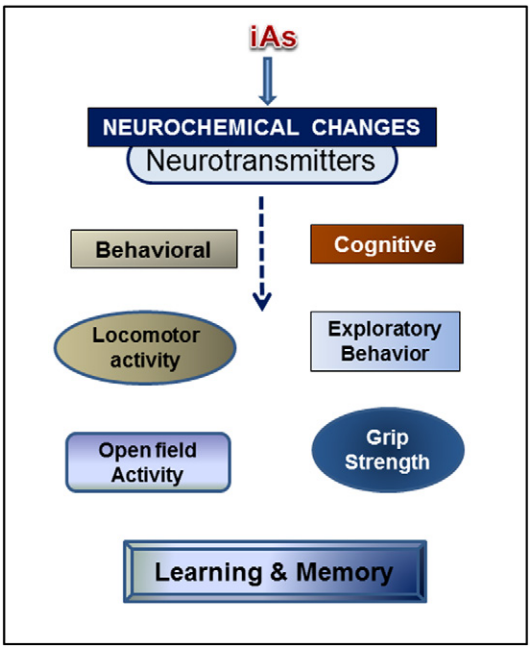

FIGURE 19–2 Arsenic-induced behavioral perturbations. Arsenic exposure is associated with behavioral and cognitive deficits. The developing brain is vulnerable to chemical insults. Exposure to arsenic causes several neurochemical alterations including disruptions in neurotransmitters. These neurochemical changes translate into behavioral deficits. Epidemiological studies show effects on visual perception, psychomotor activity, learning, and memory in children. Consistent with these observations, laboratory studies have reported behavioral and cognitive perturbations in animals treated with inorganic arsenic (iAs). Animals treated with arsenic showed a decrease in locomotor activity, exploratory behavior, open field activity, and grip strength. They also showed poor performance in learning and memory assessed by the Morris water maze experiments.

not exhibit consistent cognitive defects [97,98]. However, some studies show that impaired cognition becomes more evident during adulthood. These results suggest that timing, dose, and duration of exposure determine the extent of neurological damage, with cumulative arsenic intake being an important risk factor compared to acute exposure [99,100]. Several recent studies have also shown the association of arsenic exposure and negative impacts on adult cognition and symptoms of Alzheimer's disease [101,102].

19.4 Conclusions

Arsenic is a xenobiotic environmental metalloid with serious health concerns worldwide. WHO lists arsenic among the top 10 chemicals with major global public health concerns. Acute or chronic exposure to arsenic compounds (especially inorganic arsenic) has detrimental effects on all major organs; however, carcinogenic and neurotoxic effects are more prominent and greatly impact public life. Arsenic causes neuropathy leading to several neurological dysfunctions, and affects learning and memory. It is also clear that the developing

brain is vulnerable to the toxic effects of arsenic [103]. Animal studies assessing the localized effects of arsenic neurotoxicity have shown differential effects on various parts of the brain. In children, often perturbations in psychomotor activity and attentiveness are mild, while verbal learning and memory seem to be severely affected by arsenic poisoning [13].

The precise mechanisms associated with the neurotic effects of arsenic have been investigated. A number of studies published show the association of neurotoxic effects of arsenic with neurochemical alterations in neurotransmitter (e.g., cholinergic, GABAergic, and monoaminergic) systems, induction of ROS, modulations in signaling (MAPK and PI3/Akt) pathways, and disruption of neuronal outgrowth. Arsenic exposure and its subsequent toxicity can be mediated through multiple mechanisms including alteration in epigenetic profiles, uncoupling of oxidative phosphorylation, increased ROS, inhibition of thiol-containing enzymes, altered signal transduction, and cell proliferation [104,105]. Arsenic exposure contributes to dysfunction of the hippocampus; glutamatergic, glucocorticoid, collinergic, and monoaminergic signaling; Alzheimer's disease pathways; and synaptic plasticity (neurogenesis). Studies using animal models have demonstrated that arsenic exposure leads to alterations in hippocampal-dependent tasks, which correlate with the results from epidemiological studies in humans [106,107]. Similarly, arsenic exposure in animals leads to altered synaptic activity and synapse-related gene expression such as reduced NMDA receptor component NR2A [108], and reduced pERK1/2 in the hippocampus [109].

The hypothalamus-pituitary-adrenal axis (HPA) axis regulates neuroendocrine and behavioral responses and its proper functioning is critical for normal physiology and cognition. Corticosterone is a stress hormone that plays a role in mediating the effects of the HPA in response to stress signals. Recent studies indicate that arsenic interferes with the functioning of key components of the HPA axis. Alteration in the HPA axis is observed in neuropsychiatric illness and is connected to depression like symptoms [110,111], which correlates with decreased behavioral ability and increased depressive symptoms in arsenic-exposed animals. Arsenic has multiple effects on the HPA: modification of the levels of catecholamine norepinephrine in the hypothalamus, which regulate corticotrophin-releasing factor [112]; increases in plasma adrenocorticotrophin hormone [113]; and increases in plasma corticosterone levels [114]. Signaling via corticosterone is mediated by the mineralocorticoid and glucocorticoid (GR) receptors. Studies have shown that mice exposed to arsenic have lower levels of GR in hippocampal tissue; this alters the expression of H-Ras and Raf-1, and in turn leads to decreased expression of ERK [115], whose targets play an important role in learning and memory. A recent study also demonstrated the susceptibility of the glucocorticoid receptor to arsenic, which in turn affects MAPK activation and the HPA axis, which are important components for hippocampal-related functions like learning and memory [116]. Arsenic exposure in rodents also affects motor learning, cholinergic signaling, and locomotion possibly through reduction in acetylcholinesterase and choline acetyltransferase activity [48].

Metal chelators are routinely used in the treatment of heavy metal toxicity. Chelating agents such as British Anti Lewisite (BAL), sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), meso-2,3-dimercaptosuccinic acid (DMSA), and MiADMSA have been tested for treating arsenic toxicity [91–93]. Supplementation of essential metals Ca or Zn and chelating agent MiADMSA

has reversed the arsenic-induced neurochemical alterations in various brain regions in rats. Interestingly, combined supplementation of essential metals and chelating agent resulted in higher recovery [33]. The brain is highly susceptible to oxidative damage due to higher utilization of oxygen and the presence of high iron content and unsaturated fatty acids [63,64], hence many studies attributed oxidative stress as a major mechanism in arsenic-induced neurotoxicity [52–55]. Since the association of ROS induction in arsenic poisoning is an accepted fact, apart from chelating agents, several therapeutic strategies have been employed, along with the combination of antioxidant supplements. Laboratory studies were performed using antioxidants including resveratrol and natural products such as *Centella asiatica* aqueous extract and *Moringa oleifera* seed powder, all of which showed some promising results [54,117]. Further studies are under way to identify effective therapeutic agents including natural compounds; also novel strategies are being developed to prevent exposure to this xenobiotic metal.

Neurobehavioral disorders affect approximately 15% of all births. Environmental factors are believed to play major roles in the developmental of such disorders and arsenic is among the five industrial chemicals that are identified as developmental intoxicants in a systematic review conducted by Grandjean and Landrigan in 2006 [118]. These investigators also later postulated that additional candidates may join the list after further screening [103]. The research conducted on assessing toxicity of arsenic using rodent models delineated the effects on multiple systems and specific pathways associated with learning and memory; however, further epidemiological studies are recommended for evaluating the status of the existing standard dose (10 μg/L) corresponding to the maximum limit for arsenic exposure [94,119]. While research on understanding the toxicity of arsenic has identified specific pathways, further studies should focus more on fulfilling the need for developing therapeutic strategies to overcome the arsenic-induced developmental perturbations in cognitive functions.

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Arsenic and the Cardiovascular System

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CHAPTER OUTLINE

20.1 Introduction

Arsenic (As), bearing atomic number 33 with a relative mass of 74.92, has physical and chemical properties in between metals and non-metals, and is thus often referred to as a metalloid. This existing metalloid belongs to Group VA of the periodic table with four oxidation states (−3, 0, +3, and +5), out of which arsenite (As^{III}) and arsenate (As^{V}) are the most prevalent forms found in the environment. As is ubiquitous and can be found naturally in water, soil, rocks, and living organisms in concentrations ranging from parts per million (ppm) to parts per billion (ppb). Interestingly, it is very rare to find As in free natural form; instead As combines with a variety of other elements such as oxygen, hydrogen, sulfur, nickel, cobalt, copper, iron, lead, calcium, magnesium, and zinc, and many more. This versatility of binding or coexistence of As renders its presence ubiquitous, and it is therefore impossible for animals, including humans, to avoid exposure to it from natural sources. Thus, exposure to arsenic is inevitable.

From a biological and toxicological perspective, arsenic exists in three major forms: inorganic, organic, and arsine gas. Of the inorganic forms arsenic trioxide, sodium arsenite ($NaAsO_2$), and arsenic trichloride ($AsCl_3$) are the most common trivalent forms, whereas

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arsenic pentaoxide (As_2O_5), arsenic acid (H_3AsO_4), and arsenates (AsO_4^{3-}) (such as lead arsenate) are the most common pentavalent forms. Organic arsenic compounds include arsanilic acid ($C_6H_8AsNO_3$), methylarsonic acid (CH_5AsO_3), dimethylarsenic acid ($C_2H_7AsO_2$), and arsenobetaine ($C_5H_{11}AsO_2$).

Arsenic is the 20th most abundant element in Earth's crust but the major sources of arsenic exposure to humans are drinking water and occupational exposure. Arsenic in water usually comes from natural sources with varying concentrations; however, high arsenic levels occur in hot water springs, and in ground water in areas of thermal activities or rocky areas with high salt concentrations. Further, the arsenic form and its concentrations depend on various factors. These include the level of oxygen in the water, the degree of biological activity, the type of water (fresh, sea, or ground) and the proximity to arsenic-rich geographical locations. Fresh water arsenic concentrations are usually less than 10 μg/L but can be as high as 5 mg/L near As-rich locations. Similarly, ground water averages 1–2 μg/L but can be as high as 3 mg/L near volcanic regions (WHO, 2001). Exposure to high levels of arsenic in drinking water is well reported from around the globe, notably in China, India, Bangladesh, Taiwan, and to a lesser extent in Argentina, Australia, Chile, Mexico, the USA, Vietnam, Japan, Thailand, and Brazil. The levels in some of these regions are astonishingly high, from tens to a few thousand micrograms per liter. It is estimated that more than 100 million people worldwide are exposed to arsenic levels exceeding 50 μg/L [1]. Exposure also takes place during certain operations such as mining, smelting, and burning fossil fuels. Furthermore, exposure could also occur through As-based pesticides, insecticides, food products, and herbal medicines, but exposure from these sources has now declined considerably as per Environmental Protection Agency (US EPA) guidelines [2].

Arsenic exposure and its association with numerous diseases such as blackfoot disease, cancers, central nervous system dysfunction, anemia, and cardiovascular disease (CVD) including coronary heart disease (CHD), stroke, and peripheral arterial disease (PAD) is well reported in the literature [1]. While chronic arsenic exposure has been linked to numerous diseases, the most well-established mechanism for arsenic toxicity in biological species is oxidative stress. Arsenic increases the production of reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide, superoxide radicals, and many more (reviewed by Flora [3]). These ROS specifically disrupt the mitochondrial membrane potential and activate translocation of cytochrome c and caspase, resulting in cellular apoptosis. Apart from cellular apoptosis, arsenic also interacts with numerous key enzymes, resulting in physiological dysfunctioning.

The only respite patients have from arsenic poisoning is chelation therapy. The use of chelation therapy dates back more than 100 years. There are not many chelating agents available for therapeutic purposes. British anti-Lewisite was developed as an antidote to Lewisite (dichlorovinyl arsine) during World War II but it has numerous side effects including essential metal loss and toxicity [4]. DMSA (meso-2,3-dimercaptosuccinic acid), a dithiol chelator with minimal side effects, was effective in treating chronic arsenic poisoning in animals, but double blinded randomized clinical trials did not yield any beneficial results [5]. Recently, MiADMSA, an isoamyl ester of DMSA, has shown to be more promising than the parental compound [4,6–10]. Phase I clinical trials for MiADMSA have been approved and their results are awaited. However, currently there is no literature that shows the effect of chelation therapy on the cardiovascular system.

Although there have been numerous studies on arsenic and its toxicity in human and animal models, most of these are focused on arsenic-induced oxidative stress and recovery of biochemical variables post-therapy with chelating agents or antioxidants or their combination. Studies have also shown linkages of arsenic to various diseases but most of the focus has been on neuronal, hepatic, and cancer studies. In-depth and systematic studies investigating the effects of As on the cardiovascular system are limited. In this chapter, we shall focus on the effects of arsenic on this system. The chapter has been divided based on the major constituents of the cardiovascular system (blood, vasculature, and heart); however, an overlap of information may be present as it would be hard to delineate the effects arsenic has on individual tissues.

20.2 Cardiovascular System

In general, the cardiovascular system, also known as the circulatory system, consists of heart, a network of blood vessels (arteries, veins, and capillaries), and approximately 5 liters of blood, which are responsible for transporting oxygen and nutrients throughout the body. It is also known to be the hardest working organ of the human body. While transportation of materials such as oxygen and nutrients is one of the key functions of this system, it also protects the body through circulating white blood cells in case of infections/diseases and for cellular debris clean-up. The cardiovascular system is, in addition, instrumental in maintaining of body homeostasis by regulating body temperature, pH, and osmotic concentrations.

Chronic arsenic exposure is well documented to induce cancers of the skin, bladder, and lungs but the association between chronic arsenic exposure and cardiovascular disease has been less well studied. However, in the recent past, epidemiological studies have provided highly indicative data supporting the role of arsenic in cardiovascular disease. Although not conclusive, recent studies have shown that the linkage or disease aggravation may be more prominent in arsenic-exposed populations. It is now becoming more evident that arsenic is linked with vascular diseases, endothelial dysfunctioning, blood pressure, hypertension, ischemic heart disease, atherosclerosis, calcium overload, and arrhythmias. Furthermore, oxidative stress in tissues caused by the burden of arsenic leads to dysfunction of structural and functional proteins, further elevating cardiovascular disease. Early exposure to arsenic during pre- or neonatal developmental stages increases mortality in infants due to acute myocardial infarction and arterial diseases. Moreover, arsenic exposure is also known to alter micro-RNA (miRNA), a group of small RNAs that regulate gene expression and are known to play a critical role in modulating embryonic and fetal development. Changes in miRNA levels during development are associated with alterations of histone modifications and DNA methylation status, which regulates gene expression at the epigenetic level. In the following sections, we shall discuss in detail the effects of arsenic on heart, blood, and vasculature in animal models and humans and attempt to gain a better understanding of the role of arsenic in the cardiovascular system. In the last part of the chapter, we showcase a new technology that has induced pluripotent stem cells or embryonic stem cells that can now provide us with both a better model to study human development and also humanized models to study cardiac development in arsenic-exposed conditions.

20.3 Arsenic Effects on Blood

In this section, we focus on the toxic effects of arsenic on one of the major components of the cardiovascular system: blood. While oxidative stress is currently the most well-documented mechanism of arsenic toxicity, it is believed that arsenic's direct interaction with components of blood such as hemoglobin may also play a pivotal role in mediating its toxicity. Blood, being the first line of cellular defense, is directly affected by arsenic. Arsenic enters the bloodstream as arsenate and is metabolized within hours, being reduced to arsenite before entering the cell. Since blood is one of the most easily accessible biological fluids and can provide systemic information on the biological effects of arsenic, a plethora of literature exists where blood is used as a means to understanding arsenic poisoning. While it would be impossible to cover all these studies in this chapter, we summarize the major findings that have helped researchers in understanding the role of arsenic in blood.

20.3.1 Erythrocytes

Hemoglobin (Hb) is one of the main constituents of red blood cells and its interaction with arsenic is well reported. Hb is rich in –SH groups and it is suggested that arsenic and its metabolites may have a propensity to bind to Hb and reduce its binding affinity to oxygen. Recently, Mondal et al. [11] studied the binding affinity of trivalent arsenic to Hb and its consequential modification of the structure and function of Hb. Isolated Hb from arsenicosis patients had a binding affinity constant of $0.256\,\mu M^{-1}$ with a Hill coefficient of +2.961, suggesting that arsenic molecules modify and lower the binding affinity to oxygen. The study also showed that Hb has a higher binding affinity to arsenic than to oxygen. Similarly, Lu et al. [12] evaluated the binding affinities of four trivalent As species: iAs, MMA, DMA, and PhAsO in rat and human Hb. Rat Hb demonstrated a 3–16 times stronger binding to arsenic than did human Hb but most of it was found in protein-bound form. In two early studies, Breton et al. [13] investigated the role of Hb in the development of arsenic-induced skin lesions in 900 case–control pairs from Bangladesh using conditional logistic regression. The second study examined the association between toenail arsenic, urinary arsenic, and Hb within a cohort of 184 individuals from 50 families in the same region who did not have arsenic-induced skin lesions. While Hb showed a significant association with skin lesions in the first study that was gender specific for males, no association was observed between toenail, urinary arsenic, and Hb levels in the second study. These studies show that in individuals with skin lesions, there was a clear relationship between arsenic binding and Hb levels.

A plethora of studies performed in animals by Flora and his group have shown that arsenic is known to inhibit the rate limiting enzyme δ-aminolevulinic acid dehydratase (ALAD), a sulfhydryl-rich enzyme of the heme biosynthesis pathway [14–18]. In mammalian and avian tissues the principal product of this pathway is heme (ferro-protoporphyrin IX), an essential component of various biological functions including oxygen transport, mixed function oxidative reactions, and other oxidative metabolic processes. In one such study, Bhadauria and Flora [17] showed that dose-dependent exposure of As^{III} or As^{V}, *in vitro* or *in vivo*,

demonstrated a significant loss of ALAD activity. It was, however, interesting to note that at higher doses, As^{III} showed a more toxic effect than As^{V}. These effects were attributed to the attachment of arsenic to –SH groups of the enzyme since As has a strong affinity for these groups [14,19], which renders the enzyme inactive. It is also suggested that inhibition of ALAD results in an accumulation of ALA, which may play a role in arsenic-mediated ROS generation in blood [17]. These studies provide evidence that arsenic interferes with heme biosynthesis and ALAD may be a sensitive biomarker to evaluate heavy metal poisoning. One of the major components of the antioxidant defense system is glutathione, a cysteine-rich, primary intracellular antioxidant molecule. While glutathione may act in multiple pathways, the two important pathways in this context are its donation of an electron to ROS (simultaneously converting to GSSG) (glutathione disulfide) and donation of an electron to reduce arsenate to arsenite. Yildiz and Cakir [20] demonstrated that arsenic exposure causes an efflux of glutathione from erythrocytes in a dose- and time-dependent manner. In another cross-sectional study, a total of 378 participants from areas with different arsenic water concentrations were recruited and their blood GSH and GSSG were evaluated through HPLC. The results showed that the interquartile range increase in water arsenic was negatively associated with blood GSH and urine arsenic levels [21]. These observations are consistent with other observations in animal studies where arsenic exposure influences GSH levels in blood [22,23]. These results suggest that arsenic not only has disruptive effects, by direct interactions with heme biosynthesis, but also has important antioxidant molecules like GSH in blood. Apart from these biochemical changes, arsenic has also been shown to induce morphological changes in red blood cells (erythrocytes). Winski and Carter [24] demonstrated that sodium arsenite or arsenate induced significant death of red blood cells (hemolysis). Furthermore, morphological changes such as classical discocyte–echinocyte transformation to sphero-echinocytes were observed in a dose-dependent manner in arsenate groups but not extensively in arsenite groups. Furthermore, these changes coincided with ATP depletion in these cells and it was suggested that erythrocytes are 1000 times more sensitive to arsenate than arsenite. A decade later, Biswas et al. [25] showed that a decreased lifespan of erythrocytes in arsenic-exposed populations resulted in anemia. This decrease in lifespan was due to significant morphological changes where human erythrocytes transformed from smooth discoid red cells to echinocytic to irreversible spheroechinocytes. Furthermore, arsenic exposure decreased cellular ATP along with increased microviscosity and an elevated cholesterol/phospholipid ratio that hampered the flexibility of the erythrocytes. These cumulative effects together triggered apoptotic removal of cells from the system, depicting the involvement of both erythrophagocytosis and hemolysis in the destruction of human erythrocytes during chronic arsenic exposure.

In a separate study the same group [26] also showed that chronic arsenic exposure in rats led to a gradual accumulation of arsenic in erythrocytes and 4-hydroxylnonenal (4-HNE) contributed significantly to the pathological events observed during oxidative stress. 4-HNE triggered death signal cascade, which was initiated by the generation and accumulation of various reactive radicals (e.g., H_2O_2, superoxide, and hydroxyl radicals) that eventually increased caspase activity leading to cellular apoptosis in rat erythrocytes. Interestingly, antioxidants such as NAC could attenuate these effects. The study also demonstrated that arsenic accumulation

caused degradation of band 3 leading to its aggregation on the surface of erythrocytes, which resulted in the activation of the complement system to eliminate the affected cells from the circulation. These studies indicate that arsenic could reduce the lifespan of red blood cells in circulation. Exposure of erythrocytes for 5 minutes to arsine, the simplest yet most toxic form of arsenic, at 1 mM results in hemolysis, accompanied with loss of cell volume, leakage of potassium, and influx of sodium. However, no significant changes in ATP levels were observed [27]. Interestingly, Biswas and co-workers [28] demonstrated the role of cholesterol in promoting ROS-mediated Fas signaling-dependent apoptosis via caspase-3 in erythrocytes post-arsenic exposure, suggesting a new pathway for cellular apoptosis in red blood cells.

While the above studies demonstrate the direct binding effect of arsenic in red blood cells in humans and animals, arsenic may also indirectly exert its toxic effect through oxidative stress. Flora and co-workers [29] demonstrated that chronic exposure of rats to arsenic at 25 ppm through drinking water for 24 weeks significantly reduced blood ALAD levels, enhanced zinc protoporphyrin (ZPP), and also altered various clinical hematological variables like WBC, MCH, MCHC, and platelet counts. Furthermore, these changes were accompanied by decreased SOD and increased catalase activities. Recently, Prabu and Sumedha [30] demonstrated that rats orally administered with 5 mg/kg of arsenic for 28 days showed significant alterations in plasma marker enzymes, plasma and erythrocyte membrane oxidative stress markers, erythrocyte membrane antioxidant enzymes, and non-antioxidant enzymes indicative of oxidative stress in erythrocytes. Arsenic exposure also reduced the activity of membrane bound ATPase and increased the percentage of DNA tail length in lymphocytes, suggesting DNA damage. Further, in a population-based study, Caciari and co-workers [31] determined that there was a correlation between chronic low-dose arsenic exposure and alterations in the hematopoietic system. The authors recruited 349 policemen and divided them into three groups based on urinary As levels. The subjects were further divided according to gender, smoking habits, and work task. Based on multiple linear regression and correlation analysis, a significant correlation and regression was seen between urinary arsenic, RBC, WBC, Hb, hematocrit, and reticulocytes. While arsenic causes dysfunction of blood through ROS generation, Chou and colleagues [32] suggested that this may be beneficial for the therapeutic regimes of acute myelocytic leukemia (APL) patients. The authors suggested that arsenic mediated ROS could induce apoptosis in cancer cells. Using interference RNA and genetically engineered cells, they demonstrated that NAPDH oxidase is a prime target of ROS, which is important for antibacterial function of WBCs, which may play a critical role in the treatment of APL patients.

20.3.2 Leukocytes

Apart from red blood cells, other cell types are also affected by arsenic. A microarray-based genome-wide analysis of expression patterns from peripheral blood of 21 samples showed significant differences in the abundance of transcripts between high- and low-arsenic exposure groups. The major genes that were altered belonged to defense response, immune function, cell growth, apoptosis, cell-cycle regulation, T-cell receptor signaling pathway, and diabetes. Furthermore, groups exposed to higher arsenic concentrations exhibited several killer cell

immunoglobulin-like receptors that could inhibit natural killer cell activities [33]. Banerjee and co-workers [34] compared the degree of apoptosis in peripheral blood of 30 arsenic exposed individuals with skin lesions with that in 28 unexposed individuals and observed that the percentage of apoptotic cells was significantly higher ($p < 0.001$) in the former group. The mechanism for cellular apoptosis in these peripheral blood mononuclear cells (PBMCs) was shown to be ROS driven and mitochondrial dependent. Furthermore, arsenic-exposed PBMCs showed cell cycle arrest at the G0/G1 phase. Two independent studies by Avani and Rao [35] and Pant and Rao [36] showed that exposure to arsenic caused peripheral blood lymphocytes and blood cultures to significantly increase the incidence of chromatid exchange and alter the cell-cycle proliferative index. More recently, Sharma and co-workers [37] reported on one of the probable mechanisms of how arsenic could impair cell-cycle regulation. Low-dose arsenic exposure of PBMCs resulted in overexpression of miR-2909. This overexpression of miR-2909 was shown to regulate *cyclin D1* expression within these cells, by inducing splice switching of tumor suppresser *CYLD* as well as modulation of SP1 activity through repression of *KLF4* expression at the translational level. Furthermore, As-dependent regulation of *ATF* and *BCL3* was also modulated through its capacity to induce miR-2909 expression. This may be a critical step in how arsenic modulates cell-cycle regulation in lymphocytes.

While apoptosis and cell-cycle regulation may be more general effects of arsenic, the subtype population with the lymphocytes shows alterations of various pathways. Gupta and co-workers [38] showed that As_2O_3 at clinically achievable therapeutic concentrations induces apoptosis in normal T cells by increasing the ROS burden, decreasing mitochondrial membrane potential and intracellular GSH, down-regulating Bcl-2, translocating cytochrome c, and activating caspases. Furthermore, addition of exogenous GSH or overexpression of Bcl-2 inhibited As_2O_3-mediated apoptosis in T cells. Yu and co-workers [39] investigated T-cell survival and function in mononuclear cells and showed that arsenic at 1 μM dose induces TNF-α release and had cytotoxic effects on T cells, especially $CD4^+$ cells inducing apoptosis. This enhanced TNFR1 expression along with death domain proteins and caspases was more prominently observed in $CD4^+$ cells as compared to other mononuclear cells, suggesting the role of TNFR1 signals in arsenic-induced T helper cell apoptosis. In a similar study, dose-dependent exposure to sodium arsenite (0.001, 0.1, and 1 μM) showed that 1 μM was more toxic to T-helper ($CD4^+$) than T-cytotoxic ($CD8^+$) cells in human PBMCs. Furthermore, PBMCs from females demonstrated a more reduced T-lymphocyte cell proliferation than males, suggesting that females may be more susceptible to arsenic toxicity [40]. The study by Morzadec et al. [41] demonstrated that non-cytotoxic concentrations of NaAs (0.25–2 μM) significantly reduced T cell proliferation by increasing the percentage of non-dividing cells via causing a block in the G1 phase and preventing cyclin D3 and *CDC25A* expression, which may be IL-2 independent. Contrary to this, Martin-Chouly and colleagues [42] showed that arsenic reduces IL-2 secretion and T cell proliferation. They also showed that arsenic blocks monocyte-dependent accessory signals by PHA resulting in reduced expression of monocyte-derived cytokines such as IL-1, IL-6, and TNF-α. Moreover, microarray studies showed that 35 genes related to the immune system and stress factors were also significantly up-regulated. These results showed that arsenic interacts with T cells directly.

Apart from T cells that constitute the immunological system, arsenic is known to impair macrophages too. Effects of inorganic and methylated arsenicals were evaluated in murine macrophages *in vitro*. Interestingly, both inorganic arsenicals (arsenite and arsenate) induced activation of inflammatory cytokines, TNF-α, and necrotic death (80%) with limited apoptosis (20%) at low doses and were strongly toxic to macrophages. In contrast, the methylated arsenical, DMMA suppressed TNF-α levels and exerted cell death through apoptosis at significantly higher doses than inorganic arsenicals. MAA and TMAO did not show any significant toxicity at even higher concentrations, suggesting that methylation of arsenicals may reduce the inflammatory and immunological responses of inorganic arsenicals [43]. Arsenic trioxide also significantly increases superoxide levels in a time-dependent manner in human monocyte-derived macrophages. Phosphorylation of p38 kinase followed by phosphorylation and membrane translocation of NADPH oxidase subunit p47(phox) and translocation of Rac1 and p67(phox) has been noted. Using selective inhibitors of NAPDH oxidase (Apocynin) or p38 (SB203580) these changes could be reversed in macrophages, suggesting that arsenic induces alterations in Rho-kinase/p38-kinase pathways, which impairs macrophage function [44]. In humans, hematopoietic cells from 70 individuals with skin lesions that were chronically arsenic exposed were compared with 64 controls [45]. Arsenic-exposed macrophages from these individuals showed cell rounding with a significant ($p < 0.0001$) loss of cell adhesion capacity, decrease in NO production, impaired phagocytic capacity, and decreased CD54 and F-actin expression, with affected RhoA-ROCK pathway, suggestive of immunosuppression in these individuals [45]. Microarray studies on human monocyte-derived macrophages following arsenic trioxide treatment for 72 h show up-regulation of 32 genes and repression of 91 genes. Twenty-six genes altered in these studies were specifically related to the differentiation program of macrophages. The prominently altered genes were *MMP9*, *MMP12*, *CCL22*, *SPON2*, and *CXCL2*, which are an integral part of macrophage function [46]. These microarray studies have been supported by research investigating chronic exposure as well. Prolonged exposure to arsenic alters macrophage-specific (*MMP9*, *CCL22* and *CXCL2*), stress-specific (*HMOX1* and *GCLM*) genes and nuclear levels of Nrf2 and Bach1, and represses the expression of transcriptional factor EGR2, a protein involved in macrophage differentiation [47]. More recently, Wang et al. [48] showed that non-lethal concentration of arsenic trioxide (1 and 2.5 μM) markedly inhibited monocyte-to-macrophage differentiation together with expression of macrophage markers. However, these changes could be reversed following treatment with α-lipoic acid. Taken together, these studies suggest that arsenic alters signaling pathways important for differentiation as well as the function of macrophages and thus indirectly compromises the cellular immune system.

Like macrophages, lymphocytes show differential effects towards arsenic species. For instance, Colognato et al. [49] evaluated the genotoxic effects of six different As compounds (As^{III}, As^{V}, $MMAs^{III}$, $MMAs^{V}$, $DMAs^{V}$, and $TMAO^{V}$) on lymphocytes. While 4 μM of As^{III} and 32 μM of As^{V} increased micronuclei frequency, a statistically significant increase in micronucleus frequency was also seen with 2 μM of MMA^{III} and 500 μM of MMA^{V}. No significant micronuclei were observed with DMA^{V} and $TMAO^{V}$, suggesting that MMA^{III} had aneuploidogenic properties and could induce geno- and cytotoxic effects. A study of 87 children aged 6–10 years evaluating the effects of arsenic exposure on immune cells using multiple linear

regression analysis showed iAs had a positive association with basal NO^- in PBMCs ($p = 0.049$) and monocytes ($p = 0.04$), while basal O_2^- had an association with DMA ($p = 0.046$) [50]. Interestingly, in activated monocytes, a positive association was also observed with iAs, MMA, DMA, and tAs, suggesting that activated and inactivated cells could have differential responses [50]. Apart from T cells and monocytes, macrophages also play a vital role in the human immune defense system.

In one of the early pilot studies, analysis of blood and urine samples from 11 individuals chronically exposed and 13 individuals with lower exposure to arsenic was performed [51]. Interestingly, percentages of chromosomal aberrations and the frequencies of sister-chromatid exchanges were similar in both groups. However, complex aberrations were more frequent in the highly exposed group, which also showed a higher average variation frequency in the HGPRT assay. However, these observations were not statistically significant [51]. Later, *in vitro* studies evaluating lymphocytes from control, symptomatic (having As-induced lesions), and asymptomatic (no skin lesions but exposed to As) samples found significant changes in chromosomal aberrations [52]. While both symptomatic and asymptomatic samples accumulated more arsenic in nails and hair, excreted less in urine, and showed chromosomal aberrations as compared to the controls, significantly higher incidences of aberrations were observed in the symptomatic group [52]. Similarly to this study, lymphocytes of 30 individuals exposed to high levels of As ($247 \pm 18.93\,\mu g/L$) through drinking water in West Bengal, India, when compared with 30 unexposed ($7.6 \pm 0.5\,\mu g/L$) individuals, showed significant signs of DNA damage ($p < 0.01$) in lymphocytes. The comet tail lengths showed a positive correlation with the arsenic levels [53]. In another study, telomere length and expression of genes related to telomeres were evaluated in a cohort of 202 women exposed to a wide range of arsenic (3.5–200 µg/L) [54]. A significant correlation was observed with telomere length and urinary arsenic levels. Moreover, individuals with above median fractions of iAs and MMA showed longer telomere lengths than those below the median [54]. As evidenced by these reports, DNA-based tools could be used as a sensitive biomarker for arsenic poisoning.

20.3.3 Methyltransferases

Although arsenic is a potent toxicant, the efficiency by which the biological system metabolizes arsenic may govern differences between the toxic effects of arsenic across various populations. This complex process is governed by the arsenic methyltransferase gene (*AS3MT*), and more recently a putative N-6-adenine-specific DNA methyltransferase 1 (*N6AMT1*) has been reported to methylate arsenic *in vitro* [55]. A large number of studies in recent years have been done using lymphocytes/blood to demonstrate that changes in epigenetic levels may regulate the toxic manifestations of arsenic. In one study, Chervona and colleagues [56] evaluated global histone methylation patterns of H3K9me2, H3K9ac, H3K27me3, H3K27ac, H3K18ac, and H3K4me3 in peripheral blood from a subset of folate clinical trial participants ($n = 40$). The study reported that total urinary arsenic levels correlated with H3K9me2 ($p = 0.02$) and inversely with H3K9ac ($p = 0.002$). Interestingly, other methylation patterns showed a gender bias, where H3K4me3 and H3K27me3 positively correlated with females, whereas H3K27ac

and H3K18ac negatively correlated with females, indicating that arsenic exposure affects the whole genome methylation pattern and there may be a gender-specific pattern of histone association marks showing linkage to epigenetic dysregulation. However, most of the studies are focused on specific methyltransferase genes. Engström et al. [57] evaluated the impact of polymorphism of five methyltransferase genes (*AS3MT*, *DNMT1a*, *DNMT3b*, *PEMT*, and *BHMT*) on As metabolism in peripheral blood of two groups (South American ($n = 172$) and Southeast Asian ($n = 361$)) with urinary arsenic levels 200 µg/L and 100 µg/L, respectively. The results showed that six *AS3MT* polymorphisms were significantly associated with As metabolite patterns in both groups ($p < 0.01$). Interestingly, the frequency of the *AS3MT* haplotype in Bangladesh was prominently associated with MMA% whereas the most frequent haplotype in the Argentina group was associated with DMA% and not MMA%. More specifically, *AS3MT* Met287Thr allele frequencies were evaluated and a micronucleus assay was performed on 207 subjects exposed to different arsenic levels. While peripheral blood micronucleus frequencies poorly correlated with arsenic levels, *AS3MT* Met287Thr genotypes carrying the variant allele showed significant correlation with micronucleus frequencies ($p = 0.0003$) [58].

In a recent study, arsenic methylation efficiency in 188 women exposed to arsenic contaminated drinking water (≈200 µg/L) in the Argentinean Andes was genotyped for *N6AMT1* and *AS3MT* polymorphism in blood samples [55]. Five *N6AMT1* SNPs (single nucleotide polymorphisms) and two *N6AMT1* haplotypes were significantly associated with the MMA% in urine. The MMA% increased uniformly according to the number of alleles for each SNP (mean MMA% was 7.5% for GG, 8.8% for GT, and 9.7% for TT carriers). Recently, Gribble et al. [59] evaluated methylation status of 48 CpG loci at the *AS3MT* promoter region from 48 participants. Based on linear regression analysis a hypomethylated region in the *AS3MT* promoter was associated with higher arsenic exposure. These results suggest that not only the gene polymorphism but also the promoter regulation is disrupted by arsenic [59]. These studies clearly suggest that governance of arsenic metabolism may be playing a very important role that could provide indications on the level of toxicity arsenic would induce on prolonged exposure. These studies pave the way to develop better diagnostic tools for predicting population susceptibility to various arsenic-linked diseases such as cancers.

20.4 Arsenic Effects on the Vascular System

20.4.1 Clinical Studies

Many studies have been conducted that show a direct correlation between As exposure and increased risk of cardiovascular disease (CVD). Wang et al. conducted a study where 605 Taiwanese residents were evaluated for development of carotid atherosclerosis due to As exposure [60]. Residents who fell into the following categories of carotid artery intima-media (IMT) thickness >1.0 mm, plaque score ≥ or stenosis >50% were diagnosed as having carotid atherosclerosis. During the study a dose-dependent relationship between As exposure and increased risk of carotid atherosclerosis was observed. Residents who had been exposed to high levels of As (>50 µg/L) had an age- and gender-adjusted odds ratio (OR) of 3.3 for developing carotid

atherosclerosis while those who had been exposed to low (≤10 μg/L) and mid-levels (10.1–50 μg/L) of As had an OR of 1.0 and 1.4, respectively [60]. Similarly, Hsieh and co-workers also showed a dose-dependent relationship between As exposure and increased risk of carotid atherosclerosis in Taiwan [61]. Residents who had been exposed to high levels of As in drinking water (≥50 μg/L) with a cumulative arsenic exposure (CAE) of ≥1.1 mg/L/year had an age- and gender-adjusted OR of 2.4 and 1.9 for developing carotid atherosclerosis, respectively ($p < 0.05$). Apart from carotid atherosclerosis, hypertension has also been associated with As exposure. Huang and colleagues showed that inefficient As methylation (the ability to metabolize and excrete As) may lead to increased risk of hypertension. Based on a positive history or measured systolic blood pressure (≥140 mm Hg) and/or diastolic blood pressure (≥90 mm Hg), 372 out of 871 As-exposed Taiwanese residents were diagnosed as having hypertension. The primary As methylation index [PMI, defined as monomethylarsonic acid (MMA^V) divided by (As^{III}+As^V)] and secondary arsenic methylation index [SMI, defined as dimethylarsinic acid (DMA^V) divided by MMA^V] were the indicators used to determine the level of As methylation [62]. Interestingly, although the residents had stopped consuming artesian well water for 20–30 years, the level of As in urine was still directly related with CAE ($p = 0.02$), indicating the long-term effects of As exposure. Residents with hypertension had higher percentages of MMA^V and lower SMI than those that did not have the disease. However, residents having CAE >0 mg/L/year had a higher risk of hypertension than those who had CAE = 0 mg/L/year regardless of the methylation index [62].

A study of 11,746 residents in Bangladesh was evaluated for a period of 6 years to investigate the affiliation of As exposure and CVD mortality rate [63]. A dose-dependent relationship was observed between As exposure and CVD mortality rate. Residents who had been exposed to low (<12.0 μg/L) and high levels of As (≥12.0 μg/L) had mortality rates of 214.3 and 271.1 per 100,000 person years, respectively. When As exposure was further categorized into low (0.1–12.0 μg/L), mid- (12.1–62.0 μg/L), high (62.1–148.0 μg/L), and very high levels (148.1–864.0 μg/L), the hazard ratios for ischemic heart disease (IHD) and other CVDs were 1.00, 1.22, 1.35, and 1.92, respectively ($p = 0.0019$ for trend). A synergistic interaction between As exposure and cigarette smoking was also associated with CVD mortality. This joint effect was instrumental in increasing CVD mortality in that the hazard ratio of moderate As exposure levels (mean 63.5 μg/L) and cigarette smoking was greater than the sum of the hazard ratios associated with individual effects ($p = 0.010$) [63]. While most of these studies were conducted in the Asian continent, studies from European regions also show similar trends. Medrano and colleagues investigated the association of As concentrations with CVD mortality by analyzing the standardized mortality rates (SMR) for cardiovascular, coronary, and cerebrovascular disease at the municipal level [64] between 1998 and 2002. For municipals having As concentrations >10 μg/L, the SMR for cardiovascular, coronary, and cerebrovascular disease were 1.10, 1.18, and 1.04, respectively. Further, municipals having As concentrations between 1–10 μg/L and >10 μg/L showed an increase of 2.2% and 2.6%, respectively in cardiovascular mortality rates when compared to municipals having very low As concentrations (<1 μg/L) (p-value for trend 0.032). For coronary heart disease the increase in mortality rate was 5.2% and 1.5% while for cerebrovascular disease it was 0.3% and 1.7%, respectively. These ecological studies demonstrate that prolonged As exposure could result in elevated CVD risk [64].

20.4.2 Atherosclerosis

It is well documented that prolonged As exposure leads to atherosclerotic vascular diseases [65,66] by inducing formation of ROS. Arsenic augments the levels of oxidative stress allowing for the accumulation of oxidized lipoproteins [67]. Since it was previously reported that these oxidized low-density lipoproteins (LDLs) can induce cytotoxicity [68], it is now speculated that atherosclerotic lesions along with endothelial cell dysfunction are caused by LDLs. Recently, Hossain and colleagues [69] demonstrated that sodium treatment significantly increased lectin-like oxidized LDL (oxLDL) receptor (LOX-1) levels in mouse aortic endothelial cells. Furthermore, there was significant induction in the phosphorylated forms of nuclear factor kappa-light polypeptide gene enhancer (NF-κB)/p65 levels in cells, suggesting an important role of the LOX-1 signaling pathway in arsenic-induced atherosclerosis. While LOX-1 signaling is activated, FasL ligand is repressed by arsenite but not by arsenate in ECV304 cells through ROS [70]. Moreover, these effects could be moderated through specific blockers or antioxidants like NAC.

20.4.3 Genetic Polymorphisms

Therefore, antioxidant enzymes and As metabolizing enzymes are pivotal for As excretion and for preventing oxidative stress. However, genetic polymorphisms (variants) in the genes that code for these enzymes have been reported to result in decreased enzyme activity and with it increased vascular toxicity. When genetic variants in *GSTM1*, *GSTT1*, and *GSTP1* as well as *p53* were investigated for their role in As-induced carotid atherosclerosis, it was observed that individuals who were exposed to high levels of As (>50 μg/L), having a *GSTP1* (Ile/Val and Val/Val) or *p53* (Arg/Pro and Pro/Pro) double variant, increased their risk of developing carotid atherosclerosis, with an age- and gender-adjusted OR of 6.0 and 3.1, respectively ($p < 0.001$) [60]. No risk was associated with individuals having *GSTM1* and *GSTT1* variants. Furthermore, individuals with either one *GSTP1* or *p53* variant and those with two variants had an OR of 2.8 and 6.1 for developing carotid atherosclerosis, respectively. Interestingly, even though they had variants of *GSTP1* and *p53*, individuals exposed to low levels of As (≤50 μg/L) were not at risk for carotid atherosclerosis. When other risk factors were taken into consideration (age, hypertension, variant genotypes of *GSTP1* and *p53*, and As concentration), individuals with double variants of *GSTP1* and *p53* still had a higher risk of developing carotid atherosclerosis, with a multivariate-adjusted odds ratio of 3.4 ($p < 0.001$) [60]. On similar lines, polymorphism within lipid metabolism and inflammation genes has also been shown to be linked to CVD. The relationship of polymorphism in *APOE* (lipid metabolism gene) and *MCP-1* (inflammation gene) with the risk of carotid atherosclerosis was evaluated in 479 arsenic-exposed residents of Taiwan [61]. Residents with a carotid artery IMT thickness of ≥1.0 mm or with observable plaque in the extra-cranial carotid artery were diagnosed as having carotid arteriosclerosis. Moreover, residents with ε4-allele of *APOE* had higher levels of LDL cholesterol compared with those residents who lacked this allele (116.1 ± 25.2 mg/dL vs. 132.9 ± 143.5 mg/dL, $p = 0.0004$), increasing the risk of carotid atherosclerosis by two-fold. On the other hand, individuals with *MCP-1* risk genotypes (A/G or G/G) had only a borderline chance of manifesting the disease. However, presence of both *APOE* and *MCP-1* risk genotypes increased the risk of carotid

atherosclerosis by 2.5-fold ($p < 0.01$). Further, individuals who had both risk genotypes and who had also been exposed to As concentrations of >10 μg/L or had CAE of >0.22 mg/L/year had a 10.3- and 15.7-fold higher risk of obtaining the disease, respectively ($p < 0.05$). When other risk factors were taken into consideration (gender, cigarette smoking, diabetes mellitus, cholesterol, and triglyceride), individuals with both *APOE* and *MCP-1* risk genotypes were still at a higher risk of developing carotid atherosclerosis. These studies indicate the joint effects of genetic polymorphisms and As exposure with regard to the development of carotid atherosclerosis. This study highlights that the coexistence of at least two risk genotypes may exacerbate the effects of As exposure and increase the risk of CVD.

While the above studies highlight the detrimental effects of high levels of As exposure, low levels of As exposure together with certain genetic polymorphisms can also elevate risk of CVD. A study in the rural Texas counties of Cochran, Palmer, and Bailey was conducted to investigate the effects of low levels of As exposure and a single nucleotide polymorphism (SNP) in *AS3MT* on coronary heart disease, hypertension, and hyperlipidemia [71]. The function of AS3MT is to catalyze methylation from MMA^{III} to DMA^{V}—an important pathway involved in As metabolism. A higher MMA^{III}/DMA^{V} ratio indicates toxicity while the inverse indicates detoxification. Therefore, 499 residents were evaluated for the presence of an A35991G SNP (rs10748835) within *AS3MT*. In this cohort the estimated groundwater As concentrations ranged from 2.2 to 15.3 μg/L (mean of 6.2 μg/L). After adjustments were made for age, ethnicity, gender, education, smoking status, alcoholism, and anti-hyperlipidemia medication, it was found that coronary heart disease was associated with both low levels of As exposure ($p < 0.05$) and the G/G genotype of *AS3MT* ($p < 0.05$). While hypertension was associated with low levels of As exposure ($p < 0.05$), hyperlipidemia was only associated with the A/G genotype of *AS3MT* ($p < 0.05$) [71]. Although hyperlipidemia was not found to be associated with low levels of As, a study conducted by Das et al. showed that mice administered with high doses of sodium arsenite (10 mg/kg, orally) for 10 consecutive days exhibited significant oxidative stress as well as developing hyperlipidemia [72]. Apart from oxidative stress, recently it was shown that arsenic exposure significantly increased the expression of two angiotensin II type I receptors (AT1R A and B) in mouse aortic endothelial cells [73]. This in turn increased phosphorylation of JNK and activated AP-1, providing evidence of how arsenic may modulate pathogenesis of hypertension.

Apart from *AS3MT*, *PNP*, *GSTO1*, and *GSTO2* were evaluated as well. In Taiwan, 863 residents who had been genotyped and for whom the severity of carotid atherosclerosis had been determined were included in this study. Just like in the previous studies, a dose-dependent relationship was observed between arsenic concentration and increased risk of carotid atherosclerosis ($p = 0.04$). Interestingly, a significant association was not found between genetic risk polymorphisms of *PNP* (Gly51Ser, Pro57Pro), *AS3MT* (Met287Thr), *GSTO1* (Ala140Asp), and *GSTO2* (A183G) and risk of carotid atherosclerosis in low level As exposure groups. However, in high-level As exposure groups (>50 μg/L), residents who carried the *PNP* AT haplotype, *AS3MT* T/C SNP and *GSTO* CAA/CAG or AGG haplotype had a higher risk of carotid atherosclerosis ($p \leq 0.05$). Additionally, coexistence of the *PNP* risk haplotype with either the *AS3MT* risk SNP or the *GSTO* risk haplotype severely increased the risk of the disease as indicative of

a multivariate-adjusted OR of 6.43 ($p = 0.004$). In support, residents who carried the *PNP* risk haplotype or the *AS3MT* risk SNP also had a significantly lower DMA/MMA ratio, indicating a reduction in As metabolism [74].

Furthermore, the occurrence of a GT-repeat polymorphism within the *HO-1* promoter was evaluated in 504 participants in Taiwan who were followed for 10 years for the occurrences of CVD-related deaths, including coronary heart disease, cerebrovascular disease, and peripheral arterial disease [75]. Individuals who had <27 GT-repeats were grouped into the S-allele category and those with ≥27 GT-repeats were grouped into the L-allele category. Individuals who carried the S-allele had a lower risk of CVD mortality than those who carried the L-allele. This was indicated by the crude mortalities for S/S, S/L, and L/L genotypes, which were 2.85, 3.10, and 8.42 cases per 1000 person-years, respectively. This study showed that longer repeats within the promoter of *HO-1* had detrimental effects and increased the risk of CVD. Supporting this study, association of GT-repeat polymorphism with blood pressure (BP) and their interaction on CVD mortality risk in 894 As-exposed participants showed that carriers of the S-allele had lower diastolic BP (L/S genotypes, $p = 0.014$) and a reduced risk of being hypertensive (L/S genotypes, $p = 0.048$) [76]. After a 10-year follow-up, hypertensive individuals who carried the S-allele had a reduced risk of CVD mortality ($p = 0.007$). However, those who did not carry the S-allele had a 5.23-fold increased risk ($p = 0.0008$) of CVD mortality [76]. While both these studies showed a link between the *HO-1* genotype and CVD in As-exposed populations, the researchers did not investigate the relationship of *HO-1* GT-repeat polymorphisms with regard to As concentrations.

20.4.4 Endothelial Cells

Apart from genotype polymorphism, dysfunction of the endothelial cells may also be responsible for CVDs. In order to understand the mechanisms of As-induced CVD, *in vitro* and *in vivo* studies have also been conducted both in human and in animal models. Treatment of porcine aorta endothelial cells (PAECs) with arsenic trioxide (ATO) and $NaAsO_2$ at 20 μM resulted in a decrease in viability and diminished G0/G1 phase and increased apoptosis [77]. Although both forms of As induced similar cytotoxic effects, the mode at which they exerted their effects was different. For instance, $NaAsO_2$ treatment increased the G2/M phase whereas ATO treatment led to necrosis. Further, $NaAsO_2$ treatment led to p53 up-regulation whereas ATO treatment led to both p53 and caspase-3 up-regulation. In another study, $NaAsO_2$ treatment (0.1–50 μM for 24 h) decreased the viability of human umbilical vein endothelial cells (HUVEC) in a time- and dose-dependent manner [78]. A cell cycle and apoptosis regulatory protein, $p21^{Cip1/Waf1}$, was activated by approximately 10-fold during this treatment. $NaAsO_2$ also stimulated and activated EGF and ErbB2 receptors, which appeared to be an upstream event leading to $p21^{Cip1/Waf1}$ activation as small molecule inhibitors (and RNAi) of the receptors could block $p21^{Cip1/Waf1}$ activation. Treatment with $NaAsO_2$ also activated JNK and p38 MAPK, which could be inhibited with SP-600125 and SB-203580, respectively. This inhibition also diminished activation of $p21^{Cip1/Waf1}$, which prevented apoptosis. Tsou et al. showed an alternative mechanism in HUVECs, whereby arsenite, through regulation of AP-1 and NF-κB, augmented TNF-α-induced VCAM-1 expression in a GSH-sensitive manner [79]. This has led to speculations that As-induced vascular toxicity may also be a result of coronary arterial endothelial cell apoptosis [80].

A more comprehensive study in SVEC4-10 mouse endothelial cells showed the effects of ATO with regard to cytotoxicity, intracellular ROS, gene expression, and signaling pathways [81]. ATO treatment reduced the viability of SVEC4-10 cells while simultaneously upregulated *HO-1*, *IL-6*, *MCP-1*, *Nrf2*, and *VEGF* transcript levels. Treatment also led to an increased activation of NF-κB. Interestingly, an HO-1 inhibitor (zinc protoporphyrin) reduced As-induced cell death in an inverted-U dose–response curve suggesting a biphasic role of HO-1. Targeted reduction of HO-1 or p38-MAPK decreased As-induced increase in *VEGF* expression. This study indicated that ATO activates *HO-1* expression through Nrf2-, NF-κB-, and p38 MAPK-dependent signaling pathways, which in turn act as upstream regulators of VEGF [81]. Moreover, experiments in mice exposed to low to moderately high-levels of As^{III} showed altered expression of angiogenic or cardiac tissue remodeling genes, such as *VEGF*, *VEGFR*, plasminogen activator inhibitor-1, and MMP-9, as well as cell–cell junction proteins, VE-cadherin and ZO-1, indicating the probable role of arsenic in vascular remodeling [82,83]. Conversely, Roboz and colleagues in 2000 showed that the therapeutic potential of arsenic against PML is the result of causing apoptosis in both normal and cancerous cells as well as inhibiting VEGF production [84]. Non-lethal doses of arsenic apart from increasing intracellular oxidant levels also increased the nuclear retention of NF-κB binding proteins. This retention was mainly regulated by p65/p50 heterodimers in endothelial cells [85]. In another study, it was shown that arsenic causes a release of a neuropeptide (substance P), which activates endothelial neurokinin-1 (NK-1) triggering a neurogenic inflammatory process *in vivo* that causes damage to blood vessels. Moreover, the study did not find any role of histamine release that may further regulate this process [86]. These studies suggest that multiple cellular pathways may be involved directly or indirectly through ROS during arsenic exposure.

20.4.5 Smooth Muscle Cells

Apart from inorganic forms, methylated forms of arsenic may also have a detrimental effect on blood vessels. Bae et al. conducted a study that investigated the effect of MMA^{III} on vasomotor tone of blood vessels. When phenylephrine (PE), serotonin, and endothelin-1 were used to induce vasoconstriction in rat thoracic aorta and small mesenteric arteries, MMA^{III} irreversibly suppressed this vasoconstriction [87]. Further, MMA^{III} directly interfered with the contractile function of vascular smooth muscle, as inhibition of vasoconstriction was still retained in aortic rings lacking endothelium. This phenomenon was a result of MMA^{III} inhibiting the Ca^{2+} increase, which was initially induced by PE via the intracellular store and L-type Ca^{2+} channel. MMA^{III} was also shown to directly block this channel as demonstrated in *Xenopus* oocytes. In an *in vivo* rat model, MMA^{III} also inhibited the increase in blood pressure that was induced by PE [87]. This study showed that apart from endothelial cells, metabolites of As could also induce dysfunction in smooth muscle cells, thereby contributing to vascular toxicity.

While these pathways and epidemiologic studies have shown association of arsenic with atherosclerosis and vascular dysfunction, Simeonova and colleagues in 2003 showed the role of apolipoprotein E (ApoE) in wild and $ApoE^{-/-}$ mice models. Exposure to 20 or 100 μg/mL arsenite for 24 weeks showed a marked increase in lesions in the intimal area of the aorta in $ApoE^{-/-}$ mice but no change was observed in cholesterol levels [88]. Moreover, *in utero* arsenic

exposure of pregnant ApoE-deficient mice showed a two-fold increase in lesion formation in the aortic roots and arch as well as defects in vasorelaxation in response to acetylcholine response in offspring. While triglyceride levels decreased, no change was observed in cholesterol, phospholipids, and VLDL or HDL levels, suggesting endothelial signaling disturbances [89]. These studies in short demonstrate that endothelial cells may be one of the main targets within the vasculature system that is affected by high and low dose exposure to arsenic.

20.5 Arsenic Effects on the Heart

In this section, we focus on the detrimental effects of arsenic on one of the major constituents of the heart: the myocytes. While the toxic effects of arsenic exposure on liver or brain are well documented in the literature, the cardiotoxic effects are not well classified. However, it is now clear that there is a relationship between prolonged arsenic exposure and cardiotoxicity or heart injury [90,91]. As a result, researchers have investigated a plethora of factors responsible for As-induced cardiotoxicity. These include age, sex, race, demography, As concentration, and genetic polymorphisms. Insight has therefore been gained with regard to the risks associated with As exposure.

20.5.1 QT Prolongation

One of the earliest reports in the 1970s indicated that a female patient was admitted for ventricular fibrillation, which was due to chronic arsenic poisoning. With post-arsenic management with dimercaprol (BAL) and procainamide hydrochloride therapy, the episode of ventricular fibrillation subsided; the ECG showed non-specific ST segment changes and prolongation of the heart rate corrected (QTc) interval [92]. In a typical ECG trace, the cardiac cycle consists of a P wave, a QRS complex, and a T wave. The distance between the QT interval (shortened or prolonged) is used as a marker for determining the potential for ventricular tachycardia (VT), a type of arrhythmia that could result in sudden death. Thus, it would be no surprise that chronic arsenic poisoning could result in heart rhythm issues such as long QT or VT [93,94]. It was interesting to note that most of the heart rhythm changes were seen in patients under cancer management.

Arsenic trioxide (ATO/As_2O_3) is commonly used to treat patients with acute promyelocytic leukemia (APL) owing to its ability to induce apoptosis and suppress proliferation of cancer cells as well as reduce angiogenesis. In a clinical trial of ATO in the treatment of relapsed and resistant cases of acute promyelocytic leukemia, adverse effects from arsenic were observed. The ATO therapy caused prolongation of corrected QT interval and three patients developed TdP (torsades de pointes, a form of ventricular tachycardia) [93]. Another study, which investigated the effects of ATO in 99 patients with advanced malignancies with APL, who received 170 courses of arsenic trioxide in either a phase I or phase II investigational study, reported that 38 patients developed a QT interval prolongation with ≈70% being >500 ms (normal QT interval is ≤400 ms) [95]. When normalized against the heart rate (corrected QT/QTc), the increase in QTc for 36.6% of patients was between 30 and 60 ms and for 35.4% of patients the increase was more than 60 ms. Interestingly, following the withdrawal of ATO, QTc intervals returned to normal suggesting that ATO does not permanently prolong the QT interval. However, the degree of

prolongations was higher in men than in women during the first course of the therapy and in patients with hypokalemia [95].

In another ATO study, Yamakazi and co-workers [96] investigated the incidence and mechanism of arrythmogenesis caused by ATO in 20 APL patients. As_2O_3 (0.15 mg/kg) significantly prolonged the corrected QT interval (QTc: 445 ± 7 to 517 ± 17 ms, $p < 0.01$), and also increased the QTc dispersion and transmural dispersion of repolarization. While four patients showed non-sustained VTs, one had TdP [96]. Ohnishi et al. [97] also observed that all of the eight patients on ATO therapy developed QT prolongations, out of which four developed non-sustained VT. Similarly, in another multicentric ATO study in the USA, 63% of the patients showed QT prolongations but only one of them had an absolute QT interval of >500 ms together with an asymptomatic 7-beat run of TdP [98].

Mumford et al. [99] studied the effects of arsenic exposure in 313 residents of Ba Men, Inner Mongolia, who were chronically exposed to arsenic via consumption of water. The residents with a mean arsenic exposure of 15 years were divided into three groups of low (≤21 μg/L), medium (100–300 μg/L), and high (430–690 μg/L) and their ECG evaluated. A direct dose-dependent relationship was observed between As exposure levels and QT interval prolongation ($p = 0.001$). When categorized into low, medium, and high As exposure groups, individuals displayed an increase in QT interval by 3.9, 11.1, and 20.6%, respectively. In this study, although As-induced QT prolongation was more prevalent in females ($p < 0.0001$) no correlation was observed with regard to age ($p = 0.486$) or smoking ($p = 0.1018$) [99].

The elevated risk of females to As-induced QT prolongation was also reported by Chen et al. [100] in a study evaluating the effects of long-term As exposure from drinking water (0.1–790 μg/L). Another study, where the authors performed a cross-sectional analysis in elderly men in Boston, MA, between 2000 and 2002 or in 2006 from the Normative Aging Study to analyze associations between toenail arsenic and QT/QTc durations using linear regression, found the interquartile range increase in arsenic concentration was associated with a 3.8 ms increase in QT (95% confidence interval: 0.82, 6.8) and a 2.5 ms increase in QTc (95% confidence interval: 0.11, 4.9) [101].

Wang and colleagues [102] studied 280 men and 355 women living in the endemic area of arsenicosis in southwestern Taiwan to evaluate a correlation between QT intervals with ICH and carotid atherosclerosis. The authors found significant associations of the QTc duration with ischemic heart disease and carotid intima-medium thickness after adjustment for various risk factors in the multiple linear regression analysis (all p-values <0.05). Three indices of chronic arsenic exposure were all significantly associated with the risk of QTc prolongation showing dose–response relationships ($p < 0.001$) in the study population [102].

20.5.2 Ischemic Heart Disease

While QT prolongation is one of the main effects that are observed on cardiomyocytes following chronic arsenic exposure, other cardiac diseases like ischemic heart disease and atherosclerosis too have shown linkage to arsenic. A study in 462 people living in blackfoot disease-hyperendemic villages of Taiwan showed that long-term exposure to As increased the risk

of IHD. IHD was diagnosed by coding the resting electrocardiograms with the Minnesota code. Among the subjects, 78 cases (16.9%) were diagnosed as having IHD. The prevalence rates of IHD for the age groups of 30–39, 40–49, 50–59, and >60 years were 4.9, 7.5, 16.8, and 30.7%, respectively. It was noted that for individuals with a cumulative As exposure (CAE) rating of 0, 0.1–14.9, and ≤15 mg/L-years, the prevalence rates of IHD were 5.2, 10.9, and 24.1%, respectively ($p < 0.001$) [103]. Chen et al. [104] evaluated the mortality rates of residents of 60 villages in Taiwan with endemic arseniasis, from 1973 through 1986. Based on 1,355,915 person-years and 217 IHD deaths, the cumulative IHD mortalities from birth to age 79 years were ranged from 3.4 to 6.6% for residents who lived in villages in which the median arsenic concentrations in drinking water were <0.1 to ≥0.6 mg/L, indicating that higher arsenic levels resulted in higher mortality. Furthermore, the group showed that a cohort of 263 patients suffering from blackfoot disease and 2293 non-affected residents from these areas showed a gradient relationship between arsenic exposure and IHD. The relative risk was 2.5 (0.1 to 9.9 mg/L), 4.0 (10.0 to 19.9 mg/L), and 6.5 (≥20 mg/L) compared to zero arsenic exposure. The risk for blackfoot disease patients was much higher than for non-blackfoot disease patients [104].

In Bangladesh, the mortality rate for cardiovascular disease was 214.3 per 100,000 person-years in people drinking water containing <12.0 μg/L arsenic, compared with 271.1 where arsenic levels were ≥12.0 μg/L. Furthermore, a dose–response relation between exposure to arsenic and mortality from IHD and other heart disease could be established. The hazard ratios in increasing quarters of arsenic concentration were reported to be 1 (0.1–12.0 μg/L), 1.22 (12.1–62.0 μg/L), 1.35 (62.1–148.0 μg/L), and 1.92 (148.1–864.0 μg/L) ($p = 0.0019$ for trend) [63]. In another study in arsenic endemic villages of Taiwan, a cohort of 74 patients suffering from IHD and 193 age- and sex-matched healthy controls were evaluated for serum levels of micronutrients by HPLC. The study not only demonstrated a significant biological gradient between the risk of IHD and the duration of consuming high-arsenic water but also a reverse dose–response relationship of arsenic-related IHD with serum level of α- and β-carotene, but not for serum levels of retinol, lycopene, and α-tocopherol [105].

These studies clearly show that long-term low dose exposure could have detrimental effects on cardiomyocytes that can alter the heart rhythm and, in association with cardiac vasculature, could be associated with cellular death of cardiac tissue as in IHD. However, most of our understanding on the mechanism of the action of arsenic on myocytes is based on animal studies.

20.5.3 Ion Channels

There have been a large number of studies to understand the mechanism of how arsenic induces changes in signaling cascades in myocytes and causes cellular damage. From the above human studies, it is very clear that one of the effects of arsenic on myocytes is prolonged depolarization of the cardiomyocytes resulting in long QT manifestations. A study in a human cardiomyocyte/fibroblast fusion line (AC16) reported that As exposure resulted in a down-regulation of several ion channel genes including *CACNA1*, *KCNH2*, *KCNQ1*, and *KCNE1* [106]. These genes code for ion channel proteins, which play a pivotal role in maintaining normal cardiac electrophysiology. Yamazaki et al. [96] evaluated the action potentials and isometric

contractions in guinea pig papillary muscles post-ATO perfusion (350 micromol/L). They observed an increase in APD_{90} from 150 ± 11 to 195 ± 12 ms at 60 mins, $p < 0.01$. Further, perfusion in low K^+ with a low stimulation rate augmented the prolongation of APD, and provoked early after-depolarizations. Prolonged exposure to ATO induced muscle contracture, after-contractions, and triggered activities, which could be partially prevented with tetrodotoxin [96]. Similarly, using conventional action potential recording techniques, Chiang et al. [107] found that ATO dose dependently prolonged APD in guinea pig muscle with slow pacing frequency. Moreover, parenteral as well as intravenous infusions of ATO induced prolonged QT intervals in guinea pig heart, showing direct effects of ATO on cardiac repolarization similar to findings in humans [107]. Similar effects are also observed in rabbits. Wu et al. [108] utilized Langendorff perfusion to determine the direct effects of ATO on electrophysiological changes in rabbit heart after acute and chronic (0.2 mg/kg/day i.v. for 30 days) treatments. While acute treatment did not have any effects on cardiac conduction and repolarization, chronic treatment with 30 μM demonstrated polymorphic VT (14%) together with prolonged QTc. Nevertheless, an extremely high dose of 300 μM exposure showed prolonged QTc. It could thus be concluded that extremely high doses such as in suicide cases can result in QTc prolongations but the more common low dose chronic exposure could also alter cardiac rhythms [108].

Changes in the QT interval or cardiac repolarization are mainly dependent on the potassium current across the cellular membranes. *KCNH2*/hERG (ether-go-go-related gene) and *KCNQ1* genes code for the potassium currents I_{Kr} and I_{Ks} respectively, which are solely responsible for maintaining normal QT intervals in cardiomyocytes. Disruption of any of these proteins is one of the major reasons for QT prolongations. Ficker et al. showed that ATO treatment at clinically relevant doses of 0.1–1.5 μM also led to a lack of hERG trafficking to the cellular membrane in ventricular myocytes [109]. During trafficking to the membrane, hERG forms a complex with Hsp70 and Hsp90 [110], which assists in its maturation. Hsp90 consists of a highly conserved thiol pair and since ATO has the ability to modify thiol groups, this chaperone may lose its functionality. Hence, by inhibiting the hERG–chaperone complex, ATO prevented its membrane localization and the consequences were that of a prolonged QT interval.

In a recent study, Shan et al. [111] demonstrated that ATO administration in guinea pigs increases muscle-specific miR-1 and miR-133 ($p < 0.001$). Overexpression of miR-133 and miR-1 led to QT prolongation as well as down-regulation of hERG (encoded by *KCNH2*) and Kir2.1 (encoded by *KCNJ2*), respectively, which simultaneously led to a decrease in I_{Kr} and I_{K1} currents. However, treatment with the antisense molecules of these miRNAs abrogated the electrophysiological disorders. These studies indicate that arsenic not only directly interacts with hERG protein and affects its function but may also interact with the molecules governing its regulation. Drolet et al. [112] demonstrated that in *KCNH2*- or *KCNQ1*-transfected CHO cells, As_2O_3 caused a concentration-dependent block of both the I_{Kr} and the I_{Ks} current with an IC_{50} for tail currents block of 0.14 ± 0.01 and 1.13 ± 0.06 for I_{Kr} and I_{Ks}, respectively [112]. Ficker and co-workers in 2004 showed that ATO treatment also induces calcium (Ca^{2+}) overload, which leads to an increase in Ca^{2+} currents [109]. Similarly, Sun and colleagues [113] demonstrated that As_2O_3 not only increased QT prolongation in a dose- and time-dependent manner but also enhanced L-type calcium currents (I_{CaL}) and increased intracellular calcium

concentrations in ventricular myocytes. Moreover, the authors also demonstrated that choline treatment could reverse these As_2O_3-mediated changes [113].

Chen and colleagues [114] recently showed that rats treated with 0.8 mg/kg/day As_2O_3, intravenously for 7 d, had prolonged QTc of 36% in the animals and prolonged APDs in isolated ventricular myocytes. Moreover, the authors also demonstrated that As_2O_3 suppressed I_{K1} and shifted the reversal potential to a more positive direction. Furthermore, the treatment significantly augmented Ca^{2+} current densities making the steady state activation curves more negative. However, these changes did not alter the inactivation and reactivation curves of calcium currents. These results together suggest that As_2O_3 perturbs the fine balance for the transmembrane currents that may result in prolongation of APDs or QTc [114]. However, while these results indicate that in general myocytes change in electrophysiological variables, these changes may be species dependent.

Lu and co-workers [115] evaluated the direct electrophysiological effects of As_2O_3 in cardiac tissues isolated from four different species (guinea pig, dog, rabbit, and pig). These authors demonstrated that arsenic (after 30 to 95 min perfusion) at a 10 μM dose caused prolonged APD_{90}, and increased triangulation of the AP and elicited EADs only in guinea pig and dog Purkinje fibers and not in those of rabbit and pig. Furthermore, they did not observe the occurrence of EADs in papillary muscles of guinea pigs and rabbits. This study clearly indicates that the choice of the species as well as the cell type is quite important for performing such studies [115].

20.5.4 Cellular Signaling

Arsenic is well documented to induce ROS-mediated apoptosis in various cell types such as those of liver and brain. However, there is limited information on arsenic's effect on cardiomyocytes. While it would be tempting to speculate that the same action may be seen in cardiomyocytes, there is limited literature to support such claims. Raghu and Cherian showed that when treated with different doses of ATO (30, 60, and 90 μM) for 24, 48, and 72 hours, rat cardiomyocytes underwent apoptosis in a dose- and time-dependent manner [116]. Similarly, Zhao et al. [117] showed that As_2O_3 (2–10 μM) reduced the viability of H9c2 cardiomyocytes in a dose-dependent manner. They showed that increased ROS and calcium overload with increased caspase-3 activity resulted in cell shrinkage and cellular apoptosis. These changes could be reversed using the caspase-3 inhibitor Ac-DEVD-CHO and antioxidant vitamin E [117]. Additionally, Bessho et al. showed that ATO triggers apoptosis via a ROCK-dependent pathway, since using a ROCK inhibitor (Y-27632) resulted in the protection of H9c2 cardioblastoma cells from caspase-mediated apoptosis [118].These studies show that ROS-mediated apoptosis may play a pivotal role in cellular regulation of cardiomyocytes. Arsenic-induced cellular death may also play a part in progression of heart failure or IHD. While apoptosis is a key event regulating survival of cardiomyocytes post-arsenic exposure, there are a number of signaling pathways that are altered in this process.

Using whole-genome microarray, Park and Park showed that just 24 hours following ATO treatment, H9c2 cardiomyocytes up-regulated 405 genes and down-regulated 499 genes by more than two-fold indicating the large physiological changes As exposure can induce in a relatively short period of time [119]. In this study, ATO treatment at a subcytotoxic dose (0.5 ppm)

increased ROS generation, which led to oxidative stress as indicated by up-regulation of *HO-1*, *GST*, metallothionein (MT), and catalase. Despite ROS generation and oxidative stress, As exposure also induces cardiotoxicity by modulating ion channels [119]. Metallothionein is a well-known endogenous antioxidant that plays a vital role in regulating heavy metals in biological systems [4]. Although modulation of MT is well reported in the literature with reference to cadmium toxicity, not much is known about its role in arsenic poisoning. Miao et al. [120] demonstrated that MT overexpressing H9c2 cardiac cells and transgenic mice (MT-TG), when exposed to clinically relevant doses of arsenic, showed significant protection against arsenic-induced cellular apoptosis as compared to control animals. Moreover, MT overexpressing cells and animals prevented activation of MAPK members such as ERK1/2, JNK, and p38 that were highly up-regulated in control groups [120]. This study clearly indicated that endogenous antioxidants like MT have a major role in regulating signal pathways like MAPK that are important for arsenic-induced cellular death. Recently, Fan et al. [121] showed that arsenic-induced ROS and calcium overload could increase phosphorylated levels of MAPK members like JNK and p38 in an As-induced long QT model. However, using an antioxidant (genistein) these activations could be significantly reduced, which indirectly resulted in suppression of caspase-3 activity, preventing cellular apoptosis. Similarly, Ghosh and co-workers [122] demonstrated that sodium arsenite ($NaAsO_2$) heightened the phosphorylation of IKK and NF-κB in rat neonatal myocytes, which was also accompanied by an increase in phosphorylated p38-MAPK and JNK. This study further highlights the involvement of the MAPK signaling pathway in As-induced cardiotoxicity. Interestingly, when using an IKK inhibitor (PS-1145), cleavage of caspase-3 and PARP was abolished. Additionally, a JNK inhibitor (SP600125) and p38-MAPK inhibitor (SB203580) could also decrease phosphorylation of IKK and NF-κB, thereby reducing apoptosis. Furthermore, when animals were treated with a potent antioxidant, taurine, most of the variables demonstrated reversals and prevented apoptosis [122].

Another pathway shown to be affected in cardiomyocytes is TGF-β. Chu and co-workers [123] showed that when ATO was administrated to guinea pigs, they developed prolonged QT and severe myocardial fibrosis. This was accompanied by an increase in TGF-β1 secretion. Interestingly, it was also found that cardiac fibroblasts (and not cardiomyocytes) were responsible for this increase in TGF-β1 secretion, which eventually led to the paracrine down-regulation of hERG and Kir2.1—two K^+ channels required for I_{kr} and I_{k1} currents, respectively. However, through the use of a TGF-β signaling antagonist (LY364947), interstitial fibrosis and LQTS were eliminated. Simultaneously, the abnormal levels of TGF-β1, hERG, and Kir2.1 returned to normal [123].

Arsenic has also been reported to interfere with redox homeostasis (Nrf2-HO-1 pathway). Nrf2 is a transcription factor responsible for the up-regulation of several antioxidant genes, which include but are not limited to *GST*, *GCL*, *HO-1*, and *MRP*. Sumi and colleagues showed that when compared to cells derived from other sources (e.g., liver; LC_{25} 25 μM, kidney; LC_{25} 112 μM, and brain; LC_{25} 49–73 μM), rat cardiomyocytes (LC_{25} 4 μM) were the most sensitive to As^{III} [124]. Interestingly, although Nrf2 was up-regulated in these other cell lines following As^{III} treatment, it was poorly activated in cardiomyocytes. Levels of GCL and MRP were also lower in cardiomyocytes suggesting that they have a reduced ability to metabolize and excrete As.

Furthermore, antioxidants such as resveratrol also possess similar effects as they were shown to prevent the down-regulation of *Nrf2* and *HO-1* in rats administered with ATO [125]. Resveratrol also decreased ROS generation and oxidative DNA damage. These studies show that small molecule inhibitors of various signaling pathways and antioxidants such as genistein and taurine may have potential therapeutic value against As-induced cardiotoxicity.

Looking at the current published data, it is evident that multiple signaling pathways are involved in arsenic-induced cardiotoxicity *in vivo* as well as *in vitro*, and antioxidant therapy has to some extent been able to reverse these effects. It would be hard to state that antioxidants alone would be a complete therapy for treating cardiotoxic effects of arsenic, as these changes may be temporary and the toxic metal may still be present in the cells. However, there have been studies that show that various antioxidants from synthetic sources or plants could have beneficial effects on cardiomyocytes exposed to arsenic.

Zhang et al. showed that matrine and oxymatrine, two compounds that can be isolated from the *Sophora* genus, could shorten APD duration, which was prolonged due to ATO treatment in both guinea pig ventricular myocytes and a human heterologous HEK overexpression system [126]. However, while low doses (1 μM and 10 μM) of matrine led to an increase in I_{Kr} currents, a high dose (100 μM) resulted in a decrease. Following long-term treatment, both these compounds (1 μM) could reverse the QT prolongation by up-regulating Sp1 (a transactivator of *KCNH2*), which in turn led to an increase of hERG on both the transcriptome and proteome levels. This was followed by an increase of hERG on the cell membrane and with it a simultaneous increase in I_{kr} currents. Further, salvianolic acid B (Sal B), a component of *Salviae miltiorrhizae*, has also been reported to protect against ATO-induced apoptosis in H9c2 cardiomyocytes via PI3K/Akt, a signaling pathway known for exerting cardio-protective effects [127]. The PI3K/Akt signaling pathway is known to confer a protective effect by modulating the balance between anti-apoptotic proteins (Bcl-2 and Bcl-xL) and pro-apoptotic proteins (Bax, Bad, and Bak). Sturlan et al. showed that ATO-induced apoptosis occurs due to the down-regulation of Bcl-2 and simultaneous up-regulation of Bax [128]. Treatment with Sal B increased Bcl-2 and Bcl-xL and returned Bax to normal levels. However, when using a PI3K inhibitor (LY294002), the protective effect of Sal B was lost and cardiomyocytes underwent apoptosis following ATO treatment [127].

In another study, silibinin, an extract of milk thistle seeds, has also been shown to ameliorate As-induced ROS generation in rats [129]. This compound had the ability to recover the activities of heart mitochondrial enzymes (ICDH, SDH, MDH, a-KDH, and NADH) which were lost following As exposure. Leaves from *Corchorus olitorius*, a popular crop in Bangladesh and West Bengal, are another form of protection against As-induced cardiotoxicity [72]. Rats administered with $NaAsO_2$ developed significant oxidative stress in their myocardial tissue. This was signified by the down-regulation of several antioxidant enzymes such as SOD, CAT, GST, and GPx. However, treating these rats with aqueous extract of *C. olitorius* leaves 15 days prior to $NaAsO_2$ administration protected their cardiac tissue from As-induced cardiotoxicity by maintaining the activities of the aforementioned enzymes. Ethanolic extract of *Boerhavia diffusa* (BDE) was also found to protect H9c2 myoblasts against ATO-induced cardiotoxicity [130]. In this study, ATO treatment at varying concentrations (5, 7.5, and 10 μM) reduced the

integrity of mitochondria, initiated ER stress, disrupted cytoskeletal networks, inhibited antioxidant enzymes, and increased both ROS generation and Ca^{2+} load. However, BDE could reverse the pathophysiological properties of ATO-treated cells par at high doses (10 μM).

20.5.5 Chelation Therapy

Chelating agents are the mainstay for heavy metal poisoning. Chelating agents have been reported to reduce arsenic poisoning in small animals [3,131–137]. These agents bind to As and prevent it from interacting with other molecules and enzymes within the body. Simultaneously upon interaction with chelating agents, As is converted into a water-soluble form, which can be excreted more readily [138]. Kathirgamanathan et al. reported that progressive, multilevel cardiac conduction block as a result of ATO treatment could be rectified through chelation treatment with dimercaprol (British anti-Lewisite/BAL) [139]. Further, Kumazaki et al. showed that treatment with BAL and α-lipoic acid (LA) could reverse ATO-mediated QT prolongation in guinea pigs [140]. Chelation treatment, however, generally results in toxicity and side effects such as nausea, diarrhea, and the appearances of rashes, and even death can occur. It has therefore been reported that chelation treatment together with antioxidant supplementation may provide a safer alternative [141].

20.6 Human Pluripotent Stem Cells: Understanding Arsenic Toxicity

While animal models and human heterologous overexpression systems have provided basic understanding with regard to mechanisms associated with As-induced cardiotoxicity, the true cause of heart injury still eludes us due to the lack of an ideal human model. Human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), through defined culture conditions, can be differentiated into various cell types, including cardiomyocytes [142]. Therefore, human ES- and iPSC-derived cardiomyocytes provide a more suitable alternative to animal models. Further, unlike human heterologous overexpression systems, which only express one ion channel, pluripotent stem cell (PSC)-derived cardiomyocytes concurrently express all ion channels in a similar fashion as in the human heart. Human PSC-derived cardiomyocytes are hence an ideal model for studying the effects of As exposure with regard to ion channel dysregulation. Additionally, since human PSCs can be cultured indefinitely, a large number of cardiomyocytes can be generated at any given time. This would be ideal for studying other mechanisms and signal transduction pathways involved in As-induced cardiotoxicity. However, reports have shown that there are inherent differences within various pluripotent stem cell lines that may affect their differentiation abilities to various lineages [143]. However, having efficient differentiation protocols could resolve these inherent differences [142,144].

In a proof of principle study, Mehta and colleagues [145] utilized this model to study development toxicity. In the study, human embryonic stem cell-derived embryoid bodies (EBs) were generated that mimic human embryonic development and these EBs were exposed to

various drugs to test their developmental toxicity effects. Our results suggested that using this sensitive method, one could evaluate and classify compounds as non-, weakly, and strongly toxic. This study demonstrated the potential application of this technology in drug toxicity screening [145]. Based on this model, Flora and Mehta [146] studied the developmental toxicity of arsenic. In the study, human embryonic stem cell-derived embryoid bodies (EBs) were exposed to various concentrations of arsenic. These arsenic exposed EBs were found to be highly sensitive to arsenic treatment in comparison to fibroblasts with IC_{50} values of 0.005 and 0.31 μg/mL, respectively. Gene expression analysis of markers indicative of cardiovascular lineage development such as T, Gata4, actinin (cardiac and muscle), myosin light chain, and CD31 (endothelial cell adhesion) showed significant down-regulation in a dose-dependent fashion. Moreover, genes involved in cell cycle regulation (CCND1, CCRK), apoptosis (Bcl-2, CASP3), methylation (DNMT3B), signaling pathways (BMPs, FGF, NODAL, NOTCH, WNT), and gap junction (GJA1) also showed significant alterations post-arsenic treatment [146]. These results as a proof of principle show that hESC-derived EBs mimic similar effects as reported by previous studies on different human cell types [146]. This technology provides a method through which researchers could gain access to human cell types like cardiomyocytes or neurons that are impossible to be collected from humans.

The emergence of human-induced pluripotent stem cell technology provides a new dimension to evaluate various diseases. Induced pluripotent stem cells are similar to human embryonic stem cells in almost all aspects, except that they are generated by reprogramming of fibroblasts with transcriptional factors (Oct4, Sox2, klf4, c-myc) as previously demonstrated by Takahashi and Yamanaka in 2006 [147]. We and others have previously demonstrated that fibroblast cells could be reprogrammed to generate hiPSCs from various sources and these human iPSCs can be differentiated into cardiomyocytes [148–150]. Moreover, these cardiomyocytes respond appropriately to anti-arrhythmic drugs in a similar fashion to human cardiac cells [151], suggesting that these iPSC-derived cells mimic human cardiomyocytes not only structurally but also functionally. However, the one limitation seen in these myocytes is that they are immature and resemble the shape of a 16-week-old fetus. Having said this, these myocytes have still been able to unravel mechanisms of various diseases. For instance, we recently demonstrated that fibroblasts isolated from a patient with long QT syndrome 2 (mutation in *KCNH2* gene) could be reprogrammed to hiPSC state and subsequently differentiated into patient-specific cardiomyocytes, which manifest long QT intervals as revealed by multielectrode array analysis (*in vitro* system similar to ECG) as well as by whole-cell patch clamp studies [152]. Moreover, we demonstrated that QT prolongation was due to reduced I_{Kr} currents in these patient-specific cardiomyocytes and there was a trafficking defect due to which mutated hERG could not be transported to the membrane, thus reducing the overall presence of functional hERG on the membrane of these diseased myocytes [153,154]. Such studies to understand pathways or mechanisms of disease manifestation, which were previously not possible, are easily possible today. The use of such technologies may provide better understanding of disease pathogenesis and since they are being applied in humanized models they may show a better clinical relationship when translated into clinical setups.

20.7 Conclusions

In conclusion, inorganic and methylated forms of arsenic have detrimental effects on the cardiovascular system. While most of these harmful effects are a result of oxidative stress-induced cell death, studies have also shown that arsenic has the ability to bind to certain macromolecules like hemoglobin, as well as various proteins in the cardiovascular system, thereby interfering with their biological function. Further, these molecular associations, in turn, could lead to a complete disarray of signaling cascades that alter various biochemical pathways within a cellular system. Although a large number of clinical studies have shown a significant correlation between arsenic exposure and indices of various cardiovascular diseases such as hypertension, atherosclerosis, and IHD, these aspects have not been critically evaluated or supported by compelling evidence. Perhaps one of the reasons for this could be the fact that all these diseases are multifactorial and are not linked to a single gene. Moreover, due to disparate mechanisms, the differential response exhibited by cell types to arsenic, together with genetic polymorphisms, complicates the understanding of its toxicity. On the other hand, by directly or indirectly interacting with hERG (the protein that regulates the critical I_{Kr} current in cardiomyocytes), arsenic has been shown to alter normal heart rhythm (prolongation of QT intervals). Recently, increasing numbers of studies have been published identifying mechanisms, biochemical pathways, and potential candidate molecules that are perturbed during chronic arsenicosis. Most of these studies have been aimed at developing better and more accurate diagnostic tools for arsenicosis. In general, comprehensive studies accurately dissecting the changes in signaling and biochemical pathways as a result of arsenic exposure are yet to be reported. Such studies could yield a better understanding of how arsenic impairs the cardiovascular system. Moreover, newer technologies like pluripotent stem cells could provide a better opportunity to study the effects of arsenic in a cell type-specific manner. The development of efficient and accurate tools is pivotal to providing a solution for millions exposed to arsenic on a daily basis.

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Immunotoxic Effects of Arsenic Exposure

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CHAPTER OUTLINE

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21.1 Introduction

Arsenic, a toxicant of natural occurrence in mineral deposits, is used in many human activities such as manufacturing, agriculture, and medicine [1]. Arsenical compounds are transported into the environment mainly by water from wells drilled into the arsenic-rich geologic strata or by ambient air during smelting and burning of coal [1,2]. The main route of arsenic exposure for the general population is via drinking water. Endemic arsenic exposure emerged as a single catastrophe affecting millions of people mostly living in Bangladesh, India, Mexico, Taiwan, and South America. In these regions, the concentrations of arsenic amount to several hundred micrograms per liter, which considerably exceed the standard of 50 μg/L recommended by the World Health Organization for drinking water in Bangladesh and India [1]. In Bangladesh, approximately 85 million people are at risk of drinking arsenic-contaminated water from about 2 million tubewells that supply waters containing more than 50 μg/L of arsenic [3].

In humans, the liver rapidly detoxifies inorganic arsenic that is consumed in drinking water by transforming it into organic forms called monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) that are rapidly excreted in the urine [4], and to give an overall arsenic half-life in the body of about 30 hours [5]. Thus, following exposure to arsenate (valency state V), the first step in biotransformation is the reduction to arsenite (valency state III), a process that may be considered a bioactivation [6]. There is evidence that the methylating capacity differs among individuals and populations and that different capacities would result in variation in tissue retention of arsenic. It has been found that arsenic causes the depletion of *S*-adenosylmethionine, the main cellular methyl donor [7]. Environmental factors, particularly diet, might be important in explaining susceptibility to arsenic toxicity [8]. Also its metabolism can be influenced by the concentration of arsenic that is being ingested and by gender, as has been found in boys compared to girls of the same age [9].

The immune system is a network of white blood cells and their soluble mediators, tissues, and organs that work together to defend the body against attacks by foreign invaders. The foreign pathogens are primarily microbes such as bacteria, parasites, viruses, and fungi that can cause infections. Microorganisms or toxins that successfully enter an organism encounter immunocompetent cells and mechanisms of the immune system that keep them out or, failing this, seek them out and destroy them. The functions of the immune system are modulated by a number of chemical and biological substances, environmental factors, and trace elements (metals) including aluminum [10], magnesium, selenium, and zinc [11], and many toxic pollutants such as mercury, lead, cadmium, and arsenic [12]. Human peripheral blood mononuclear cells incubated with 15 μM arsenic and 65 μM cadmium showed that both are able to induce apoptosis of lymphoid cells with arsenic inducing a significant level, while lead as high as 500 μM is non-toxic and did not induce a significant degree of apoptosis [12].

Metals and metal compounds occur naturally in mineral deposits whose distribution depends on their natural sources (e.g., volcanoes or erosion) and use in human activities such as industry, agriculture, and even medicine. They are transformed naturally by bacterial activity with formation of organic species that influence their mobility and accumulation in abiotic as well as biotic systems. Due to their widespread use, different metal species are released into

the environment, posing a serious threat to human health. The use and actions of selected metal species of scientific concern (arsenic, selenium, and platinum) showed that numerous health risks may be associated with exposure to these substances [13].

High concentrations of natural arsenic are generally associated with different geochemical environments [14]. Geological arsenic can stay in solid phases as minerals, amorphous solids, and sedimentary organic matter that supply dissolved arsenic. Release from iron oxide and sulfide minerals appears to be the most common cause of widespread arsenic contamination in ground water. Mobilization of arsenic in sedimentary aquifers may be, in part, a result of changes in the geochemical environment due to agricultural irrigation. In the deeper subsurface, elevated arsenic concentrations are associated with compaction caused by excessive groundwater withdrawals [15].

There is strong evidence from epidemiological studies of an association between chronic exposure to inorganic arsenic and hyperpigmentation, hyperkeratosis, and neoplasia in the skin as well as other diseases [16]. A higher prevalence rate of arsenical skin lesions with a clear dose–response relationship has been found among Bangladeshi populations drinking arsenic-contaminated well water [17], and callus-like growths all over the extremities with changes in skin pigmentation have been reported [18]. The general adverse health effects associated with human exposure to arsenicals include cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, alteration of metabolic enzyme functions, diabetes, fibrosis of the liver and lung, and hematological disorders [19,20].

Several studies have found that anemia, leukopenia, and thrombocytopenia are common effects of arsenic poisoning in humans following acute [21] and chronic oral exposures [22] at doses of 50 μg/kg/day or more. These effects may be due to both a direct cytotoxic or hemolytic effect on the blood cells [21,23] and a suppression of erythropoiesis [23]. The magnitude of exposure to patients examined by these researchers is not specified, although arsenic concentration in some of the wells in the area exceeded 1000 μg/L. However, there are reports that hematological effects are not observed in all cases of acute poisoning with arsenic [24,25], and these abnormalities are reversible within weeks of termination of arsenic exposure [26]. Such hematological effect has been observed in imprinting control region (ICR) mice where the mean corpuscular volume of red blood cells decreased significantly when the animals were challenged with drinking water containing high levels of arsenic that replicated the levels detected in streams and soils in Buruli ulcer endemic communities of Ghana [27].

It has been shown that the immune system is among the targets for arsenic. Recent studies on the mechanisms of arsenic toxicity suggest that the metal toxicant can alter the delicate balance and regulation of the immune cells and can increase the incidence of certain diseases [28]. It has been known for a long time that arsenic is the immunosuppressive component of gallium arsenide (GaAs) that alters macrophage function and T lymphocyte proliferation [29,30]. A single intratracheal dose of (GaAs) at 200 mg/kg was reported to decrease the percentages of Thy 1.2 positive T cells in mice, and the IgM and IgG antibody-forming cells in the spleen were reduced by 66% and 48%, respectively [31]. It has been further reported that gallium arsenide selectively alters the expression of CD25 (IL-2R/p55) [32]. Another study showed that GaAs affected both humoral and cellular immune parameters in B6C3F1 female mice and impaired the ability of the immune system to protect against B16F10 tumor challenge [31].

21.2 Influence of Nutritional Factors

An association of clinical complications of arsenic toxicity with the duration of exposure and nutritional status of the affected subjects based on a study conducted on a total of 115 exposed individuals diagnosed as arsenicosis patients who were randomly selected from four known arsenic endemic villages in Bangladesh, and age-matched 120 unexposed (control) subjects [33] has been reported. The duration of arsenic exposure in about 37% of the patients was at least 10 years, while the population's mean and range were 7.6 ± 5.2 years and 1–25 years, respectively. The mean arsenic concentrations in the drinking water for the exposed and unexposed populations were 218.1 μg/L and 11.3 μg/L, respectively. The spot urine of the arsenicosis patients, considered a reliable marker of recent exposure to arsenic, contained an average of 234.6 μg/L arsenic. It was found that adverse clinical symptoms of melanosis, keratosis on palms and soles with cracks and nodule formation, severe skin irritation, and lump formation on feet developed with the increase in duration of arsenic exposure. About 15% of the controls had body mass index (BMI) lower than 18.5, the cut-off point for malnutrition (underweight); the corresponding value among the arsenicosis patients was about 28%; they had more severe arsenical symptoms and complications compared to the overweight patients, having BMI 25.0 or above. These findings demonstrate that the poor nutritional status of patients increases the complications of chronic arsenic toxicity [33].

21.3 Effects on Blood Leukocytes

It has been reported that arsenicosis patients have a significantly lower white blood cell (WBC) count in their peripheral blood compared to As-unexposed healthy subjects. About 16% of the subjects chronically exposed to arsenic via drinking water showed leukopenia, having WBC count below 4 million cells per mL. About 26% of the patients showed neutropenia, having less than 50% neutrophils in the peripheral blood (normal range: 50–70%), and 39% had more than 40% lymphocytes. Prevalence of neutropenia and lymphocytosis was observed in patients with chronic exposure to high levels of arsenic in drinking water. It was observed that many of the chronically exposed subjects were suffering from anemia (26%) and weakness (about 33%). Also, the incidences of leukopenia and anemia were more common in the female patients who were malnourished (BMI <18.5). These findings demonstrate a higher prevalence of anemia, leukopenia, and lymphocytosis in chronic arsenic toxicity [33]. A previous study conducted in West Bengal, India, reported that anemia was present in 47% of 156 subjects with arsenical skin lesions [34].

21.4 Interruption of Energy Production

It is known that arsenic is able to exert its toxic effects on the metabolic pathways by modulating the antioxidant defense system, interrupting the glycolytic pathway and citric acid cycle, and thus inhibiting oxidative phosphorylation. Many inorganic compounds have been

found to compete with phosphorus in the oxidative phosphorylation process [35], mainly in their pentavalent form [36]. We found that the levels of inorganic phosphorus in the serum of arsenicosis patients were significantly higher than in the unexposed control subjects [20]. Arsenic can compete with phosphorus in the oxidative phosphorylation process by replacing phosphorus in the cells, thereby causing an increase in the levels of unutilized inorganic phosphate in the blood. It has been reported that arsenic is an uncoupler of mitochondrial oxidative phosphorylation that induces generation of reactive oxygen species (ROS) [37]. Thus, replacement of phosphorus by arsenic in the crucial energy yielding steps may explain why arsenicosis patients suffer from weakness, as phosphate becomes unavailable inside the cells for utilization for the synthesis of high-energy compounds.

21.5 Effects on ROS Production

Production of ROS such as nitric oxide anion (NO^-) and superoxide anion (O_2^-) by activated monocytes is an important innate immune response to destroy invading microbes. It was found in Mexican children drinking arsenic-contaminated water that arsenic exposure was positively associated with O_2^- production by mitogen-stimulated monocytes [38]. These data suggest that arsenic could impair the function of monocytes. For example, elevated ROS levels in unstimulated monocytes indicate arsenic-induced oxidative stress; ROS overproduction by activated cells could cause oxidative damage to surrounding tissues, whereas diminished ROS production could weaken monocyte defense against pathogens. It has been suggested in another study that arsenic alters cellular glutathione levels either by utilizing this electron donor for the conversion of pentavalent to trivalent arsenicals or directly binding with it or by oxidizing glutathione via arsenic-induced free radical generation [39].

21.6 Genotoxic and Carcinogenic Potentials

It has been shown that arsenic is a potent gene and chromosomal mutagen whose effects are mediated through the induction of ROS [40]. Recent investigations found that mitochondrial damage plays a crucial role in arsenic mutagenicity, and that mitochondrial damage can lead to the release of superoxide anions, which then react with nitric oxide to produce the highly reactive peroxynitrites. These data suggest that mitochondria are a primary target in the arsenic-induced genotoxic response [41]. Another study hypothesized that arsenic is a well-recognized human carcinogen that has been thought to act through epigenetic mechanisms by modifying DNA methylation patterns, perhaps in conjunction with DNA-damaging agents. Arsenic causes the depletion of *S*-adenosylmethionine, the main cellular methyl donor, and represses the expression of the DNA methyltransferase genes *DNMT1* and *DNMT3A*. It is possible that as a consequence of these two complementary mechanisms, long-term exposure to arsenic results in DNA hypomethylation [7]. In a recent review article, Flora [39] supports the hypothesis that as a carcinogen arsenic acts through epigenetic mechanisms rather than as a classical mutagen, and states that the carcinogenic potential of arsenic may be attributed

to activation of redox-sensitive transcription factors and other signaling pathways involving nuclear factor κB, activator protein-1, and p53.

21.7 Hematological Effects on Experimental Animals

The concentration of toxicant present in the environment and the duration of exposure appear to be important for the deleterious health effects in the experimental animals and humans to be manifested. In a study conducted in Haryana, India, on crossbred (Friesian × Sahiwal) male calves of 3–4 months age supplemented daily with 50 ppm arsenic (as As_2O_3) for up to 90 days in their diet in an attempt to investigate certain hematological parameters, no change in blood total leukocyte counts, differential leukocyte counts, packed cell volume (PCV), and hemoglobin was observed when comparing with the corresponding non-supplemented group [42]. In these experiments, calves of both groups were fed as per ICAR standards [43] and their requirements were fulfilled by feeding concentrate mixture and green oats. All calves were kept under similar conditions, and blood samples were collected at fortnightly intervals to estimate various parameters. The reasons why no adverse effects of arsenic had been observed in the exposed calves were two-fold: first, in this study although the ruminant animals were challenged with a high concentration of arsenic, 50 ppm (50 mg/L), their toxicity level in drinking water was not known; second, the study period was too short, only 90 days.

There could be species difference and degree of adversity on toxic effects of environmental pollutants among the directly exposed animals. To study the effect on birds, arsenic-induced toxicities were studied in research conducted at the Bangladesh Agricultural University, Mymensingh. In this study, ducklings were given arsenic trioxide at 100 mg/L in the drinking water daily for 90 days starting from day 15. One group of same-age ducklings was kept as control. Five birds were sacrificed from each group at 15-day intervals and toxic signs, body weight, and hematological parameters were recorded. It was found that ducks drinking a high concentrations showed signs of depression with dullness and reduced feed intake, and had ruffled feathers. About 15% of these birds did not gain body weight. There were reductions in total erythrocyte count, hemoglobin content, and PCV values, and the rise of erythrocyte sedimentation rate (ESR) values was significant ($p < 0.01$) in the arsenic-treated group [44].

In the study of Gyasi et al. [27], ICR mice were challenged with 0.8–4.8 mg/L arsenic via their drinking water, the As concentration being identical to that found in the streams and soils in Buruli ulcer endemic communities of the Amansie West District of Ghana. The mice developed inflammation, erythema, and open ulcers on skin (with scab formation) that were negative for acid-fast bacilli. Histopathological studies revealed liver and spleen damage with hepatic cell swelling and degenerative change showing cytoplasmic vacuolation with nuclear blebbing and gradual cell loss. The spleen developed a lymphoid background with multinucleate cell formation. Hematological examination revealed significant dose-dependent decrements in white blood cells indicating a detrimental effect on the body's immune system, a situation that makes the body susceptible to infections including *M. ulcerans*. The mean corpuscular volume of red blood cells also decreased significantly indicating microcytosis. High levels of arsenic in tissue (possibly from accumulation) caused inflammation, erythema, and

open ulcers on the skin; the presence of As at such levels has the potential to cause liver and spleen damage, reduced immune system function, and red blood cell microcytosis in experimental ICR mice.

21.8 Effect on Heme Synthesis

In research carried out by Woods and Fowler, it was found that chronic exposure of both rats and mice to sodium arsenate at 20, 40, or 85 ppm in drinking water resulted in depression of hepatic δ-aminolevulinic acid synthetase and heme synthetase, the first and last enzymes in heme biosynthesis, respectively. Concomitantly, urinary uroporphyrin and coproporphyrin concentrations were elevated in each of the exposed groups of rats and mice compared to their corresponding control values. In contrast, no changes were observed in the activities of cytochrome oxidase or cytochrome P450, indicators of mitochondrial and microsomal hemoprotein function, respectively. These results demonstrate that prolonged exposure to low levels of arsenic results in selective alteration of hepatic heme biosynthetic pathway enzymes, with concomitant increases in urinary porphyrin concentrations. Changes in hepatic hemoprotein function may serve as a specific indicator of pretoxic arsenic exposure in both rats and mice [45].

21.9 Hepatic Effects and Lipid Peroxidation

A cross-sectional epidemiological study was reported by Guha Mazumder [46] on 7683 people residing in arsenic-affected districts of West Bengal, India. Of these, 3467 people (control group) consumed drinking water containing safe levels of arsenic (less than 50 μg/L), and 4216 people consumed drinking water containing arsenic levels above 50 μg/L (arsenic-exposed group). The prevalence of hepatomegaly was significantly higher in arsenic-exposed people (10.2%) compared to controls (2.99%, $p < 0.001$). The incidence of hepatomegaly was found to have a linear relationship proportionate to increasing exposure of arsenic in drinking water in both sexes ($p < 0.001$). From hospital-based studies on 248 cases of arsenicosis, hepatomegaly was found in about 77% of the patients, and non-cirrhotic portal fibrosis was the predominant lesions in 63 out of 69 patients who underwent liver biopsy [46].

In a similar study conducted by the same investigator, BALB/C mice were given water contaminated with arsenic (3.2 mg/L) *ad libitum* for 15 months, the animals being sacrificed at 3-month intervals. There was progressive reduction of hepatic glutathione and enzymes of the antioxidative defense system associated with lipid peroxidation [46]. Liver histology showed fatty infiltration at 12 months and hepatic fibrosis at 15 months. In another study, pregnant Wistar rats were subchronically exposed to arsenic (50 mg/L) through drinking water, and changes occurred in the blood of arsenic-exposed pups that were examined during gestation and lactation. There was normocytic and normochromic anemia as well as a significant increase in hemolysis, enhanced lipid peroxidation (a measure of oxidative stress, such as thiobarbituric acid reactive substances (TBARS) production), and catalase activity in the blood of arsenic-intoxicated pups [47]. These studies on arsenic-affected people as well as experimental animals show that prolong drinking of arsenic-contaminated water is associated with

hepatomegaly. The predominant lesion of hepatic fibrosis appears to be caused by arsenic-induced oxidative stress.

21.10 Effects on Immune Responses in Fish

21.10.1 Innate Immune Response

The innate immune response, which is the first line of defense against invading pathogens, can be affected by environmental toxicants such as arsenic. In zebrafish infected with snakehead rhabdovirus, arsenic decreased induction and altered the kinetics of the antiviral cytokines interferon (IFN) and Mx, and inhibited tumor necrosis factor-α (TNF-α) priming of the respiratory burst response [48]. In another study, it was found that exposure to 2 and 10 ppb arsenic, both considered safe levels in drinking water, resulted in a greater than 50-fold increase in viral load and at least a 17-fold increase in bacterial load in embryos of zebrafish. Presence of arsenic lowered the overall innate immune response of the fish as evidenced by reductions in respiratory burst activity. Viral infection, after arsenic exposure, showed decreases of up to 13- and 1.5-fold changes in interferon and Mx mRNA expression, respectively. Bacterial infection, post-arsenic exposure, demonstrated at least 2.5- and four-fold declines in interleukin-1β and TNF-α mRNA levels, respectively. Maximum expression of these essential cytokines was also delayed upon arsenic exposure. These data indicated that arsenic exposure, at concentrations deemed safe in drinking water, suppressed the overall innate immune function in zebrafish [49].

21.10.2 Humoral Immune Response

The effects of long-term arsenic exposure (150 days) on the head kidney (HK) and ensuing humoral immune responses were studied in *Clarias batrachus* L. [49]. Exposure to non-lethal concentrations of arsenic (42.42 μM) resulted in significant time-dependent alterations in HK cell numbers, eventually affecting the HK somatic index. Prolonged exposure to arsenic also suppressed HK-B cell proliferation and led to significant reduction in serum immunoglobulin levels and antigen-specific serum bacterial agglutinin titers. Arsenic exposure inhibited the release of "IL-4 like factors" from HK-T cells. Histological studies documented extensive lymphocytopenia and a decrease in the melano-macrophage population, and hemosiderin accumulation. From exposure-challenge studies with *Aeromonas hydrophila* it was evident that pathogens could efficiently disseminate and colonize distant host tissues in the exposed fish. Thus, long-term exposure to non-lethal concentrations of arsenic affects HK and interferes with the humoral immune response of *C. batrachus* rendering them immunocompromised and susceptible to pathogenic challenge [50].

Chronic exposure (30 days) to arsenic (As_2O_3, 0.50 μM) in the same species of fish led to a significant increase in arsenic content in the HK accompanied by reduction in both head kidney macrophage (HKM) number and head kidney somatic index. Transmission electron microscopy of arsenic-exposed HKM revealed prominent endoplasmic reticulum, chromatin condensation, and loss in structural integrity of nuclear membrane. HKM from exposed fish demonstrated significant levels of superoxide anions but on infection with *Aeromonas*

hydrophila the fish were unable to clear the intracellular bacteria and died. Exposure–challenge experiments with *A. hydrophila* revealed that arsenic interfered with the phagocytic potential of HKM, and helped in intracellular survival of the ingested bacteria inside the HKM thereby inducing significant HKM cytotoxicity. The immunosuppressive effect of arsenic was further evident from the ability of *A. hydrophila* to colonize and disseminate efficiently in exposed fish. Further, arsenic suppressed the production of pro-inflammatory "IL-1β-like" factors from HKM. These data suggest that arsenic even at very low concentrations is immunotoxic to fish and the changes observed in HKM may provide a useful early biomarker of low-level As exposure [51].

21.11 Effects on Immune Responses in Laboratory Animals

21.11.1 Humoral Immune Response

Male Wistar rats were treated for 4, 8, and 12 weeks with 3.33, 6.66, 13.3, or 26.6 mg/kg of inorganic arsenic ($NaAsO_2$) per os by gavage [51]. Changes in humoral immune response, which was tested after 4 weeks of treatment, were not marked. After prolonged exposure to arsenic, the weight of thymus, spleen, and adrenals, which are organs responsible for immune response, was significantly reduced. The animals showed reduced delayed-type hypersensitivity (DTH) reaction and mean cell volume (MCV) of red blood cells, a hematological parameter. Plaque-forming cell assay proved to be insensitive in this short-time exposure. These results suggest that subchronic low-level exposure to arsenic can affect immune responses in rats [52]. In an earlier study [52], adult male cotton rats were exposed to 5 or 10 ppm sodium arsenite in drinking water for 6 weeks. The daily food intake of the animals decreased in a dose-dependent manner. Masses of liver, kidney, adrenals, popliteal lymph nodes, spleen, and seminal vesicles and selected hematological parameters were unaffected by arsenic exposure (see also next section).

21.11.2 Cell-Mediated Immune Response

In the study by Savabieasfahani et al. mentioned above [52], cell-mediated immunity, as measured by a phytohemagglutinin (PHA)-hypersensitivity response to an intradermal challenge, was suppressed 30% in the low-dose group compared to controls. Arsenic treatment did not have a measurable impact on lymphoproliferative responses of cultured splenocytes to the mitogens concanavalin A (Con A) and pokeweed mitogen, or to interleukin-2. Also no impact of low-level arsenic exposure was observed on macrophage phagocytic activity and tumoricidal activity of lymphokine-activated killer cells *in vitro* [53]. The effects of a single intratracheal instillation at doses of 50, 100, and 200 mg/kg gallium arsenide (GaAs) exposure on immunocompetence of B6C3F1 female mice were investigated. Fourteen days after exposure, the mitogenic response of splenic T cells to Con A and PHA was found unaffected by GaAs, but that of B cells to lipopolysaccharide (LPS) was increased by 52%. The DTH response to keyhole limpet hemocyanin (KLH) and mixed lymphocyte response were significantly reduced in a dose-dependent manner by GaAs exposure. The natural killer cell activity against the YAC-1 mouse lymphoma was enhanced in the GaAs-treated mice [31].

Following GaAs exposure, analysis of peritoneal exudate cells (PEC) revealed a dose-dependent decrease in number and a shift in the composition of PECs with increased percentage of monocyte and decreased percentage of lymphocyte populations. The adherent PECs demonstrated decreased phagocytosis of covaspheres and increased phagocytosis of chicken erythrocytes. GaAs exposure had no effect on host resistance to *Plasmodium yoelii* or *Streptococcus pneumoniae*, but dose dependently increased resistance of the mouse to *Listeria monocytogenes*. These observations suggested that GaAs affected the cellular immune parameters in mice [31].

In another study, mice exposed to 50 mg/L arsenic in the drinking water for 4 weeks demonstrated a reduction in lymph node cell proliferation and ear swelling following sensitization with 2,4-dinitrofluorobenzene (DNFB). Langerhans cells (LC) and T-cell populations in the draining lymph nodes of DNFB-sensitized mice were evaluated by fluorescence-activated cell sorting. The number of activated LC was reduced in cervical lymph nodes, suggesting altered LC migration following arsenic exposure. Lymphocytes from arsenic-treated animals sensitized with fluorescein isothiocyanate (FITC) exhibited reduced proliferative responses following T-cell mitogen stimulation *in vitro*; however, lymphocyte proliferation in non-sensitized, arsenic-treated mice was comparable to that in controls. Arsenic exposure also reduced the number of thioglycollate-induced peritoneal macrophages and circulating neutrophils [53]. This study, along with earlier studies by the same group, demonstrates that repeated, prolonged exposure to non-toxic concentrations of sodium arsenite alters immune cell populations and results in functional changes in immune responses in contact hypersensitivity [54].

21.12 Effects of Arsenic in Drinking Water on Human Health

Exposure to arsenic through drinking water is related to many health effects that have different incidences depending on the geographical area, ethnic group, age, and gender, as well as nutritional status of the affected subjects [33,55–60]. The variations could be due to different valence states and level of toxicities of the ionic species of arsenic. The most evident effects of arsenic exposure in Bangladesh and India are often linked to arsenical skin lesions including hyperkeratosis, hypopigmentosis, and cancer development on skin, alteration of the functions of major organs such as liver, bladder, kidney or lung, and ultimate formation of cancer in these organs [17,18,61,62]. Chronic exposure to arsenic in drinking water has been found associated with cancer development and blackfoot disease in Taiwan, some parts of Mongolia, China, and Mexico [63], and with diabetes in the USA [64]. Other manifestations of chronic exposure of arsenic via drinking water include neurological effects, obstetric problems, high blood pressure, and diseases of the respiratory system and blood vessels including the cardiovascular system [65].

In Bangladesh and India, most of the arsenic in drinking water comes from natural sources. In these regions the ground water is contaminated with geological arsenic that is brought to the surface by digging tubewells (in order to collect microorganism-free drinking water), which is the main cause for human exposure. Many of these tubewells supply very high levels of arsenic. In the northern district of Chapainowabganj of Bangladesh, we found the levels of arsenic in the waters

of different tubewells in one area to vary from 3 to 875 μg/L [33]. The levels of arsenic in the ground water of different states of Mexico vary from 66 to 725 μg/L [66], while the aquifers of Lagunera in northern Mexico vary from 8 to 624 μg/L [67]. In Mexico, most of the ground water is on the top of volcanic rocks that contain several forms of arsenical compounds that are liberated in to the water, together with other potential toxic substances, causing the water in each region to have different effects on human health. In the central zone and its adjacent states of Mexico where arsenic comes from the mining processes, the affected subjects suffer mainly from diabetes [68], infertility [69], and reduced IQ [70] rather than skin cancer. These observations suggest the influence of environmental factors including nutritional status and lifestyle on the toxic effects of arsenic.

In the United States, researchers are taking a much closer look at drinking water, from southwestern states like Nevada, where wells sometimes contain arsenic at more than 500 μg/L, to the upper Midwest and New England, where a belt of arsenic-infused bedrock taints aquifers in stretches from the coast of Maine to a point midway through Massachusetts. Water in parts of the Central Valley of California has been found to be tainted with arsenic as well [14]. Depending on local environmental conditions, arsenic can leach from soils or mineral deposits into ground water. Some private wells in Michigan and Connecticut have been found to contain arsenic in the range of 10 to 100 ppb [71]. While municipal water suppliers are required to meet the safety standard of 10 ppb or 10 μg/L of the Environmental Protection Agency (EPA) for arsenic in drinking water, no such regulation exists for private wells. In the USA, about 13 million people get drinking water from private wells with arsenic levels above the federal standard. There have been increased mortality rates linked to everything from diabetes to heart disease among arsenic-affected subjects in Michigan [72].

21.13 Immunotoxic Effects of Organic Arsenicals in Foods

The immunotoxic effects of organic arsenic compounds, namely, arsenocholine (AsCho), arsenobetaine (AsBe), and the tetramethylarsonium ion (TetMA), on the principal immune effector cells, macrophages, and lymphocytes of marine animals were studied, by comparing them with the effects of inorganic arsenicals *in vitro* [73]. The inorganic arsenicals arsenite and arsenate showed strong cytotoxicity to both macrophages and lymphocytes. The concentration of arsenite that reduced the number of surviving cells to 50% (IC_{50}) of that in untreated controls was 3–5 μM dm^{-3}, while the cytotoxicity of arsenate was lower ($IC_{50} = 100\,\mu^{-1}$ mM dm^{-3}) than that of arsenite. Compared with these findings, AsCho and AsBe were less toxic even at a concentration over 10 mM dm^{-3} to both macrophages and lymphocytes; however, TetMA had weak, but significant cytotoxicity to these cells (IC_{50} was about 6 mM dm^{-3}).

In another study, synthetic AsBe was orally administered to CDF_1 mice at a dose of 1.625 g/kg weight once a day on days −6, −4, −2 and 0 (four times, total 6.5 g/kg mouse weight), and its effect on the immune organs and immune effector cells were assessed until day 8. Orally administered AsBe was found temporally distributed to the spleen and thymus, and to immune effector cells, splenocytes, thymocytes, Peyer's patch lymphocytes, and peritoneal

macrophages, but was not very toxic either quantitatively or qualitatively. These findings suggest that ingestion of AsBe contained in marine animals is relatively safe in relation to the health of people who often consume marine foods in their daily diet [74].

21.14 Medicinal Use of Arsenic and Its Mechanism of Action

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) with distinctive biologic and clinical features. Most patients with APL are young and exhibit a life-threatening coagulopathy, which is the most notorious manifestation of the disease. The cells from almost all patients have a balanced reciprocal translocation between chromosomes 15 and 17 [75], which generates a fusion transcript joining the *PML* (promyelocyte) and *RAR*-α (retinoic acid receptor-α) genes [76]. In children, the disease is often associated with a high white blood cell count (WBC >10,000/μL), the microgranular variant (M3V), and all-*trans* retinoic acid (ATRA)-related toxicities [77]. Intensive studies of the biology and treatment of the disease have resulted in a remarkably thorough understanding of its pathogenesis [78]. Low dose arsenic trioxide (As_2O_3) has shown substantial efficacy in treating both newly diagnosed and relapsed patients with APL [79]. This achievement reflects collaborative laboratory and clinical studies, led initially by innovative investigators in China [80] and then France [81] and subsequently by many other cooperative oncology groups throughout the world.

The successes with arsenic trioxide have prompted investigations to elucidate the mechanisms of action underlying the clinical responses. Substantial data show that arsenic trioxide produces remissions in patients with APL at least in part through a mechanism that results in the degradation of the aberrant PML (promyelocyte)-retinoic acid receptor-α fusion protein. As a single agent, it induces complete remissions, causing few adverse effects and only minimal myelosuppression [81]. The combination of ATRA and arsenic trioxide, with minimal chemotherapy to control leukocytosis, is very effective therapy for newly diagnosed APL patients [83]. Studies have also investigated concerns about the toxicity and potential carcinogenicity of long-term exposure to environmental arsenic. Arsenic apparently affects numerous intracellular signal transduction pathways and causes many alterations in cellular function. These actions of arsenic may result in the induction of apoptosis, inhibition of growth and angiogenesis, and the promotion of differentiation [82].

21.15 Effects of Arsenic Compounds on Human Cells in Culture

The toxicities of inorganic arsenicals (iAs) and trivalent and pentavalent methylated arsenicals were examined in cultured human cells derived from tissues that are considered a major site for iAs methylation (liver) or targets for carcinogenic effects associated with exposure to iAs (skin, urinary bladder, and lung). Among the arsenicals examined, trivalent monomethylated species were the most cytotoxic in all cell types, and trivalent dimethylated arsenicals were at

least as cytotoxic as trivalent iAs (arsenite) for most cell types. Pentavalent arsenicals were significantly less cytotoxic than their trivalent analogs. Among the cell types, the greatest methylation capacity for iAs was exhibited by primary human hepatocytes, followed by epidermal keratinocytes, and bronchial epithelial cells. Cells derived from human bladder did not methylate iAs. There was no apparent correlation between susceptibility of cells to arsenic toxicity and their capacity to methylate iAs. These results suggest that the trivalent methylated arsenicals may significantly contribute to the adverse effects associated with exposure to iAs; and high methylation capacity does not protect cells from the acute toxicity of trivalent arsenicals [84].

It is generally accepted that methylation is a mechanism of arsenic detoxification; recent studies have suggested that methylated arsenicals also have deleterious biological effects. In the study of Vega et al. [16] the effects of inorganic arsenicals and trivalent and pentavalent methylated arsenicals were tested in human keratinocyte cultures. Viability testing showed that the relative toxicities of the arsenicals were as follows: $iAs^{III} > MAs^{III}O > DMAs^{III}GS > DMAs^{V} > MAs^{V} > iAs^{V}$. The trivalent arsenicals induced an increase in cell proliferation at concentrations in the 0.001 to 0.01 μM range, while at high concentrations (>0.5 μM) cell proliferation was inhibited. The pentavalent arsenicals did not stimulate cell proliferation. The methylated forms of As^{V} were found more cytotoxic than iAs^{V}. These data suggest that methylated arsenicals, products of the metabolic conversion of inorganic arsenic, can significantly affect viability and proliferation of human keratinocytes.

21.15.1 Effects on Growth-Promoting Cytokines and Growth Factors

It was found that exposure of human keratinocytes in culture to low doses of trivalent arsenicals stimulated secretion of the growth-promoting cytokines, granulocyte-macrophage colony stimulating factor (GM-CSF) and TNF-α. The dimethylarsinic acid ($DMAs^{V}$) reduced cytokine secretion at concentrations in which cell proliferation and viability were not affected [16]. Epidemiological studies have shown that inorganic arsenicals are human skin carcinogens that induce hyperproliferation and hyperkeratosis, but there is currently no known mechanism for their action or an established animal model for its study. Germolec et al. [85] observed increased mRNA transcripts and secretion of keratinocyte growth factors, including GM-CSF and transforming growth factor-α (TGF-α) and the proinflammatory cytokine TNF-α in primary human epidermal keratinocytes cultured in the presence of low micromolar concentrations of sodium arsenite. Treatment with sodium arsenite resulted in a significant increase in cell proliferation, as indicated by increases in cell numbers, *c-myc* gene expression, and incorporation of [^{3}H]-thymidine into cellular DNA. Studies of transcriptional regulation indicate that the rate of GM-CSF mRNA transcription is increased, while the elevated TGF-α is likely the result of message stabilization. While a number of cytokine regulatory networks exist in the skin, studies utilizing neutralizing antibodies against the growth factors indicate that inhibition of the arsenic-induced increase in TGF-α results in a corresponding decrease in the gene expression and secretion of GM-CSF. These results demonstrate that growth-promoting cytokines and growth factors are induced in keratinocytes following treatment with arsenic and could play a significant role in arsenic-induced skin cancer [85].

21.15.2 Effects on Human T-Cell Functional Responses

Previous workers have reported impaired T-cell functional responses in arsenic-exposed subjects. A study on 11 exposed and 13 control individuals in Mexico indicated that chronic exposure to arsenic via drinking water (390 μg/L) significantly decreased the phytohemagglutinin (PHA)-induced proliferative response of lymphocytes [86]. A later study showed that arsenic exposure leads to a diminution of PHA-stimulated T-cell proliferation in humans [87]. In that study, the evaluation of the peripheral blood lymphocyte proliferation was done at different culture time intervals. The mitotic index (MI) and labeling index (LI) in 72 h cultures in the exposed subjects with mean arsenic level in drinking water at 412 μg/L showing arsenical skin lesions were significantly lower than in the unexposed individuals without lesions who drank water with a mean arsenic level of 37.2 μg/L. Correlations between LI and MI showed that progression from the initial S- to M-phase is altered in exposed individuals [84].

Arsenic was evaluated on the expression of CD25 and IL-2 secretion in human peripheral blood mononuclear cells by proliferation assay [86]. Most of the donors did not show any change in the expression of CD25, but IL-2 secretion was inhibited in six of the seven donors tested. The determination of the mRNA of IL-2 and the intracellular IL-2 levels demonstrated that the inhibition was not at the transcriptional level. Electron microscopy studies revealed that cellular ultrastructure in Golgi apparatus, mitochondria, cytoskeleton, and perinuclear membrane was altered due to arsenic. These alterations suggested that sodium arsenite affected the cytoskeleton and the intracellular secretion of proteins including IL-2, leading to an impaired proliferation of the T cells when stimulated with PHA [88] (see Table 21–1).

21.16 Immunotoxic Effects on Murine and Human Monocytes/Macrophages

Inorganic arsenicals, both arsenite and arsenate, are strongly toxic to macrophages, and the concentration that decreased the number of surviving cells to 50% of that in untreated controls (IC_{50}) was 5 and 500 μM, respectively [89]. These inorganic arsenicals mainly caused necrotic cell death of about 80% cells, while 20% underwent apoptotic cell death. The inorganic arsenicals also induced marked release of an inflammatory cytokine TNF-α, at cytotoxic doses. The strong cytotoxicity of arsenite might be mediated via reactive oxygen and protease activation because it was inhibited by antioxidants superoxide dismutase (SOD), catalase, and reduced glutathione (GSH), or by a peptide inhibitor of interleukin-1β-converting enzyme (ICE). It was suggested that these immunotoxic effects of inorganic arsenicals might evoke both immunosuppression and inflammation, and they might be central factors causing carcinogenesis and severe inflammatory responses, such as hepatomegaly and splenomegaly, in chronic arsenicosis patients drinking arsenic-contaminated well water [89].

It was found that arsenite sensitively inhibited the colony-stimulating factor (CSF)-induced *in vitro* maturation of monocytes into macrophages at nM levels, and it also induced small, non-adhesive and $CD14^+$ abnormal macrophage generation from monocytes with granulocyte-macrophage CSF (GM-CSF) at 50–500 nM without cell death. The addition of other

Table 21–1 Immunotoxic Effects of Arsenic Exposure in Human Populations / Human Cells

Immunotoxic Effects	Arsenic/Study Place	Reference
Impaired PHA-induced T-cell proliferation	DW: 390 μg/L, Mexico	[86]
Altered replication of lymphocytes from the S- to M-phase	DW: 412 μg/L, Mexico	[87]
Overexpression of growth factors in human epidermal keratinocytes	Na-arsenite: low μM	[85]
Higher prevalence rate of arsenical skin lesions with clear dose-response relationship	DW: 10–2040 μg/L, Bangladesh	[17]
Ultrastructure in Golgi apparatus, mitochondria, cytoskeleton and perinuclear membrane altered; intracellular secretion of IL-2 affected	Sodium arsenite	[88]
Neuropathy in adults; increases fetal loss and premature delivery in women	DW: >300 μg/L, India	[100]
Associated with poor nutritional status; lower WBC counts; causes neutropenia and lymphocytosis	DW: 218 μg/L, ME: 7.6 yrs, Bangladesh	[33]
$CD4^+$ Th cells more sensitive than $CD8^+$ Tc cells in PHA-activated PBMCs	Sodium arsenite, 1 μM	[56]
Induces oxidative stress by producing nitric oxide and superoxide anions in PBMCs and monocytes	AsU: 12.3–1411 μg/g creatinine, Mexico	[38]
Mitochondria are a primary target in arsenic-induced genotoxic response in human-hamster hybrid A_L cells	Na-arsenite: 1–2 μg/mL	[41]
Inhibition of GM-CSF-induced maturation of monocytes into macrophages; caused nonadhesive, small, abnormal $CD14^+$ macrophage generation	Na-arsenite: 50–500 nM	[90]
Rapid cell rounding of macrophages with loss of adhesion at non-toxic arsenic dose	Arsenic trioxide, As_2O_3: 1 μM	[91]
Inhibition of lymphocyte proliferation in PHA; inhibition of IL-2 secretion	DW: 412 μg/L, Mexico	[94]
Increases respiratory diseases - asthma, bronchitis, cough; significantly elevated levels of IgG, IgE, and IgA in serum	DW: 216 μg/L, ME: 7.4 yrs, Bangladesh	[106]
Significant decrease in levels of IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ secreted by T cells; increases opportunistic infections	DW; skin lesions, Kolkata, India	[95]
Decreases IL-7 and lactoferrin in breast milk; increases ARI in infants	DW; maternal AsU high, Bangladesh	[96]
Increases lower respiratory tract infection and diarrhea in infants	Maternal AsU high, Bangladesh	[101]
Lower complement-mediated bactericidal activity	DW: 216 μg/L, AsU: 223 μg/L, Bangladesh	[110]

DW; drinking water; AsU: arsenic in urine; ME: mean exposure to As; ARI: acute respiratory infection.

metallic compounds including chromium, selenium, mercury, cadmium, nickel, copper, zinc, cobalt, manganese, and other pentavalent arsenic metabolites, such as inorganic arsenate, monomethylarsonic acid and dimethylarsinic acid, could not induce the same abnormal cell generation from monocytes with CSFs at any concentration and any additional time schedules; they showed only simple cytolethality in monocytes and macrophages at nM–mM levels accompanied by cell death [90].

21.16.1 Effects on Monocyte/Macrophage Functional Responses

Treatment of human macrophages with a non-cytotoxic concentration of 1 μM arsenic trioxide (As_2O_3) induced a rapid cell rounding and a subsequent loss of adhesion [89]. These morphologic alterations were associated with a marked reorganization of actin cytoskeleton, which included retraction of peripheral actin extensions and formation of a cortical actin ring. In addition, As_2O_3 reduced expression of various macrophagic surface markers, enhanced that of the monocytic marker CD14, and altered both endocytosis and phagocytosis. Exposure of macrophages to the metalloid also strongly potentiated expression of TNF-α and IL-8 induced by LPS; like monocytes, As_2O_3-treated macrophages can be differentiated into dendritic-like cells. Impairment of macrophage function by As_2O_3 mainly resulted from activation of a RhoA/Rho-associated kinase pathway; indeed, pretreatment of macrophages with the Rho-associated kinase inhibitor Y-27632 prevented the metalloid effects on cytoskeleton and phagocytosis. These findings suggested that human macrophages constitute sensitive targets of inorganic arsenic, which may contribute to immunotoxicity on these cells [91].

Impaired functions were also observed in monocyte-derived macrophages from exposed subjects with skin lesions ($n = 70$) compared to the unexposed individuals ($n = 64$) in another study conducted in West Bengal, India, in which the macrophages of arsenic-exposed individuals showed cell rounding with a significant ($p < 0.001$) loss of adhesion capacity, together with decreased nitric oxide production, and impaired phagocytic function. There was decreased CD54 and F-actin expression. Additionally, chronic arsenic exposure affected the RhoA–ROCK pathway, which in turn impaired macrophage functions [92]. These combined effects could contribute significantly to arsenic-induced immunosuppression observed in the exposed individuals.

21.16.2 Impairment of Macrophage-Functional Genes

Using a pan-genomic microarray, Bourdonnay et al. [93] found that exposure of human blood monocyte-derived macrophages to 1 μM As_2O_3 for 72 h, a non-cytototoxic concentration, resulted in up-regulation of 32 genes and repression of 91 genes. Among these genes, 26 are specifically related to a differentiation program of human macrophages. In particular, As_2O_3 strongly alters expression of *MMP9, MMP12, CCL22, SPON2*, and *CXCL2* genes, which contribute to major macrophagic functions. It was interesting to note that most of the effects of the major environmental contaminant were reversed when As_2O_3-treated macrophages were next cultured in arsenic-free medium. Further, it was found that As_2O_3 similarly regulated expression of this macrophagic gene subset in human alveolar macrophages, the phenotype of which closely resembles that of blood monocyte-derived macrophages. This study demonstrates that environmentally relevant concentrations of As_2O_3 impair expression of macrophage-specific genes, which fully supports the theory of the interference of the metalloid with the differentiation program of human macrophages [93].

21.17 Immunotoxic Effects on Murine Mononuclear Cells

The effect of arsenic exposure on the activation of splenic mononuclear cells (SMCs) in male CD57BL6N mice was evaluated [27]. Intra-gastric exposure to arsenic (as sodium arsenite) for 30 days (1, 0.1, or 0.01 mg/kg/day) reduced the proportion of $CD4^+$ cells and the $CD4^+$/$CD8^+$ ratio in the spleen, increasing the proportion of $CD11b^+$ cells. Arsenic exposure did not modify the proportion of B cells. SMC showed an increased level of phosphorylation of lck and fyn kinases (first kinases associated with TCR complex when activated). Although normal levels of apoptosis were observed on freshly isolated SMC, an increase in apoptotic cells related with the increase in phosphorylation of lck and fyn was observed when SMCs were activated with Con A. Arsenic exposure reduced the proliferative response of SMCs to Con A, and also reduced secretion of IL-2, IL-6, IL-12, and IFN-γ. No effect was observed on IL-4 and IL-10 secretion. The same effects were observed when SMCs of exposed animals were activated with anti-CD3/CD28 antibodies for 24 h, but these effects were recovered after 72 h of stimulation. These findings suggest that repeated and prolonged exposure to arsenic alters cell populations and produces functional changes depending on the specific activation pathway, and could be related to the phosphorylation status of lck and fyn kinases [28]. Further, arsenic exposure in mice inhibits T cell proliferation and macrophage activity, decreases $CD4^+$ T cells in the spleen, and down-regulates contact hypersensitivity response to 2,4-dinitrofluorobenzene (DNFB) [94].

21.18 Decreased Cytokine Production by Human T Cells

It has been found that arsenic can produce an inhibition of IL-2 secretion in human lymphocytes *in vitro*. In a study conducted on Mexican adults, exposure to 412 μg/L of arsenic in drinking water was found to inhibit lymphocyte proliferation in response to PHA. Arsenic exposure increases the incidence of autoimmune-mediated diseases such as diabetes mellitus and other diseases related to immunosuppression such as the presence of skin cancer [94]. In a study conducted in Kolkata, India, the effect of arsenic on T-cell proliferation and cytokine secretion in 20 individuals with arsenic-induced skin lesions was investigated and compared with the results in 18 arsenic-unexposed individuals. There was a significant dose-dependent suppression of Con A-induced T-cell proliferation in the arsenic-exposed individuals compared with the unexposed individuals ($p < 0.001$). This correlated with a significant decrease in the levels of secreted cytokines by the T cells (IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ) in the exposed individuals ($p < 0.001$). Thus, it can be inferred that arsenic exposure can cause immunosuppression in humans [95].

21.19 Effects of *In Utero* Exposure on Infant Immune System

Little is known about early-life effects of arsenic on immunity; the impact of *in utero* exposure of arsenic on infant immune parameters and morbidity was evaluated in a pilot study in Matlab, a rural area of Bangladesh. Pregnant women ($n = 140$) extensively affected by arsenic from tubewell water were enrolled at 6–10 weeks of gestation. Maternal urinary arsenic during pregnancy showed significant negative correlation with interleukin-7 (IL-7) and lactoferrin (Ltf) in breast milk and child thymic index (TI). Urinary arsenic was also positively associated with fever and diarrhea during pregnancy and acute respiratory infections (ARIs) in the infants. The effect of arsenic exposure on ARI was only evident in male children. The findings suggest that *in utero* arsenic exposure impaired child thymic development and enhanced morbidity, probably via immunosuppression [96]. Because of its ability to readily cross the placenta [97], arsenic could potentially impair fetal development; indeed, gestational arsenic exposure is linked to increased fetal loss and infant mortality in birth cohort studies in Bangladesh [98,99]. The effect of *in utero* arsenic exposure seemed to be partially gender dependent, and arsenic exposure also affected breast milk content of trophic factors and maternal morbidity [96]. In an evaluation of arsenic exposure among the villagers in the Middle Ganges Plain, Bihar, India, where 57% of the 206 tubewells supply water with arsenic concentrations exceeding 50 μg/L, with about 20% containing >300 μg/L, arsenic-typical neuropathy was diagnosed in 63% of the adults, and there was apparent increase in fetal loss and premature delivery in the women with the highest concentrations of arsenic in their drinking water [100].

There may be an association between prenatal arsenic exposure and increased risk of infant mortality caused by infectious diseases, but no epidemiological study was available until recently in support of or against this observation. A recent prospective population-based cohort study was conducted in the arsenic hyperendemic region of Matlab, Bangladesh, that included 1552 live-born infants of women exposed to arsenic. The concentrations of metabolites of inorganic arsenic were determined in maternal urine samples collected at gestational weeks 8 and 30, and information on symptoms of lower respiratory tract infection (LRTI) and diarrhea in infants was collected during a 12-month follow-up period. The estimated risk of LRTI and severe LRTI increased by 69% [adjusted relative risk (RR) = 1.69; 95% confidence interval (CI), 1.36–2.09)] and 54% (RR = 1.54; 95% CI, 1.21–1.97), respectively, for infants of mothers with urinary arsenic concentrations in the highest quintile (average of arsenic concentrations measured in early and late gestation, 262–977 μg/L) relative to those with exposure in the lowest quintile (<39 μg/L). The corresponding figure for diarrhea was 20% (RR = 1.20; 95% CI, 1.01–1.43). These observations suggested that arsenic exposure during pregnancy was associated with increased morbidity of infectious diseases during infancy [101].

21.20 Gender-Related Immunotoxic Effects in Human

To evaluate whether arsenic effects are gender related, the *in vitro* toxicity of sodium arsenite on human T lymphocyte subpopulations from men and women were investigated. Peripheral

blood mononuclear cells (PBMCs) obtained from healthy young men and women were treated with sodium arsenite (0.01, 0.1, and 1 μM). The cell viability, cell proliferation, and the proportion of $CD4^+$ Th and $CD8^+$ Tc cells after 48 or 72 h of arsenic exposure in resting and PHA-activated PBMC were assessed. It was observed that sodium arsenite at 1 μM was more toxic for Th than for Tc cells in PBMCs from women. In addition, T lymphocytes from women were more affected by the cell proliferation inhibition induced by arsenic, suggesting that women could be more susceptible to the immunotoxic effects caused by arsenic exposure [56]. Gender differences in the biotransformation of arsenic by methylation have been reported, and men seem to be more affected by arsenic-related skin effects than are women. Experimental studies indicate major gender differences in arsenic-induced cancer [60]. Also, tuberculosis mortality rate ratios in arsenic-exposed men were found significantly higher for region II of Chile compared with findings in As exposed women of the same region during the years 1982–1986 (deaths: 359 among men and 95 among women) [102]. Maternal exposure to arsenic during gestation has been found to be associated with ARI, and the effects have been more prevalent in male than in female children [96].

21.21 Effects of Chronic Exposure on Immune Response

21.21.1 Effects of Chronic Exposure on Mice and Human Lungs

Exposure to environmentally relevant concentrations of arsenic is known to be associated with an increased risk of lung disease, making As a unique toxicant, because lung toxicity is usually associated with inhalation rather than ingestion. To study the changes in the lungs, C57BL/6J mice fed a casein-based AIN-76A defined diet were exposed to 10 or 100 ppb arsenic in drinking water or food for 5–6 weeks. The whole genome transcriptome profiling of mice lungs revealed significant alterations in the expression of many genes with functions in cell adhesion and migration, channels, receptors, differentiation, and proliferation that are associated with the innate immune response. Confirmation of mRNA and protein expression changes revealed that genes for interleukin-1β, interleukin-1 receptor, a number of Toll-like receptors, and several cytokines and cytokine receptors were significantly altered in the lungs of arsenic-exposed mice. These findings indicate that chronic low dose arsenic exposure can elicit effects on the regulation of innate immunity, which may contribute to altered disease risk, particularly in the lung [103]. Epidemiological studies carried out on 7683 subjects in West Bengal showed that the prevalence of non-malignant pulmonary effects were markedly increased for participants with arsenic-induced skin lesions who also had high levels of arsenic in their drinking water [104].

To investigate whether arsenic exposure is a significant contributor to the increased risk of lung disease, C57BL/6J mice were exposed to 100 ppb arsenic in drinking water for 5 weeks, followed by intranasal inoculation with a sublethal dose of respiratory influenza A (H1N1) virus. Arsenic was associated with a number of significant changes in response to influenza, including an increase in morbidity and higher pulmonary influenza virus titers on day 7 post-infection. There was a decrease in the number of dendritic cells in the mediastinal lymph

nodes early in the course of infection. The study findings indicate that chronic arsenic exposure significantly compromises the immune response to infection. Alterations in response to repeated lung infection may also contribute to other chronic illnesses, such as bronchiectasis, which is elevated by arsenic exposure in epidemiological studies [105]. Similarly to this observation, high prevalence of respiratory diseases including asthma and bronchitis has been reported among Bangladeshi subjects suffering from drinking-water arsenic toxicity [106].

21.21.2 Effects of Chronic Exposure on Humoral Immune Response in Humans

In a cross-sectional epidemiological study, the humoral immune response was evaluated in Bangladeshi subjects ($n = 125$) chronically exposed to drinking water arsenic by measuring serum immunoglobulin profiles [106]. The mean duration of exposure in the patients was 7.4 ± 5.3 years, and the levels of arsenic in the drinking water and urine samples were 216 ± 211 and $223 \pm 302\,\mu g/L$, respectively, compared to 11 ± 20 and $29 \pm 19\,\mu g/L$, respectively, in the unexposed subjects. The exposed subjects had highly significantly elevated levels of IgG and IgE compared to the control unexposed subjects. The mean level of IgG in the arsenicosis patients was $24.3 \pm 7.5\,g/L$ while the value in the controls was $13.8 \pm 7.8\,g/L$ ($p < 0.001$), and the mean level of IgE in the patients was $600 \pm 265\,IU/mL$ compared to $186 \pm 221\,IU/mL$ in the control subjects ($p < 0.001$). The levels of IgA were significantly higher ($2.7 \pm 1.5\,g/L$ vs. $2.2 \pm 0.7\,g/L$, $p < 0.005$) but levels of IgM were similar to those in the controls [106]. In contrast to the findings of this study, no abnormalities were detected in the serum immunoglobulin IgG, IgA, or IgM levels in workers ($n = 47$) exposed to arsenic through inhalation in a coal-burning power plant in an earlier study [107]. However, the levels of arsenic or duration of exposure were not measured in this study and they might have been too small to cause significant damage to the immune system.

It has been revealed after analysis of the clinical symptoms based on skin manifestations that the levels of both IgG and IgE were significantly elevated during the initial stages while IgE were further elevated with the duration of arsenic exposure [106]. It might be possible that arsenical skin lesions (dermatosis) caused by chronic exposure played a significant role in eliciting highly elevated levels of serum IgG and IgE in the exposed subjects. Higher incidences of opportunistic infections are found in arsenic-exposed individuals, indicating that their immune systems may be functionally impaired [95]. It is expected that a healthy and functional immune system would be necessary to provide adequate immune protection against tumor cells and an adequate response to infectious agents.

21.22 Association with Respiratory Complications and Impaired Immune Responses

It has been reported that subjects chronically exposed to arsenic in drinking water have higher prevalence of respiratory diseases (60%), gastric and abdominal pain (59%), weakness (33%), and itchy rash (28%), among other complications [33]. Prevalence of respiratory effects has been reported in other studies conducted in West Bengal, India [104,108], and in

Bangladesh [109]. The relationship between chronic arsenic exposure through drinking-water and respiratory complications and the immune response was assessed in Bangladeshi subjects chronically exposed to arsenic [106]. The respiratory symptoms included breathing problems, chest sounds, asthma, bronchitis, and cough. Among the exposed subjects with arsenicosis, the mean serum IgE level was 706 ± 211 IU/mL in those with respiratory complications compared to 542 ± 241 IU/mL in those without apparent involvement with the respiratory system ($p < 0.01$). The eosinophil counts in the exposed population did not differ significantly from the counts in the control unexposed subjects, indicating that elevated levels of serum IgE might not be due to allergic diseases; rather they could be due to direct effects of arsenic. There were significant linear relationships between the levels of serum IgE and inorganic phosphorus ($p < 0.05$), and the levels of serum IgA, and urinary excretion of arsenic ($p < 0.001$). These observations suggested that arsenic toxicity caused respiratory complications, induced changes in the humoral as well as mucosal immune responses.

21.23 Effects of Chronic Exposure on Serum Complement Function

Functions of serum complement were evaluated in B6C3F1 female mice following exposure of gallium arsenide as a single intratracheal instillation at doses of 50, 100, and 200 mg/kg. Fourteen days after exposure, the levels of the serum complement protein C3 were measured; they were increased by as much as 16%. There was no significant change in the classical pathway hemolytic activity as measured by CH50 levels [31]. However, the effect on the complement system of a single inhalation exposure to arsenic has not been studied in other animals to compare these results.

In a recent cross-sectional study, serum complement function was evaluated in Bangladeshi subjects ($n = 125$) chronically exposed to drinking-water arsenic, by measuring bactericidal activity [110]. The mean levels of arsenic in drinking water and urine samples of the exposed subjects were 216 ± 211 and 223 ± 302 µg/L, respectively, and the mean duration of exposure was 7.4 ± 5.3 years. The exposed subjects had significantly lower complement-mediated bactericidal activity than the unexposed controls ($p < 0.01$). The levels of complement C4 in about 20% of the exposed subjects were above normal. In the exposed subjects, the mean complement C3 was 1.56 g/L, and C4 was 0.29 g/L compared to 1.68 g/L and 0.25 g/L, respectively, in the unexposed controls. The mean IgG level in the exposed subjects was 24.3 g/L and was highly significantly elevated ($p < 0.001$). The exposed subjects showed a significant direct correlation between C3 and bactericidal activity ($p = 0.014$); this observation together with elevated levels of C4 indicated underutilization and possibly impaired activity of the classical complement pathway in drinking-water arsenic toxicity [110].

21.24 Conclusions

Studies carried out on *in vivo* and *in vitro* systems indicate that arsenic can act as a modulator of the body's immune defense system that could render the host immunocompromised.

A suboptimal or non-protective immune system could pose increased risk of infections and development of several cancers as observed in subjects chronically exposed to arsenic. Exposure of experimental animals to arsenic via drinking water or through nasal challenge resulted in alterations of the cellular and cell-mediated immunity that generally agree with immunological outcomes in humans. Exposures to very low doses (<10 ppb) could elicit significant effects on innate immune mediators. Some studies evaluated the effects of the metalloid on cultured murine and human cells. However, only few experiments have been done to study the effects of arsenic on the humoral immune response. More work needs to be done for better understanding the risk of human immunotoxicity. Some affected subjects in different parts of the world have been consuming arsenic-contaminated water for some 10–20 years or more. It would be difficult to construct animal models to study such long-term chronic effects. Moreover, inconsistencies in epidemiological findings, possibly due to differences in dose, sampling method, genetic background, and environmental/nutritional factors, as well as variation in arsenic compounds present in natural sources, indicate the need for larger study-participant numbers and diverse ethnic populations. Also, populations experiencing variations in exposure levels should be evaluated for better understanding of dose-dependent relationships. Comprehensive data will be critical for identifying the potential molecular targets of arsenic to elucidate mechanisms of immunotoxicity.

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22

Arsenic and Developmental Toxicity and Reproductive Disorders

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CHAPTER OUTLINE

22.1 Introduction

Arsenic is a naturally occurring metalloid that is present at trace levels in soil, rock, water, and air. It has a complex biogeochemical cycle, existing in several oxidation states (0, +3, and +5) and in inorganic and organic forms. Ingestion is the most common route of exposure. Arsenic levels can become elevated in natural waters from the weathering and dissolution of arsenic-containing minerals, volcanic activity, microbial activity, and anthropogenic activities [1]. The concentration of arsenic in water is highly dependent upon the environmental and geological conditions of the aquifer. Due to its increased mobility and solubility under the pH and redox conditions, arsenic is one of the most difficult contaminants to remove from drinking water. Redox potentials also influence inorganic arsenic's valence state, which in turn can influence its toxicity [2]. Under oxidizing conditions, the dominant form of inorganic arsenic is pentavalent arsenate (As^{+5}), a less toxic and less soluble species. When redox potentials decrease, arsenate is reduced to trivalent arsenite (As^{+3}), a more soluble and more toxic species.

There are approximately 20 countries including Bangladesh, Taiwan, Mexico, Chile, Argentina, Vietnam, Laos, India, China, Romania, and the United States that have groundwater aquifers that are naturally contaminated with arsenic at levels exceeding the World

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Health Organization's drinking water recommendation of 10 μg/L [1]. Many of these aquifers are positioned below densely populated regions and are used as a potable water source leading to millions of people being chronically exposed to arsenic [3]. In the absence of drinking arsenic-contaminated water, diet is the primary route of exposure for arsenic [4]. Agricultural produce and livestock can accumulate arsenic from contaminated soil [5], water [6], pesticides [7], forage [8], and feed supplements [9]. Rice and cereal grains are considered the primary food sources for arsenic exposure because arsenic is readily taken up by the silicon transport system in these plants [10]. Daily dietary intake of inorganic arsenic will depend on dietary patterns and life stage. Diet studies have estimated that certain ethnic groups with higher rice consumption can have an average daily arsenic intake of 1 μg/kg-day [11]. Market basket surveys have also shown that infants are at risk of high arsenic exposure from rice cereal [12].

Once ingested, inorganic arsenic is metabolized through a series of reduction and oxidative methylation reactions forming two methylated metabolites: monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) [13]. In humans, approximately 60–70% of the ingested dose is excreted in the urine within 3 days, but the methylation process does not happen uniformly and urine contains, on average, 10–30% inorganic arsenic, 10–20% MMA, and 60–70% DMA [14–16]. This makes urinary arsenic a good biomarker for recent exposure to arsenic. The percentage of each metabolite, or their ratios (MMA/inorganic or DMA/MMA), is also commonly used as a biomarker of an individual's methylation capacity, which can be an independent risk factor for arsenic toxicity [17].

Arsenic can also be measured in other biological tissue including blood, hair, nails, and placental tissue. It is important to consider the half-life of arsenic in each of these biological tissues and individual exposure histories to select and interpret biomarker data. For instance, a single dose of arsenic is cleared from the bloodstream within several hours and excreted via the kidney. Subsequently, blood arsenic levels may only provide useful exposure information for individuals who have a blood test immediately after an exposure, or in those who are chronically exposed to consistently high levels of arsenic [18]. Recently, methods have been developed for measuring arsenic and other heavy metals in dried blood spots that are collected on filter paper [19]. Collecting blood spots from newborns is common for screening newborns for metabolic diseases and this new analytical technique provides a new tool for epidemiological studies to examine *in utero* exposure to arsenic.

Hair and toenails are useful biomarkers for historical exposures. Inorganic arsenic binds to keratin and becomes isolated from the metabolic activity that would lead to the excretion of arsenic in the urine. Any inorganic arsenic that is circulating in the blood will present itself quickly in the base of the hair shaft and nail bed. It takes several months to a year for arsenic to reach the distal tip of the nail where it can be collected. The growth rate of hair and nails is approximately 1.25 centimeters per month and 1.6 millimeters per month, respectively [20,21]. Subsequently inorganic arsenic detected in hair and nails will reflect exposures that accumulated in these matrixes during their growth [22]. Taking individual segments of each matrix can provide a timeline for individual exposures. Alternatively, samples can be pooled to provide an indication of the cumulative past exposures.

Many studies have shown that arsenic can be taken up and stored by the human placenta, as well as transported through the placenta [23–25]. Arsenic concentrations in maternal blood are highly correlated with arsenic concentrations in cord blood, demonstrating that arsenic readily crosses the placenta [26]. By examining the concentration of arsenic species in the urine of pregnant women, epidemiologists have shown that arsenic metabolism shifts during pregnancy and women excrete lower levels of inorganic arsenic species and higher levels of methylated arsenic metabolites [27,28]. This phenomenon has also been observed in mice [29]. These data demonstrate that the developing fetus is likely exposed to inorganic arsenic, MMA, and DMA. This raises concerns about arsenic's developmental toxicity.

This chapter will describe arsenic's developmental toxicity and its role in contributing to reproductive disorders including birth defects, infant mortality, low birth weight, and fertility. It will also discuss the evidence that developmental exposure to arsenic contributes to an increased risk of disease later in life.

22.2 Developmental Toxicity

22.2.1 Neural Tube Defects

22.2.1.1 Animal Models

Arsenic is highly teratogenic in laboratory animals. The most frequently reported anomalies include neural tube defects and similar disorders of the developing nervous system. Arsenic induces neural tube defects in several animal models, including mouse [30–33], rat [34], hamster [35–37], and chick [38,39]. Animal studies have demonstrated that arsenic crosses the placenta and preferentially accumulates in the neuroepithelium of the developing hamster, mouse, and monkey embryos [40,41]. Parental (intravenous or intraperitoneal) treatment is associated with greater frequencies of neural tube defects than oral treatment, but experimental studies have demonstrated that oral treatment is also teratogenic at higher doses [32].

The mechanisms of arsenic-induced neural tube defects in these models remain unknown. Several studies have reported that arsenic administration increases the production of reactive oxygen species, leading to oxidative stress and cytotoxicity, which may contribute to neural tube defects through oxidative damage to the developing neural plate [42,43]. Folate deficiency may exacerbate the direct toxic effects of arsenic on the neural plate by inhibiting the reduction of arsenic into less reactive subspecies. Mice with specific defects in folate transport and folate metabolism have higher rates of neural tube defects after *in utero* arsenic exposure compared to wild-type mice similarly exposed [44,45].

There is also some evidence from mice models to support the hypothesis that arsenic's effects on the neural tube are mediated through maternal hyperglycemia. This has been shown in experiments that delivered an intraperitoneal dose of arsenate and observed an elevation of glucose during pregnancy as well as increased rates of neural tube defects in the offspring [46]. This theory has been explored through *in silico* modeling, which computationally predicts that inhibition of the glucocorticoid receptor pathway would prevent arsenic-induced neural tube defects in chick embryos [47].

There is also increasing evidence that DNA methylation plays an important role in early embryonic development. DNA methylation is an epigenetic modification that involves the covalent addition of a methyl group to a cytosine at the 5′-position of a CpG dinucleotide [48]. CpG dinucleotides are clustered in the promoter regions of genes and in highly repeated elements such as long interspersed nucleotide elements [49,50]. When CpG sites in promoter regions are not methylated normal gene transcription occurs, but when CpG sites are methylated gene transcription is silenced [51]. Similarly, when long interspersed nucleotide elements are methylated they remain silenced, but when they become less methylated they can be transposable; that is, expression can lead to insertion into other genomic regions, which can result in gene silencing and chromosomal instability [52,53].

In vitro studies have demonstrated that high doses of inorganic arsenic alter DNA methylation in human lung, kidney, and keratinocytes [54–58]. In mice, prenatal inorganic arsenic exposure alters DNA methylation in fetal tissues [58–62]. There is also some evidence that aberrant DNA methylation is an underlying mechanism of neural tube defects. Experimental evidence demonstrated that arsenic-treated chick embryos exhibited significantly lower global DNA methylation as measured by 5-meC content [43].

22.2.1.1.1 HUMAN STUDIES

Few studies have investigated the relationship between arsenic exposure during pregnancy and risk of birth defects in humans. Archeologists report that the prevalence of spina bifida occulta among ancient mummies recovered in an area of northern Chile characterized by high environmental arsenic levels was 13.5%, compared with 2.4% in a low-arsenic area believed to have had no other differences in diet or other factors [63]. Investigation of a cluster of anencephalic births near the Texas–Mexico border found that maternal and paternal exposure to arsenic via drinking water was associated with higher risk of neural tube defects, but this finding was not statistically significant [64]. In a series of studies conducted in the same area of Texas, self-reported pesticide exposures were associated with neural tube defect risk. Though qualitative data on the specific pesticide were not obtained, given the widespread use of arsenical compounds in agriculture, the possibility that this association may be attributable to arsenic should be considered [64,65]. Ecological studies have linked soil arsenic levels to neural tube defects in China [66]. In a study of 2000 pregnant women in rural Bangladesh that used individual measures of exposure, Kwok et al. [67] reported a weak but statistically significant association between arsenic concentrations in drinking water and all birth defects, but no other adverse effects on pregnancy outcomes.

22.2.1.1.2 OTHER BIRTH DEFECTS

At least two studies have proposed an association between maternal arsenic exposure in drinking water and increased risk of congenital heart malformations in exposed offspring. In a case–control study in Massachusetts, Zierler et al. studied 270 cases of congenital heart anomalies compared to 665 randomly selected controls [68]. Arsenic concentration in drinking water at any detectable level was associated with a three-fold increase in risk of coarctation of the aorta

(odds ratio (OR) 3.4, 95% confidence interval (CI): 1.3–8.9). The arsenic level was, however, very low, with the maximum concentration 2.1 μg/L. In an ecological study using mortality data from 30 US counties from 1968 to 1984 and population-weighted mean arsenic concentration in public drinking water supplies, standard mortality ratios (SMR) for congenital heart disease appeared to be elevated for counties with mean arsenic concentrations exceeding 20 μg/L [69].

22.3 Reproductive Toxicity

22.3.1 Infant Mortality

There is epidemiological evidence that arsenic exposure is associated with higher rates of infant mortality. In Inner Mongolia, China, a large cross-sectional study ($n = 9989$) that used data from governmental reproductive permits observed that drinking-water arsenic concentrations greater than 50 μg/L during pregnancy was associated with an increased risk of neonatal death (OR 2.01, 95% CI: 1.12, 3.59). Because all women received the same government provided prenatal and postnatal healthcare, this study was able to control for many confounders [70]. Interestingly, this study also observed that maternal drinking-water arsenic concentrations above 50 μg/L was associated with a 0.05 kg higher term birth weight (95% CI: 0.02, 0.08 kg).

In a retrospective study in Chile, elevated infant mortality rates were observed in Antofagasta, a city that has a well-documented history of high arsenic exposure (approximately 860 μg/L) in its publically supplied drinking water [71]. Compared to Valparaiso, a city with lower arsenic concentrations in drinking water (below 20 μg/L), the rates of late fetal mortality [rate ratio (RR) = 1.7; 95% CI: 1.5–1.9], neonatal mortality (RR = 1.53; CI: 1.4–1.7), and post-neonatal mortality (RR = 1.26; CI: 1.2–1.3) were higher in Antofagasta after adjustment for location and calendar time.

In Bangladesh, a cross-sectional study ($n = 533$) observed excess risk for spontaneous abortion (OR = 2.5, 95% CI: 1.5, 4.3) and stillbirth (OR = 2.5, 95% CI: 1.3, 4.9) among pregnant women who were exposed to drinking water containing As at more than 50 μg/L compared to less than 50 μg/L after adjusting for participant's height, history of hypertension and diabetes, and (for neonatal death only) age at first pregnancy [72]. This study, however, did not observe a statistically significant elevated risk for neonatal death (OR = 1.8, 95% CI: 0.9, 3.6). However, a large prospective study in Bangladesh ($n = 2924$) determined that women with the highest total urinary arsenic concentrations measured at 8 weeks' gestation have a 1.4-fold increase in spontaneous abortion (95% CI: 0.96–2.2) compared to women with the lowest concentrations of total urinary arsenic in their urine [73].

22.3.2 Birth Weight

Few human studies have examined the relationship between arsenic exposure and low birth weight. These studies were conducted in different populations and used different study

designs and different sample sizes. For instance, an ecological study in Taiwan that used an extreme points contrast design observed that infants born into arsenic-exposed villages were, on average, 29 grams (95% CI: 13.6–44.6 g) lighter than infants born into non-arsenic-exposed villages after adjusting for maternal age, marital status, maternal education, and infant sex [74]. Yet, a cross-sectional ecological study performed in Mongolia ($n = 9890$) observed that infants born in villages with the highest arsenic levels in public water wells were on average 50 grams heavier than infants born in villages with lower arsenic concentrations in their drinking water wells [70].

In Bangladesh, a small prospective cohort study ($n = 54$) observed that arsenic measured in maternal hair collected at <20 weeks' gestational age was associated with decreased birth weight ($\beta = -193.5 \pm 90.0$ grams, p-value $= 0.04$) after adjusting for many confounders [75]. A much larger birth cohort study that was also recruited in Bangladesh ($n = 1578$) also reported a negative association between arsenic and birth weight. Rahman et al. measured total urinary arsenic concentrations in maternal samples collected at gestational weeks 8 and 30. Linear regression models estimated that for each 1-μg/L increase of arsenic in urine, birth weight decreased by 1.68 grams (standard error, 0.62) but only when urinary arsenic was <100 μg/L [76].

22.4 Early-Life Exposures and Delayed Health Effects

Several epidemiological studies have observed that exposure to arsenic during fetal development is associated with increased risk of chronic diseases later in life. A large ecological study by Smith et al. in Antofagasta, Chile, reported that the SMR for lung cancer at age 30–49 years was 7.0 (95% CI: 5.4–8.9; $p < 0.001$) for people who were conceived when their mothers were exposed to high concentrations of arsenic from drinking water compared to the rest of Chile [77]. This group also reported that the SMR for bronchiectasis was 12.4 (95% CI: 3.3–31.7; $p < 0.001$) in the same study indicating that the early life exposure to arsenic had long-lasting impacts on the pulmonary system.

There is also some evidence that prenatal exposure to arsenic impacts the immune system. In Bangladesh, a prospective birth cohort study observed that infants born to mothers with the highest quintile of urinary arsenic concentrations measured at 8 and 30 weeks of gestation had an increased risk of lower respiratory tract infection and diarrhea in the first year of life compared to infants born to mothers with the lowest quintile of urinary arsenic [78]. Data from a subset of this study's population also showed a negative correlation between maternal urinary arsenic and thymic size at age 2, 6, and 12 months [79]. The thymus is an organ believed to play an important role in the development of cells of the immune system. The relationship between developmental exposure to arsenic and increased risk of infection in early childhood has also been observed in the United States. Farzan et al. recruited a prospective birth cohort in New Hampshire ($n = 214$) and observed that maternal total urinary arsenic concentrations measured at 24–28 weeks' gestation was related to an increased risk in the total number of infections requiring a physician visit at 4 months of age (RR per one-fold increase in total urinary arsenic = 1.5, 95% CI = 1.0, 2.1) [80]. These investigators also observed that maternal urinary arsenic concentrations were associated with an increased risk of lower respiratory infections

treated with prescription medication (RR = 3.3, 95% CI = 1.2, 9.0), upper respiratory infections (RR = 1.6, 95% CI = 1.0, 2.5), and colds treated with prescription medication (RR = 2.3, 95% CI = 1.0, 5.2) [80].

While it is not fully understood how early-life exposure to arsenic would lead to increased susceptibility to pulmonary diseases and infectious disease later in life, there is growing evidence that developmental exposure to arsenic influences epigenetic mechanisms [81–83]. For instance, DNA methylation patterns are established during early fetal development and prenatal exposure to arsenic has been shown to influence DNA methylation extracted from cord blood [81,82]. This human evidence that environmentally relevant exposure to arsenic during development is supported by experimental studies, shows that inorganic arsenic causes both global DNA hypomethylation [57,84,85] and gene-specific DNA hypermethylation [57,86]. This effect might be explained by the overlap between inorganic arsenic metabolism and DNA methylation processes. Both consume S-adenosylmethionine, the universal methyl donor, which is a critical cofactor for both DNA methylation and the methylation of xenobiotics. Excess inorganic arsenic could reduce the availability or alter the activity of biological processes utilizing methyl groups, including DNA methylation and glutathione reduction [84]. It is possible that arsenic could initiate a cascade of events that alters DNA methylation, resulting in aberrant gene expression and also diminished glutathione activity leaving cells more vulnerable to oxidative stress.

22.5 Reproductive Disorders

22.5.1 Male Infertility

22.5.1.1 Animal Studies

While little is known regarding arsenic's ability to cause female infertility, there is fairly consistent evidence from rodent studies demonstrating that high doses of inorganic arsenic impacts spermatogenesis and decreases sperm motility and viability [87–89]. The effect of inorganic arsenic on sperm motility is believed to be due to inorganic arsenic's affinity to thiols, which are abundant in sperm flagella. It is also possible that inorganic arsenic damages Sertoli cells (cells in the testicles that support sperm development) [90].

22.5.1.2 Human Studies

There is also evidence in epidemiological studies that arsenic exposure influences male fertility. In a small cross-sectional study ($n = 94$) of male partners in infertile couples, higher arsenic exposure, which was defined as above the median DMA concentration in urine, was significantly associated with below-reference sperm concentration (ORs: 1.0–7.2; $p = 0.02$) after adjusting for age, BMI, abstinence, smoking, and drinking [91]. A hospital case–control study in China observed that males with elevated urinary inorganic arsenic had a greater probability of being infertile. Specifically, the odds of exhibiting male idiopathic infertility was 4.9-fold higher (95% CI: 1.8–13.6) and 13.6-fold higher (95% CI: 4.8–38.6) at the third and fourth quartiles of urinary inorganic arsenic [92].

22.6 Conclusions

Inorganic arsenic and its metabolites are developmental toxicants and contribute to the prevalence of reproductive disorders. Developmental exposure to inorganic arsenic and its metabolites is associated with increased risk of neural tube defects, neonatal mortality, and decreased birth weight. There is also growing evidence that developmental exposure to arsenic increases susceptibility to infectious diseases early in life, as well as chronic pulmonary diseases in adulthood. While the mechanisms linking these exposures and outcomes are poorly understood, there is evidence that epigenetic dysregulation may play an important role. Arsenic is also associated with reduced sperm quality and male infertility in adults.

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23 Arsenic and Cancer

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CHAPTER OUTLINE

23.1 Arsenic and Arsenic-Containing Compounds

Arsenic is a metalloid (atomic number, 33; relative atomic mass, 74.92) widely distributed in Earth's crust at an average concentration of 2 mg/kg. It occurs in trace quantities in all rock, soil, water, and air. Arsenic can exist in four oxidation states: −3 (e.g., arsine gas), 0 (metalloid arsenic), +3 (trivalent), and +5 (pentavalent) [1]. Arsenic compounds from a biological and toxicological point of view are divided into three major groups: inorganic arsenic compounds, organic arsenic compounds, and arsine gas [2,3]. Arsenic compounds like other chemicals are best identified using the CAS (Chemical Abstracts Service) registry number [2].

Atmospheric conditions affect the prevalence of arsenic forms; for example arsenite (As(III)) is the dominant form under reducing conditions, while arsenate (As(V)) is the stable form in oxygenated environments [2,3]. More than 200 mineral species contain arsenic, with 60% comprising arsenate and 20% sulfide and sulfosalts, while the rest (20%) consist of arsenides, arsenites, oxides, and elemental arsenic [4]. Arsenic is found associated with many types of mineral deposits, especially those including sulfide mineralization, and the most common of the arsenic minerals is arsenopyrite (FeAsS). The most prevalent trivalent inorganic arsenic compounds are arsenic trioxide, sodium arsenite, and sodium trichlorite, while arsenic pentoxide, arsenic acid, and arsenates (e.g., sodium arsenate, lead arsenate, and calcium arsenate)

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are the most common pentavalent inorganic arsenic compounds [2]. There are many organic arsenic compounds but the following are mostly encountered: arsenalic acid, methylarsonic acid, dimethylarsinic (cacodylic acid), and arsenobetaine [5]. Arsenic species differ in their toxicity and biochemical and environmental behaviors. Arsenate [As(V)] is the most common environmental form of inorganic arsenic, but arsenite [As(III)] is more toxic and probably the more carcinogenic species [6]. A list of various arsenic compounds of environmental and human health relevance is presented in Table 23–1.

Arsenic can be found in nature as a pure elemental crystal or in conjunction with sulfur or other metals [7]. Arsenic oxidizes to arsenic trioxide (As_2O_3) when heated in air, giving out garlic odor-like fumes. As_2O_3 can also be produced as a by-product of metal smelting operations [8]. Elemental arsenic is not soluble in water. Arsenic salts exhibit a wide range of solubility depending on pH and the ionic environment [2,9].

Table 23–1 Arsenic Compounds of Environmental and Human Health Relevance

Chemical Name	CAS Reg. No.	Synonyms	Formula
Arsanilic acid	98-50-0	Arsonic acid, (4-aminophenyl)-	$C_6H_8AsNO_3$
Arsenic monoxide	12005-99-1	Arsenic monoxide	AsO
Arsenic	7440-38-2	Metallic arsenic	As
Arsenic(V) pentoxide	1303-28-2	Arsenic oxide [As_2O_5]	As_2O_5
Arsenic trisulfide	1303-33-9	Arsenic tersulfide	As_2S_3
Arsenic diiodide	13770-56-4	15942-63-9	As_2I_4
Arsenic(III) sulfide	1303-33-9	Arsenic sulfide [As_2S_3]	As_2S_3
Diarsenic tetrahydride	15942-63-9	Diarsine	As_2H_4
Arsenic(III) trichloride	7784-34-1	Arsenic chloride [$AsCl_3$]	$AsCl_3$
Arsenic(III) trioxide	1327-53-3	Arsenic oxide [As_2O_3]	As_2O_3
Arsenobetaine	64436-13-1	Arsonium (carboxymethyl), trimethyl-hydroxide, inner salt; 2-(trimethylarsonio) acetate	$C_5H_{11}AsO_2$
Arsine	7784-42-1	Arsenic hydride [AsH_3]	AsH_3
Calcium arsenate	7778-44-1	Arsenic acid [H_3AsO_4], calcium salt (2:3)	$As_2Ca_3O_8$
Dimethylarsinic acid	75-60-5	Cacodylic acid	$C_2H_7AsO_2$
Lead arsenate	7784-40-9	Arsenic acid [H_3AsO_4], lead (2+) salt (1:1)	$AsHO_4Pb$
Methanearsonic acid, disodium salt	144-21-8	Arsonic acid, methyl-, disodium salt	$CH_3AsO_3{\cdot}2Na$
Methanearsonic acid, monosodium salt	2163-80-6	Arsonic acid, methyl-, monosodium salt	CH_4AsO_3Na
Potassium arsenate	7784-41-0	Arsenic acid [H_2AsO_4K], monopotassium salt	KH_2AsO_4
Potassium arsenite	13464-35-2	Arsenous acid, potassium salt [AsO_2K]	$KAsO_2$
Sodium arsenate	7631-89-2	Arsenic acid [H_2AsO_4Na], monosodium salt	Na_3AsO_4
Sodium arsenite	7784-46-5	Arsenous acid, sodium salt [AsO_2Na]	$NaAsO_2$
Sodium dimethylarsinate	124-65-2	Arsinic acid, dimethyl-, sodium salt, sodium cacodylate [$C_2H_6AsO_2Na$]	$(CH_3)_2AsNaO_23H_2O$
Gallium arsenide	1303-00-0	Gallium monoarsenide	AsGa

Absorption of arsenic in inhaled airborne particles is highly dependent on the solubility and size of particles. Both pentavalent and trivalent soluble arsenic compounds are rapidly and easily absorbed from the gastrointestinal tract. Arsenic metabolism often involves two major types of reactions: reduction reactions of pentavalent to trivalent arsenic [10] and oxidative methylation reactions in which trivalent forms of arsenic are sequentially methylated to form mono-, di-, and trimethylated products using S-adenosyl methionine (SAM) as the methyl donor and glutathione (GSH) as an essential co-factor. The methylation of inorganic arsenic facilitates the excretion of inorganic arsenic from the body, as MMA and DMA in urine [11].

23.2 Sources of Arsenic and Potential for Human Exposure

Arsenic is ubiquitous in nature and is introduced into the environment from both natural and anthropogenic activities. Human activities that contribute to environmental contamination by arsenic include mining, industrial smelting of metals, power generation with coal, and applications of arsenic-containing pesticides and herbicides [12]. Human exposure may occur through ingestion of contaminated food, drinking water, or medication; inhalation of dust particles or aerosols; and/or direct dermal contact [9,13]. It is important to understand the environmental sources and levels of arsenic in order to prevent human exposure and the consequent deleterious effects.

23.2.1 Arsenic and Drinking Water Contamination

Water is the major source of human exposure to arsenic compounds [13]. Underground water and water aquifers are contaminated when arsenic-bearing minerals undergo oxidation and release arsenic into water. The reduction of Fe/As oxyhydroxides could also be a source for arsenic contamination in ground water [14]. Some regions across the world have been observed to have arsenic drinking water levels exceeding the standards set by the US Public Health Service and the World Health Organization—10 μg/L for the USA and Canada and 50 μg/L for developing countries with limited access to potable water and larger populations exposed to arsenic [15]. Bangladesh and West Bengal (India) are the most affected areas of the world with arsenic concentration in ground water in some areas up to 3200 μg/L [16]. The inorganic form of arsenic is the most common species found in water and can be stable as both arsenite or trivalent [As(III)] and arsenate or pentavalent [As(V)] inorganic arsenicals [17]. As(III) is the most prevalent species in ground water, while As(V) species are more significant in the surface water of rivers [18].

23.2.2 Arsenic and Food Contamination

Arsenic was intentionally added to food as a preservative in the mid-19th century before it was discovered that it has dangerous effects on human health. Sea food is the major source

of arsenic exposure to humans [19]. A level of arsenic of 1.3 ppm above the United States Environmental Protection Agency (EPA) regulatory limit was observed in commercial fish (Chilean sea bass, croaker, flounder, porgie, and whiting) in New Jersey, USA [20]. Diet is second to water as the major source of both inorganic and organic arsenic. Estimates of dietary inorganic arsenic intakes vary. Arsenic could enter the food cycle by growing a crop on arsenic-contaminated soil or by irrigating the farm with arsenic-contaminated water [21]. The US average daily consumption of arsenic by adults and children is 3.2 μg/day with a range of 1–20 μg/day [22], while it is 0.13 to 0.56 μg/kg/day for average consumers; it is 9.1 to 39.2 μg/day for a 70-kg adult in Europe [23]. In India, it was observed that cooked foods had higher levels of arsenic than raw foods. Daily dietary intakes of arsenic from the foodstuffs for adults were from 171.20 to 189.13 μg/day, while the range for children was 91.89–101.63 μg/day [24]. Arsenobetaine is the most common form of organic arsenic that naturally accumulates in marine organisms with the exception of algae that contain mostly dimethylarsinoylribosides. Humans are thus exposed to these arsenic compounds when they consume any diet that includes seafood [1,9].

23.2.3 Arsenic and Soil Contamination

Arsenic in soil comes mainly from the parent soil. The nature of arsenic in soil is affected by the lithology of parent rock materials, volcanic activity, bioactivity, weathering history, transport, sorption, and precipitation [25]. The natural content of arsenic in soils globally ranges from 0.01 to over 600 mg/kg, with an average of about 2 to 20 mg/kg. Biotransformation of arsenic species occurs in the soil and the three major modes of biotransformation observed include: the biosynthesis of organoarsenic compounds, redox transformation between arsenite and arsenate, and the reduction and methylation of arsenic [26]. Arable lands could be contaminated with arsenic from run-off water and the use of arsenic-rich ground water for irrigation. Inorganic arsenic is the major form of arsenic in soil but high levels of organic forms are seen in soils with anthropogenic activities such as mining and manufacturing, and application of arsenic-containing pesticides [27]. Pentavalent inorganic arsenic is more commonly seen in soil because trivalent arsenicals are easily oxidized [28]. Human exposures by inhalation of soil particles blown by wind and dermal absorption are not considered major sources of arsenic because the amount of arsenic in ambient air is low; also arsenic is poorly absorbed through the skin from the soil [29]. However, incidental ingestion of arsenic in soil is considered significant though far less than the amount in drinking water and diet [30]. This is likely because there is a reduced amount of inorganic arsenic as well as reduced bioavailability of arsenic in soil compared to water [27].

23.2.4 Arsenic and Air Pollution

Inorganic arsenic is the major form of arsenic found in the air and it is very low in concentration, contributing to less than 15% of all forms of exposure including that from food, water, and soil [31]. The concentration of arsenic in air due to non-human activities ranges from 1 to 3 ng/m^3, and the

range of the concentration in urban areas is 20 to 100 ng/m^3 [9]. The European Commission [31] reports that levels of arsenic in air range between 0 and 1 ng/m^3 in remote areas, 0.2 and 1.5 ng/m^3 in rural areas, and 0.5 and 3 ng/m^3 in urban areas, with levels up to about 50 ng/m^3 in the vicinity of industrial sites.

23.2.5 Arsenic Exposure from Medication

Arsenic has been used to treat various ailments for more than 2400 years including toothaches, ulcers, and abscesses [32]. Fowler's solution, which consists of 1% solution of potassium arsenite, was discovered in 1786 and was used to treat diseases such as malaria, syphilis, asthma, chorea, eczema, and psoriasis [33]. Fowler's solution was reported to lower white blood cell count in leukemia patients [34] and was also used as a tonic for anemia and to treat rheumatism, dermatitis herpetiformis, Hodgkin's disease, pemphigus, and pernicious anemia. [32] Salvarsan, which is an organic arsenical, was introduced in 1910 by Paul Ehrlich for treating syphilis and trypanosomiasis [35]. The United States Food and Drug Administration (FDA) in September 2000 approved the use of arsenic trioxide for the treatment of relapsed or refractory acute promyelocytic leukemia (APL) cases resistant to all-trans retinoic acid (ATRA) [34]. The therapeutic effect of arsenic trioxide on other types of cancers such as non-APL acute myeloid leukemia and myelodysplastic syndromes has been explored [36]. There are also ongoing researches on the combination of arsenic trioxide with other agents such as vitamin C (ascorbic acid) for the treatment of lymphoproliferative disorders [37] and multiple myeloma [38]. Arsenic trioxide was the drug of choice before the discovery of metronidazole in 1959 for the treatment of *Trichomonas vaginalis* infection (trichomoniasis), which causes vaginal discharge [32]. In traditional Chinese medicine, arsenic derivatives are still used to benumb the pulp of a painful tooth [39]. Organic arsenic compound melarsoprol is still the drug of choice for the treatment of the protozoan parasite disease trypanosomiasis [40]. However, the use of arsenic derivatives in medication has been drastically reduced due to the perceived toxic properties.

23.2.6 Occupational Exposure to Arsenic

Arsenic is an essential component of a wide variety of industrial products, such as wood preservatives, herbicides, insecticides, pesticides, fungicides, high-emitting diodes, and semiconductors. Workers at factories manufacturing arsenic-containing products are prone to exposure directly through inhalation and dermal contact [9]. Arsenic was first used for tick control in South Africa in 1893 and since then over a thousand cattle dipping vats have been constructed throughout the country, arsenic being applied as the sodium salt of arsenous acid [41]. Old mines and abandoned metal ores are reported to have high arsenic levels resulting from the smelting process and coal burning. These processes generate stack dust and flue gas that contaminate the soil and water with arsenic [42]. The amount of airborne arsenic inhaled depends on the characteristics, matrix composition, and particle size distribution of the compound [43]. Studies on orchard workers, who were becoming ill after exposure to lead arsenate,

used in pesticides for apple and cherry orchards, provided a basis for understanding some of the long-term effects of occupational exposure to arsenic. However, definitive links between arsenic, a by-product of copper smelting, and lung cancer through inhalation was established studying copper smelting workers who were ill [44]. The association of arsenic exposure with occupational health defects has led to discontinuation of most inorganic arsenic in agriculture and most industrial processes and the establishment of stricter limits by government regulation agencies.

23.3 Molecular Mechanisms of Arsenic-Induced Carcinogenesis

Several mechanisms of action of arsenic have been described and they are summarized in Figure 23–1. The mechanisms of arsenic toxicity and carcinogenicity have been reported for various cell types [45]. Several lines of evidence support stimulation of cell proliferation, alteration of DNA methylation, and perturbation of signaling cascades [46], oxidative stress, and

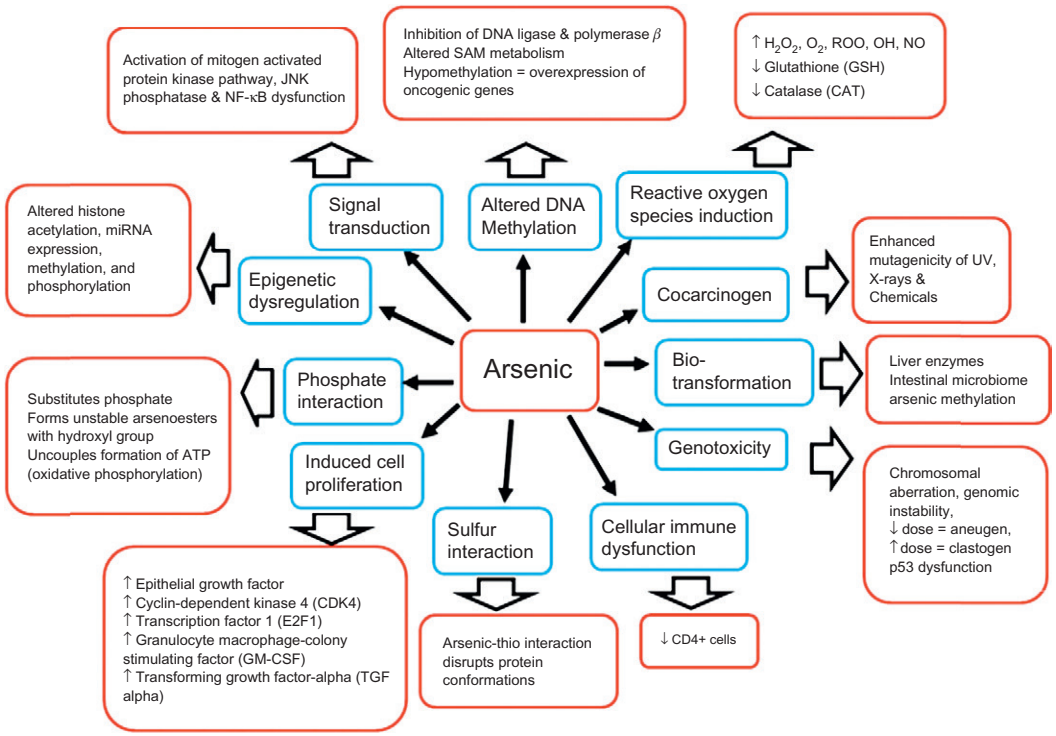


FIGURE 23–1 Potential mechanisms of arsenic-induced carcinogenesis.

chromosomal aberrations [47]. Disruption of transcriptional activity following arsenic exposure with extensive changes in global gene expression, and disruption of diverse regulatory mechanisms of gene expression [21,48,49] have been reported. Epigenetic dysregulation and arsenic metabolic activities involving human microbiome and liver enzymes have also been reported [50,51].

Although much progress has been recently made in the area of arsenic's possible mode(s) of carcinogenic action, a scientific consensus has not yet been reached. A recent review discusses nine different possible modes of action of arsenic carcinogensis: induced chromosomal abnormalities, oxidative stress, altered DNA repair, altered DNA methylation patterns, altered growth factors, enhanced cell proliferation, promotion/progression, suppression of p53, and gene amplification [52]. Presently, three modes (chromosomal abnormality, oxidative stress, and altered growth factors) of arsenic carcinogenesis have shown a degree of positive evidence, both in experimental systems (animal and human cells) and in human tissues. The remaining possible modes of carcinogenic action (progression of carcinogenesis, altered DNA repair, p53 suppression, altered DNA methylation patterns, and gene amplification) do not have as much evidence, particularly from *in vivo* studies with laboratory animals, *in vitro* studies with cultured human cells, or human data from case or population studies. Thus, the mode-of-action studies suggest that arsenic might be acting as a cocarcinogen, a promoter, or a progressor of carcinogenesis.

23.3.1 Arsenic Perturbation of Keratin Expression

Aberrant and progressive alterations in cytokeratin (CK) expression are a common feature observed in human skin lesions, including hyperkeratosis and squamous cell carcinoma (SCC) [53]. Thus, the expression of simple epithelial keratins could be used as a biomarker for monitoring malignancy of keratinocytes and to determine tumor invasion and/or changes in epithelial–mesenchymal interactions [54]. Sun et al., in their report, showed that the expression of CK in HaCaT keratinocytes was impaired by chronic arsenic exposure [53]. Keratins are the major abundant and structural proteins of the epidermal layer of epithelial cells. It has been suggested that the expression of CK in arsenic carcinogenesis of skin may be exacerbated by an increase in the expression of other proteins such as MMP-9 (matrix metalloproteinase-9 secretion, an enzyme often secreted by cancer cells to help them invade through the local extracellular matrix) [53]. The transcripts of various keratin genes used as markers of different stages of cell differentiation have been reported to be increased in arsenic-treated HaCaT cells. These include *CK1*, associated with hyperkeratosis, *CK10*, always co-expressed with *CK1*, *CK13*, a biomarker for dermal cancer progression, *CK8* and *CK18* [54], differentiation marker genes in simple epithelial tissues as they are the earliest keratin genes expressed during embryogenesis, filaggrin (linked to proliferation) [55], involucrin (an early differentiation marker) and loricrin (late differentiation gene), *CK6* (induced in response to stressful stimuli, such as wounding), and *CK 6/16* and *7/17* (stress response, adaptive response to arsenic) [53].

23.3.2 Arsenic-Induced Genotoxicity

Disruptions in gene expression are responsible for many diseases [56] and the amount and nature of mRNA produced by a cell have been used in gene expression studies to understand genomic stability and chromosomal aberration by observing genes that were either overexpressed or suppressed as a result of exposure to arsenic [47,48]. Inorganic arsenic is said to modulate expression and DNA-binding activities of several key transcription factors, including nuclear factor kappa B, tumor suppressor protein (p53), and activating protein-1 (AP-1) [57]. Arsenic activates AP-1 stimulating the mitogen-activated protein kinase (MAPK) cascade, which leads to an increase in the expression and/or phosphorylation of the two major AP-1 constituents, *c-jun* and *c-fos* [58]. Also, high doses of arsenic have been demonstrated to be clastogenic, while low doses are aneugenic and induce sister chromatid exchanges in a variety of mammalian cells *in vitro* [59]. One of the suggested mechanisms of arsenic skin carcinogenesis is the disruption of cycle check point proteins and inhibition of DNA repairs, causing production of hydroxyl radicals ($^{\bullet}$HO), DNA damage [60], and aberrant CK expression [53].

23.3.3 Arsenic-Induced Aberration of Gene Expression

Induction of aberrations in gene expression is regarded as an important mechanism of action of arsenic. Genotoxic and cytotoxic properties of arsenic have been demonstrated using a cultured human skin keratinocyte model [61]. Gene expression studies involve the application of advanced technologies such as microarray, which generates high-throughput data and provides a platform to simultaneously examine multiple mechanisms based on alterations in expression of target genes. Gene expression studies on arsenic toxicity/carcinogenicity include: skin [62], keratinocytes [45], kidney [63], myeloma [64], peripheral lymphocytes [62], neural tube [65], liver [66], and urogenital cells [67].

23.3.4 Arsenic Dysregulation of Cellular Immune Function

Arsenic exposure induces dysfunction of the cellular immune system by targeting the $CD4^+$ cells with concomitant reduction in $CD4^+$ cells in epidermal keratinocytes, which may trigger arsenic-induced skin cancer [68,69]. There are reports of induction of aberration in the expression of immune response genes including up-regulation of inflammatory signals like cytokines and TNF-alpha in HaCaT keratinocytes chronically exposed to a low dose of arsenic trioxide [48]. *AKR1C3* (aldo-keto reductase family 1, member C3), *IGFL1* (insulin growth factor-like family member 1), *IL1R2* (interleukin 1 receptor, type 2), and *TNFSF18* (tumor necrosis factor [ligand] superfamily, member 18) have been up-regulated in HaCaT cells exposed to low doses of arsenic trioxide with down-regulation of *RGS2* (regulator of G-protein signaling 2) [48]. *IL1R2* improves cell migration, which increases its oncogenic potential [70]. Arsenic induces up-regulation of *IGFL1*. The *IGF*-like (*IGFL1*) genes encode proteins that contain 11 conserved cysteine residues at fixed positions including two CC motifs. Since *IGLF1* encodes proteins rich in cysteine residues, it could be vital in arsenic binding and responsiveness in keratinocytes [71].

23.3.5 Arsenic Distortion of Protein Structure

Arsenic impairs cellular respiration by inhibiting various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation. Toxic by-products are released when arsenic interacts with sulfhydryl groups of proteins and enzymes, and substitutes phosphorus in a variety of biochemical reactions [72]. For example, dihydrolipoyl dehydrogenase and thiolase enzymes are inactivated when arsenic reacts with their sulfhydryl groups causing inhibited oxidation of pyruvate and beta-oxidation of fatty acids. Arsenic also distorts the conformation of protein structure by attacking the disulfide bonds and thiol groups and binding to vicinal cysteines [73]. Arsenites interfere with sulfhydryl group of amino acids and disturb protein structure, while arsenates substitute for phosphate, affecting cellular processes such as ATP and DNA synthesis [74].

23.3.6 Arsenic Induction of Cell Proliferation

Induction of cell growth by low concentration of arsenic has been observed by many scientists. In human keratinocyte studies, arsenic was observed to induce overexpression of growth factors [75], cyclin A with increase in S-phase population of cells in cell cycle [46], cyclin-dependent kinase 4 (CDK4), transcription factor 1 (E2F1), granulocyte macrophage-colony stimulating factor (GM-CSF), and transforming growth factor-alpha (TGF-alpha) [46,75]. Low concentrations (0.5 to 1 ppm) of AsO_3 stimulate keratinocyte proliferation but induce cell death at higher concentrations (>1 ppm) [76]. The cell death may have been caused by the activation of caspase-3, which triggers cell cycle arrest at the G2-M phase [61]. *IGFL1* is known to be up-regulated in skin conditions such as psoriasis that promote the abnormal proliferation and differentiation of epidermal keratinocytes [77]. IGFL1, like other related IGFs, is involved in cellular energy metabolism, growth and development, and promotion of cell division [71]. Also, increase in cell proliferation by arsenic could be attributed to induction of *cyclin D1* transcription [78]. Cyclin D1 stimulates growth by shifting the G1 growth phase into the S/G2 cell cycle compartment.

23.3.7 Arsenic Dysregulation of Epigenetic Mechanisms

An epigenetic trait is a stably inherited phenotype resulting from changes in a chromosome without alterations in the DNA sequence [79]. Both genetic and epigenetic mechanisms can regulate gene expression and they must be put into perspective during a study on the toxicogenomics and cell-transforming ability of arsenic and other toxicants capable of causing aberrations in gene expression and cancer. The three major epigenetic mechanisms reported in arsenic toxicity and carcinogenicity are: DNA methylation [80], histone modification (acetylation, methylation, and phosphorylation) [81], and microRNA (miRNA) expression [82]. Hypomethylation of DNA may cause overexpression of oncogenic genes [83] and decrease in DNA repair, stress defense mechanisms, and apoptosis [45,83].

23.3.8 Arsenic Cocarcinogenicity

Arsenic has also been considered as a weak mutagen and may not initiate but potentiate the mutagenicity, cytotoxicity, and clastogenicity of other carcinogens such as UV

radiation, X-rays, and other heavy metals by acting as a tumor promoter, or cocarcinogen, in skin cancer development. According to Yu et al. [69], As and UVB stimulated caspase pathways, and caspase-9 and caspase-8 signaling, respectively, which induced apoptosis in keratinocytes [69].

23.3.9 Arsenic Interference with Signal Transduction

Cell cycle regulation is crucial for proper cellular homeostasis. Communication between or within a cell is done through cell signaling and a change in the activity of the cell is sent as a signal that may trigger a cascade of reaction for the body to respond accordingly. Any process that affects this homeostasis affects directly or indirectly the entire cellular processes such as transcription and cell metabolism, regulated by cell signal transduction [84]. Arsenic is known to affect the signaling pathways and the severity may depend on the oxidation state of the arsenic species and cell characteristics [85]. Arsenic perturbs the activation of the mitogen-activated protein kinase pathway, c-Jun N-terminal kinases (JNK), phosphatase, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) dysfunction [46,68,84]. NF-κB dysfunction occurs when arsenic blocks inflammatory signal transduction by inhibiting inhibitors of nuclear factor kappa-B kinase (IKK) required to activate pro-inflammatory transcription factor NF-κB [68]. The induction of NF-κB is one of the strong supports of arsenic as a carcinogen since NF-κB promotes proliferation and angiogenesis. Arsenites also induce the expression of proteins that reduce the toxic effect of arsenic, for instance, the translocation of protein kinase C (PKC) isoforms (PKCepsilon, PKCdelta, and PKCalpha) from cytosol to membranes where these enzymes interact with MAP kinase (Erks, JNKs, and p38 kinases) [86].

23.3.10 Arsenic Induction of Reactive Oxygen Species

An imbalance between the production of cellular oxidant species and the capability of the cells to produce antioxidants results in oxidative stress [87]. Oxidative stress creation is one of the carcinogenesis processes and arsenic is proposed to induce skin carcinogenesis by induction of oxidative stress/reactive oxygen species (ROS) [88]. Arsenic induces morphologic alterations in mitochondrial integrity, which leads to inactivation of mitochondrial enzymes and loss of mitochondrial membrane potential. Arsenite may serve also as a bypass for electrons from the respiratory chain that facilitate the formation of superoxide anion radicals and generation of ROS (H_2O_2, O_2, ROO, OH, and NO) [60] as well as a reduction in activity of an important cellular antioxidant, glutathione (gamma-glutamylcysteinylglycine, GSH) [89]. Arsenite reduces oxygen directly to H_2O_2 and/or formation of arsenic peroxyl radicals, which are mediators of DNA damage [60], mitosis disrupters, and apoptosis promoters [88]. In his studies, Aposhian [90] observed that inorganic arsenate stimulates a rapid burst of oxidative stress in mammalian cells as a result of the repetitive reduction of pentavalent to trivalent arsenic followed by the oxidative methylation of trivalent arsenic [90]. Although ROS are known to trigger signals that enhance cell proliferation such as activation of transcription factor AP-1 and nuclear factor NF-κB [91], they have also induced apoptosis through prolonged activation of JNK/AP-1 and

SAPK/JNK signaling. SAPK/JNK signaling activates AP-1, which stimulates apoptosis [92]. AP-1 activity could be very important in explaining the discrepancy observed between the proliferative effect of low dose arsenic and the pro-inflammatory high dose [48,76].

23.4 Health Effects Associated with Arsenic Exposure

Research reports have shown that arsenic is a systemic toxicant that affects almost all the organs of the body and induces various forms of health effects. The adverse effects on health due to chronic arsenic exposure vary and may be influenced by the population groups, age, gender, cumulative dose of arsenic, nutritional status, genetic factors, lifestyle, individual susceptibility, and different chemical forms of arsenic in drinking water [93]. Arsenic is linked with clastogenic damage in different cell types with different endpoints in exposed individuals and in cancer patients. Soluble inorganic arsenic such as As_3O_2 is easily absorbed by the digestive system and may cause acute arsenicosis (arsenic poisoning). Also, inhalation and ingestion of large doses may lead to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, and eventually death [8]. Exposure to arsenic has been strongly associated with cancer in human organs including the bladder and kidney [63], liver [66], prostate and lung [94], bone marrow (myeloma), [64], peripheral lymphocytes [62], neural tube [65], urogenital cells [67], and especially the skin [95]. Other arsenicosis diseases are blackfoot disease, atherosclerosis [64], cerebral infraction, hypertension, diabetes mellitus [96], ventricular fibrillation, skin lesions, portal fibrosis of the liver, lung fibrosis, hematologic effects (anemia, leukopenia, and eosinophilia), hearing loss, and neurologic damage (peripheral neuropathy, encephalopathy, and intellectual deficits) in children [9]. The severity of adverse health effects is related to the chemical form of arsenic, and is also time and dose dependent [97,98]. Table 23–2 presents a summary of health effects associated with arsenic exposure.

23.4.1 Arsenic and Keratosis

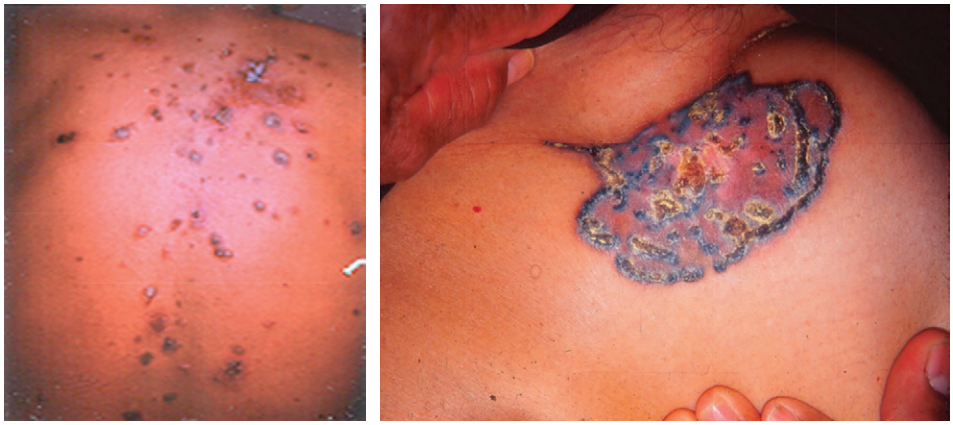
Exposure to arsenic induces a growth of keratin on the skin referred to as arsenical keratosis. Keratosis is considered as the pre-malignant stage of squamous cell carcinoma *in situ* (Bowen's disease). Other pathological features that lead to Bowen's disease are hyperkeratosis, parakeratosis, and arsenical pigmentation. Two distinct gross variations are observable: first, multiple, punctate, hard, yellow, corn-like papules within areas of friction such as on the soles and palms, and second, slightly elevated, scaly, erythematous or pigmented patches often seen in non-sun-exposed areas.

Since exposure to sun is the major cause of skin cancer and other related skin diseases, appearance of bowenoid lesions in non-sun-exposed areas suggest that arsenic could be the cause. Arsenic bowenoid lesions are non-invasive and can be found in both sun-exposed and non-exposed areas of the skin with characteristic atypical epithelial cells occupying the full thickness of the surface epithelium. Arsenical keratosis and Bowen's

Table 23–2 Clinical and Pathological Manifestations of Acute and Chronic Arsenic Poisoning [97,99]

Organ/Tissue Level	Acute Effects	Chronic Effects
• Dermatologic	• Capillary flush • Contact dermatitis • Folliculitis • Hair loss	• Melanosis • Bowen's disease • Facial edema • Palmoplantar hyperkeratosis • Cutaneous malignancies • Hyperpigmentation • Desquamation
• Neurologic	• Hyperpyrexia • Convulsions • Tremor/coma • Disorientation	• Encephalopathy • Headache • Peripheral neuropathy • Axonal degeneration
• Gastrointestinal/hepatic	• Abdominal pain • Dysphagia • Vomiting • Bloody/rice water diarrhea • Garlicky odor to breath • Mucosal erosions • Fatty liver • Cholangitis • Cholecystitis	• Nausea • Vomiting • Diarrhea • Anorexia • Weight loss • Hepatomegaly • Jaundice • Pancreatitis • Cirrhosis • Liver cancer
• Renal	• Tubular damage • Glomerular damage • Oligura • Uremia	• Nephritis • Proteinuria
• Hematologic	• Anemia • Thrombocytopenia	• Bone marrow hypoplasia • Anemia • Thrombocytopenia • Basophilic stippling • Karyorrhexis
• Cardiovascular	• Ventricular fibrillation • Tachycardia	• Arrhythmias • Pericarditis • Acrocyanosis • Raynaud's and gangrene
• Respiratory	• Pulmonary edema • Bronchial pneumonia • Tracheobronchitis	• Cough • Pulmonary fibrosis • Lung cancer

disease can become invasive squamous cell carcinoma after many years. During this period, changes in skin color occur as illustrated in Figure 23–2, such as hyperpigmentation (darker color) and hypopigmented (lighter color) raindrop-like macules ranging in size from 1 to 2 millimeters [99].



(A) (B)

FIGURE 23–2 Basal cell carcinoma. (A) Multifocal basal cell carcinoma of the back. (B) Patient with a single lesion of the left groin [99].

23.4.2 Arsenic and Skin Cancer

There is increased risk of developing epithelial cancers of the skin such as intraepidermal carcinomas (Bowen's disease), squamous cell carcinomas (SCC), basal cell carcinomas (BCC), and Merkel cell carcinoma (MCC) in areas endemic with arsenic-contaminated water with exposure to low dose arsenic over a long period of time [1]. Manifestations of arsenical keratosis, hyperkeratosis, hyperpigmentation, and multiple cutaneous malignancies are used as markers for epithelial cancers of internal organs as they often coexist and also because the internal organ cancers have no characteristic histologic features that will infer arsenical etiology [100,101]. SCC and BCC are the most common types of malignant skin cancers. SCC lesions are invasive skin tumors that arise from the surface epidermal layer. Histologically, they look like normal surface squamous cells but with atypical features and growth patterns. Typically, SCC is seen in sun-exposed areas of the body, but could appear in sun-protected areas due to chronic arsenic ingestion. BCC is most often seen in the trunk and scrotum and is associated with arsenic exposure [102]. It arises from the lower part of the epidermis and shows a variety of growth patterns including adenoid, reticulated, trichoepitheliomatous, and hyperpigmented. Histologically, there could be multinucleated giant cells present; some may have bizarre appearance and atypical mitoses [103]. Figure 23–2 illustrates two cases of arsenic-induced basal cell carcinoma [99].

Malignant melanomas account for the majority of deaths due to skin cancer as they can spread to other tissues, unlike SCC and BCC. Melanomas begin in the pigment-making cells, melanocytes located along the base of the squamous epithelium. There is evidence that arsenic can induce melanoma directly or as a cocarcinogen [104]. However, more cases of arsenic-related skin cancers are linked with SCC and BCC than with melanoma [105].

23.4.3 Arsenic and Liver Cancer

The liver is a very important organ that performs many essential functions related to digestion, metabolism, immunity, and the storage of nutrients, and especially detoxification of substances entering the body. Exposure to toxic agents may cause temporary hepatic lesions that may resolve when the toxin is removed, or evolve to hepatic failure, malignancy, or death. Chronic arsenic exposure leads to liver injury when the cell's organelles are damaged. Chronic arsenic exposure has been associated with a variety of hepatic dysfunctions including macrovesicular steatosis, phospholipidosis, cholestatic lesions, steatohepatitis, granulomatous reactions, fibrosis, cirrhosis, vascular lesions, and/or neoplasms, depending on the dose and exposure conditions. Arsenic has been reported to enhance hepatic morphological and biochemical changes in phenobarbital-pretreated rats. Hydrophic degeneration, total loss of glycogen, necrosis in some centrolobular zones, and an increase in lipid vacuoles around the periportal area have been observed [106]. Also, several other hepatic histopathological changes such as disruption of hepatic cord, sinusoidal dilation, fatty infiltration, and altered expression of cyclin D1, p27, JLK, PTEN, and beta-catenin in the liver have been linked to subchronic exposure to arsenic [107].

Malignant transformation of the sinusoidal endothelial cells is referred to as angiosarcoma, and is linked to exposure to inorganic arsenic as well as vinyl chloride, Fowler's solution, anabolic steroids, and Thorotrast [108]. Histologically, there are two growth patterns of angiocarcinoma: cavernous and solid. The cavernous areas are characterized by plump, atypical endothelial cells that line the dilated sinusoids, while solid areas have sheets of anaplastic-spindled tumor cells that may be accompanied by areas with hemorrhage and necrosis.

Ingestion of arsenic has been linked to hepatocellular carcinoma (HCC), which is another type of malignant transformation of hepatocytes [109]. HCC have been observed in different populations exposed to elevated levels of arsenic in drinking water including Japan, Mexico, Chile, Germany, Argentina, Taiwan, China, India, and Bangladesh. It is suggested that arsenic could be interacting with other risk factors, including hepatitis B and C infection, aflatoxin, alcohol abuse, and genetic hemochromatosis [94,110]. Histologically, it could be difficult to differentiate between hepatocellular carcinoma and normal liver, except that a well-differentiated HCC lacks acinar architecture, and it is positive for sinusoidal staining with CD34 (Q-Bend-10) immunostain, which is increased in neoplastic liver; also the growth pattern may be varied [111]. Moderately differentiated HCC has increased nuclear pleomorphism, mitoses, growth pattern abnormalities (thickened trabeculae), and lacks normal acinar architecture, while diagnosis of poorly differentiated HCC depends on clinical manifestations to exclude metastatic disease. Figure 23–3 shows the histological sections of a normal liver (A) and neoplasia associated with chronic exposure to arsenic, including hepatocellular carcinoma (B) and liver angiosarcoma (C) [99].

23.4.4 Arsenic and Kidney Cancer

Chronic arsenic exposure is capable of causing chronic renal insufficiency from cortical necrosis. Acute arsenic poisoning may cause acute tubular necrosis, with acute renal failure

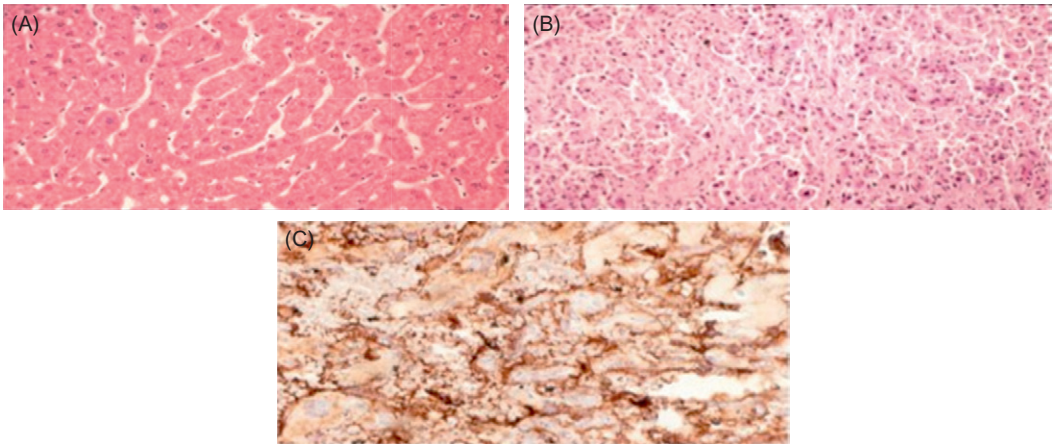


FIGURE 23–3 Histological sections of a normal liver (A), and neoplasia associated with chronic exposure to arsenic, including hepatocellular carcinoma (B) and liver angiosarcoma (C) [99].

commonly being seen. Arsine gas and arsenic are known to cause tubular necrosis but arsine gas is more nephrotoxic. There is no strong evidence to prove that arsenic directly causes kidney cancer; however, a study of smelter workers in Tacoma, Washington (USA), showed a 30% increase in incidence of renal carcinoma in those who were chronically exposed to arsenic as a by-product of smelting non-ferrous metal ores [1,9,112,113]. The mining workers and copper smelters also showed increased rates of lung cancer, gastrointestinal cancer, and hematolymphatic malignancies [112].

23.4.5 Arsenic and Urinary Bladder Cancer

Transitional cell carcinoma (TCC), also called urothelial cell carcinoma (UCC), typically involves the urinary system. TCC has been associated with arsenic exposure in drinking water in Argentina, Chile, and Taiwan [110,114]. Experimental research has also shown that arsenic affects urothelial cells [67], and the development of TCC is dose related [114]. TCC has a latent period of about 8 to 20 years and arises from the lining of epithelial cells of the renal pelvis, ureters, and urinary bladder. There are two forms of UCC: a low-grade papillary tumor, which may be transient and recurs; and a high-grade malignancy, which develops into dysplasia or carcinoma *in situ*, and may also be invasive [115].

23.4.6 Arsenic and Lung Cancer

There are several experimental and epidemiological reports linking arsenic exposure and lung cancer [44]. It is estimated that 1.5 million workers in the United States are occupationally

exposed to arsenic in industries that manufacture glass, pigments, pesticides, and paints. Studies have shown a dose-related association between arsenic in drinking water and both kidney and lung cancers [110]. In arsenic-exposed workers, there is a systematic gradient in lung cancer mortality rate, which depends on the duration and intensity of exposure and also on exposure to radiation, asbestos, radon, and inhaled substances such as nickel, chromates, arsenic, and genetic factors. But exposures to cigarette smoke and sulfur dioxide have not been confirmed as co-factors in arsenic-induced lung cancer [1,9,112].

Arsenic has a predilection for epithelial cells and in the lung it causes a malignant epithelial neoplasm called pulmonary adenocarcinoma, which has features of glandular differentiation. Pulmonary adenocarcinoma is seen mostly in the periphery of the lung, and women are more affected than men. Histologically, pulmonary adenocarcinoma is recognized based on the observation of intracellular mucin production. In well-differentiated adenocarcinoma, the glands are lined by tall columnar or mucinous epithelium with ample cytoplasm, basal nuclei, prominent nucleoli, and frequent mitoses. As differentiation decreases, there is increased nuclear atypia and mitosis [116]. SCC can also be found in the lung apart from the skin. In the lung, it is found in the bronchus, close to the hilum [116].

23.4.7 Arsenic and Gastrointestinal Cancer

Gastric carcinoma is the term for stomach cancer and adenocarcinoma is the most common form of cancer found in the gastrointestinal tract. Literature is replete with conflicting reports linking carcinoma of the gastrointestinal tract and arsenic ingestion [2]. Observations were made mostly among factory workers who had developed multiple cancers many years after initial exposure. For example, a study of Swedish glass-blowers exposed to high consumption of lead, arsenic, antimony, and manganese revealed increased risk of death from cancer of the stomach, lung, and colon [117]. Also, a study of 839 copper smelters in Japan found significant increase in mortalities from lung and colon cancers [118]. In Ontario, Canada, excess mortality from stomach cancer was observed among gold miners [119].

23.4.8 Arsenic and Brain Cancer

The primary malignant brain tumor is referred to as glioma because it originates from the glial cells of the nervous system. In the brain, gliomas are commonly found in the cerebral hemispheres but other areas may be affected including the optic nerve, the brain stem, and, particularly among children, the cerebellum. An occupational exposure study in Sweden revealed an increased incidence of gliomas in both men and women and also an increased incidence of meningiomas in women due to exposure to chemicals such as arsenic, mercury, and petroleum products [120]. About 50% of all primary brain tumors and about 20% of all primary spinal cord tumors are gliomas. There exist different groups of gliomas based on the type of glial cell involved. Astrocytomas develop from astrocytes (star-shaped glial cells). Astrocytomas, the most common type of glioma, are also the most common type of primary brain tumor. Arsenic-exposed patients may develop destruction of axonal cylinders, leading to peripheral neuropathy [1].

23.5 Conclusions

This review of published scientific literature indicates that both anthropogenic and geogenic contamination of the environment constitute a major public health problem of global proportions. Human exposure to arsenic may occur via several pathways including oral ingestion, inhalation, and dermal contact. Although arsenic is considered a systemic toxicant that affects many target organs and tissues causing adverse dermatologic, neurologic, gastrointestinal and hepatic, renal, hematologic, cardiovascular, reproductive, and respiratory effects, the most critical damage it gives rise to is associated with its ability to cause a variety of human cancers. Several epidemiological studies have reported significant increases in incidence and mortality rates related to skin, lung, liver, kidney, urinary bladder, and colon neoplasms. Research has also elucidated several important mechanisms associated with arsenic carcinogenesis including induction of oxidative stress and activation of proto-oncogenes, down-regulation of pro-apoptotic genes, modulation of signal transduction, and alterations of cell cycle control genes.

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24

The Association between Chronic Arsenic Exposure and Type 2 Diabetes: A Meta-Analysis

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CHAPTER OUTLINE

24.1 Introduction

Arsenic exposure has been considered a serious public health problem in many countries [1]. It is widespread in the environment and some inorganic species can cause detrimental effects on human health even at low exposure levels [2]. As a naturally occurring metalloid, we constantly encounter arsenic in our daily lives and, fortunately, most of these environmental exposures do not pose health effects [3]. Again, arsenic has been used for long time in many ways such as medicine and commercial products [3]. Importantly, the medicinal use of arsenic is notable and has been widely acknowledged for a number of centuries, although it is often referred as the "king of poisons" [3]. It has been used in the Far East and in the ancient Roman and Greek civilizations as a Chinese traditional medicine [4]. William Withering, the

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scientist who discovered digitalis, proposed arsenic-based therapies in the 15th century and argued that "poisons in small doses are the best medicine; and the best medicine in too large doses is poisonous" [5]. In 1786, a British physician, Dr. Thomas J. Fowler, discovered a solution of potassium arsenite (known as Fowler's solution) and it was used empirically during the 18th, 19th, and 20th centuries as a curative for many systemic illnesses such as malaria, syphilis, asthma, cholera, eczema, and psoriasis [3,5,6]. Arsenic was reported as a white blood cell count reducing agent in 1878 and as an effective treatment for chronic myeloid leukemia in 1930 [5].

Despite all of this, in 1887, Jonathan Hutchison identified arsenic-induced skin lesions and cancer in patients who were treated with arsenic [7]. A number of studies have been conducted to investigate the effects of chronic arsenic exposure on human health in most parts of the world, especially in countries where arsenic in drinking water is a major concern. Until today, inorganic arsenic exposure was found to be associated with many chronic and systemic diseases [8–12], and type 2 diabetes (T2D)/non-insulin dependent diabetes mellitus (T1D) is one of those diseases that is inconclusively reported to be associated with chronic arsenic exposure. Unlike T1D where insulin production is completely stopped, in T2D the human body is unable to use insulin initially because of insulin resistance but subsequently there is an impaired insulin secretion by the pancreas. The mechanism by which arsenic influences diabetes is unclear. Evidence suggests that chronic exposure to arsenic induces oxidative stress and up-regulates the expression of a few cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6), and these are well-known inducers for insulin resistant [13]. Furthermore, oxidative stress may cause pancreatic β-cell dysfunction, thus lowering insulin secretion [13,14].

However, drinking water containing high amounts of inorganic arsenic is the commonest form of exposure in humans [15]. It is demonstrated that low to high amounts of arsenic in drinking water is associated with higher risk of T2D in many populations, and many of the observational studies reported a dose–response pattern [10,16–18]. Del Razo et al. confirmed this association in 2011 and reports the link between risk of diabetes and production of one of the most toxic inorganic arsenic metabolites, dimethylarsinite (DMA) in urine [19]. Population-specific ecological data showed a positive association between total urinary arsenic and the prevalence of diabetes mellitus [20–22]. Nevertheless, review articles on the association of arsenic with diabetes provide an unconvincing result. One of the reviews of the association studies between arsenic and diabetes reveals that prolonged exposure is associated with T2D [23], while another systematic review reports a lack of evidence to establish a causal role of arsenic in diabetes [24]. Recently, Wang et al. performed a meta-analysis that included studies published between 1990 and 2013. Surprisingly, they did not include all the studies in their meta-analysis. Moreover, inclusion of a study by Wang et al. might have influenced the overall risk [25]. With this conflicting outcome and since there are available many observational and ecological studies from most parts of the world, we decided to conduct a meta-analysis to investigate whether there is a true relationship between arsenic exposure and T2D.

24.2 Methods and Materials

24.2.1 Literature Search

We used a comprehensive search strategy to identify all relevant studies. The search was carried out in Ovid MEDLINE, EMBASE, PubMed, Google Scholar, and Web of Knowledge without limitation on the time of publication. The research question was defined as "What are the associations between chronic arsenic exposure (both in general and for specific by-products) and type 2 diabetes?" This question was then broken down to cover specific search terms such as "diabetes/diabetes mellitus," "arsenic," "arsenic in water," "arsenic pollution," "environmental exposure," "inorganic arsenic," "diabetes," "DM," and "T2D." Each term was included in one or more searches. We also hand-searched for additional relevant studies and cross-checked reference lists of prior review articles to identify any additional papers that had not been retrieved through the electronic databases.

24.2.2 Selection of Studies

The included studies are (1) cross-sectional, (2) case-control, nd (3) cohort studies of chronic arsenic exposure reporting a relative risk (RR) or odds ratio (with 95% confidence interval or *p*-value to be used to calculate the 95% CI). We also included studies that did not report OR/RR but reported numbers to be used to calculate those measures. We screened the initial list of articles to identify articles that were irrelevant. We developed a standard data extraction format to extract relevant information from all the included papers.

Two investigators (T.A. and A.H.) initially screened the list of articles to identify the irrelevant articles for exclusion from the list. To facilitate faster screening, articles were first assessed on the basis of their titles to discard the irrelevant articles. Three investigators (T.A., B.R., and A.H.M.) then read the abstracts of the remaining articles to ensure that the main subject of the study was related to arsenic exposure and T2D. Articles whose abstracts showed that the study was not relevant to the objective of the meta-analysis were discarded. Final screening of the articles was based on the full content. After the screening, we extracted information from the selected articles and evaluated the quality of each study.

24.2.2.1 Quality of the Studies

Each article was evaluated in a standardized manner to assess its individual quantity. Quality assessment was facilitated through the use of a standardized questionnaire, which was previously developed for a meta-analysis of arsenic exposure and hypertension [26] and amended for the outcome assessment of diabetes for this study. This scoring instrument has a good reliability (intra-class correlation coefficient of 0.88 for total quality score) and has been described in details elsewhere [26]. Because of the already established high reliability we did not validate it in this study and one scorer scored all the individual studies under four domains: selection of participants 20%, measurement of exposure 30% and outcome 20%, adjustment of confounders 20%, and appropriate statistical analysis 10%.

24.2.2.2 Statistical Analysis

To conduct the meta-analysis, ORs were extracted from case–control and cross-sectional studies and RRs from cohort studies.

For studies where several measures of association were reported, associations that related diabetes to amount of arsenic intake or to arsenic intake combined with duration of exposure were considered. Pooled effect measures were estimated by comparing the highest exposure category to the lowest one combined for males and females.

Effect estimates (OR and RR) were pooled using the inverse-variance-weighted random effects method [27]. Between-studies heterogeneity was measured using I^2-statistics, which measures the proportion of total variation in study estimates due to heterogeneity [28]. A sensitive analysis was carried out to evaluate the key studies that have substantial impact on the between-study heterogeneity by using the "leave one-out" method [29]. The influence of each study on the pooled effect measure was examined by repeating the meta-analysis while omitting each study one by one [30]. We tested for possible publication bias using Begg's and Egger's tests [31,32]. We also generated a contour enhanced funnel plot to visually inspect asymmetry in the plot and explain the underlying cause [33,34]. Where asymmetry was identified, we adopted the "trim and fill" method to see the effect of correcting the publication bias [35]. This method first identifies the asymmetry in the funnel plot and trims those studies that cause the asymmetry. The pooled estimate with the remaining studies is calculated and then the funnel plot is filled in by replacing the trimmed plots and adding their mirror images in the plot. The final pooled results come from an analysis using all the true estimates and the simulated mirror images.

Meta-regressions of the logs of the effect measures, weighted by the inverse of their variances, on the total quality scores and their subcategories were undertaken to assess the possible impact of study quality on the effect measures. These regressions fitted a random effects model with two additive variance components (within and between studies). We conducted a separate meta-analysis excluding studies that were of very poor quality (<25% of the total possible quality score).

We conducted a random-effects dose–response meta-analysis. A restricted cubic spline model with four knots at the 5th, 35th, 65th, and 95th centiles of the levels of arsenic concentration in drinking water was estimated using generalized least squares regression, taking into account the correlation within each set of published effect measures [36]. Statistical significance of the non-linearity of the dose–response relationship was examined by testing the null hypothesis that the coefficients of the second and third spline were equal. All statistical analyses were performed with STATA V13.

24.3 Results

24.3.1 Literature Search

Our search identified 226 studies, of which 33 reported associations between chronic arsenic exposure and T2D. Five of these articles were excluded from the meta-analysis as none had

reported any relative risk or odds ratio, or whether the risk was calculable (see Table 24–1). Therefore, our final meta-analysis included a total of 27 studies: 18 cross-sectional, five cohort, and four case–control studies.

24.3.2 Quality Scoring

The total and domain-specific quality scores are presented in Table 24–2. Overall, cross-sectional studies scored a little higher than case–control and cohort studies, although cohort studies scored higher on selection. Potential confounders were adjusted more in cross-sectional studies than in the other two designs. All the studies scored poorly on exposure measurement as arsenic exposure measurement remains a challenge to date. In the majority of the studies, a single exposure measurement has been used as a proxy measure for total arsenic exposure for study participants.

We assessed quality with respect to confounding by listing all potential confounders relevant to studies of chronic arsenic exposure and T2D, and counted how many were adjusted for, or shown not to be confounders, in each study. All the study types performed poorly in this aspect too. Analysis was better conducted in cross-sectional studies than cohort and case–control studies. Only two [17,37] out of five cohort studies estimated a hazard ratio or RR with CIs in categories of age, sex, or other relevant variables. Outcome assessment was relatively good in all three study types.

We finally incorporated 27 studies in the analysis including 15 cross-sectional, four case–control, and three cohort studies reporting OR, and three cross-sectional and two cohort studies reporting RR. The pooled OR estimate was 1.78 (95% CI: 1.38, 2.30), and the pooled RR was 1.28 (95% CI: 1.03, 1.59). After combining all three types of studies the pooled estimate was 1.76 (95% CI: 1.38, 2.25) with overall between-studies heterogeneity measured by I^2 statistics of 98.3% (not shown in the analysis).

We repeated the meta-analysis after dropping the study by Wang et al. [25] because in the original paper we find neither an effect measure reported nor any numbers that could be used to estimate one. The overall effect slightly reduced to 1.50 (95% CI: 1.33–1.69) and the I^2 statistics reduced to 75.5% from 98.3%. We considered this as our final meta-analysis (Figure 24–1). Note that for some of the studies (e.g., [37]) the 95% CI of the effect measures reported in Table 24–1 are slightly different from that in Figure 24–1. This discrepancy occurred for log transformation of the study-specific estimates and their confidence intervals to calculate standard error for doing the meta-analysis and then recalculating the confidence intervals from the calculated standard errors.

24.3.3 Sources of Heterogeneity

The initial meta-analysis demonstrates strong evidence of heterogeneity among studies for chronic arsenic exposure and T2D ($I^2 = 75.5\%$). We conducted a sensitivity analysis to examine the contribution of individual studies to the overall heterogeneity. After excluding the study by Jovanovic et al. [16], the between-study heterogeneity reduced ($I^2 = 68.06\%$). After excluding the studies by Jovanovic et al. and Rahman et al. [38], the between-study heterogeneity reduced

Table 24–1 Summary of the Studies included in the Meta-analysis of Chronic Arsenic Exposure and Type 2 Diabetes

Author, Year	Study Design	Study Population and Location	Diabetes Diagnosis	Arsenic Exposure Categories	Cases/ Non-cases	Odds Ratio/ Relative Risk of T2D (95% CI)	Variables Adjusted for
Lagerkvist and Zetturland, 1994 [49]	Cross-sectional	43 copper smelter workers exposed to iAs; 46 referents worked at a car factory, Sweden	Self-reported T2D	Mean urinary iAs, MMAA and DMAA for arsenic workers = 39 μg/L (range 5–520 μg/L) or 14.3 μg/g creatinine); mean 9 μg/L (range 5–15 μg/L) for reference population	4/85	9.61 (0.53–173.62)	Crude
Lai et al., 1994 [18]	Cross-sectional	891 adult residents in high-arsenic area, Taiwan	Oral glucose tolerance test (OGTT) or self-reported T2D	Cumulative arsenic exposure (ppm-years): 0 (referent), 0.1–15.0, ≥15.1	86/805	10.05 (1.3–77.9)	Age, sex, BMI, and physical activity
Rahman and Axelson, 1995 [50]	Case–control	Copper smelter workers, Sweden	Death certificate, medical record	Arsenic in air: <0.5 mg/m^3, ≅0.5 mg/m^3, and >0.5 mg/m^3	12/31	3.3 (0.5–30)	Age
Rahman et al., 1996 [51]	Cross-sectional	2456 deaths in glass industry area, 240 exposed and 2216 unexposed to arsenic, Sweden	Death certificate	Exposed vs. unexposed workers	240/2216	1.4 (0.9–2.1)	Age
Jensen and Hansen, 1998 [52]	Cross-sectional	Taxidermists, wood workers, other jobs, Denmark	HbA1C	All arsenic workers (***n*** = 40): sum of urinary arsenic, MMAA and DMAA-mean 35.9 (46.2), median 22.3, range 11.5–294.5 nmol of arsenic/ mmol of creatinine	5/59	4.43 (0.47–41.88)	Age
Rahman et al., 1998 [38]	Cross-sectional	Participants in high and low arsenic area, Bangladesh	Self-reported symptoms + glucosuria + oral glucose tolerance test (OGTT)	Time-weighted arsenic concentration (mg/L): unexposed (referent), <0.5, 0.5–10, >1.0	46/971	5.9 (2.9–11.6)	Age, sex, BMI
Tsai et al., 1999 [53]	Retrospective cohort study	Deaths in 1977–1994, Taiwan	Death certificate	High arsenic area (median = 0.78 ppm arsenic) vs. local and national reference group	531 deaths due to DM/20067 total deaths	1.46 (1.28–1.67)	Age, sex

Rahman et al., 1999 [41]	Cross-sectional	Participants in high-arsenic area, Bangladesh	Glucosuria	Arsenic in drinking water: <0.5 mg/L, 0.5–1.0 mg/L and >1.0 mg/L; time-weighted arsenic concentration: <1.0, 1.0–5.0, >5.0–10.0 and >10.0 mg-years/L	263/1332	2.1 (1.1–4.2)	Age, sex
Tseng et al., 2000 [37]	Cohort	Participants in high-arsenic area, Taiwan	OGTT	Cumulative arsenic exposure: ≥17 vs. <17 mg/L-years	41/405	Ref 2.1 (1.1–4.2)	Age, sex, BMI
Wang et al., 2003* [25]	Cross-sectional	National Health Insurance database, Taiwan	ICD-9 250 ICD-9 A181	Arseniasis endemic area vs. non-endemic area	27543/678791		Age, sex
Zierold et al., 2004 [54]	Case–control	Participants with private wells	Self-reported	Water arsenic concentration: <2, 2–10 and >10 μg/L	67/1118	1.02 (0.49–2.15)	Age, sex, smoking status, and BMI
Coronado-Gonzalez et al., 2007 [55]	Case–control	400 participants living in areas with high arsenic in drinking water, Mexico	Fasting blood glucose	Urinary total arsenic: <35, 35–100 and >100 μg/g	200/200	2.16 (1.23–3.79)	Age, sex, triglycerides, BMI, hypertension, and family history of diabetes
Ettinger et al., 2009 [40]	Prospective cohort study	532 women lived proximate to the Tar Creek Superfund site, USA	Oral glucose tolerance test (OGTT)	Hair arsenic (μg/L): Q1: 0.23–0.92 Q2: 0.93–1.39 Q3: 1.40–2.08 Q4: 2.09–24.07	22/507	1.03 (0.50–2.10) 2.21 (1.14–4.29) 2.35 (1.18–4.69)	Age, race, BMI, Medicaid use, and marital status
Navas-Acien et al., 2008 [21]	Cross-sectional	788 participants from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 survey, USA	Fasting blood glucose, self- reported physician diagnosis	Urinary arsenic (μg/L): 20th (referent) vs. 80th centile of arsenic exposure	93/695	4.26 (0.83–21.8)	Age, sex, race/ethnicity, urinary creatinine, education, cotinine, and hypertension medication
Steinmaus et al., 2009 [56]	Cross-sectional	Participants from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 survey, USA	A self-reported physician diagnosis, fasting serum glucose	Urinary arsenic (μg/L): ≤3.5, ≥18.3	77/718	0.88 (0.39–1.97)	Sex, age, ethinicity, education, BMI, serum cotinine, and current use of hypertension medications

Table 24–1 (Continued)

Author, Year	Study Design	Study Population and Location	Diabetes Diagnosis	Arsenic Exposure Categories	Cases/ Non-cases	Odds Ratio/ Relative Risk of T2D (95% CI)	Variables Adjusted for
Chen et al., 2010 [42]	Cross-sectional	11,319 participants from the "Health Effects of Arsenic Longitudinal Study," Bangladesh	A self-reported physician diagnosis, taking insulin or hypoglycemic medication	Urinary arsenic concentration (μg/L): 1–36 (referent), 37–66, 67–114, 115–204, ≥205; time-weighted arsenic concentration in drinking water (μg/L): 0.1–8.0 (referent), 8.1–41.0, 41.2–91.7, 91.8–176.2–864.0	241/11078	1.11 (0.73–1.69)	Age, sex, BMI, smoking status, educational attainment, and urinary creatinine
Kim & Lee, 2011 [22]	Cross-sectional	National Health and Nutrition Examination Survey (NHANES) participants, Korea	Fasting blood sugar, self-reported physician diagnosis, self-reported use of insulin or oral hypoglycemic medication	Urinary arsenic concentration (μg/g creatinine)	156/1521	1.31 (1.04–1.66)	Age, sex, BMI, smoking status, drinking status, educational level
Del Razo et al., 2011 [19]	Cross-sectional	Residents of Zimapán and Lagunera regions, Mexico	Fasting glucose test,75-g oral glucose tolerance test, a self-reported physician diagnosis, taking insulin or hypoglycemic medication	Urinary arsenic concentration (ng/mL); cumulative arsenic exposure in drinking water (ppm-years)	25/233	1.13 (1.05–1.22)	Age, sex, obesity, and hypertension
Gribble et al., 2012 [57]	Cross-sectional	3925 American Indian adults aged 45–74 years, USA	Fasting glucose test,75-g oral glucose tolerance test, HbA1C, taking insulin or oral hypoglycemic medication	Urinary arsenic concentration (μg/L): <7.9 (referent), 7.9–14.1, 14.1–24.2, ≥24.2	1939/1986	1.14 (1.08–1.21)	Urinary creatinine, age, sex, educational level, alcohol consumption, smoking, BMI, and region
Islam et al., 2012 [10]	Cross-sectional	High and low arsenic contaminated area, Bangladesh	Fasting glucose test using glucometer, a self-reported physician diagnosis	Arsenic concentration in drinking water (μg/L): ≤50 (referent), >50; <22(referent), 23–32, 33–261, ≥262	89/915	1.9 (1.1–3.5)	Age, sex, education, BMI, and family history of diabetes

Makris et al., 2012 [58]	Cross-sectional study	The small community of Mammari in the Nicosia prefecture of Cyprus	A self-reported physician diagnosis	Cumulative arsenic exposure index (mg As): 1st quintile (referent), 2nd vs. 1st, 3rd vs. 1st, 4th vs. 1st, 5th vs. 1st	21/296	1.86 (0.30–11.59)	Age, sex, and smoking status
Jovanovic et al., 2013 [16]	Cross-sectional study	Arsenic-exposed and -unexposed population in middle Banat region, Serbia	Fasting glucose test, 75-g oral glucose tolerance test	Arsenic in drinking water (μg/L): 0–10.0 (referent); <100 (referent), ≥100	1730/771747	1.62 (1.46–1.80)	–
Kim et al., 2013 [20]	Nested case–control	American Indians, USA	75-g oral glucose tolerance test	Urinary arsenic concentration (μg/L): 0–10.0 (referent)	150/150	1.16 (0.89–1.53)	Age, sex, creatinine level, BMI, and urinary
Li et al., 2013 [43]	Cross-sectional	Arsenic-contaminated area of Inner Mongolia, China	Fasting glucose level, a self-reported physician diagnosis, taking insulin or hypoglycemic drugs	Arsenic in drinking water (μg/L): <10 (referent), 10–50, >50	42/627	1.6 (0.59–4.32)	Gender, age, alcohol consumption, cigarette smoking, BMI, and cumulative arsenic exposure
James et al., 2013 [17]	Case-cohort	San Luis Valley Diabetes Study (SLVDS), USA	Fasting glucose test, 75-g oral glucose tolerance test, a self-reported physician diagnosis	Time-weighted arsenic concentration (μg/L-yr): ≤4, 4.0–8, >8–20, >20	141/347	1.27 (1.01–1.59)	Ethnicity, gender, socio-economic status, first degree family history, BMI, smoking, alcohol, and physical activity
Rhee et al., 2013 [14]	Cross-sectional	National Health and Nutrition Examination Survey (NHANES) participants, Korea	Fasting glucose test,75-g oral glucose tolerance test, taking insulin or oral hypoglycemic medication	Urinary arsenic concentration (μg/g creatinine): <70.68 (referent), 70.7–117.7, 117.7–193.4, ≥193.4	309/3293	1.56 (1.03–2.36)	Sex, age, residence area, smoking, alcohol, occupation, and serum mercury level
Pan et al., 2013 [39]	Case–control	Individuals with arsenical skin lesions and controls, Bangladesh	HbA1C	Arsenic in drinking water (μg/L): ≤1.7 (referent), 1.8–15.5, 15.6–170.0, ≥170.1; toenail arsenic (μg/g): ≤0.93 (referent), 0.94–2.12, 2.13–6.18, ≥6.19	84/849	4.51 (2.01–10.10)	Age, sex, BMI, cigarette smoking, skin lesions, arsenic in drinking water, toenail arsenic

*Not included in the meta-analysis.

Table 24–2 Average Quality Scores for Studies included in the Meta-analysis

Categories of Quality Scoring (maximum points value)	Average (%) of the Maximum Category Quality Score (range)		
	Cross-Sectional Studies ($n = 18$)	Cohort Studies ($n = 5$)	Case–Control Studies ($n = 4$)
Selection (20 points)	15.6 (10–20)	18.2 (15–20)	15 (10–20)
Exposure (30 points)	16.6 (5–20)	13.2 (5–20)	14 (5–20)
Outcome (20 points)	15.3 (10–20)	14.1 (10–20)	14 (10–20)
Confounders (20 points)	14.1 (0–17)	10.2 (7–17)	13 (7–17)
Analysis (20 points)	8.1 (5–10)	6.9 (5–10)	8 (5–10)
Total quality score	69.7 (40–87)	62.6 (47–87)	64 (37–87)

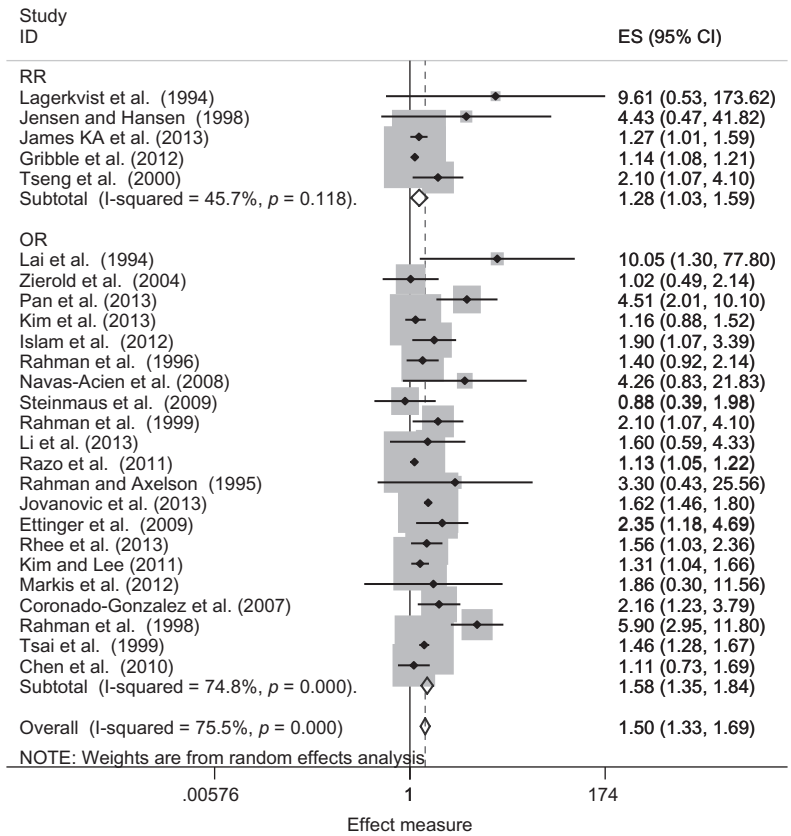

FIGURE 24–1 Forest plots of meta-analysis of chronic arsenic exposure and T2D.

further ($I^2 = 58.1\%$). After excluding the study by Pan et al. [39] along with these two studies, the I^2 statistic reduced to 50.57%. The between-study variability reduced further after excluding the study by Rahman et al. [38,41] along with the three studies ($I^2 = 49.73\%$). In the study by Jovanovic et al., comparisons were made between two populations exposed and unexposed

to arsenic [16]. Exposure to arsenic was measured from public water supply systems; therefore, individual arsenic exposure level as well as the duration of arsenic exposure was not available for the study participants. Information on T2D was obtained from the national registry of diabetes. The study also did not adjust for any potential confounders. Rahman et al. in their study compared the prevalence of T2D among participants from arsenic-exposed and -unexposed populations [38]. In this study, due to absence of information on individual arsenic exposure level, the indication of "keratosis" was considered as the sign of arsenic toxicity. Therefore, misclassification of exposure might have occurred. Diagnosis of T2D was not uniform for all the participants. Individuals with possible symptoms of diabetes who had two positive samples with glucometric strips were examined for hyperglycemia after overnight fasting and 2 hours after 75-mg glucose intake. This might have reduced the sensitivity of the diagnosis. Pan et al. recruited cases and controls for their study from a previous case–control study that recruited cases with arsenical skin lesions [39]. Therefore, this recruitment process limits the generalizability of the study finding. They measured HbA1C for the outcome measurement, i.e., diagnosis of T2D, which is different from the procedures used in most of the studies. Furthermore, there is an uncertainty about excluding all the prevalent self-reported cases of T2D in 2001–2003. This may have biased the reported association between chronic arsenic exposure and T2D. In the study of Rahman et al. [38], arsenic measurement was obtained from various sources. To assess the outcome, urine samples were analyzed for glucosuria as the proxy measure for T2D using a glucometer. This might have introduced misclassification of the outcome. A number of potential confounders were also not adjusted for in the analysis [41]. Therefore, when we dropped these four studies from the meta-analysis, between-study heterogeneity was within the acceptable level ($I^2 = 49.7\%$).

24.3.4 Meta-Regression and Sensitivity Analysis

We performed a random effects meta-regression to verify the dependency of the outcome measure on the quality scores that was obtained during quality assessment. Only the quality of exposure assessment was found to be significantly associated with the outcome measure ($p = 0.006$) where for a 1% increase in the quality score the effect measure would decrease by 4% (95% CI: 1–7). When we restricted our meta-analysis to the studies that had a quality of at least 75%, the overall risk reduced further to 1.30 (95% CI: 1.16 to 1.46) (see Figure 24–2).

24.3.5 Influence Analysis

The influence analysis did not detect any single study influencing the pooled estimate (data not shown).

24.3.6 Publication Bias

First, we conducted the tests for funnel asymmetry using the Begg ($p = 0.252$) and Egger ($p = 0.001$) tests. Although the Egger test showed significant funnel asymmetry, it is a very sensitive test and when it disagrees with the Begg test the latter should be considered.

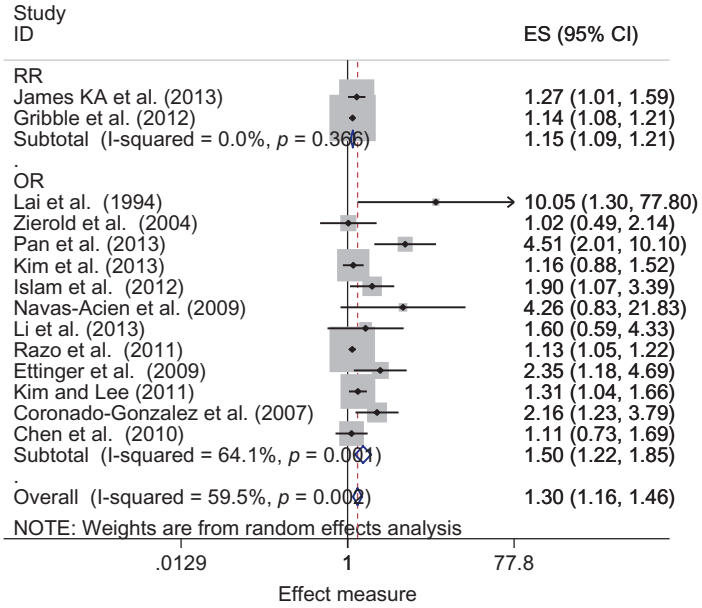

FIGURE 24–2 Meta-analysis of chronic arsenic exposure and T2D with studies having quality ≥75%.

We investigated the cause of the apparent funnel asymmetry by doing a contour funnel plot. We found that most of the studies were missing on the left side of the funnel at a high significant region (Figure 24–3). This indicates that publication bias is unlikely to occur because of funnel asymmetry.

We adopted the trim and fill method to correct for publication bias and examined its effect on the pooled estimate. The method did not fill any study to deal with the apparent asymmetry and the pooled estimate was unchanged. This also demonstrated that there was no evidence of publication bias in this study.

24.3.7 Dose–Response

We included five studies in the dose–response meta-analysis [10,16,18,38,39,42,43]. For each 100 unit increase in arsenic concentration, the effect measure increases by 23%, although this dose–response was not significant (95% CI: −29, 111). There was also no statistical evidence of a non-linear dose–response trend ($p = 0.054$) (not shown in the analysis). We excluded the study of Ettinger et al. [40] as they measured arsenic in blood and in hair samples of a subset of the study participants, which is different from other studies we included in this dose–response meta-analysis.

24.4 Discussion

In this meta-analysis of 28 studies, we observed an overall association between chronic arsenic exposure and T2D. However, the association varies according to the study designs. The pooled

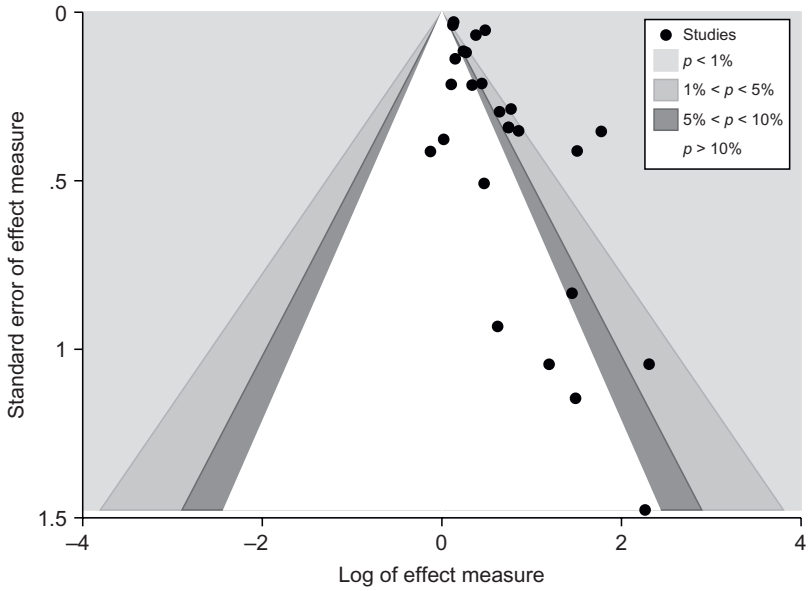

FIGURE 24–3 Funnel plot of random effects meta-analysis of arsenic exposure and T2D.

OR estimate was 1.58 (95% CI: 1.35–1.84), the pooled RR was 1.28 (95% CI: 1.03–1.59), and the overall pooled estimate of risk was 1.50 (95% CI: 1.33, 1.69).

It is essential to investigate the presence of between-study heterogeneity in a meta-analysis [44]. In this meta-analysis, between-study heterogeneity was very high. This observed heterogeneity may be attributable to variations among study participants' characteristics such as age, sex, body mass index, family history of T2D, and other co-morbidities. However, excluding the study by Wang et al. [25] reduces the between-study heterogeneity substantially. Between-study variability reduced further when we restricted the meta-analysis within total study quality ≥75% ($I^2 = 59.5\%$).

The meta-regression revealed a significant inverse association with exposure assessment, which is the most important characteristic of an observational study. Thus, with improved exposure measurement we can expect diminishing association. This evidence is also supported by the subgroup analysis where weaker association was found in the meta-analysis with overall better study quality. Compared to the previous meta-analysis [45], this study did not get a significant dose–response association after including more studies. Moreover, when we restricted our dose–response analysis to the same studies by Wang et al. [45], we could not reproduce the significant dose–response they reported. Thus, the dose–response association is also not conclusive. This may be due to the differences in the way they handled the exposure categories in the regression model. We used the median exposure level as the independent variable in the model but they did not mention how they did it. Moreover, in the meta-analysis by Wang and co-workers [45] they included the large study by Wang and co-workers [25] with a very tight confidence interval (OR = 2.69, 95% CI: 2.65 to 2.73), which forced the pooled estimate away

from the null. We did not include this study in our analysis because we did not get that effect measure reported in the original study.

Adverse effects of arsenic on T2D have been demonstrated in previous animal model experimentation. These experiments suggest that arsenic impairs pancreatic β-cell function, thus affecting insulin synthesis and secretion. The molecular mechanism for this effect may be through adverse effects on insulin signal transduction and the inhibition of gene transcription factors [46–48]. Furthermore, a number of other non-specific mechanisms such as inflammation, apoptosis or oxidative stress may also increase the risk of T2D.

The main strength of our study is that we did not get any evidence of publication bias in the meta-analysis, but we did get inclusion of all the studies fulfilling eligibility criteria, comprehensive assessment of quality, and the use of the quality score in a meta-regression and subgroup analysis to assess the effect of quality scores on the study findings.

Nevertheless, the meta-analysis also has a few limitations. Measurement of arsenic exposure was not uniform across the studies; therefore, the observed association between chronic arsenic exposure and T2D may be underestimated. Outcome measurement also varied among the studies, in particular there was a concern about diagnosis of T2D using a glucometer. The validity of the glucometer had not been assessed earlier. Finally, observed findings could be explained by a number of unknown confounders, which could not be adjusted for, and this may have over- or underestimated the risk estimates.

In conclusion, this meta-analysis suggests that chronic arsenic exposure is likely to increase the risk of T2D. However, this finding is limited by high between-study heterogeneity. Therefore, the hypothesized association needs further validation through well-conducted, large prospective cohort studies with better exposure assessment.

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Arsenic Biosensors: Challenges and Opportunities for High-Throughput Detection

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CHAPTER OUTLINE

25.1 Arsenic: The Toxic Metalloid

Arsenic (As) is a toxic metalloid distributed in nature and the incidence of its poisoning among human populations from various parts of the world is well documented [1]. These instances were reported to occur mainly through contaminated water and food sources, which have been attributed to activity that pertains to use of arsenical pesticides, digging of sediment-rich wells, and leaching near thermal plants, which significantly adds to arsenic exposure to the environment posing a serious threat to life [2,3]. It is established that arsenic is a potent human carcinogen, prolonged exposure to which leads to a wide range of physiological implications affecting skin, gastrointestinal, hepatic, neurological, and respiratory organ systems mediated through oxidative stress and genetic manifestations [4,5]. The highest prevalence of arsenic in soil and water is reported from Bangladesh and West Bengal state in India [6,7]. Two of arsenic's inorganic forms, the trivalent As(III) and the pentavalent As(V), are predominant in occurrence, the former being more toxic [8]. The World Health Organization recommended the maximum permissible concentration of arsenic in drinking water to be 10 μg/L, which corresponds to 0.1 μM As [9]. Consequently, it is of utmost importance that water, food, and soil, particularly in suspected regions, be monitored regularly for any possible exposure for which

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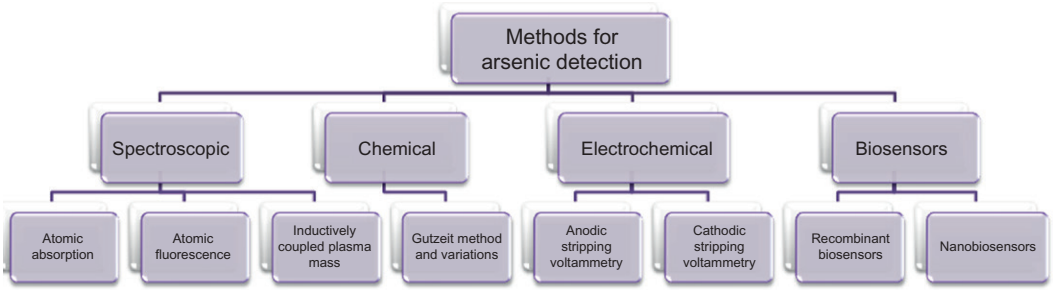

FIGURE 25–1 Classification of arsenic detection methods.

efficient, reliable, and high-throughput arsenic detection methods are desired. The methods for arsenic detection are broadly classified in Figure 25–1.

Established laboratory methods are the spectrometric methods. These techniques, though highly accurate, are costly, time intensive, need acidic pretreatment of samples before analysis, and are essentially laboratory bound. However, these methods are reference methods and are used for validation of newer techniques. Most arsenic detection kits developed for on-field application and used for drinking water and ground water analysis are chemical kits based on the Gutzeit method. Some research groups have evaluated the efficacy of these kits [10–12]. Though some commercial kits furnish satisfactory responses, it was noticed that most of them exhibit a high percentage of false negatives and false positives, which is attributed to interference by other metals and ions found in real samples; the reliability of these kits is therefore questioned. In addition, the chemical methods entail generation of the highly toxic arsine gas, which raises safety concerns. More recently, electrochemical methods of arsenic detection have shown promise in terms of safety and portability in measurements [13,14]. Anodic and cathodic stripping voltammetry rely on specific working electrodes. There are concerns about measurements due to peak currents overlapping with those of interfering species, which is a keen research focus for many groups, and nanoparticles may seem to address those issues [15]. High-throughput on-site detection with these methods may still be a challenge since they need either gold or mercury working electrodes, which raise cost and disposal concerns. Alongside the above-mentioned methods and challenges, biological assays have emerged as promising alternative methods for arsenic detection. In particular, biosensors with whole cells and oligonucleotides as biorecognition elements enable ultrasensitive detection owing to biological origins that respond to environmental stress conditions by evolving inherent mechanisms of safety, which have been carefully developed by bioanalytical chemists into arsenic diagnostic assays, the features of which are discussed below.

25.2 Arsenic Biosensors

In recent years, biosensors for arsenic have been reported based on physiological changes such as pH in response to the presence of the analyte, a chromogenic induction, or inhibitory

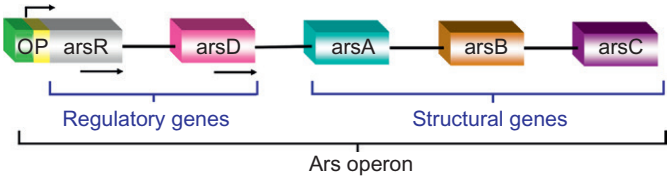

FIGURE 25–2 The *ars* operon.

action of the metal ion on the enzyme acetylcholinesterase [16–19]. However, it was noticed that these biosensors were lacking in selectivity and had very high detection limits, which clearly jeopardized their applicability. This led researchers to explore the possibility of engineering microorganisms such that they elicit a specific response in the presence of arsenic, the basis of which lay in the ability of some of the microorganisms to develop a natural self-defense mechanism for survival in metal-laden toxic environments, and this led to a plethora of interesting works on recombinant arsenic biosensors.

25.2.1 Evolution of a Natural Self-Defense Mechanism—the *ars* Operon

The microorganisms in their response to toxicity posed by arsenic have evolved a natural self-defense mechanism in which the metalloid is pumped out of the cell by an efflux pump that is genetically regulated by a complement of five genes that comprise *ars* operon as shown in Figure 25–2.

The three structural genes *arsA*, *arsB*, and *arsC* code for membrane bound ATPase, a transmembrane protein, and arsenate reductase, respectively, to form an efflux pump responsible for removing arsenite from the cell, deriving energy for efflux from ATP hydrolysis. The cells that are incapable of effluxing arsenate depend upon *arsC* encoded arsenate reductase to generate arsenite, which induces *arsAB* thereby extruding arsenite out of the cell. *arsR* and *arsD* are the up-regulatory genes responsible for repression and controlled expression of structural genes [20]. Figure 25–3 depicts the arsenite efflux pump in cells.

In the presence of arsenite the *arsR* encoded repressor protein undergoes a conformational change leading to its dissociation from operator/promoter, thereby allowing the commencement of transcription to yield the defense by switching on expression. Many workers have utilized this phenomenon to quantify arsenite by fusion of *arsR* with a reporter gene to generate a response that is dependent on arsenite concentration, which is established as a highly specific recombinant biosensing system [21] as illustrated in Figure 25–4. Three of the most used arsenite concentration-dependent recombinant plasmid-borne hosts are those of *Escherichia coli*, *Bacillus subtilis*, and *Rhodopseudomonas palustris* [22–25]. For detailed information on the performance of these biosensors, readers are referred to the work of Diesel et al. [26].

25.2.2 Recombinant *Escherichia coli*—the Favored Biorecognition Element

Though many bacterial genera have been reported for use in arsenic biosensing systems, it is observed that most of these did not furnish an appreciable sensitivity of arsenic detection,

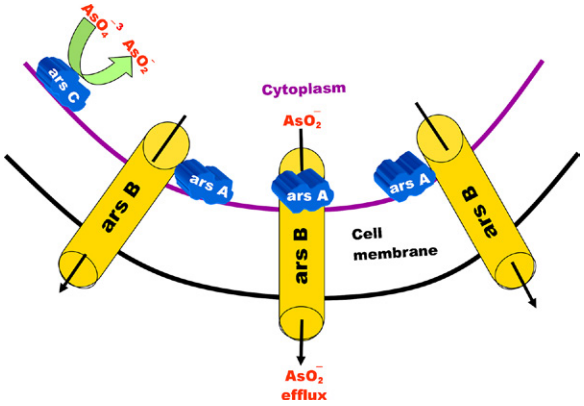

FIGURE 25–3 The arsenite efflux pump.

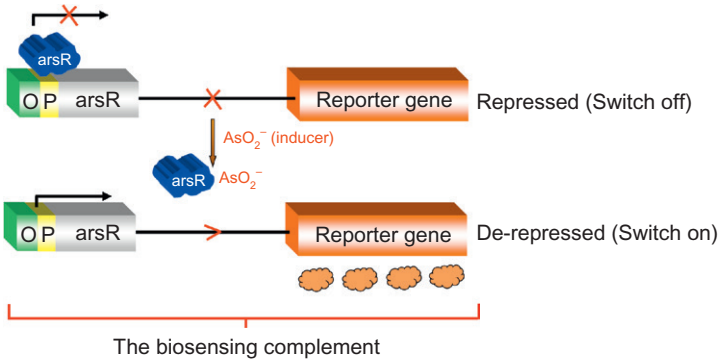

FIGURE 25–4 The recombinant biosensing phenomenon.

the lowest detectable concentration being 0.3 μM. Due to arsenic toxicity and the WHO established safe limit of 0.1 μM, it is incumbent for biosensors to exhibit ultrasensitive responses, a feature that justifies the objectives of biosensors over conventional techniques of detection, which bear certain limitations as mentioned earlier. Due to its evolved mechanism to survive under extreme chemical stress environments and the ease in manipulating its genetic makeup, *E. coli* has been a favored host for recombinant constructs and has proven this ability in the realm of arsenic detection [26]. Much like other bacterial strains, in some of the initial works reported with *E. coli* for arsenic the limit of detection was not sensitive enough. However, with continued re-engineering and with understandings of newer reporter genes that furnished autofluorescent and reagentless expressions, a significant improvement in the sensitivity of detection, as low as subnanomolar concentration, was observed. In addition, the coupling of electrochemical and optical transducers for sensitive quantification of genetic expression further refined the analytics in recombinant biosensors by the sending of read-out signals that paved the way for on-the-spot field detection of this toxic metalloid.

Table 25–1 Response Characteristics of Recombinant *E. coli* Biosensors for Arsenic

Plasmid	Reporter Gene	Induction Time	Output Signal	Limit of Detection	Reference
pJAMA-arsR-ABS	*luxAB*	1 h	Bioluminescence	0.05 μM	[21]
pPR-arsR-ABS	*gfp*	1–5 h	Fluorescence	0.12 μM	[21]
pMV-arsR-ABS	*lacZ*	30 min	Colorimetric	qualitative	[21]
pRLUX	*luxAB*	3 h	Bioluminescence	1.0 fM	[27]
pBGD23	*lacZ*	30 min	Chemiluminescence	1.0 fM	[28]
pBGD23	*lacZ*	17 h	Electrochemical	0.1 μM	[28]
pSD10	*gfp*	30 min	Fluorescence	8.0 μM	[29]
pPR-arsR-ABS	*egfp*	1 h	Laser-induced fluorescence	0.1 μM	[30]
pJAMA-arsR-ABS	*luxAB*	2 h	Bioluminescence	6 ng/g dry wt. (rice)	[31]
arsR-lacZ strains 1580	*lacz*	8 h	Colorimetric	<0.08 μM	[32]
arsR-ccp strains 1971, 1981	*ccp*	4 h	Colorimetric	<0.01 μM	[32]

Many workers have investigated recombinant *E. coli* for arsenic detection, of which two research groups have made significant contributions: Daunert and van der Meer. In one of their pioneering works Daunert's group engineered a plasmid pRLUX by fusion of *arsD* with reporter genes *luxA* and *luxB*. The expression was regulated by *arsR* upstream of *arsD*. *arsR* responds to arsenic in a mechanism described previously to yield bioluminescence in transformed cells of *E. coli*. A significant feature of this work is the remarkable sensitivity of detection at subattomolar concentrations [27]. In another study, a different reporter gene *lacZ* was fused with *arsD* to yield a plasmid pBGD23 to look for any improvement in sensitivity characteristics. The transformed *E. coli* that furnished a chemiluminescent signal exhibited similar sensitivity of detection as in the previous work, though it conferred a higher selectivity of response as investigated with a number of metal ions. Even though the biosensor had a shorter induction time, reporting with *lacZ* bears a limitation since the need to be lysed before a response can be generated, which limits its real-time applicability and also the effect of the cationic peptide used as a lysing agent, cannot be ignored [28]. With the same recombinant, the group for the first time reported electrochemical transduction in which *lacZ* expression was quantified by generation of an electroactive product that was measured at a peak oxidation current with a gold working electrode at a potential of +0.23 V vs. Ag/AgCl. A limit of 10^{-7} M arsenic detection was observed; however, the unusually high induction time followed by a long incubation prompts alternative modes of detection. In such an advancement the group in their subsequent work reported a recombinant with a reporter gene coding for a mutant green fluorescent protein GFPuv, a reagentless autofluorescent reporter fused with *arsR* to get pSD10 plasmid construct. *E. coli* transformed with pSD10 generated autofluorescence upon induction with arsenite and the limit of detection was observed to be 8×10^{-6} M. A significant feature of this work was the adaptation of the biosensing system onto a microfluidic platform with the purpose of miniaturization, which would afford portability and hence wider applicability to the sensing device [29]. Table 25–1 summarizes the response characteristics of some of the significant recombinant *E. coli*-based biosensors developed for arsenic detection.

Van der Meer's investigations reported a new dimension in improved response characteristics of recombinant *E. coli* biosensors for arsenic by diminishing the expression of ArsR protein, which would then enhance the relative expression of reporter proteins thereby yielding a higher sensitivity of detection. To carry this out, a recombinant was engineered by inserting a 72 bp DNA fragment downstream of *arsR* as a secondary binding site for ArsR protein, which then bound to both sites. This was shown to exhibit higher titers of reporter proteins by reducing background expression of *arsR*. Three plasmid constructs pJAMA-arsR-ABS, pPR-arsR-ABS, and pMV-arsR-ABS with *luxAB*, *gfp*, and *lacZ*, respectively, which furnished bioluminescence, autofluorescence, and colorimetric responses, were investigated for their performance. The bioluminescence and fluorescence biosensors exhibited a higher sensitivity of detection of 0.05 and 0.12 μM arsenic. The colorimetric biosensor was developed into a qualitative paper strip test able to convey the safety/toxicity of samples. A significant reduction in induction times was observed from the previously reported 3 h to 1 h in this work, which is attributed to the secondary binding site introduced in the genetic construct, though a higher induction time would further lower the detection limit. As expected, the adsorption of cells onto a paper strip did leach out the same into the solution and therefore cell confinement onto biocompatible matrices was required, though the method proved to be useful as a qualitative and disposable diagnostic for on-site analysis. In *gfp* harboring cells, autofluorescence was generated after 1 h induction, though a higher time was suggested based on cell counts. The read-out signal was measured by epifluorescence microscopy, which allows the monitoring of individual cells [21,33]. In an advanced detection method laser-induced fluorescence confocal spectroscopy was used to measure arsenite-induced expression of the enhanced green fluorescent protein egfp. The technique furnished a similar ultrasensitive response of 1.0 mM arsenic detection increase with single molecule detection [30].

Further, the pJAMA-arsR-based biosensor was validated for application of real-time groundwater samples by negating the effect of Fe(II) and Fe(III) on arsenic detection since iron in aqueous environments forms colloidal hydroxides that strongly adsorb arsenic up to 80–90%, which decreases the bioavailability of the metalloid for detection by the biosensor leading to generation of a false response. To reduce the effect of presence of Fe(II) and Fe(III), acidification and chelation strategies were opted for and it was observed that use of EDTA had a detrimental effect on the cells due to chelation of ions in the cell walls causing apoptosis. Mineral acids were therefore used to maintain a highly acidic environment in which Fe(II) and Fe(III) would remain in solution, but a challenge was the survival of cells under pH stress and so a strategy for sequential increase in pH was optimized in which, upon acidification and instant incubation with cells, neutralization was achieved with pyrophosphate, thus maintaining the viability of the cell suspension and also keeping arsenic bioavailable. The response of the biosensor was very promising with negligible false responses, and it was successfully used for on-field detection of arsenic and proposed for high-throughput detection [34]. A subsequent study investigated the interference in biosensor response due to phosphate and silicate, if any. It was reported that in the presence of arsenite the biosensor responses could be normalized and false positives and negatives could be countered by competitive binding and desequestration, thereby sustaining the biosensor response [35]. Later, the same recombinant

was used for detection of arsenic in rice where it showed a limit of detection as low as 6 ng/g dry wt. of rice [31]. Since the intensity of reporter protein expression is clearly dependent on induction time and cell density as well as on the physiological state of the recombinants, particularly for colorimetric responses, a variation in reproducibility was a concern. To address this, different strains of one recombinant were engineered and used simultaneously comprising a cell line that responded to a range of arsenic concentrations. For *lacZ* reporters the intervening sequence between *arsR* and *lacZ* was modified to modulate expression to get four strains 1580, 1595, 2245, and 2066, which yielded colorimetric responses upon arsenic exposure in concentration ranges <0.001, 0.001–0.1, 0.1–0.67, and >0.67 μM arsenic, respectively. Likewise, another cell line with variation in cytochrome c peroxidase (ccp) expression was engineered with strains 1971, 1981, 1982, 2031, 2332, and 2340 that too responded to arsenic over specific ranges with a lowest detectable concentration of <0.01 μM. This work proposed an internal calibration of the biosensing complement to eliminate limitations mentioned earlier and suggested easier and more practical application for paper strip assays [32].

To accomplish expression of reporter genes, an initial induction by arsenite, the time of which may be up to a few hours as seen in Table 25–1, is necessary, followed by harvesting of cells and treating with an appropriate substrate, in most cases to elicit a measurable response. In the evolving concepts of biosensing such laboratory bound sensing, which is dependent on the shelf-life of microorganisms and their regeneration behavior, limits their applicability. More importantly, the use of genetically modified organisms for on-field detection entails risk assessment and regulatory approvals and any untoward mutation caused by environmental stress, which may lead to physiological implications, cannot be ignored. In addition, a rapid quantitative response would entail proximate coupling of a transducer with the biorecognition element, which was not observed in the above reported works; therefore, a high-throughput detection of arsenic remains unjustified. For this, three necessary inclusions are envisaged: (1) a biorecognition element that negates pretreatment to furnish a response, (2) a confinement matrix for the biorecognition element that compounds the elicited response, and (3) proximate coupling of the biorecognition element with a transducer that affords ultrasensitive read-out. Keeping this in view, it is appropriate to bring attention to nanosensor platforms. Conducting polymers, sol–gels, and metal nanoparticle-impregnated graphenes have been a recent choice for bioanalytical chemists. These, along with new age biorecognition elements, are promising future directions for high-throughput detection of arsenic. One class of such biorecognition elements that has shown tremendous features and has just begun to be used for arsenic detection is *aptamers*, and one class of matrix that has remarkable properties of sensitivity is *graphenes*.

25.3 Nanosensor Platforms—Towards High-Throughput Detection

A class of biosensors, the immunosensors—based on antigen–antibody complex formation—are by far the most specific biosensors reported for protein detection. The unbeatable specificity of

antibodies makes them suitable candidates for biomolecular recognition elements. However, there are some limitations of antibodies that need consideration, such as liability to degrade due to thermal instability, *in vivo* production, labeling that may lead to a loss of affinity, and being raised only against immunogenic molecules. A class of biomolecular recognition elements able to completely overcome these limitations and affording remarkable specificity was reported in 1990 [36,37], the potential of which is being explored in analytics. These molecules, called *aptamers*, are synthetic ssDNA/RNA sequences selected from oligonucleotide libraries by an *in vitro* selection process called SELEX (Systematic Evolution of Ligands by Exponential enrichment) [38], a well-established procedure that selects aptamers employing a combinatorial approach based on their affinity for a particular target. These, along with peptide aptamers, i.e., small peptides with recognition properties and a recent variant of these affinity biomolecules, have significant advantages [39]:

- very high specificities and affinities for targets
- besides proteins and whole cells, ability to synthesize aptamers for non-immunogenic targets like drugs, metals, and inorganic compounds
- *in vitro* synthesis and small in size (usually 15–80 mers)
- terminal tagging without loss of affinity
- long-term stability.

In recent years there have been reports of the use of aptamers, particularly for pathogen detection, antibiotic and toxin testing, clinical diagnostics, and more recently for arsenic detection. It is noteworthy that the sensitivities of detection reported in these works is promising, from nM down to pM concentrations [40,41]. In 2009, the first DNA aptamer for arsenic was reported. Of the nine aptamers synthesized by SELEX, Ars-3, which is an oligonucleotide with a sequence 5′-GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTT TACAGAACAACCAACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTGCAATCT-3′, had the highest binding affinity for As(III). This aptamer was used for arsenic removal from groundwater samples as a treatment technology [42]. More recently, aptamer Ars-3 was used for sensitive detection of As(III) when used in a combination with water soluble cationic polymer polydiallyldimethyl ammonium PDDA and gold nanoparticles, AuNPs. Incubation of Ars-3 and PDDA led to electrostatic coupling in both, thus starving the AuNPs leaving them in a dispersed phase. However, in the presence of As(III) the aptamer/As(III) complex forms instantaneously owing to remarkable binding affinity of the aptamer for arsenic making PDDA available for AuNP aggregation, which was measured spectrophotometrically. The biosensor showed a detection limit of 0.07 μM arsenic. Also, the sensor showed a high selectivity for As(III) against other metal ions, a minor interference caused only by As(V) [43]. In a similar work cationic surfactants were investigated for their efficiency in aggregating AuNPs and also their preferential binding for Ars-3 aptamer. It was observed that cationic surfactant cetyl trimethylammonium bromide (CTAB) had the highest efficiency, attributed to its alkyl chain length. A remarkable feature of this work was a rapid response of less than 2 min and a significantly low detection limit of 0.008 μM As(III) [44]. In yet another nanoparticle-based assay using the same aptamer Ars-3, arsenic was detected by resonance Rayleigh scattering (RRS),

the intensity of which was dependent on nanoassembly of the aptamer assisted by a cationic dye crystal violet to form differently sized nanoparticles, which upon interaction with arsenic changed the size of the nanoparticles due to As(III)-Ars binding to elicit a change in RRS intensity. The limit of detection achieved in this assay of 0.001 μM As(III) along with a near instantaneous response is an outstanding achievement in arsenic biosensors [45]. The precision of these tools in groundwater samples is yet to be established; however, the possibility that they offer rapid and highly selective ultrasensitive detection of arsenic is ascertained.

For all these reasons, aptamers are already a clear choice for biorecognition elements. In the wake of the above and with the focus on arsenic, two kinds of biosensors may be developed using aptamers: one in which the aptamer is synthesized for arsenic, and one in which the aptamer is synthesized for the reporter protein. Since the reporter protein expression entails long induction times, to justify the remarkable capability of aptamers the first option would be the obvious choice. Figure 25–5 shows a schematic that proposes a futuristic approach to arsenic biosensing.

Graphene has attracted a keen interest among analysts due to its tremendous charge carrier ability, large surface area augmenting functionalization, and unique mechanical properties [46]. This exceptional conductivity of graphene gives it the potential to be explored in electrochemical biosensing applications. Coupled with this, this carbon material has the ability to impart extremely high sensitivity of detection due to its 2D structure, wherein even a single molecule adsorbed on its surface can cause resistance to charge transfer. The use of graphene in biosensors is just beginning and it is reported that graphene confers much higher sensitivity than its nanotube counterparts [47,48], a property of immense value in clinical diagnostics. A technical challenge posed with subsequent real-time application of these biosensors would be the occurrence of interfering ions in *in vivo* environments, which would generate overlapping profiles. To resolve the responses, data processing tools like artificial neural networks (ANNs) to perform pattern recognition and quantification of specific analytes among different ions can be used. The coupling of biosensor arrays and data processing tools is known as electronic tongues. The chemometric tools serve to build multivariate response models in such a way that every considered metal ion will generate a signal that is independent of others, thus accounting for cross-selectivity, leading to a consolidated response. These tools have been used in analytics and have made significant contributions to generate electronic tongues [49–52]. Considering the binding affinity of selected aptamers and accounting for false positives for various ions, these chemometric tools are expected to generate data that would identify arsenic with a high degree of accuracy and also differentiate arsenite and arsenate.

The suggested approach for arsenic detection therefore advocates electrochemical transduction using aptamer graphene nanoensembles for ultrasensitive and high-throughput detection. It may be added here that optical transducers may be equally competent in achieving the same, for which Forster resonance energy transfer (FRET) may be used by capping the aptamer with a fluorophore, which upon conjugation with arsenic elicits a conformational change leading to FRET since graphene is an efficient quencher too. Further, these devices have the capability of miniaturization and can therefore be developed into handheld devices, with satisfies the objectives of biosensor development studies.

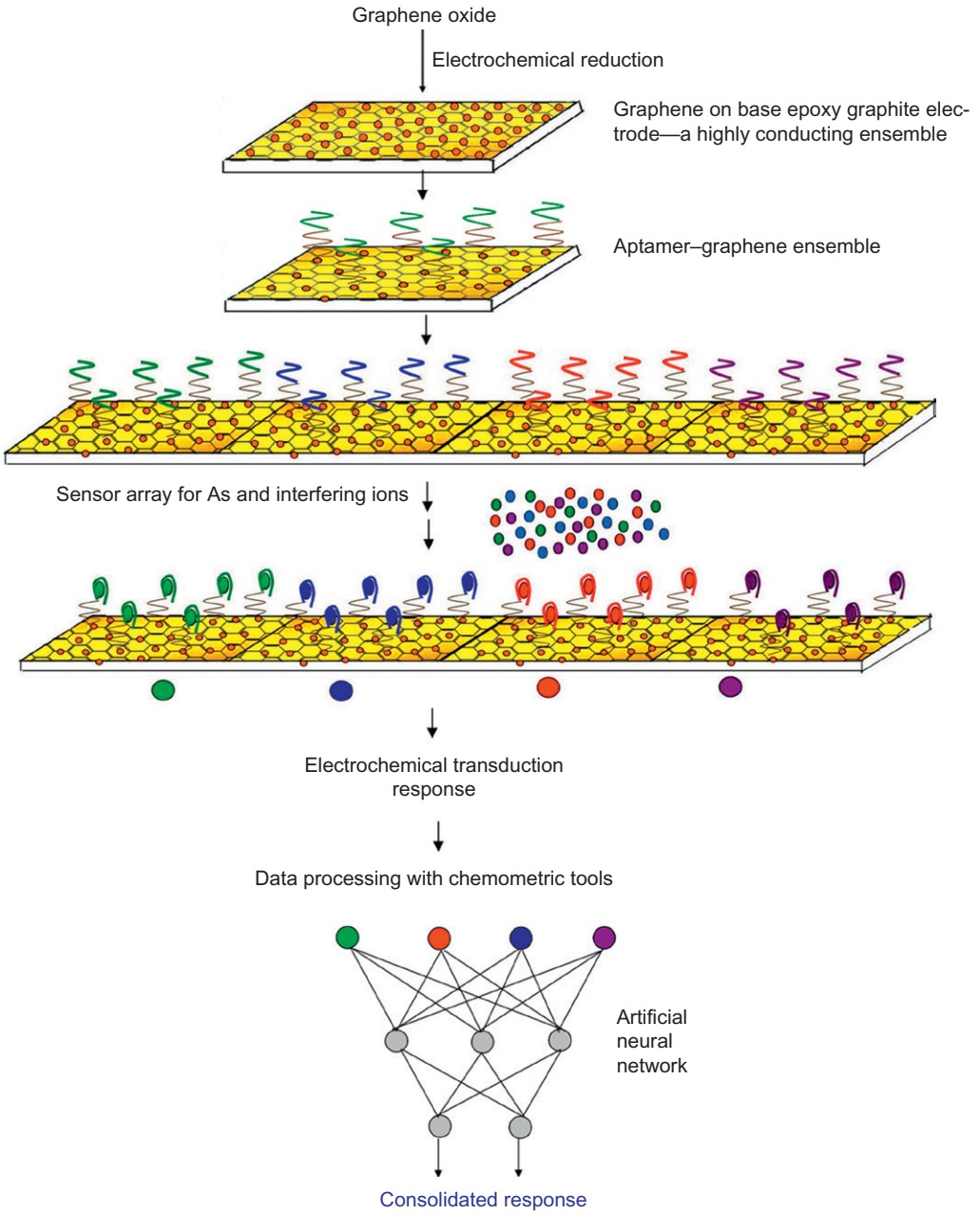

FIGURE 25–5 A schematic depicting a future approach in arsenic biosensing.

25.4 Conclusions and Future Directions

Arsenic poisoning leads to long-term implications that are detrimental to human health and such incidences through consumption of arsenic-contaminated water and food are well known. It is imperative then to ensure sensitive, rapid, and accurate high-throughput detection of this toxic metalloid so that any possible consequence may be dealt with quickly. For this, robust and cost-efficient methods of arsenic detection are needed which entail minimum sample preparation and high selectivity, and which are able to detect arsenic at ultra-low concentrations to ensure regulatory compliance. Where the laboratory bound conventional detection methods pose obvious practical implications for on-site application and hence high-throughput analyses, the chemical methods pose a significant challenge to reliability. The electrochemical methods offer sensitive responses; however, their need for specific working electrodes questions their onsite capability, though these methods are the focus of much research and may provide opportunity in arsenic diagnostics. In this light, the role of biosensors as potential alternatives capable of addressing the aforementioned concerns has been established. Among them, recombinant whole-cell biosensors have offered valuable characteristics particularly in terms of sensitivity. These recombinants, which host engineered plasmids comprising regulatory regions of the *ars* operon fused with reporter genes, were able to elicit responses dependent on arsenic concentration. These biosensors saw further improvements in response characteristics with the advent of newer reporter genes that negated the need for substrates to furnish responses. Some of these biosensors were validated for application in groundwater samples since in these real samples the bioavailability of arsenic is diminished due to the presence of iron oxides, phosphates, and silicates, which has led to false responses. Owing to its biological nature, the whole cell biosensor responses are largely dependent on their physiological state and regeneration characteristics, which challenge the reproducibility of response, and though cell-based systems still provide a platform for biomonitoring, their very long induction times defeat the objective of their development into diagnostic devices. Nanotechnology offers many avenues for improvement, and nanobiosensors, which have impacted diagnostics significantly, are envisaged as the new generation of sensors capable of rapid and ultrasensitive responses offering a clear opportunity for high-throughput detection. Aptamers are such new generation biorecognition elements, which offer immense selectivity, and one aptamer for As(III) has been reported and used as an arsenic sensor showing promising outcomes. These biorecognition elements, which elicit instantaneous responses, are capable of differentiating As(III) from As(V), which highlights the level of selectivity offered by them. It is further suggested to combine the efficacy of aptamers with the remarkable properties of a conducting matrix such as graphene, which will add a new dimension in arsenic analytics towards miniaturized devices for high-throughput on-site detection. It may be reiterated here that no standalone method can offer significant working results, but these new-generation, easy-to-use devices are capable of supporting and augmenting the conventional detection methods and subsequently contribute to methods that can be considered when establishing reference points of action for risk assessments.

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26

Medical Countermeasures—Chelation Therapy

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CHAPTER OUTLINE

26.1 Introduction

Arsenic is a well-known environmental toxicant and long-term exposure thereto may lead to severe clinical manifestations, particularly lung and skin cancer [1]. Various regulatory agencies such as the European Commission [2], the United States Environmental Protection Agency (USEPA) [3], and the World Health Organization (WHO) [4] have revised the maximum concentration of arsenic in drinking water by decreasing it from 50 to 10 μg/L. Chelation therapy is the only promising clinical treatment required for reducing the toxic effects of arsenic. Chelation therapy refers to the administration of a chemical agent to remove toxic metal from the body. The term chelation originated from the Greek word *chele*, which means claw of a lobster, thus representing the concept of clutching or holding with a strong grasp. The major objective of

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chelation therapy is to inhibit the action of intruding metal by sequestering it through the formation of complexes that are rapidly excreted from intracellular and extracellular spaces of the body. Chelating agents can alter the effects of metal toxicity by mobilizing the toxic metal mainly into the urine. A chelating agent forms a stable complex with toxic metal, shielding biological targets, and thus removes the specific toxic metal ion from the desired site in the body. The chemical nature and biological responses differ with different chelating agents.

One of the key features of chelation therapy is to have good chelating agents. It has been suggested that an ideal chelator should have high solubility in water, be resistant to biotransformation, have the ability to reach the sites of metal storage, retain chelating capability at the pH of body fluids, and, most importantly, form metal complexes that are less toxic than the free metal ion [5]. Although chelating agents are the only clinical option available to limit arsenic toxicity, their use is often limited by various side effects and lack of selectivity and specificity. Thus, it becomes imperative to identify new novel chemical compounds that are effective in excreting excess toxic metal/metalloid from the body without altering the physiological ionic homeostasis.

A chelating agent binds to metals at two or more sites, as chelating molecules possess electrons required to form bonds with positively charged metal ions. It can be attached to the metal ion by two or more bonds leading to the formation of a ring referred as a "chelate ring" [6]. However, the complex formed by the metal-chelating agent in the human body is influenced by various factors, including competing metals and ligands, dynamics of circulation, compartmentalization, and metabolism of the chelating agent. Thus, chelation therapy is the most promising medical regime in the field of metal toxicity. This chapter focuses on the use of chelating agents and various other therapeutic strategies available for the treatment of arsenic intoxication.

26.2 Clinical Aspects of Arsenic

Arsenic exists in two oxidative forms, mainly as arsenite (As^{III}) and pentavalent arsenate (As^{V}). When it combines with elements like oxygen, sulfur, and chlorine, it is referred to as inorganic arsenic, and with hydrogen and carbon it is known as organic arsenic. Inorganic arsenic is generally more toxic than organic arsenic [7]. Among inorganic forms, trivalent arsenic is most toxic and reacts with protein thiol groups, whereas the pentavalent form is comparatively less toxic and uncouples oxidative phosphorylation. Moreover, the pentavalent form converts to the trivalent form of arsenic through reduction [8]. Clinically, arsenic affects all organs but it preferentially interferes with the central nervous system, hematopoietic system, and the hepatic and renal systems [9,10]. Globally, more than 200 million people are at risk, residing in areas where groundwater arsenic concentrations exceed the WHO maximum permissible level of 50 mg/L [11], with about 50% of these people residing in Bangladesh and West Bengal, India, who suffer from arsenicosis [12].

It is well reported that arsenic metabolism in cells leads to the generation of various types of reactive oxygen species (ROS). Oxidative stress has been associated with the progression of

arsenic-induced pathological conditions such as carcinogenicity. Reactive nitrogen species (RNS) are also produced in response to arsenic and cause oxidative damage to lipids, proteins, and DNA in cells [13,14]. Depending on the oxidative state of arsenic, it affects the cells by one of two main mechanisms [15]: (1) trivalent arsenite (As^{III}) binds with sulfhydryl groups of critical enzymes that deplete lipoate, which plays a critical role in the synthesis of Krebs cycle intermediates—depletion of lipoate inhibits Krebs cycle and oxidative phosphorylation resulting in ATP depletion [16]; and (2) pentavalent arsenate (As^{V}), on the other hand, replaces the stable phosphate ester bond in ATP resulting in uncoupling of oxidative phosphorylation and depleted ATP stores [16,17]. Experimental evidence suggests that arsenic-induced generation of free radicals can cause cell death through the activation of various oxidative-sensitive signaling pathways [18].

Arsenic is a carcinogenic toxicant and its chronic exposure leads to severe health complications such as tumors of the lung, skin, liver, bladder and kidney, skin lesions, peripheral neuropathy, and anemia [19]. Das et al. [20] reported cardiovascular disorders following oral exposure to arsenic. There is also evidence from epidemiological trials that inhaled inorganic arsenic may affect the cardiovascular system. Epidemiological evidence on the association between arsenic and cardiovascular outcomes in Taiwan has been conducted and has estimated the chances of relative risks for coronary disease, stroke, and for peripheral arterial disease [21].

There is another interesting study in which the association between intellectual deficiencies in children and exposure to arsenic has been reported. Adolescents from various regions of Taiwan and China, exposed to low (0.0017–0.0018 mg As/kg/day) levels of inorganic arsenic in drinking water, showed decreased performance in the switching attention task, while children in the high exposure group (0.0034–0.0042 mg As/kg/day) showed decreased performance in both the switching attention task and tests of pattern memory, relative to unexposed controls [22]. Moreover, excessive ingestion of arsenic compounds like arsenic trisulfide could act as suicide agents [23].

26.3 Diagnosis

Since arsenic is a ubiquitously found metalloid, escaping from its exposure is inevitable, especially in areas endemic to arsenic-contaminated drinking water. Thus, diagnosis of arsenic levels in humans is an important aspect of chelation therapy. Arsenic levels can be measured from various body fluids; urine and blood, but also hair and nail samples are the most common sources. These sources act as good biomarkers for arsenic poisoning, for instance: (1) urine is the most preferred biomarker for arsenic exposure with a reference value of <25 μg/dL. For the measurement of arsenic concentration, 8–24-hour urine should be collected. A "spot" of urine sample is not considered but it may be helpful in acute poisoning. Generally, two methods are employed for measuring arsenic in urine samples—fractionated (speciated) measurement for inorganic and organic arsenic, and total arsenic level. However, this test could be carried out within 1 week after seafood abstinence to avoid false-positive results. (2) Blood

arsenic levels are rarely used because of the short half-life of arsenic in blood as well as background exposure levels of arsenic in normal blood. Blood arsenic levels are <3 µg/dL. (3) While hair and nails are considered to be good indicators for chronic arsenic exposure the levels of As therein have limited significance due to external contamination.

26.4 Chelation Therapy

26.4.1 Concept

Chelating agents are chemical compounds whose structures permit the attachment of their two or more donor atoms (or sites) to the same metal ion simultaneously and produce one or more rings. These molecules are also called "chelates" or chelating groups, and the formation of rings is called "chelation." These metal complexes have the ability to resolve into optically active (R&L) forms. Stability of metal complexes differs with pattern of complex formation and difference in stability becomes more relevant in the increasingly dilute solutions in biological systems such as serum or tissue. The toxicokinetics and toxicodynamics of metal and chelating agents are an integral part of an effective chelation therapy in addition to the following criteria, which also need to be fulfilled:

- High affinity for the toxic metal
- Low affinity for essential metals
- Minimal toxicity
- Lipid solubility
- Good absorbability from the gastrointestinal tract.

These criteria are not easy to fulfill as there is yet no available chelating agent that exhibits all the above-mentioned points. Lipid soluble chelators easily cross the cell membrane and bind metals within the cell but chelators that are not lipid soluble are usually more toxic. Thus, it is difficult for lipophobic chelators to find optimal conditions for binding specific toxic metal with minimal risk of adverse effects. Most of the metals encountered generally react with O-, S-, and N-containing ligands because they contain lone pairs of electrons (Figure 26–1). A simplified mechanism by which a chelating agent can detoxify a toxic metal may be depicted as:

$$M^{n+} + \text{chelating agent} = M^{n+} - \text{chelating agent complex}$$

where M^{n+} is the toxic metal cation that binds to a chelating agent and forms an insoluble complex *in vivo*.

The process of chelation depends on both the nature and the properties of the metal and those of the chelating agent, such as ionic diameter, ring size and deformability, and hardness or softness of the electron donors and acceptors. It also depends on factors such as route of administration, bioavailability, metabolism, organ and intra/extracellular compartmentalization, and excretion [24].

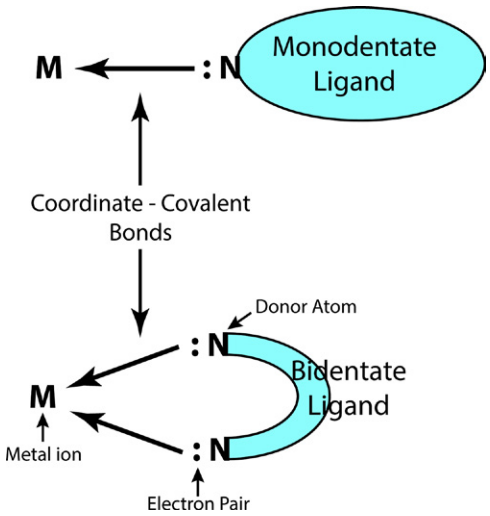


FIGURE 26–1 Showing the monodentate and bidentate ligands along with donor atom and metal.

26.4.2 Chemistry

The chemistry involved provides an understanding about metal transport, metal binding constants, and ion specificity in relation to metal-ion exchange kinetics. These processes/parameters introduce opportunities for metal-ion-related therapy, which goes beyond traditional chelate-based metal-ion detoxification. Hardness–softness character and the chelating effect determine the following properties of the chelator–metal complex: toxicity of chelator, side effects of the chelator, hydrophilicity/lipophilicity of the chelating agent, hydrophilicity/lipophilicity of the resulting metal complex, and stability of the metal–chelator complex. To design a chelating agent for a toxic metal ion, the following characteristics must be kept in mind about the toxic metal:

- Coordination number
- Net ionic charge
- Stereochemistry.

The chelating agent occupies a coordination position in a metal ion. It generally forms a complex of greater stability compared to those complexes with chelating agents that occupy fewer positions. Metal ion forms bond with ligands, which can share electron pairs and the formation represents the "coordination sphere" of the metal ion. Metal atoms can form complexes with a coordination number from 2 to 8. The atoms present in the coordination sphere undergo an alteration when a toxic metal is consumed and absorbed by any organism. The bond formation between the metal ions and several types of donor atoms takes place to acquire a more stable characteristic coordination sphere in the biological system. Most commonly found donor atoms in living systems include oxygen atoms (water, carbonyl and phenolic groups of amino acids, phosphate groups, etc.), nitrogen atoms (amino acids, nucleotides,

etc.), and sulfur atoms (cysteine- and thiol-containing compounds such as methionine, etc.). A toxic metal coordinates with ligands of a physiologically essential molecule like an enzyme, messenger molecule, or DNA resulting in their decreased reactivity. These changes in the reactivity of the essential molecules are responsible for the biologically adverse effects of toxic metal.

Chelating agents excrete intracellular deposits of toxic metals by four common pathways: (1) non-polar chelators passing through the lipid portion of the cellular membrane, (2) electrically neutral polar chelators passing through the cellular membrane, (3) mono-anions passing through appropriate mono-anion transport systems, and (4) di-anions passing through appropriate di-anion transport systems. The use of a chelating agent restricted to the extracellular space leads to a large reduction of the toxic metal, which usually favors the diffusion of some of the toxic metal from intracellular sites to extracellular sites [25] (Figure 26–2).

The clinical efficacy of a chelating agent is governed by its ability to shift the toxic metal ion from biomolecules such as enzymes and DNA, and subsequently reactivate. In this process, the toxic metal–chelate complex, which is lipophilic in nature, is excreted from the body and so leaves the metal unavailable for attaching to another biomolecule.

26.4.3 Chemical Considerations

26.4.3.1 Thermodynamics of Metal Chelation

In complexation reactions of metals with "n" molecules of the monodentate ligand L or with one molecule of the n dentate ligand represented by Ψ:

$$M + nL \rightarrow ML_n \tag{26.1}$$

$$M + \Psi \rightarrow M\Psi \tag{26.2}$$

The overall stability constants can be expressed by

$$\beta_L = \frac{[ML_n]}{[M][L]^n} \tag{26.3}$$

$$\beta_\Psi = \frac{[M\Psi]}{[M][\Psi]^n} \tag{26.4}$$

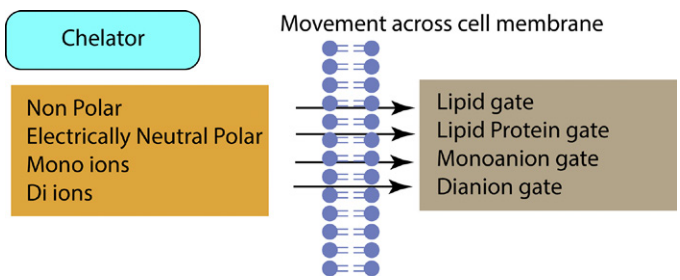


FIGURE 26–2 Excretion of chelate compounds from intracellular sites to extracellular sites is regulated by various channels. The channels are specific for chelators on the basis of polarity and ionic charge.

The stability of a complex depends on

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT\ln\beta \tag{26.5}$$

During complex formation of a metal with n monodentate ligands, the following changes take place:

- Enthalpy changes associated with bonding, which is proportional to the free energy
- Entropy changes, directly proportional to the ordering of ligands around the ion, which counteract the entropy effect of desolvation.

If the number of ligands enters one molecule of a multi-dentate ligand, the entropy support from desolvation and the complex stability may increase.

26.4.3.2 Kinetic Considerations in Metal Chelation

Chelating agents possess "ligand," which binds atoms capable of forming two covalent or coordinate linkages in the case of bidentate chelates. Ligands comprise atoms like S, N, and O, which function in the form of chemical groups like –SH, –S–S, $-NH_2$, =NH, –OH, $-OPO_3H$, or >C=O [26] (Figure 26–3). Bidentate or multi-dentate ligands form ring structures, which include metal ion and the two ligand atoms. Most of the donors act as bidentate ligands [5].

26.4.3.3 In Vivo Efficacy of Chelating Agents

Toxic metals are generally present in "free" concentrations because of small biological ligands available in the biological system, which may form mixed aqua-bioligand complexes with metals. Complexation reactions *in vivo* most often occur as a series of ligand or metal exchange reactions between toxic metals and chelating agents. Equilibrium constant and the complex formation are highly favorable but are limited due to factors like (1) rate effects, (2) competition with other ligands/metals, and (3) systemic transport kinetics of the chelator. Formation of a complex between chelator and toxic metal and quantitative urinary excretion of the ML complex (at equilibrium), and the efficiency E of a chelating agent for mobilizing a toxic metal, can be described as:

$$M + L \leftrightarrow [ML] \tag{26.6}$$

$$E = \frac{ML}{M} \tag{26.7}$$

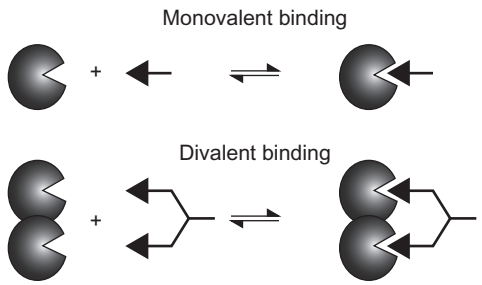


FIGURE 26–3 Binding of the monodentate and bidentate.

However, the potential for mobilizing metal depends on the degree of formation of the *ML* complex. In this context, considering one of the important biological competing metals, Ca(II), and total chelator concentration, L_t, the conditions for a large E can be described from the standard stability constant [27]:

$$\frac{ML}{M} = \beta_{ML}[L] \tag{26.8}$$

Chelation efficiency depends on the chemical and biological characteristics of the metal, the chelator, and the organism. The main focus in most clinical uses is mainly on the mobilization of the toxic metal. Therefore, a chelating agent forms a stable complex with a toxic metal and shields the biological targets from the metal ions and reduce the toxicity soon after administration when mobilization has not yet occurred. It may expose the metal to the biological environment and prevent the metal from being scavenged by biological protective mechanisms, which leads to increased toxicity of the metal.

Metal ions have a critical role in the biological system, either beneficial or pathological. However, there are a few misconceptions about a chelating agent: for example, it will inhibit biological activity and selectivity, and the outcome. In complex biological environments such as exists in humans, it is very difficult to have an exquisite selectivity for one specific metal ion over other metals. In addition, the metal ion sequestered by the chelators leads to the formation of a metal complex, which itself may have some biological effect [28].

Chelation plays an important role in analytical chemistry as it finds a place in toxicity, mechanisms, and therapy. Arsenic exerts its adverse effects by forming complexes with enzymes, DNA, and other biomolecules in the cell. Chelating agents *per se* are added exogenously to a biological system and may bind to endogenous ligands for the removal of arsenic. They must also effectively compete for the removal of arsenic *in vivo*. A chemical molecule that qualifies as an ideal chelator in an *in vitro* model may not necessarily also act in a similar way *in vivo.* There might be number of other considerations including toxicity or competition with other endogenous substances such as hemoglobin and cytochromes, which also bind metals and thus might also act as chelators. Selectivity of a chelator *in vivo* is an important factor to be considered in chelation therapy. pH is another important factor, which may influence complex formation and stability. Many chelating agents are shown to be unstable at low pH; while at higher pH, metals tend to form insoluble hydroxides, which become less accessible to chelating agents. This condition is particularly significant in pathological conditions leading to acidosis or alkalosis.

26.4.4 Toxicokinetics of Chelation

Chelating agents are rapidly excreted over a few hours or days while the toxic metals accumulate for prolonged periods and thus are distributed to various organs. Thus, it is not easy for all the chelating agents to reach the desired site. A chelating agent will mobilize the most readily available metal first, typically in the plasma, kidney, and liver, and then to a lesser extent

in bone and the central nervous system. The most readily accessed "pools" of toxic elements will be depleted due to repeated doses, but re-equilibration/redistribution slowly replenishes the toxic elements in more accessible body compartments. This is evident from the rebound levels of arsenic in the blood after discontinuation of administration of a chelator, which highlights three important facts: (1) blood and urine are poor substitutes to measure toxins accrued over the lifetime, although they are the most commonly used biomarkers to detect arsenic. (2) Sequestration of toxic metals in bone and soft tissues does not signify their complete immobilization; they return to the bloodstream and tissues where they once again exert toxic effects. Thus it is necessary to acquire an understanding of the quantities of biologically accessible toxic elements within the body that are not necessarily reflected in baseline blood or urine levels, before proceeding with chelation therapy. (3) Chelation therapy induces a shift of both essential and toxic cations in the body [29] (Figure 26–4).

The toxicokinetics of a chelating agent depends on various factors such as (1) hydrophilicity/lipophilicity of the compound, (2) whether it chelates extracellular or intracellular metal deposits, (3) the best route of administration (oral vs. intravenous infusion). Moreover, it also determines the hydrophilicity/lipophilicity of the formed metal complex. Urinary excretion is the main mode to remove lipophilic complexes, but due to reabsorption, excretion of these complexes is decreased, which alters the metal's organ distribution. Moreover, chelators forming lipophilic metal complexes enhance intestinal metal uptake, which potentially enhances the toxicity of the metal [27]. The oral route for administration of chelating agents is preferred and comprises emptying of the GI system of the toxic metal and removal of the subject from further exposure to avoid increased intestinal metal absorption. However, an orally administered chelating agent forms hydrophilic metal complexes, which may efficiently reduce intestinal metal uptake resulting in local toxicity after oral intoxication [30,31].

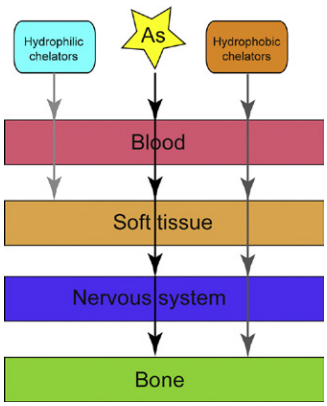


FIGURE 26–4 Toxicokinetics of hydrophilic and lipophilic chelators suggests removal of arsenic. Hydrophilic chelators are unable to cross the blood–brain barrier while lipophilic chelators can easily enter soft and hard tissues and bring about effective removal of arsenic from both extra- and intracellular sites.

26.5 Chelators in Clinical Use

Chelating agents have been used as antidotes for metal intoxication in humans since the Second World War. One of the first applied chelators was British anti-Lewisite (BAL), a chelating drug developed during World War II in the UK as an antidote for the arsenic-containing warfare agent Lewisite. Later, BAL was also used to sequester arsenicals, gold, and mercury [32]. Meso-2,3-dimercaptosuccinic acid (DMSA), sodium 2,3-dimercaptopropane 1-sulfonate (DMPS) and D-penicillamine (D-PA) were also introduced for the clinical use after BAL.

Today, these drugs together with a wide array of newly synthesized chelating agents are in clinical use or under preclinical or clinical investigations for treatment of intoxication or overload caused by various transition metals including metalloids like arsenic. However, in the case of arsenic poisoning, chelation following chronic exposure has been shown to increase excretion but in terms of potential clinical efficacy (decreased mortality and morbidity) this is still debatable. However, *in vivo* studies have shown that immediate treatment with BAL, DMPS, and DMSA can avert the adverse effects of inorganic arsenic [33].

26.5.1 Chelating Agents for Arsenic Poisoning

26.5.1.1 Dimercaprol (BAL)

26.5.1.1.1 CHEMISTRY, PHARMACOKINETICS, AND PHARMACODYNAMICS

British anti-Lewisite (BAL) or dimercaprol (2,3-dimercapto-1-propanol) is a lipophilic drug and on administration is distributed both intra- and extracellularly (Table 26–1) [34]. Its utilization during World War II minimized the risk of injury or death to the Allied infantry from Lewisite. BAL also revolutionized the treatment of heavy metal poisoning. BAL is a dithiol compound containing two sulfhydryl groups, which form a stable, relatively non-toxic five-membered heterocyclic chelate ring with arsenic.

BAL is generally administered at a dose of 3–5 mg/kg intramuscularly every 4 hours for 2–10 days depending on the toxic side effects [35]. However, in acute cases doses may be increased 4–5 mg/kg every 4 hours for the first 24 hours. BAL has a short half-life and is rapidly absorbed (30–60 min), metabolized, and excreted (within 4 hours) from the biological system [38,43]. Early administration of BAL after exposure could effectively and efficiently prevent the inhibition of sulfhydryl enzymes [26,38].

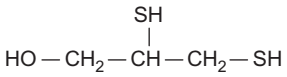
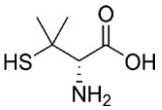
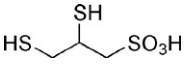
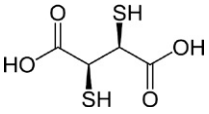
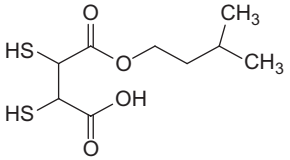
26.5.1.1.2 EFFICACY AND EXPERIMENTAL STUDIES

BAL is an effective chelator for acute arsenic poisoning (inorganic or elemental) [38,44–47]. Interestingly, BAL is the most toxic commercially available chelating agent compared to its other derivatives such as meso-DMSA and DMPS. Intraperitoneal lethal dose (LD_{50}) of BAL is 0.85 mmol/kg in rats and 1.5 mmol/kg in mice [48,49].

26.5.1.1.3 MECHANISM OF ACTION

BAL has two –SH groups in its structure and competes with the thiol groups of enzymes for binding with arsenic or other metals to form a stable metal–chelate complex. The formed complex

Table 26–1 Structures, Route of Administrations, and Doses of Chelators

Chelating Agent	Structure	Route of Administration/ Dose	Limitations	References
BAL		Administration of BAL is typically 3–5 mg/kg intramuscularly every 4 hours for 2–10 days	Low therapeutic index, painful intramuscular injection, tendency to redistribute arsenic to brain and testes	[35]
D-penicillamine		4 daily doses of 25 mg/kg/dose orally	Reversible hepatic and renal dysfunction, and autoimmune diseases	[36,37]
DMPS		200 to 400 mg daily doses orally in adults, intravenous doses of 250 mg every 4 to 6 hours and parenteral route	Headache, fatigue, nausea, taste impairment, pruritus, and rash	[38]
m-DMSA		Administered parenterally or orally in doses of 30 mg/kg/day for 5–7 days followed by 20 mg/kg/day for 1–3 weeks	DMSA not able to remove metals from hard tissues and intracellular sites	[39,40]
MiADMSA		Oral administration of MiADMSA was more effective than intraperitoneal administration and the minimum effective dose with least side effects was 50 mg/kg	Essential metal loss (copper and zinc)	[41,42]

is then excreted from the body through urine [6]. BAL forms heterocyclic ring complexes with metals as shown in Figure 26–5. Numerous *in vivo* reports suggest that BAL exhibits some side effects like nephrotoxicity and hypertension. The metabolism of BAL has not been well described but it is rapidly metabolized to inactive products such as glucuronide conjugate. If the BAL–metal complex is oxidized the metal is released and exerts its toxic effects. Thus, the dosage of BAL must be high enough to assure the excess of free BAL available in body fluids until the metal is completely excreted [50,51].

26.5.1.1.4 HUMAN CASES

Physicians recommend BAL for arsenic removal even though more novel chelating agents exist [52]. BAL therapy should continue until urinary arsenic levels are less than 50 μg/L per

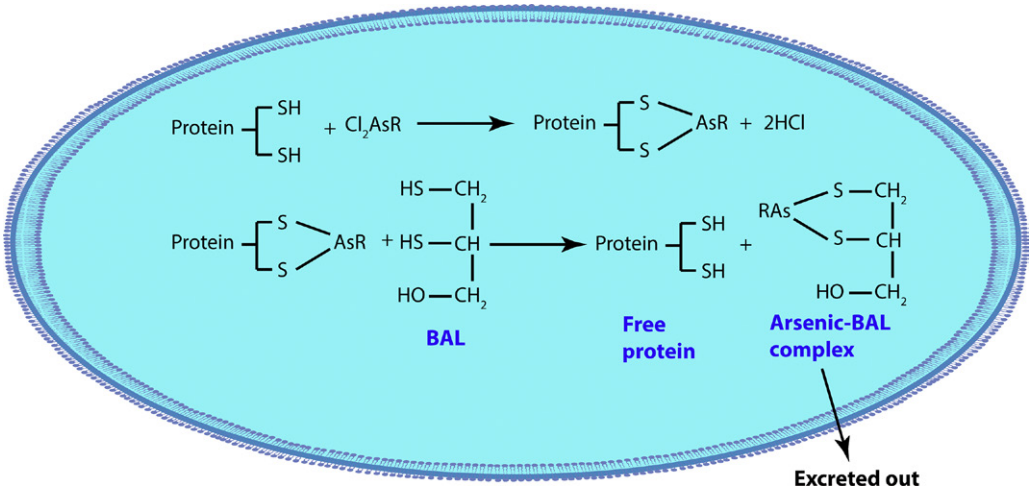


FIGURE 26–5 Mechanism of action of BAL.

24 hours. Chelation is rarely needed outside the setting of symptomatic acute poisoning. However, if chelation is prescribed, succimer is the preferred agent given at a dose of 10 mg/kg orally three times a day for 5 days, then twice a day for 14 days. Two family members who were continuously drinking arsenic-contaminated water for nearly 5 years developed several dermatological changes like hyperkeratosis and hypo- and hyperpigmented lesions (raindrops) on the abdomen, chest, and back. Histopathological examination also confirmed the inflammatory response of a papillary type. Arsenic levels in the nails and hair of both patients were found to be very high. This was probably the first report of successful treatment with dimercaprol.

26.5.1.1.5 DRAWBACKS

BAL is unstable and easily oxidized, thus it is difficult to store and requires immediate use after preparation. Besides rapid mobilization of arsenic from the body, it also causes significant elevation of brain arsenic levels [5]. Administration of BAL requires deep intramuscular injection that is extremely painful and, due to its oily nature, causative of allergy [53]. It has also been administered intraperitoneally or orally in rodents at LD_{50} values of BAL. It has a number of side effects such as: (1) injections of BAL being painful due to painful release of BAL in the tissues, (2) gastrointestinal symptoms like nausea and vomiting, dose-related hypertension, and tachycardia, and (3) nephrotoxicity, seizures, hyperpyrexia, sweating, lacrimation and rhinorrhea, urticaria, and paresthesia [38,54]. The vehicle used for BAL is peanut oil and it should be used with extreme caution in patients with peanut allergies.

Two other water soluble analogues of dimercaprol have also been studied as Lewisite antidotes. These are meso-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-1-propane sulfonic acid.

26.5.1.2 D-Penicillamine (D-PA)

26.5.1.2.1 CHEMISTRY, PHARMACOKINETICS, AND PHARMACODYNAMICS

Penicillamine is a D-B, B-dimethylcysteine, a penicillin degradation product (Table 26–1), used since 1957 as the only approved chelator for lead poisoning until DMSA became available. It can also chelate arsenic [55]. It is absorbed rapidly from the GI tract, although it has an oral bioavailability of 40 to 70% but the effects are not dose dependent [56] and various foods, antacids, and iron salts decrease its absorption from the gut. The peak concentration in blood plasma is reached within 1 to 3 hours regardless of the dose. It forms disulfide bonds with many proteins in the blood such as albumin and homocysteine, and in tissues [57]. A small portion of the parent compound is metabolized to S-methylpenicillamine in the liver and is eliminated in feces. However, the primary route of elimination is the kidneys and the half-life of unchanged penicillamine after a single dose ranges from 1.6 to 3.6 hours. The elimination of penicillamine is increased from 4 to 6 days after a steady concentration is achieved. It was suggested that penicillamine is slowly released from deep tissues and skin [58].

26.5.1.2.2 EFFICACY AND EXPERIMENTAL STUDIES

D-PA has rarely been reported as an effective agent in the treatment of arsenic poisoning. However, oral chelation therapy with D-PA has been shown to be effective in the mobilization of arsenic [59]. Furthermore, arsenic toxicity symptoms manifested post-ingestion could be augmented with D-PA therapy [59].

26.5.1.2.3 MECHANISM OF ACTION

Penicillamine contains a sulfhydryl group, which combines with toxic metals to form ring compounds and increases its elimination. This complex is further excreted through the kidneys and feces.

26.5.1.2.4 HUMAN STUDIES

In cases of acute arsenic poisoning, immediate chelation is required. A case report suggested the effectiveness of D-PA in three children exposed to arsenic and they were treated with four daily doses of 25 mg/kg each [36]. The recommended dose of penicillamine is 25 mg/kg. Compared to other chelators like DMSA, DMPS, and BAL, penicillamine is found to be less effective in arsenic poisoning [60]. The dose of penicillamine in the United States is 30 to 40 mg/kg/day or 600 to 750 mg/square meter/day for 1 to 6 months, given 2 hours before or 3 hours after meals [61].

26.5.1.2.5 DRAWBACKS

The use of penicillamine is limited due to its adverse effects, which led to the development of the thiol chelators such as DMSA and DMPS. The adverse effects include nausea, vomiting, hematuria, bone marrow depression with leukopenia, rashes, proteinuria, hemolytic anemia, gastrointestinal distress, reversible hepatic and renal dysfunction, and autoimmune diseases [37].

26.5.1.3 Meso-2,3-Dimercaptosuccinic Acid (Succimer, DMSA)

26.5.1.3.1 CHEMISTRY, PHARMACOKINETICS, AND PHARMACODYNAMICS

Succimer, meso-2,3-dimercaptosuccinic acid (DMSA), is an orally active and less toxic analogue of dimercaprol (Table 26–1). DMSA is a weak acid with four ionizable hydrogens that are ionized at pH 7.4 but lack the ability to enter cells (Table 26–1) [62]. In the meso-DMSA structure, the toxic metals can coordinate with one sulfur and one oxygen atom in the case of lead and cadmium, or with each of the two sulfur atoms in the case of mercury [63,64]. However, there are many reports regarding its effectiveness and low toxicity in the treatment of arsenic in the Soviet and Chinese literature [27]. Smith and Strupp [65] reported the efficacy and clinical utility of DMSA in rodents and non-human studies. It may be given parenterally or orally in doses of 30 mg/kg/day for 5–7 days followed by 20 mg/kg/day for 1–3 weeks; it is rapidly excreted in urine. In adults, the optimum dose is 30 mg/kg/day for 5 days [39]. It is incompletely absorbed on oral administration with peak plasma levels occurring approximately 1 to 2 hours after administration. It is extensively metabolized and only 25% of the dose is excreted in urine and remains in the feces, which represents the unabsorbed drug [38].

26.5.1.3.2 EFFICACY AND EXPERIMENTAL STUDIES

DMSA is also a preferred lead poisoning antidote, particularly in children as it is less toxic than DMPS. Meso-DMSA has multiple advantages over other chelators, such as low toxicity, oral administration, and no redistribution of metal from one organ to another. Despite its being preferred as a lead antidote, it has been shown to be successful in animal and human cases in arsenic poisoning [66,67]. DMSA has been shown to provide 80–100% survival in mice from lethal manifestations of arsenic after subcutaneous injection [62]. A significant depletion of arsenic and a significant recovery in the altered biochemical variables of chronically arsenic-exposed rats have been reported [53]. This drug thus can be effective if administered orally or intraperitoneally. Flora [68] reported that arsenic-induced oxidative stress in various tissues of rats can be mitigated by pharmacological intervention that encompasses combined treatment with N-acetylcysteine and DMSA.

The toxicity of meso-DMSA is low when given in rats intraperitoneally [69]. However, a contraindication for meso-DMSA therapy is hypersensitivity to the drug. Clinical studies indicate that meso-DMSA in therapeutic doses has no significant effect on renal excretion of essential metals like iron, calcium, and magnesium, with the exception of zinc, where excretion is doubled.

26.5.1.3.3 MECHANISM OF ACTION

Meso-DMSA binds metal cations through the thiol groups, which ionize upon complexation, as shown in Figure 26–6. The monoesters of DMSA were found to be more effective than the parent compound, DMSA. The complexes of DMSA monoesters seem to penetrate cells, which helps in targeting intracellular sites in the body and aids in the removal of arsenic in the cytosol and from organelles inside the cell.

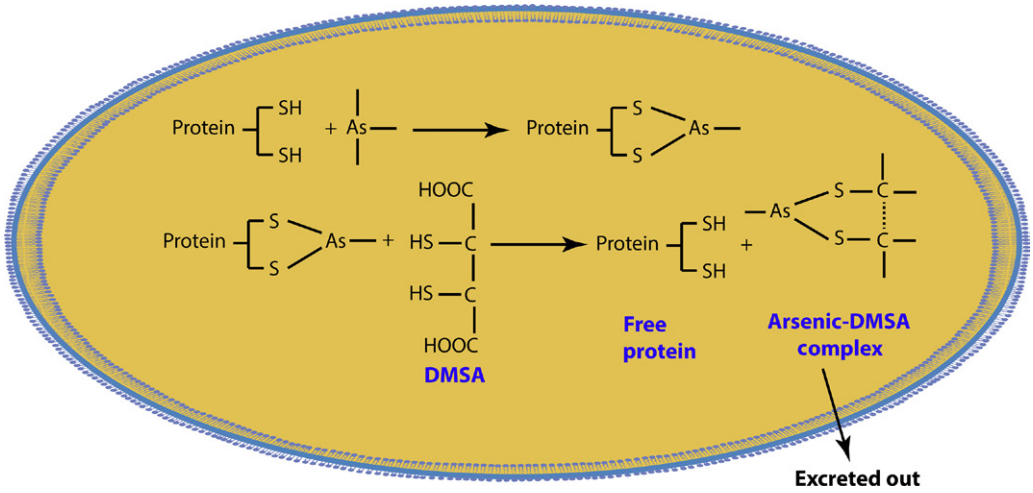


FIGURE 26–6 Mechanism of complex formation of DMSA.

26.5.1.3.4 HUMAN STUDIES

In humans, more than 90% of meso-DMSA in urine is excreted as mixed DMSA–cysteine disulfide conjugates with the remainder excreted unchanged [70]. Although the half-life of DMSA is reported to be about 2 hours, Dart et al. [71] reported that this could be up to 2 days. Meso-DMSA has been reported to be an effective chelator for arsenicosis [39,66,67,70]. In general, patient therapy with 30 mg/kg DMSA daily for 5 days has demonstrated a significant increase in arsenic excretion with clinical improvement. In cases of acute ingestion of arsenic (2 g), a therapy regime of 300 mg DMSA, orally every 6 hours for 3 days, is suggested to show better clinical recoveries. The dose could be reduced to 10 mg/kg, thrice daily for 5–7 days, followed by two daily doses of 10 mg/kg for another 10–14 days, in cases of mild arsenic poisoning. However, in cases of chronic arsenic poisoning the dose could be increased to 18 mg/kg with a similar dose regime to that for mild poisoning [72].

However, a double-blind, randomized, controlled trial study conducted on a few selected patients suffering from arsenic intoxications in West Bengal (India) with oral administration of DMSA suggested that it was not effective in producing any clinical or biochemical benefits or eliciting histopathological improvements in skin lesions [73]. Moreover, renal toxicity has also been associated with excretion of large amounts of chelated metals that pass through the renal tubules in a relatively short period during therapy.

26.5.1.3.5 DRAWBACKS

One of the main drawbacks of DMSA is its extracellular distribution, making it incapable of removing arsenic from intracellular sites. The side effects of DMSA include gastrointestinal discomfort, skin reaction, mild neutropenia, and elevated liver enzymes. Some evidence of embryo toxicity/fetal toxicity due to DMSA administration was also reported [40]. Precautions

are necessary in patients affected with renal or liver impairment, neutropenia, and glucose-6-phosphate dehydrogenase deficiency [74].

26.5.1.4 2,3-Dimercaptopropane-1-Sulfonic Acid

26.5.1.4.1 CHEMISTRY, PHARMACOKINETICS, AND PHARMACODYNAMICS

2,3-Dimercaptopropane-1-sulfonic Acid (DMPS) is a sodium salt of 2,3-dimercaptopropane-1-sulfonic acid, a derivative of BAL (Table 26–1). Presence of the sulfonic acid group renders DMPS water soluble. DMPS enters the intracellular compartment through an organic anion transport pathway since it is distributed extracellularly [34,75]. DMPS is a unithiol and could be administered orally or parenterally. It has also been administered intramuscularly as a 5% solution at a dose of 5 mg/kg, three or four times during the first 24 hours followed by two or three times on the second day, and once or twice on subsequent days. It is rapidly metabolized to acyclic and cyclic disulfides [76,77]. Half-life of the drug was found to be 10 hours [78] and after oral dosing in humans, peak plasma concentration of the total drug metabolized is reached in approximately 3.5 hours. However, if given intravenously, half-life of the parent drug is 1.8 hours and total DMPS is 20 hours [76]. In humans, arsenic and DMPS complexes are primarily removed by renal excretion [38,77]; however, in experimental animals, these complexes could also be removed by biliary excretion [79]. In adults, oral administration of 200 to 400 mg daily doses and intravenous doses of 250 mg every 4 to 6 hours [38] is recommended.

26.5.1.4.2 EFFICACY AND EXPERIMENTAL STUDIES

DMPS is an effective chelating agent for acute or chronic arsenic poisoning. It is less toxic than dimercaprol and has been used extensively in Russia and the Western world [26,34]. No major adverse effects are reported following DMPS administration in humans or animals [80]. DMPS has the ability to inhibit both arsenate-induced acute toxicity and regenerative proliferation of the rat bladder epithelium by decreasing exposure of the urothelium to trivalent arsenicals excreted in the urine [81]. Both DMSA and sodium DMPS have been reported to be useful in the prevention of arsenite- and arsenate-induced developmental toxicity in mice [82–84].

26.5.1.4.3 MECHANISM OF ACTION

Sodium DMPS may form an insoluble complex with arsenic that is firmly bound to intracellular sites. It also makes the drug effective in cases of slow, low-dose, and chronic arsenic poisoning because metal reaches the cellular compartments following the physiological barriers including the blood–brain barrier [85].

26.5.1.4.4 HUMAN STUDIES

Aposhian et al. [86] reported that DMPS has a highly specific effect on MMA metabolism or urinary excretion in humans, although the mechanism by which DMPS reduces arsenic burden is not fully established [72,87]. A study in Mongolia found that after DMPS therapy, (1) MMAIII was detected in human urine suggesting DMPS enhanced MMA excretion, (2) reduced levels of DMA were noted in human urine samples, and (3) the DMPS–MMAIII complexes were inactive as *in vitro* substrate for rabbit liver. This study concluded that DMPS

formed complexes with MMAIII, which inhibited further methylation of MMAIII to DMA and decreased DMA levels as well as increased excretion of the DMPS–MMAIII complex in the urine [88]. Guha Mazumder and co-workers observed that in the case of chronic arsenic poisoning, DMPS is superior to meso-DMSA [89]. DMPS has also been used as a challenging test for arsenic [77] and is approved for the treatment of arsenic intoxication in the People's Republic of China. Heitland and Köster [90] determined inorganic arsenic species As(III), As(V), and the organic species methylarsonate (MMA(V)), dimethylarsinate (DMA(V)), and arsenobetaine (AsB) in human urine by the fast anion exchange HPLC-ICP-MS method.

26.5.1.4.5 DRAWBACKS

Hruby and Donner [80] reported no major adverse effects following DMPS administration in humans or animals. However, a dose-dependent decrease in copper contents was found in the serum, liver, kidneys, and spleen [91]. No abnormalities in offspring with chronic oral DMPS treatment were reported. Oral administration of DMPS did not affect late gestation, parturition, or lactation in mature mice and fetal and neonatal development [40]. However, some minor adverse effects such as headache, fatigue, nausea, taste impairment, pruritus, and rash have been reported [38].

26.6 Analogues of DMSA as Potential New Arsenic Chelators

26.6.1 Monoisoamyl DMSA (MiADMSA)

In order to increase the efficacy of DMSA from just an extracellular chelator to an intracellular one, a number of DMSA analogues have been synthesized as well as tested for their efficacy against arsenic intoxication. Out of a multiplicity of analogues synthesized, monoisoamyl DMSA (MiADMSA), monomethyl DMSA (MmDMSA), and monocyclohexyl DMSA (MchDMSA) are three examples. However, MiADMSA has been shown to be the most effect analogue and is currently under phase I clinical trials. MiADMSA has been found to potentiate the synthesis of glutathione in liver and brain, along with significantly reducing the glutathione disulfide levels in tissues. MiADMSA is also shown to be capable of crossing biomembranes and is more effective in reducing the arsenic burden in acute and subchronic intoxication. MiADMSA is capable of mobilizing intracellularly bound arsenic and is seen to provide an indirect antioxidant effect.

26.6.1.1 Chemistry, Pharmacokinetics, and Pharmacodynamics

MiADMSA, a C_5 branched chain alkyl monoester of DMSA, can be synthesized by controlled esterification of DMSA with isoamyl alcohol (Table 26–1). It is lipophilic in nature compared to the parent DMSA and found to be more effective in reducing arsenic [92,93]. The plasma kinetics of MiADMSA (plasma-free drug and total drug) at 50 and 100 mg/kg p.o. was carried out. MiADMSA at a dose of 50 mg/kg administered orally provided about 45% and 75% protection against oxidative stress and lowering body arsenic burden. The toxicity profile of DMSA with

an LD_{50} of 16 mmol/kg is much lower than the toxicity of MiADMSA with an LD_{50} of 3 mmol/kg but less than that for BAL (1.1 mmole/kg). Its structural features and recent experimental evidence suggest that it is effective in chelating arsenic [5,94–96].

26.6.1.2 Efficacy and Experimental Studies

We compared the effects of a few thiol and amino chelators and a DMSA analogue, MiADMSA, on liver and tissue oxidative stress in rats. The results suggested that among the thiol and amino chelators, the latter, and in particular $CaNa_2EDTA$, were more toxic to hepatic tissue and caused a more pronounced redistribution of metals like zinc. On the other hand, MiADMSA only caused a significant loss of copper [97] with no other major side effects. Further repeated administration of MiADMSA was found to be safe in adult rats followed by young and old rats, with female rats slightly more susceptible than males [98]. We also demonstrated that oral administration was more effective than the parental route [41,99,100]. Similarly to our studies, Kreppel et al. [101] also reported the superior efficacy of MiADMSA and mono n-amyl DMSA in mice against lethal effects of arsenic. These studies support MiADMSA being a potential new, and one of the most effective vicinal classes of, metal chelator [13,92,102]. Based on these results the phase I clinical trials of MiADMSA have been initiated and results are awaited.

To date, no work has reported the protective or therapeutic efficacy of MiADMSA against Lewisite and other arsenicals. However, we reported the efficacy of MiADMSA on the reversal of gallium arsenide (GaAs)-induced changes in hepatic tissue [9]. MiADMSA was found to be better than DMSA in mobilizing arsenic and in the turnover of the GaAs-sensitive biochemical variables. We recently compared the efficacy of orally or intraperitoneally administered MiADMSA against GaAs. Our results indicated that orally administered MiADMSA reduced oxidative stress and arsenic burden by 45% and 75%, respectively, as compared to 25% and 40% through the intraperitoneal route. Furthermore, pharmacokinetic analysis supported prolonged availability of the drug through oral administration [41]. These findings clearly suggest that oral administration of MiADMSA is more effective than intraperitoneal administration and that the minimum effective dose with the fewest side effects was 50 mg/kg [41]. Generation of free radicals in the case of arsenic toxicity is known to cause cellular apoptosis through a mitochondrial-driven pathway. A study was conducted to investigate the effect of arsenic interactions with various complexes of the electron transport chain and to evaluate if the complex could trigger apoptosis. Another aspect evaluated was chelation with MiADMSA and reversal of detrimental effects. Arsenic induced free radical generation in rat neuronal cells, leading to diminished mitochondrial potential and enzyme activities of all the complexes of the electron transport chain. MiADMSA, however, was able to reverse most of these arsenic-induced altered variables to various extents, and DNA damage remained unaffected [103]. Ramkumar et al. [104] also reported that MiADMSA significantly reversed arsenic-induced alterations in behavior and biochemical variables suggestive of oxidative injury. Arsenic-exposed rats showed significant differences in behavioral functions and water maze learning. Further, biochemical studies also showed significant alteration, which was effectively restored upon MiADMSA treatment. Besides the above-mentioned *in vivo* studies, few *in vitro* studies also

supported the therapeutic efficacy of MiADMSA against arsenic. Using HaCaT cell lines, we recently reported that pretreatment with MiADMSA offered significant protection against arsenic-induced oxidative stress and apoptotic cell death. These findings are of clinical relevance and suggest MiADMSA to be a promising candidate in protecting skin against arsenic-induced toxic effects [105]. Sannadi et al. [106] also evaluated the therapeutic efficacy of MiADMSA against arsenic-induced developmental neurotoxicity. Pregnant rats exposed to sodium metaarsenite via drinking water throughout the prenatal period were treated with MiADMSA. The results suggested that MiADMSA significantly reversed arsenic-induced apoptosis and oxidative stress in the brain region, a major contributing factor to neurotoxicity.

Despite a few drawbacks (copper loss) associated with MiADMSA, the above results suggest that MiADMSA may be a future drug of choice owing to its ability to cross the blood–brain barrier, its lipophilic character, and the absence of any metal redistribution [42,97]. Moderate toxicity observed after repeated MiADMSA administration may be reversible after withdrawal of the chelating agent. MiADMSA also does not produce any developmental toxicity in mice in the absence of maternal toxicity [107]. Mehta et al. [42] also concluded that MiADMSA had no effect on length of gestation, litter size, sex ratio, viability, and lactation. Taubeneck et al. [108] showed that the developmental toxicity of DMSA is mediated mainly through disturbed copper metabolism and this may also be true for MiADMSA.

26.6.1.3 Mechanism of Action

The pronounced efficacy of MiADMSA could be attributed to its lipophilic characteristic [109]. It appears plausible that MiADMSA could decrease the oxidative stress in tissues either by removing arsenic from the target organs and/or by directly scavenging ROS via its sulfhydryl group [110].

26.6.1.4 Drawbacks

It is reported that the toxicity of DMSA is much lower than the toxicity of MiADMSA but less than that of BAL when comparing their lethal doses (LD_{50}). Flora and Mehta [100] reported that administration of MiADMSA caused no alterations in the heme synthesis pathway except for a slight rise in zinc protoporphyrin levels, suggesting mild anemia. MiADMSA seems to be slightly more toxic in terms of essential metal loss (copper and zinc) and some biochemical alterations in the hepatic tissue in female compared to male rats [42]. However, these effects were temporary and could be reversed following withdrawal of MiADMSA treatment.

26.6.2 Monomethyl DMSA (MmDMSA) and Monocyclohexyl DMSA (MchDMSA)

Apart from MiADMSA, the other two analogues of DMSA that have been tried against arsenic poisoning are MmDMSA, a straight and branched chain methyl group analogue, and MchDMSA, a cyclic carbon chain analogue. Being lipophilic, both have the ability to penetrate cells more readily than DMSA and can be administered orally. Oral administration suggests that they may possess considerable advantages in the clinical treatment of metal toxicity in the future, however; further extensive studies are required to reach a final conclusion.

It is clear from the above that most of the chelating agents have severe side effects and also have some limitations. There are summarized as follows:

1. BAL and D-PA BAL are effective antidotes for arsenic poisoning. They are liophilic in nature and distributed both in the extracellular and intracellular spaces in the cellular compartments. However, they are also toxic at doses as low at 40 µmol/kg [111]. D-PA leads to reversible hepatic and renal dysfunction and autoimmune diseases [37].
2. DMSA and DMPS: The analogues of BAL are hydrophilic in nature and more effective compared to BAL [51]. These analogues, however, also lead to essential metal loss such as of zinc and copper and extracellular distribution, and are unable to chelate metals from intracellular sites. Various studies have shown that monoesters of DMSA are far better chelating agents than parent compounds for the removal of toxic metals such as lead [112] and arsenic [101,110].
3. DMSA analogues: It has been found that newer DMSA analogues, including MiADMSA, MchDMSA, and MmDMSA, are better alternatives to DMSA as chelating agents for arsenic removal [110]. These complexes seem to penetrate cells, which helps in targeting intracellular sites in the body and aids in the removal of toxic metal ions from the cytosol and organelles inside the cell, which is not possible in the case of DMSA. Their specificity for arsenic, however, needs to be explored.

26.7 Role of Antioxidants in Preventing Arsenic Toxicity

Antioxidants are defined as substances that inhibit or delay the oxidation of biologically relevant molecules either by specifically quenching free radicals or by chelation of redox metals [113]. The most important source of antioxidants is provided by nutrition [114]. Antioxidants play an indispensable role in counteracting free radical-induced damage to macromolecules and also repair free radical-mediated cellular damage. Nutritional antioxidants may act in different compartments through different mechanisms such as by (1) directly neutralizing free radicals; (2) reducing peroxide concentrations and repairing oxide membranes; (3) quenching iron to decrease ROS production via lipid metabolism; or (4) neutralizing ROS through short-chain free fatty acids and cholesteryl esters [114].

Oxidative stress can be partially implicated in arsenic toxicity and therapeutic strategy to increase the antioxidant capacity of cells to fortify the long-term effective treatment in arsenic poisoning. This may be accomplished by either reducing the possibility of metal interacting with critical biomolecules to reduce oxidative damage, or by bolstering the cells' antioxidant defenses through endogenous supplementation of antioxidant molecules [115,116]. Ramanathan et al. [117] observed the molecular changes during arsenic exposure and possible therapeutic efficacy of antioxidants like vitamin C and vitamin E on arsenic-induced apoptosis in rats. They concluded that the administration of vitamin C and vitamin E with arsenic significantly decreased the extent of apoptosis. Antioxidants are known to regulate the expression of a number of genes and signal regulatory pathways and thereby prevent the

incidence of cell death from free radical damage. The usefulness of antioxidants in conjunction with chelation therapy has been well studied but still extensive investigations are required to delineate a specific dose, the duration of administration, and a specific and well-defined mechanism. In the following paragraphs we attempt to describe a few potential antioxidants that have been examined in the recent past for their role during chelation of arsenic.

26.7.1 Alpha-Lipoic Acid

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid; ALA), also known as thioctic acid, is an endogenous thiol antioxidant possessing powerful potential to quench reactive oxygen species, regenerate GSH, and chelate toxic metals. It is a disulfide derivative of octanoic acid and an important cofactor (prosthetic group) in various mitochondrial multienzyme complexes involved in energy production [118]. Apart from being an efficient antioxidant, alpha-lipoic acid is also a beneficial therapeutic agent in the treatment of arsenic-induced oxidative stress [119]. It is readily absorbed from the gut and easily crosses the blood–brain barrier. It is supplied through the diet, and it has been reported that exogenous supplementation increases levels of unbound lipoic acid, which acts as a potent antioxidant and reduces oxidative stress both *in vitro* and *in vivo* [119].

Alpha-lipoic acid has been shown to have substantial antioxidant properties when administered (70 mg/kg body weight) for 2 months once daily simultaneously along with arsenic [120]. ALA treatment showed significant beneficial effects in reducing oxidant production as well as lipid peroxidation levels in rat brain regions. The study demonstrated that arsenic-induced deficits in brain regions can be overcome through simultaneous treatment with lipoic acid [120].

26.7.2 N-Acetylcysteine

N-Acetylcysteine (NAC) is a thiol-containing compound, a mucolytic agent, and a precursor of reduced glutathione. It stimulates the intracellular synthesis of GSH, enhances glutathione-S-transferase activity, and acts solely as a scavenger of free radicals. It is a source of sulfhydryl-containing antioxidant that has been used to mitigate conditions of oxidative stress. A number of *in vivo* and *in vitro* studies have been conducted determining the role of NAC in combating arsenic poisoning. Reddy et al. [121] reported that the impairment of spermatogenesis in arsenic-exposed mice is mediated through oxidative stress as well as arsenic's direct effect on the germinal compartment. These authors also reported that co-administration of NAC reduces arsenic-induced oxidative stress by decreasing lipid peroxidation and activating antioxidant enzymes in the testes, thereby ameliorating arsenic-induced suppressed reproduction in male mice. In another study, NAC supplementation could significantly reduce ROS-mediated oxidative stress in liver of arsenic-exposed animals [122]. This hepatoprotective potential could also be a result of effective detoxification of electrophiles or free radicals generated by arsenic as well as its rapid elimination/excretion from the body [123]. NAC treatment could also act as an effective intervention to restore arsenic-depleted GSH stores [123].

Combined administration of NAC and succimer post-arsenic exposure led to a significant recovery in biochemical variables indicative of oxidative stress and arsenic depletion from soft organs [68,124].

In an interesting case report, a 32-year-old man was brought to the emergency department 5½ hours after ingesting a potentially lethal dose (900 mg) of sodium arsenate in a suicide attempt. The patient deteriorated progressively for 27 hours. After intramuscular dimercaprol and supportive measures failed to improve his condition, he was given NAC intravenously. The patient showed remarkable clinical improvement during the following 24 hours and was discharged from the hospital several days later [125].

26.7.3 Vitamins E and C

Vitamin E (α-tocopherol) is a fat-soluble vitamin known as one of the most potent endogenous antioxidants located in the cell membrane. α-Tocopherol comprises a group of potent, lipid soluble, chain-breaking antioxidants that prevents the propagation of free radical reactions and stabilizes cell membranes by interacting with unsaturated fatty acid chains. On the other hand, vitamin C is a water soluble antioxidant occurring in organisms as an ascorbate anion that acts as a scavenger of free radicals and plays an important role in regeneration of α-tocopherol [126]. Combined administration of ascorbic acid and α-tocopherol provides recovery by altering the extent of DNA damage by reducing tumor necrosis factor α (TNF-α) levels and inhibiting the activation of the caspase cascade in arsenic-intoxicated animals [117]. Vitamins E and C have been found to reduce the toxic manifestations of arsenic in animal models [94,116]. It was observed that vitamin E prevented arsenite-induced damage of human fibroblasts [127]. Although some studies suggested that vitamin C was better in providing clinical recoveries, vitamin E was equally efficient in decreasing arsenic burden from the tissues. Wei et al. [128] reported the involvement of oxidative stress in dimethylarsinic acid (DMA)-induced bladder toxicity and proliferation in rats, and the inhibitory action of vitamin C on the proliferative effects of DMA.

Coadministration of vitamin C or vitamin E was also investigated during chelation treatment with two thiol chelators, DMSA or MiADMSA, in combating chronic arsenic toxicity. Combined administration of vitamin C and DMSA or vitamin E plus MiADMSA led to a more pronounced depletion of brain arsenic besides significant recovery in the inhibited blood δ-aminolevulinic acid dehydratase (ALAD) activity and other oxidative stress parameters in liver, kidneys, and brain. These results suggested that coadministration of vitamin E or vitamin C may be useful in arsenic poisoning although it has only a limited role in depleting arsenic burden [129]. Supplementation of antioxidants has also led to alleviation of arsenic-induced molecular alterations. Contamination of arsenic in drinking water is associated with several human diseases including cancer. A study was conducted in which significant increase in the levels of protein oxidation, DNA strand breaks, and DNA–protein cross-links was observed in blood, liver, and kidneys of rats exposed to arsenic (100 ppm in drinking water) for 30 days. However, coadministration of ascorbic acid and α-tocopherol to arsenic-exposed rats showed a substantial reduction in the levels of arsenic-induced oxidative products of protein and DNA.

The results validate the hypothesis that free radical generation is one of the major mechanisms of arsenic-induced toxic manifestations and also suggest that ascorbic acid and α-tocopherol supplementation can ameliorate the arsenic-induced molecular alterations [130].

26.7.4 β-Carotene

β-Carotene is a member of a family of molecules known as the carotenoids, consisting of isoprene units in structure. It is a precursor of retinol (vitamin A), a lipid soluble antioxidant with properties of vitamin E [126]. The long chains of conjugated double bonds impart specific colors to carotene, and are responsible for its good antioxidative properties. It can also mop up oxygen-free radicals and dissipate their energy. A significant reverse dose–response relationship with arsenic-related ischemic heart disease was reported for serum levels of α- and β-carotene. Synergistic interaction in arsenic-related ischemic heart disease between duration of consuming artesian well water and low serum carotene levels was observed by multivariate analysis [131].

26.7.5 Taurine

Taurine (2-aminoethanesulfonic acid) is one of the major sulfur-containing free intracellular amino acids synthesized mainly in the pancreas via the cysteine sulfinic pathway. It is also found in various mammalian tissues such as muscles, brain, and heart [132]. It is a well-known, powerful antioxidant and maintains levels of cysteine, an important precursor of glutathione. The sulfonate group in taurine is a strong acid that exists as zwitter ion over the physiological pH range [133]. It has also been shown to exert protective efficacy against arsenic-induced cytotoxicity in murine hepatocytes. The cytoprotective activity of taurine against arsenic poisoning was found to be analogous to that of a known antioxidant, vitamin C. The study further suggests that taurine protects mouse hepatocytes against arsenic-induced cytotoxicity [134]. Taurine effectively reduced arsenic-induced cardiac dysfunction such as cardiomyocyte viability, ROS production, intracellular calcium overload, and apoptotic cell death. Taurine also decreased arsenic-induced activity of p38 and JNK MAPKs. Taurine effectively decreased these apoptotic actions, which is suggestive of its protective role by attenuation of p38 and JNK MAPK signaling pathways. Results showed that taurine effectively prevented arsenic-induced myocardial pathophysiology, and attenuated NF-kappaB activation via IKK, p38, and JNK MAPK signaling pathways, and hence could act as an effective therapy against arsenic-induced cardiovascular burden [135]. Flora et al. [136] also reported that administration of taurine alone significantly reduced hepatic oxidative stress in arsenic-induced toxicity in rats (100 mg/kg).

26.7.6 Melatonin

Melatonin (N-acetyl-5-methoxy tryptamine), a hormone produced by the pineal gland, is a powerful scavenger of reactive oxygen species and free radicals. It prevents the reduction of membrane fluidity from lipid peroxidation and thereby helps in scavenging free radicals [137]. It has been reported that melatonin is superior to other free radical scavengers such as

vitamin E, vitamin C, and GSH in neutralizing peroxyl radicals. Melatonin has shown to be five times superior to glutathione in scavenging free hydroxyl radicals by Pieri et al. [138]. Both the methoxy group at position 5 of the indole nucleus and the acetyl group of the side chain of melatonin are responsible for scavenging free hydroxyl radicals by donating an electron to scavenge OH and forming an indolyl cation radical, which in turn neutralizes superoxide radical [139]. Protective efficacy of melatonin against toxic metals-induced oxidative damage has been reported mostly in *in vivo* and *in vitro* studies [136,140,141]. Pal and Chatterjee [142] reported that melatonin supplementation decreased free radical-mediated cytotoxicity and thereby helps in the restoration of normal cellular antioxidant status in arsenic-exposed rats. The antioxidant property of melatonin has also been proved as a protective factor towards carcinogenesis, neurodegeneration, and aging [143].

26.7.7 Curcumin

Curcumin is a natural phenolic compound with impressive antioxidant properties. It has recently been proven that curcumin exerts its chemopreventive effects partly through the activation of nuclear factor (erythroid-2 related), factor 2 (Nrf2), and its antioxidant and phase II detoxifying enzymes [144]. Protective efficacy of curcumin against arsenic-induced hepatotoxicity and oxidative injuries has been reported. The study revealed that arsenic-induced elevation of serum transaminases and variables indicative of oxidative stress were restored to almost normal levels on treatment with curcumin [145]. The involvement of curcumin in promoting arsenic methylation and urinary elimination in this study was also confirmed. This interesting point further suggested that the potent Nrf2 activation capability might be valuable for the protective effects of curcumin against arsenic intoxication. This affords an effective useful chemopreventive dietary component for human populations [145]. Curcumin has the ability to modulate numerous signaling molecules such as pro-inflammatory cytokines, apoptotic proteins, NF-κB, cyclooxygenase-2, 5-LOX, STAT3, C-reactive protein, prostaglandin E(2), prostate-specific antigen, adhesion molecules, phosphorylase kinase, transforming growth factor-β, triglyceride, ET-1, creatinine, HO-1, AST, and ALT in human participants [145].

26.7.8 Essential Metals

26.7.8.1 Zinc

Zinc is one of the essential trace elements. Metallothionein (MT), a cysteine-rich protein, stores the cellular form of zinc in the pico- to nanomolar range. Zinc plays a crucial role in detoxification of arsenic from the body because arsenic is capable of inducing MT protein, which is responsible for arsenic detoxification. Some reports suggest that zinc pretreatment may lead to increased arsenic elimination [146,147]. Zinc supplementation also prevents damage that may chronically lead to manifestations such as cancer [148,149]. Except for a few isolated reports not much work has been done on the role of zinc against arsenic [150]. Further studies with variable doses of zinc and its effects during chelation are required against chronic arsenic poisoning.

26.7.8.2 Selenium

An antagonistic effect of selenium on arsenic has been studied in the past. Both are metalloids with very similar chemical properties but known to act differently in biological systems [151]. The possible interaction between arsenic and selenium has been reviewed by Zeng et al. [151]. Selenium is recognized for promoting biliary excretion of exogenous selenium and selenite increases the excretion of arsenic into bile [148,152]. These studies suggested that arsenic augmented the hepatobiliary transport of selenium and facilitated accumulation of selenium in red blood cells, which in turn facilitated the biliary excretion of arsenic. Glattre et al. [153] also reported distribution and interaction of arsenic and selenium in rat thyroid and suggested that both arsenic and selenium accumulate in thyroid tissue. There might be a competition between selenium and arsenic for binding with functional proteins, bioligands, and active tissue sites and the formation of a reversible compound, metal–selenide, which may indicate mechanisms for the reduction of available "free" arsenic ions in the body. Other recent evidence confirmed that selenium may play a role in arsenic elimination by forming a selenium–arsenic conjugate in the liver before excretion into the bile [151]. Recently, a study on adults and children was conducted to assess the relationship between blood selenium and urinary and blood arsenic in a population residing in a moderately arsenic-contaminated rural area in Bangladesh. Results suggested that selenium is inversely associated with biomarkers of arsenic burden in both adults and children. Moreover, this finding validates the hypothesis that selenium facilitates the biliary elimination of arsenic, possibly via the putative formation of an selenium–arsenic conjugate using a glutathione complex [154].

26.7.9 Herbal Extracts

Garlic is another well-known folk remedy for a variety of ailments. Flora et al. [155] studied the protective efficacy of aqueous garlic extract in reducing hepatic injury, tissue oxidative stress, and arsenic mobilization. Results suggested that garlic extracts contain strong antioxidant properties, which could be beneficial in preventing arsenic-induced toxicity in cells. A study was also conducted in rabbits to assess the oxidative injuries caused by arsenic toxicity and evaluate the detoxifying effects of exogenous antioxidants, vitamins, zinc, selenium (VZS), or a plant polyphenol. Results suggested that arsenic induces toxicity in rabbits associated with an increase in lipid peroxidation and nitric oxide production in the body. Administration of exogenous antioxidants such as polyphenols and a recipe of vitamins, zinc, and selenium, however, was found to be useful for arsenic detoxification [156]. Among various herbal extracts, *Moringa oleifera* seed powder has been shown to reduce significantly arsenic-induced oxidative stress and body arsenic burden. Hence, it was concluded that concomitant administration of *M. oleifera* seed powder could significantly prevent arsenic-induced toxic manifestations and thus could be used during chelation therapy [157]. Concomitant oral supplementation of *Centella asiatica* during arsenic exposure has also shown to be an effective strategy against arsenic toxicity. More extensive studies are recommended for determining the effect of coadministration of *C. asiatica* during chelation therapy with a thiol chelator [158,159]. Tossa jute, *Corchorus olitorius*, is a popular crop for arsenicosis-prone populations. A study evaluated the protective

effect of aqueous extract of *C. olitorius* leaves (AECO) against sodium arsenite-induced cardiotoxicity in experimental rats. The results concluded that treatment with AECO prior to arsenic intoxication significantly protected against arsenic-induced myocardial injury [20].

Manna et al. [160] investigated the hepatoprotective role of arjunolic acid, a triterpenoid saponin, against arsenic-induced oxidative damage in murine livers. The altered activities of hepatic marker enzymes in serum enhanced DNA fragmentation, protein carbonyl content, and lipid peroxidation end-products, and the level of oxidized glutathione responded favorably to treatment with arjunolic acid. Results suggest that arjunolic acid possesses the ability to attenuate arsenic-induced oxidative stress in murine liver probably via its antioxidant activity. *Mentha piperita* also exhibited protective efficacy against arsenic-induced liver injury in mice. Pre- and post-treatment of *Mentha* with arsenic significantly protected biochemical parameters of liver damage. The results confirmed that *Mentha* extract may be useful in reducing arsenic-induced hepatopathy [161].

26.8 Newer Strategies

26.8.1 Combination Therapy

A number of newer strategies have recently been tried to reduce the shortcomings of various chelating agents, enhance metal mobilization from the body, and lessen redistribution of toxic metals from one site (e.g., bone or liver) to more sensitive sites such as the brain [97]. In one of the most effective approaches, two chelators are used that act differently. This is based on the assumption that various chelating agents are likely to mobilize toxic metals from different tissue compartments and therefore better results could be expected [161–163]. Various strategies have been discussed in the recent past [164]. Among these strategies, combination therapy is a novel and effective approach to treat cases of metal poisoning including arsenic. As discussed earlier, treatment with MiADMSA has been found to be more effective than using most of the earlier recommended chelators. Further, we observed that combined administration of MiADMSA with another chelator like DMSA is significantly more beneficial than monotherapy with these chelators in counteracting chronic arsenic toxicity [109,162]. Results from these studies suggested that concomitant administration of DMSA, a chelator known for its extracellular distribution with lipophilic chelators like MiADMSA, plays a significant and more efficient role in abating the number of toxic effects of arsenic in animals compared to treatment with these chelators alone. We also suggested that analogues having long carbon chains (MiADMSA and MchDMSA) are better chelators than chelators with shorter carbon chains (MmDMSA) or DMSA. It is implicit that analogues of DMSA eliminate arsenic simultaneously from the cell and provide assistance in restoring GSH homeostasis towards normal levels. We also noted that combination therapy with DMSA and MiADMSA or MchDMSA proved more beneficial than combined treatment with MmDMSA and DMSA [110]. In an interesting study, we recently reported that combination therapy with two thiol chelators could reverse altered neurological functioning better than monotherapy. More significantly, neuronal cell death could also be prevented to some extent following combination therapy.

Our studies show combination therapy to be effective in providing clinical benefits and removing arsenic from the brain. However, we feel that more extensive studies are required before final conclusions can be drawn for clinical viability and effectiveness of combination treatment in chronic arsenic poisoning [105].

Coadministration of naturally occurring vitamins like vitamin E or vitamin C during administration of a thiol chelator like DMSA or MiADMSA may also be beneficial in the restoration of altered biochemical variables, although they have only a limited role in depleting arsenic burden. Various other studies, particularly from our group, have also suggested the beneficial effects of combination therapy using a strong chelating agent and a vitamin/antioxidant/essential metal or an amino acid also acting like weak chelating agents [129,165,166]. The ability of NAC to significantly provide additional beneficial effects (including arsenic mobilization) when administered with a thiol chelating agent, like DMSA, to restore hepatic and brain glutathione levels and erythrocyte enzyme to normal levels is one such example [68]. Combined administration of vitamin C with DMSA and vitamin E with MiADMSA was also found to be more effective in significant depletion of brain arsenic and useful in the restoration of altered biochemical variables, particularly the effects on heme biosynthesis and oxidative injury [129]. The effects of both DL-alpha-lipoic acid and DMSA on the antioxidants and lipid peroxidation in arsenic-treated rats revealed a significantly more pronounced depletion of reactive oxygen species formation and lipid peroxidation while antioxidant enzyme levels were restored to normal. The study further concluded that DL-alpha-lipoic acid and DMSA play a synergistic role in decreasing arsenic-induced oxidative damage by elevating the antioxidant status in liver and kidneys [167]. Taurine too when coadministered with DMSA or MiADMSA reduces total body burden of arsenic and lead [137,168].

It is evident that combination therapy is a new and better approach to treat cases of metal poisoning. Little experimental evidence is available and there is a need for in-depth investigation in this area to prompt new chelation therapy regimens for the treatment of chronic arsenic poisoning.

26.8.2 Nanoparticle Carriers to Combat Arsenic Toxicity

Focus has recently shifted from the monotherapy and combination therapy to site-specific targeted delivery of drugs using appropriate carriers such as liposome, micelles, and nanoparticles. Novel modes of drug delivery are currently available as nanocapsules, which are non-toxic, biodegradable, and non-immunogenic with sustained drug-releasing ability in biological systems. The therapeutic efficacy of drugs can be enhanced via targeting nanomaterials as carriers. Due to their small size, nanoparticles are easily accessible in the body and can be transported to different sites through the blood [169]. Nanoparticles can thus circumvent the reticuloendothelial system (RES) such as liver Kupffer cells and spleen macrophages and reach the desired site effectively [170]. This is a promising therapeutic strategy and may be effective in chelating arsenic from the intracellular space by their crossing the blood–brain barrier to adsorb apolipoprotein E from blood plasma. They may be able to mimic low density lipoprotein for having specific receptors at the surface of endothelial cells of the blood–brain barrier.

They are recognized by endothelial cell receptors. The drug may be released into these cells from the nanoparticles and diffuse into the brain. There are other processes such as tight junction modulation or P-glycoprotein inhibition, which may run in parallel or may be cooperative, thus enabling efficient drug delivery to the brain [171] (Figure 26–7).

We recently synthesized and characterized water soluble nanoparticles of curcumin and applied them as a stable detoxifying agent for arsenic poisoning. For this, chitosan nanoparticles of less than 50 nm in diameter containing curcumin were prepared. The therapeutic efficacy of the encapsulated curcumin nanoparticles (ECNPs) against arsenic-induced toxicity in rats was investigated via an oral administration. Coadministration of ECNPs along with arsenic provided pronounced beneficial effects on the adverse changes in oxidative stress parameters induced by arsenic. Results indicate that ECNPs have a better antioxidant and chelating potential compared to free curcumin. Significant neuroprotective efficacy was also observed by ECNPs against neurochemical and immunohistochemical variables. This formulation of ECNPs provides a novel therapeutic regime for preventing arsenic toxicity [172]. Ghosh et al. [136] too evaluated the therapeutic efficacy of nanocapsulated flavonoidal quercetin (QC) in combating arsenic-induced reactive oxygen species-mediated oxidative damage in rat hepatocytes and brain cells. Administration of QC provided recovery in reducing arsenic-induced oxidative damage. The authors, however, suggested that the clinical application of QC against toxicant-induced cellular damage is not possible due to its insoluble nature. A few other studies also reported the efficacy of liposome-encapsulated QC, administered intravenously to arsenic-exposed rats. These studies showed that the QC inhibited hepatic fibrogenesis and

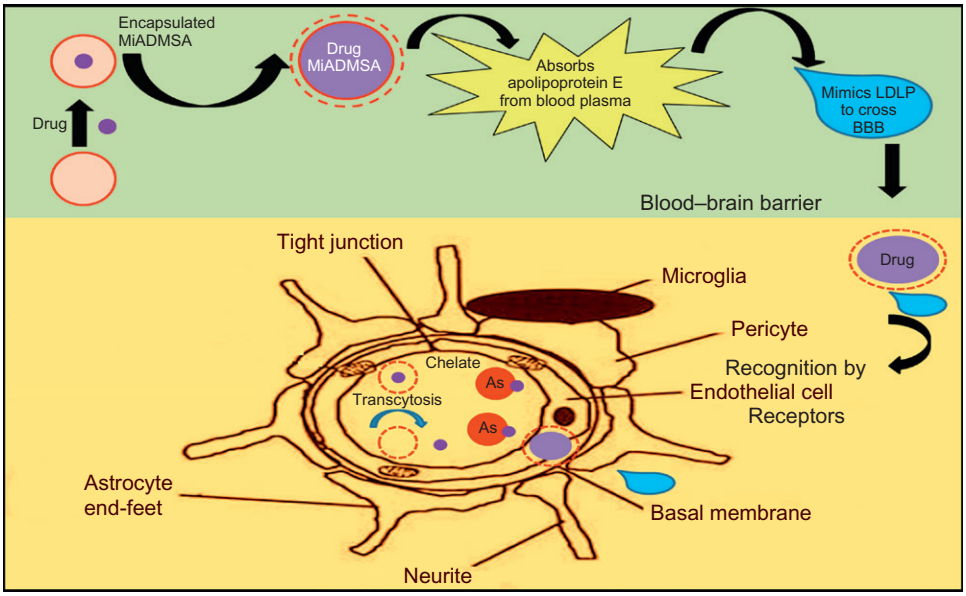


FIGURE 26–7 Mechanism of encapsulated MiADMSA in chelating arsenic from brain.

also attenuated the oxidative damage induced by ischemia-repurfusion injury in the rat brain [173,174]. Stability of the nanoparticles in the bioenvironment offers the possibility of using these vesicles as oral drug carriers. The use of nanoparticles as drug carriers has high stability in biological systems, high carrier capacity, and the feasibility of incorporation of both hydrophilic and hydrophobic to circumvent the blood–brain barrier. This colloidal carrier has the ability to cross both the GI tract mucosal barrier and the blood–brain barrier to enhance drug bioavailability via particle uptake mechanisms. Thus, nanoparticles are proven to be the most promising carriers for delivery of drug to its target site [175].

Therapeutic efficacy of nanoencapsulation of DMSA monoester against arsenic toxicity was recently reported by us [176]. Sodium arsenite-exposed mice were treated with nanoencapsulated DMSA for 5 days to evaluate alterations in blood, brain, kidney, and liver oxidative stress variables. This study also investigated the histopathological changes in tissues and the chelating potential of the nanoformulation. The study revealed a significant role of encapsulated DMSA in reducing arsenic from the tissues while eliciting positive response in altered biochemical variables, histopathological lesions, and urinary 8-OHdG levels, suggesting a better therapeutic efficacy of the novel formulation for arsenic toxicity [176,177]. Thus, we recommend nanoparticle-based drug delivery as a better approach to aid removal of arsenic from specific target sites of the biological system pending future studies (Figure 26–8).

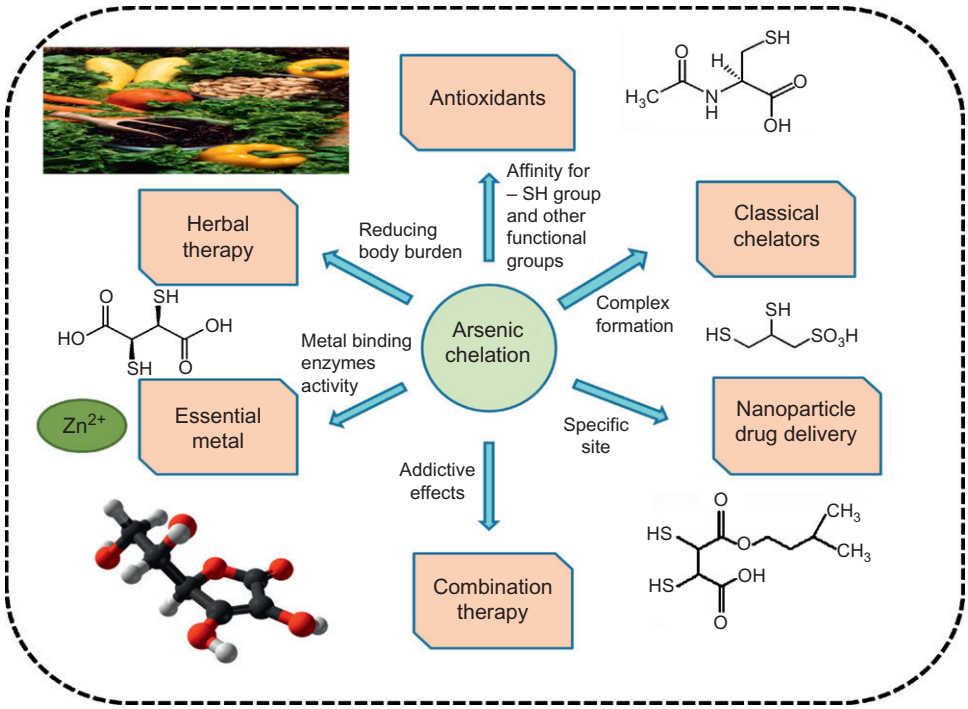


FIGURE 26–8 Various strategies of arsenic chelation.

26.9 Concluding Remarks and Future Directions

There is a growing concern these days to find a suitable, safe, and effective therapy for chronic arsenic poisoning. Various strategies that have been proposed in the recent past include synthesis of newer chelating agents, target drug delivery using nanoencapsulation of drugs, and combinational treatment, which ensures and improves trans-membrane delivery of soluble drugs to the affected cells. Nanoencapsulation of antioxidants and chelating agents ensures increases in the half-lives of these drugs in blood circulation, reducing multiple dosing and minimizing side effects. As there are very few studies available on these aspects, we recommend more detailed investigation using these strategies for treating chronic and subchronic levels of arsenic to substantiate our conclusions.

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27

Biochemical and Molecular Basis of Arsenic Toxicity and Tolerance in Microbes and Plants

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CHAPTER OUTLINE

27.1 Introduction

27.1.1 Arsenic: A Threatening Environmental Issue

Arsenic (As) is highly toxic metalloid widely distributed in Earth's crust and occurs naturally in soil, water, and air. It is the 20th most abundant element in Earth's crust. Estimated arsenic concentration in sediments ranges between 5 and 10 mg/kg [1]. Increase in arsenic concentration in soil has resulted from natural weathering of arsenic-containing rocks and minerals, as well as from anthropogenic sources such as the smelting of metal ores, arsenical pesticides, wood preservatives, fossil fuel burning, use of As-based pesticides, herbicides, and irrigation with contaminated ground water [1–4]. Arsenic can easily enter into aquatic systems through river flow and surface runoff, and As enrichment in ground water is a global concern [3,5]. Major sources of human arsenic exposure are drinking water and the food supply, leading to

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health problems such as cancer, cerebrovascular disease, diabetes mellitus, and kidney disease [6]. Arsenic is first on the Centers for Disease Control Agency for Toxic Substances and Disease Registry's Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority (Superfund) List of Hazardous Chemicals (http://www.atsdr.cdc.gov/cercla/07list.html) because of its ubiquity, health effects, and potential for human exposure. As contamination in ground water has been reported from over 70 countries of which the worst is Bangladesh [7]. Besides the use of As-contaminated ground water for drinking and household purposes, such water has extensively been used for crop irrigation, particularly paddy rice (*Oryza sativa* L.) in Bangladesh and other South and South-East Asian countries [8]. About 75% of the total cropped area and 83% of the irrigated area in Bangladesh are under rice cultivation [8]. Background levels of As in paddy soils in Bangladesh range from 4 to 8 mg/kg [9], and long-term use of As-contaminated ground water for irrigation has resulted in a significant increase of As concentration in the topsoil (about 150 mm) of paddy fields [7]. About 83 mg/kg As was found in paddy soils that had been irrigated with As-contaminated ground water [9]. Thus, the problem of As pollution is continuously worsening due to a series of natural and human activities leading to an intensification of research focus on its phytotoxicity and the mechanisms employed by organisms such as microbes (prokaryotes and eukaryotes) and plants to counter its detrimental effects. There is a need to have a complete understanding of the molecular mechanisms and genetic basis of As toxicity and detoxification to produce/identify crop plants tolerant to As or use plants for photoremediation and restoration of As-contaminated sites.

27.1.2 Worldwide Occurrence of Arsenic

Arsenic is found naturally at low concentrations in Earth's crust, but few geographical regions have been reported with high arsenic concentration in ground water due to the continuous enrichment of As through natural and anthropogenic sources, as discussed in the section above. Unlike the rare-earth elements, arsenic is commonly found concentrated in sulfide-bearing mineral deposits, especially those associated with gold mineralization or concentrated in hydrous iron oxides. Arsenic can be easily solubilized in ground waters depending on pH, redox conditions, temperature, and solution composition. Many geothermal waters or aquifers contain high concentrations of arsenic at concentrations greater than the World Health Organization—and US Environmental Protection Agency—recommended drinking water standard of 10 µg per liter. Figure 27–1 shows the major epidemic areas with high arsenic contamination in underground irrigation water and paddy soils in West Bengal (India), China, Myanmar, Nepal, Thailand, Taiwan, and Vietnam [10]. On the basis of the severity of As contamination, the Asian countries have been ranked as: Bangladesh, West Bengal (India), Inner Mongolia (PR China), and Taiwan [11]. The World Health Organization has called arsenic contamination of drinking water in Bangladesh the worst environmental catastrophe in human history, and its control is beyond the capability of many countries [12]. Coming to an Indian scenario, the first report of arsenic contamination came from West Bengal in 1983, where 33 villages in four districts were found to be affected. This number has increased to 3417 in 111 blocks in nine districts in 2008. Contamination was also detected in other states

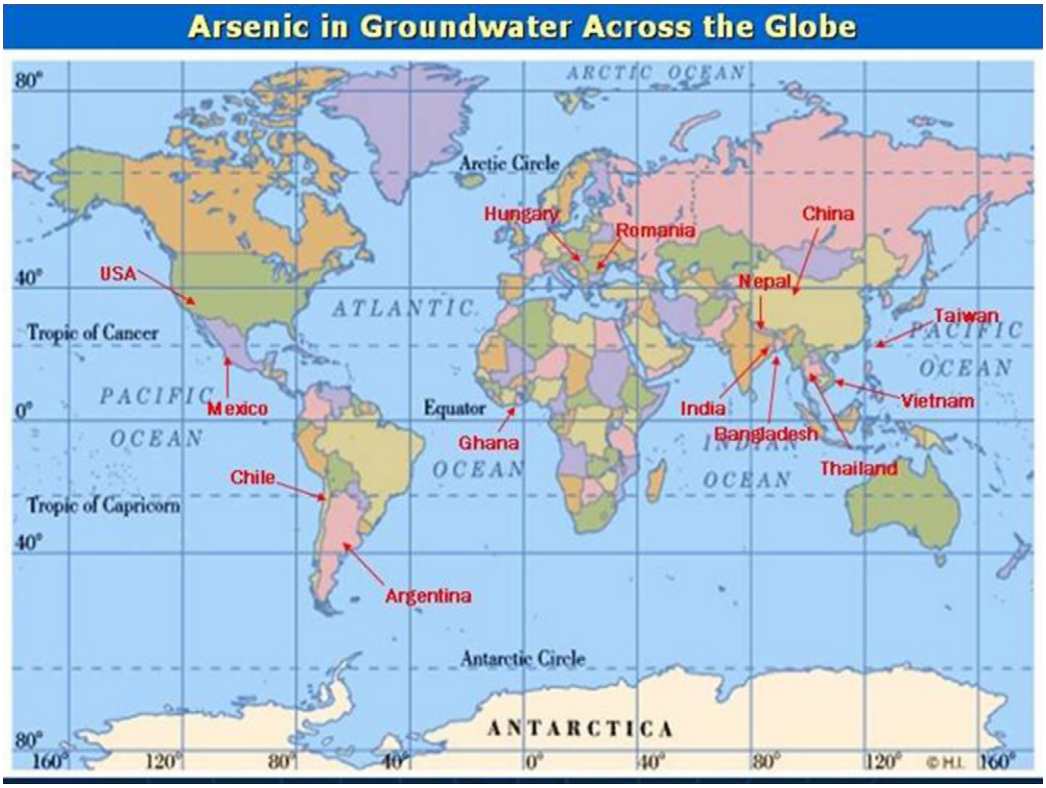

FIGURE 27–1 Worldwide occurrence of As in ground water.

such as Punjab, Bihar, Jharkhand, Orissa, Chhattisgarh, Madhya Pradesh, Uttar Pradesh, Assam, Arunachal Pradesh, Manipur, Nagaland, and Tripura [13–17]. The seven states of West Bengal, Jharkhand, Bihar, Uttar Pradesh in the flood plain of the Ganga River; Assam and Manipur in the flood plain of the Brahamaputra and Imphal rivers and Rajnandgaon village in Chhattisgarh state have so far been reported affected by As contamination in groundwater above the permissible limit of 10 μg/L. The occurrence of arsenic in groundwater in the Bengal delta plains and Gangetic plains has been recognized due to geological origins. The environmental arsenic problems, however, are the result of mobilization under natural conditions.

27.1.3 Arsenic Speciation and Mobilization

Arsenic is present in four oxidation states, −3, 0, +3, and +5, the last two being the most common in the terrestrial environment [18]. Arsenate [As(V)] is the predominant species in aerobic soils, whereas arsenite [As(III)] predominates in anaerobic environments such as submerged soils. Elemental arsenic occurs hardly ever, and arsines have been identified from fungal cultures and strongly reducing environments [19]. The relative proportions of these oxidation

states in a given environment depend on the biological processes involved in the metabolism by microorganisms [20], such as As(V) reduction, As(III) oxidation, and the various methylation reactions as well as on the local physicochemical conditions, including the redox potential (Eh) and the pH, which are important factors. Since the pKa's of arsenate (H_3AsO_4) are $pKa_1 = 2.19$, $pKa_2 = 6.94$, and $pKa_3 = 11.5$, the $H_2AsO_4^-$ form predominates in oxidative media with pH levels below 6.9, whereas the $HAsO_4^{2-}$ form predominates at higher pH. These are the most prevalent forms of arsenic in surface soil, water, and within cells, and occur primarily in aerobic environments. However, for arsenite, the lowest pKa level recorded is equal to 9.22. In most natural waters with pH levels below 9.2 as well as in slightly reductive environments, $As(OH)_3$ is the main form present [21]. Several studies have shown that the reduction of arsenate into arsenite results in the solubilization of this element [1,22]. However, arsenate may be sequestered after being co-precipitated with ferric iron or sulfur [23] or adsorbed by clay, calcite, organic matter or hydroxides, in particular ferric oxyhydroxides [24]. Generally, prokaryotes are 10 times more resistant to As(V) than to As(III) [25].

Similar to microbes, the bioavailability of As to plants is governed by edaphic properties, environmental conditions, and modification of the soil in the rhizosphere; these factors interact to influence As speciation in the soil. Analyses of a range of plant species with the aid of analytical techniques such as HPLC/ICPMS/ES-MS and synchrotron radiation-based X-ray absorption spectroscopy (XAS) have shown that both terrestrial and freshwater aquatic plants contain predominantly inorganic As [26–28] but their relative proportions vary among the plant species. As is present predominantly as trivalent species [As(III)] [29–32] and is the main species in the fronds of As-hyperaccumulating ferns [29,33,34]. Furthermore, most of the arsenite is present as complexes like GsAs(III)-PC2, As(III)-PC3, As(III)-(PC2)2, and As(III)GS3 as arsenite has a high affinity to sulfydryl groups. However, As hyperaccumulators such as *Pteris vittata* and *Pteris cretica* appear to be exceptions, containing arsenite mostly as uncomplexed species, due to low phytochelatin (PC) concentrations in tissues [35,36]. Many plant species also contain small amounts of methylated As compounds, such as MMA, DMA, TMAO, and, occasionally, the tetramethylarsonium ion [26–28]. Like As(V) and As(III), the methylated forms of As are phytotoxic [37]. Monomethylarsonous acid with As in the trivalent state [MMA(III): $CH_3As(OH)_2$] can be complexed with thiol compounds, such as MMA(III)-PC2 identified in the roots of sunflower exposed to inorganic As [31]. Although pentavalent As does not form complexes with thiol groups directly, pentavalent As in DMA can bind to glutathione (GSH) when it is activated by sulfide, forming the dimethylarsinothioyl glutathione (DMAS-GS) complex in the sulfur-rich plant species *Brassica oleracea* [38]. Arsenosugars are found in some terrestrial plant species at low levels [26,27] but whether they are synthesized by plants is not known. Root and shoot tissues of *Arabidopsis thaliana* and *Brassica juncea* are coordinated with the sulfydryl groups of thiol-rich peptides such as GSH and PCs [29,30].

27.1.4 Arsenic Epidemiology

Globally, a major fraction of the total population is exposed to arsenic due to intake of arsenic-contaminated water [10]. Arsenic produces its toxic effects by hindering cell

metabolism, growth, and proliferation. Arsenic leads to DNA damage and inhibits repair and replication machinery. Overall, arsenic acts on cells in a variety of ways, influencing numerous signal transduction pathways and resulting in a vast range of cellular effects that include apoptosis induction, growth inhibition, promotion or inhibition of differentiation, and angiogenesis. Daily intake of water containing arsenic at more than 10 µg/L for a long duration leads to many diseases. These include cancers, especially those of lungs, bladder, skin, and kidney, arsenicosis, frequent abortions, impotency, hyperkeratosis, and cardiovascular problems [13,39,40].

27.2 Arsenic Toxicity and Tolerance in Microbes

The toxicity of arsenic depends on its form and oxidation states. It was established recently that toxicity decreases as follows: arsine > arsenite > arsenoxides > arsenate > arsonium compounds > arsenic [41]. Among these, As(V) is the predominant form of As. Because As(V) is a structural analogue of phosphate, its main toxicity results from its interference with the metabolism of phosphorus. Once in the cell, As(V) substitutes for phosphate leading to the production of unstable arsenical by-products. It interferes with the normal phosphorylation processes resulting in uncoupling of oxidative phosphorylation, and thereby disruption of ATP (adenosine 5′-triphosphate) synthesis [42]. Indeed, As(V) reacts enzymatically with ADP (adenosine 5′-diphosphate) to form an arsenate–phosphate bond. The arsenical ester formed is unstable and spontaneously hydrolyses to ADP and As(V), thus preventing ATP formation. In the same way, ATP-dependent transport, glycolysis, the pentose phosphate pathway, and signal transduction pathways could be impaired [43]. Arsenic toxicity is linked with phosphate availability. Phosphate-supplemented cells reduce the uptake of arsenic. It was found that phosphate-starved cells are more sensitive to arsenic than phosphate-repleted cells [44]. Due to potential competitive inhibition between arsenate and phosphate [1,45], arsenate resistance or detoxification pathways may be particularly important in low phosphate conditions. On the other hand, As(III) has a strong affinity for protein sulfhydryl groups. Indeed, the redox status of cysteine residues can affect both the structure and the activity of numerous enzymes, receptors, and transcription factors. As(III) reacts with dithiol groups present on active sites of many enzymes and on glutathione, glutaredoxin (grx), and thioredoxin. These last three metabolites have multiple functions, including intracellular redox homeostasis, deoxyribonucleotide synthesis and repair, protein folding, sulfur metabolism, and xenobiotic detoxification. All these processes are therefore inhibited by As(III). Another cause of carcinogenicity of As(III) is likely linked to its capacity to oxidize reduced glutathione, which is the major cellular antioxidant. This oxidation leads to the increase of reactive oxygen species (ROS), known to damage macromolecules such as proteins, lipids, and DNA [46,47].

In spite of the great toxicity and widespread nature of As compounds, a considerable number of diverse organisms including archaea, bacteria, cyanobacteria, phytoplankton, and plants manage to thrive in arsenic-contaminated environments. Arsenic is a notorious metalloid that was probably more abundant on early Earth's surface than today [48]. Therefore, systems evolved by primordial organisms to cope with it still exist in prokaryotes. Not only do they resist

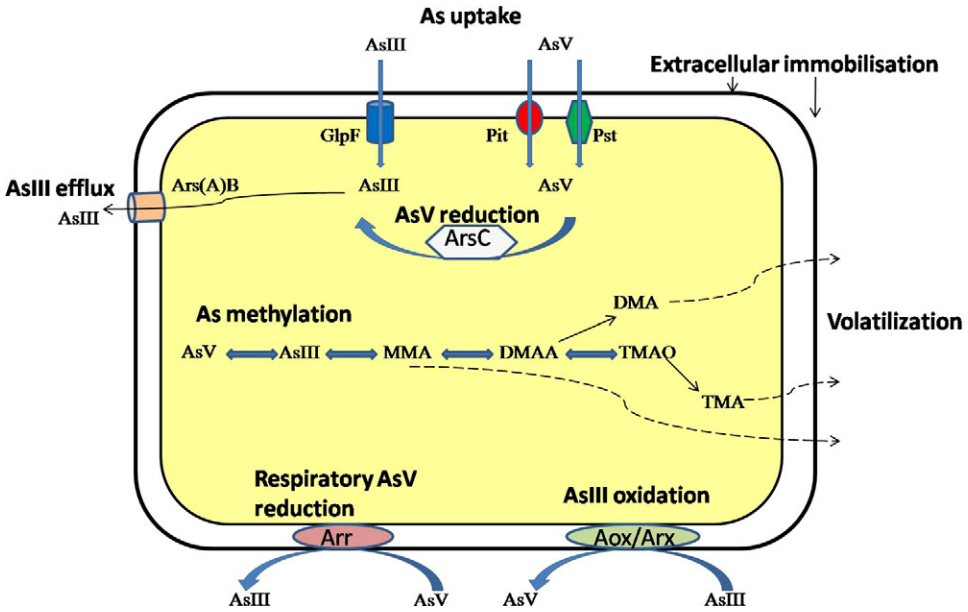

FIGURE 27–2 Schematic representation of arsenic metabolism pathways in prokaryotes. Arsenic (As) enters the cell through the phosphate transporters [arsenate, As(V)] or the aquaglyceroporins [arsenite, As(III)]; arsenic is immobilized in the environment by extracellular precipitation; once inside the cell, As(V) is reduced by an arsenate reductase, ArsC, to As(III), which is extruded out of the cell by the specific membrane pump Ars(A)B; inorganic arsenic can be transformed into organic species via a cascade of methylations; As(V) is used as an electron acceptor during respiration by the dissimilatory arsenate reductase ArrAB; As(III) can serve as an electron donor via the As(III) oxidase AoxAB or ArxAB. As, arsenic; As(III), arsenite; As(V), arsenate; Pit, phosphate (PO_4) inorganic transport system; Pst, phosphate-specific transport system; GlpF, aquaglyceroporin GlpF; ArsC, detoxifying arsenate reductase; Ars(A)B, arsenite efflux pump; MMA, monomethylarsonic acid; DMAA, dimethylarsinic acid; DMA, dimethylarsine; TMAO, trimethylarsine oxide; Aox/Arx, different arsenite oxidase types; Arr, dissimilatory arsenate reductase. *Figure modified from Paez-Espino et al.* [51]

this metalloid with a wide array of mechanisms but strikingly some of them take advantage of this toxic compound by using it as an electron donor [As(III)] or acceptor [As(V)] for energetic purposes [49–52]. It has been proposed recently that arsenic can substitute for phosphorus in proteins and nucleic acids [53]. Microbes respond to arsenic in a variety of different ways. Responses of microorganisms may include (1) extracellular sequestration [54], (2) minimizing the amount of arsenic that enters the cell, e.g., through increased specificity of phosphate uptake [55], (3) chelation mediated by glutathione, phytochelatin, and metallothionein [52,56,57], (4) reduction [49–51,58] followed by efflux [58] and methylation [59,60], and (5) oxidation [61,62] (Figure 27–2).

27.2.1 Arsenic Uptake Pathways

Due to the extreme toxicity of arsenic, specific uptake transporters have not evolved [50] to avoid its uptake. But As(III) and As(V) are taken up by glycerol and phosphate transporters, respectively,

because of their similar chemical structure. In the case of *E. coli*, two phosphate transporters, Pit and Pst, are used for As(V) uptake; Pst is the dominant uptake pathway [63]. As(III) is taken up by the glycerol transporter GlpF [64], a member of glycerol channels of the major intrinsic protein (MIP) family. Mutation in GlpF resulted in As(III)-tolerant *E. coli* strains [64].

27.2.2 Extracellular Immobilization of Arsenic

Different bacteria have been shown to have the capacity to sequester or immobilize metals outside of the cell before they enter the cytoplasm, thus preventing their entry into cells and interaction with essential components, as in the case of *Herminiimonas arsenicoxydans* [54,65]. In the presence of arsenic, this results in large amounts of exopolymers. Images obtained using transmission electron microscopy (TEM) combined with X-ray energy dispersion spectroscopy (EDS) indicated that arsenic metalloid is sequestered within the exopolysaccharide capsule [65]. Bacteria mediating Fe(II) oxidation through nitrate reduction to get energy for growth in anoxic conditions have been shown to play a key role in arsenic cycling by forming solid hydrous ferric oxide to which As(V) [66,67] or As(III) [67,68] is adsorbed. In oxic environments, the acidophilic iron oxidizer *Acidithiobacillus (At.) ferrooxidans*, when grown with Fe(II) as electron donor, scavenges As(III) with the newly produced Fe(III) to form schwertmannite or tooeleite [24,69,70].

27.2.3 Arsenic Chelation

Chelation is the mechanism for limiting As accumulation and interaction with essential cellular components after its entry into the cell. This is mediated by peptides or proteins containing thiol ligands, such as glutathione, phytochelatins, and metallothioneins [52,56,57]. In *Cyanobacteria* and *Pseudomonas* sp., metallothioneins have been detected and shown to coordinate different metals [52,71–74].

27.2.4 Arsenate Reduction

Two different mechanisms of arsenate reduction are reported in bacteria, fungi, and algae: one is widespread in all microorganisms and considered responsible for detoxification; the other is specific only for bacteria and some archaea. Microorganisms use As as a final electron acceptor in their respiratory chains, for autotrophic or heterotrophic growth.

27.2.4.1 Mechanism of Arsenic Detoxification

Pathways for arsenic detoxification exist in all organisms examined, including bacteria, yeast, plants, etc. *Escherichia coli* [75], *Staphylococcus aureus* [76], *Corynebacterium* sp. [77], *Synechocystis* sp. PCC6803 [78], and *Saccharomyces cerevisiae* [79] are a few examples of these categories. The arsenic detoxification mechanism is controlled by *ars* genes that encode proteins that facilitate As(V) reduction via a cytoplasmic enzyme arsenate reductase into As(III) followed by As removal from the cell by an efflux pump [25,80]. The two most common operons contain either five (*arsRDABC*) or three (*arsRBC*) genes. The *arsRDABC* operon is found on the plasmids of Gram-negative bacteria such as *E. coli* R773 and *arsRBC* generally on the plasmids

of Gram-positive bacteria such as *Staphylococcus aureus* pI258, or on the bacterial chromosome [75,81,82]. The *arsR* gene encodes a trans-acting repressor of the ArsR/SmtB family involved in transcriptional regulation [83], *arsB* encodes a membrane bound arsenite carrier that effluxes arsenite [84], and *arsC* encodes a cytoplasmic enzyme named arsenate reductase that converts arsenate to arsenite [85]. In contrast, the *ars* operon of *E. coli* plasmids R773 and R46 encodes two additional proteins: ArsA, an arsenite-stimulated ATPase, and ArsD, a metalloid-responsive transcriptional repressor [83,86]. Similarly, in the case of *Saccharomyces cerevisiae*, a eukaryote, a gene cluster, *ACR1*, *ACR2*, and *ACR3*, present on chromosome was shown to confer resistance to As(V) and As(III) [79]. Acr3p catalyzes extrusion of the arsenite from cells, thus conferring resistance [87].

While this fundamental strategy of conferring arsenic resistance is fairly conserved, the arsenate reductases are quite varied in different organisms. The four types of arsenate reductases characterized as yet, such as plasmid encoded R773 ArsC and pI258 ArsC from *Escherichia coli* [85,88] and *Staphylococcus aureus* [76], respectively, Acr2p from *Saccharomyces cerevisiae* [79,89], and hybrid-type arsenate reductase SynArsC from *Synechocystis* PCC6803 [78,90], possess dissimilar sequence, folds, and differ from one another in several of their physical and catalytic properties [58,90]. (1) Prokaryotic ArsC from Gram-negative bacterium *E. coli* encoded by plasmid R773 [88], which uses grx and GSH as reductant. Reduction of As(V) is mediated by three cysteine residues: one in ArsC, one in GSH, and one in glutaredoxin. As(V) binds to three arginine residues and forms a covalent bond with the single catalytic cysteine residue Cys12 (R773 ArsC). A tertiary intermediate is formed between Cys12 and a glutathione cysteine. Finally, glutaredoxin reduces As(V) and the disulfide bond leading to the release of As(III) and regeneration of reduced ArsC [58,91]. (2) The prokaryotic ArsC from Gram-positive bacterium *Staphylococcus aureus* encoded by pI258 [76] and *Bacillus subtilis* use the cysteine thiol-coupling enzyme thioredoxin as the reductant and require the presence of thioredoxin reductase and NADPH to complete the catalytic cycle. The structure of these enzymes is remarkably similar to those of low molecular weight protein tyrosine phosphate phosphatases [92] and shows a low level of phosphatase activity. Three conserved cysteine residues are essential for catalysis. As(V) is covalently bound to Cys10 (pI258 ArsC), which reduces it by nucleophilic attack. Cys82 then attacks Cys10 leading to the formation of a Cys10–Cys82 disulfide intermediate and release of As(III). Cys89 attacks Cys82 to form a Cys82–Cys89 disulfide intermediate and to regenerate Cys10 thiolate. Thioredoxin reduces the Cys82–Cys89 disulfide regenerating reduced ArsC [92]. (3) The eukaryotic arsenate reductase Acr2p from *Saccharomyces cerevisiae* [79,89], *Leishmania major* [93], and *Arabidopsis thaliana* [94] uses grx and GSH as reductant. While Acr2p and R773 ArsC obtain their reducing equivalents from glutathione and glutaredoxin, pI258 uses thioredoxin as reductant. However, in the case of *Synechocystis* PCC6803, a novel type of hybrid arsenate reductase related to thioredoxin-dependent arsenate reductases uses the GSH/glutaredoxin system for reduction [90]. This arsenate reductase is encoded by *arsC* of the *arsBHC* operon. Disruption of the arsenate reductase gene leads to arsenate hypersensitivity in both *E. coli* and *Saccharomyces cerevisiae* [58,95]. Recently, in the case of the diazotrophic cyanobacterium *Anabaena* PCC7120, two different types of arsenate reductases were characterized [96,97].

27.2.4.2 Arsenate Respiration or Dissimilatory As(V) Reduction

The arsenic respiration specific for the Bacteria and Archaea is the second mechanism for reduction of As(V). Using this mechanism, bacteria couple the reduction of oxidized arsenic compounds with the final electron acceptors from their respiratory chain. As(V) being electrochemically positive, reduction coupled with the oxidation of an electron donor with a lower redox potential can be expected to provide sufficient energy to sustain bacterial growth [98] and, indeed, microbes respiring As(V) under anaerobiosis have been found throughout the prokaryotes, including Proteobacteria, Gram-positive Eubacteria, thermophilic Eubacteria, and Crenarchaeota from different environments [48,50,51]. These microorganisms use electron donors for As(V) reduction like hydrogen and sulfide, acetate, lactate, formate, pyruvate, and complex compounds such as benzoate, phenol, and toluene [50]. Dissimilatory arsenate reduction is carried out by a terminal aresenate reductase, fundamentally different from the detoxifying arsenate reductases. To date, only the arsenate reductases from *Chrysiogenes arsenates* [99], *Bacillus selenitireducens* [100], and *Shewanella trabarsenatis* strain ANA-3 [101] have been characterized. In these cases, arsenate reductase ArrAB is a heterodimer composed of large and small subunits of about 100 kDa (ArrA) and 30 kDa (ArrB), respectively. ArrA, which belongs to the DMSO reductase family, contains the As(V) binding site and the catalytic site with a *bis*molybdopterin guanine dinucleotide cofactor and one (4Fe-4S) cluster, while ArrB is an iron–sulfur protein with four (4Fe–4S) clusters. ArrAB is translocated to the periplasm by the twin arginine translocation (TAT) pathway since a characteristic TAT signal sequence is present in the large subunit ArrA. The *C. arsenatis* and *S. trabarsenatis* enzymes reduce only arsenate whereas *B. selenitireducens* also reduces As(III), selenate, and selenite [100]. ArrB conducts the electrons from the respiratory chain to the molybdenum cofactor of the large subunit ArrA where As(V) reduction takes place. In *S. trabarsenatis* ANA-3, the membrane-associated periplasmic tetraheme *c*-type cytochrome, CymA, is required for As(V) reduction [102] and has been proposed to mediate electron transfer from the quinol pool to ArrB [103]. The ArrA and ArrB subunits are encoded in an operon. ArrA large catalytic subunit encoded by *arrA* is present upstream of the small subunit coding gene *arrB*. The third subunit encoding gene (*arrC*) has been detected upstream from *arrAB* in a large number of bacteria. Membrane-integrated ArrC has been proposed to anchor ArrAB to the cytoplasmic membrane and to mediate electron transfer from the quinone pool [50,51,102,104]. It is worth noting that no archaeal Arr representatives have been detected so far [104].

To date, the only reported study on the regulation of the *arr* operon has been performed in *S. trabarsenatis* ANA-3. This operon has been shown to be mainly expressed in anaerobiosis with further activation by As(III) and As(V), and it is repressed by oxygen and nitrate, indicating that it is regulated by at least two control systems, one sensing the cellular redox state and the other detecting specifically arsenic [105]. The *cymA* gene encoding the physiological partner of the arsenate reductase is also induced under anaerobic conditions in the presence of arsenic [102,106]. The cyclic AMP receptor protein (CRP), activated by cyclic AMP (cAMP) production under anaerobic conditions in *S. trabarsenatis* ANA-3, was found essential for *arr* expression in the absence of oxygen [106]. The As(III) sensing regulator was demonstrated to be an ArsR repressor, ArsR2, encoded by a gene located in the same locus as the *arr* operon [106]. The

model proposed could be that ArsR2 represses *arr* transcription in the absence of arsenic even when CRP is activated by cAMP under anoxic conditions [106]. Concerning the possible origin of the arsenate reductases, two hypotheses have been proposed. In one, the arsenate reductase was an "ancient" microbial enzyme that arose on primordial Earth, was functioning as an arsenite oxidase, and was responsible for the anaerobic As(III) oxidation before acquiring its As(V) reducing functions [48,107]. In contrast, Arr originates more "recently" after the Archaea/Bacteria split, likely in a *Gammaproteobacterium*, from an enzyme involved in the bioenergetic reduction of sulfur compounds (i.e., tetrathionate, thiosulfate or polysulfide reductase) followed by its dispersal over other phyla [104,108].

27.2.5 Efflux of As(III) from the Cell

The most widely distributed and the best characterized system for As(III) detoxification is the Ars system (ArsRBC or ArsRDABC). Among these systems ArsB is an integral membrane protein, which pumps As(III). ArsB when alone uses the membrane potential to drive the efflux of the As(III). But when present with ArsA, which is an ATPAse that converts membrane potential to ATP, it gets energy from ATP hydrolysis, making As(III) efflux more efficient. ArsD also functions as a chaperone of the ArsAB pump, by binding and transferring As(III) to ArsA [109]. However, in *Synorhizobium meliloti* As(III) efflux is done through an aquaglyceroporin, AqpS, encoded within the *ars* operon in place of *arsB* [110,111]. Recently, novel single arsenate resistance proteins that apparently result from recent evolutional fusion events between an aquaglyceroporin-derived arsenite channel and the C-terminal domain of an ArsC arsenate reductase in the actinobacteria *Frankia alni* and *Salinispora tropica* and between Acr3 and ArsC in *Mycobacterium tuberculosis* were reported. In these cases, a single protein generates the energy and catalyzes the efflux of As(III), which should improve the extrusion efficiency [112]. No As(V) extrusion pump has been identified to date. In fact, the less toxic As(V) is converted by the cytoplasmic arsenate reductase ArsC to the more toxic As(III), which is then pumped out of the cell as discussed in the previous section. As(V) detoxification involving its enzymatic reduction to As(III), followed by As(III) export, is conserved from bacteria to plants [113]. It was proposed that the As(III) efflux system arose first in the primordial anaerobic environments where As(III) was the predominant As species. Once Earth's atmosphere became more oxidizing with the increased production of molecular oxygen, As(V) would have become the major As species, and proteins (likely phosphatases) evolved to reduce As(V) to As(III), which was subsequently excreted from the cell via ArsB or Acr3 to mitigate the toxic effect of As(V) [58].

27.2.6 Oxidation of As(III)

A wide range of bacteria and archaea have the potential to oxidize As(III) enzymatically through arsenite oxidases. The first report of an As-oxidizing bacterium named *Bacillus arsenoxidans* was given by Green in 1918 [114]. Many studies have now reported microorganisms as arsenite oxidizers with heterotrophic or chemoautotrophic growth [61,62,65,115–117]. In these bacteria As(III) serves as an electron donor reducing oxygen or nitrate [118,119]. However, in the case of the Gammaproteobacteria *Ectothiorhodospira* sp. strain PHS-1, As(III) acts as an

electron donor for anoxygenic phototrophic growth [107]. Therefore, for As(III) oxidation two different arsenite oxidase enzymes were reported, the first being AoxAB (also named AroBA or AsoBA), widely found and extensively studied, and the second being ArxAB, found only in the Ectothiorhodospiraceae family [107,120]. The latter is phylogenetically close to respiratory arsenate reductase ArrAB than to AoxAB arsenite oxidase. The genes *aoxA* and *aoxB* encoding the arsenite oxidase AoxAB were first identified and characterized in the heterotrophic bacterium *Herminiimonas arsenicoxydans*. They have been sequenced and their operon structure has been described [121]. These genes are expressed in the presence of arsenic. Thereafter, these genes (also called *asoA* and *asoB*) were recognized and sequenced in various bacteria, including chemoautotrophic (ref. NT-26) and photoautotrophic microbes [22]. The genetic basis of arsenite oxidase was first elucidated by Muller et al. [121] in knockout mutants deficient in arsenite oxidation of strain ULPAs1. This AoxAB-type arsenite oxidase has been characterized from *Alcaligenes faecalis* [122–124], *Rhizobium* sp. strain NT-26 [116], *Hydrogenophaga* sp. strain NT-14 [125], *Arthrobacter* sp. strain 15b [126], and *Ralstonia* sp. 22 [127]. In all these cases, it consists of a large subunit arsenite oxidase B (AoxB) belonging to the DMSO reductase family with a molybdenum cofactor together with a (3Fe-4S) center and a small subunit (AoxA) containing a "Rieske" (2Fe-2S) center [104,123]. The small subunit AoxA possesses a TAT leader sequence. The difference in the quaternary structure of AoxAB-type arsenite oxidases is noticed as heterodimer α1β1 in *A. faecalis* [123] and *Arthrobacter* sp. strain 15b [126], heterotetramer α2β2 in *Rhizobium* sp. strain NT-26 [116], and heterohexamer α3β3 in *Hydrogenophaga* sp. strain NT-14 [125].

27.2.6.1 Mechanism of As(III) Oxidation

As per the proposed As(III) oxidation model [123,124], As(III) first undergoes a direct nucleophilic attack by the molybdenum cofactor Mo(VI) with the transfer of two electrons leading to the production of reduced molybdenum Mo(IV) with As(V) coordinated with the metal. As(V) is released and Mo(IV) is oxidized to Mo(VI). Then, electrons are transferred first to the (3Fe-4S) in AoxB and then to the (2Fe-2S) center of AoxA. From the small subunit, the electrons are given to the respiratory chain in the inner membrane towards the terminal electron acceptor. In the case of *A. faecalis*, the electron acceptor of AoxAB has been proposed to be azurin or a cytochrome *c* since both proteins are reduced by AoxAB in the presence of As(III) *in vitro* [122]. However, in the case of *Hydrogenophaga* sp. strain NT-14 [125], *Rhizobium* sp. strain NT-26 [128], and *Ralstonia* sp. strain 22 [127], a cytochrome *c* that accepts electrons directly from AoxAB has been characterized. In addition, in *Ochrobactrum tritici*, a cytochrome *c*, encoded by the *aox* operon, is required for arsenite oxidation [129]. The two arsenite oxidase subunits are invariably encoded in an operon with the gene encoding the small subunit *aoxA* upstream from that encoding the catalytic subunit *aoxB*. This relative gene order is conserved in all arsenite oxidizers analyzed to date like *H. arsenicoxydans* [65,121], *Rhizobium* sp. strain NT-26 [116], *Agrobacterium tumefaciens* [130], *T. arsenitoxydans* [131,132], *Achromobacter* sp. strain SY8, *Pseudomonas* sp. TS44 [133], *O. tritici* [129], and *Hydrogenobaculum* sp. strain 3684 [134]. Nevertheless, very often upstream from *aoxA*, two genes encoding the histidine kinase and the cognate response regulator of a classic

two-component signal transduction system (*aoxSR*) are detected. Downstream from *aoxB*, a gene encoding a cytochrome *c* is sometimes present in *H. arsenicoxydans* [65,121], *Rhizobium* sp. strain NT-26 [128], *A. tumefaciens* [130], *T. arsenitoxydans* [131,132,135], *Achromobacter* sp. strain SY8 [133], and *O. tritici* [129], as well as one or two genes involved in molybdenum cofactor biosynthesis *moaA* and/or *moeA*.

The *aox* operon regulation seems to be complex and to vary between species. Approximately four proteins have been shown to be involved in the *aox* operon expression: (1) a two-component signal transduction system AoxSR, which is required for *aox* expression in all the bacteria analyzed so far, (2) an ArsR/SmtB family member AoxF specific to *T. arsenitoxydans*, (3) the sigma factor σ54 (RpoN) with a binding site upstream from *aox* operon in *Rhizobium* sp. strain NT-26 [128], *O. tritici* [129], *T. arsenitoxydans* [136], and *H. arsenicoxydans* [137], and (4) DnaJ in *H. arsenicoxydans* [137]. The role of the co-chaperone DnaJ constitutes part of the Hsp70 machinery, and is believed to help in proper folding of AoxR, stabilization of RpoN, or stabilization of *aox* messenger RNA [137]. It has also been established that in some chemolithotrophic enzymes, arsenite oxidation is combined with oxygen or nitrate reduction processes (under anaerobic conditions) and that the energy thus produced serves to fix CO_2 [22]. In environments that harbored the very first forms of life on Earth, As(III) may have provided one of the main mineral energy sources required for the metabolism of the first chemolithotrophic organisms. The colonization of these primitive arsenic-rich environments by bacteria using As(III) as an electron source and transforming it into less bioavailable As(V) may have resulted in the partial detoxification of these inhospitable environments. These bacteria may therefore have contributed to extending the limits of life by making it possible for other microorganisms to survive and proliferate.

ArxAB-type arsenite oxidase has been detected in certain arsenite oxidizers, which oxidize As(III) in anoxic conditions like *Alkalimnicola ehrlichii* [117,138], *Azoarcus* sp. strain DAO1, [138] and *Ectothiorhodospira* sp. strains PHS-1 and MLP2 [107] with no *aoxAB* gene homologues known, suggesting that an alternative mechanism is utilized in these conditions. *arxA* and *arxB* genes coding for a novel arsenite oxidase were identified in *A. ehrlichii* [120,139] as well as in *Ectothiorhodospira* sp. strains PHS-1 and MLP2 [120]. ArxB has been predicted to be a small subunit with four (4Fe-4S) iron–sulfur clusters and ArxA to be a large subunit containing a molybdenum cofactor and a (4Fe-4S) cluster [120]. This new arsenite oxidase shows greater similarity with the respiratory arsenate reductase ArrAB than with the arsenite oxidase AoxAB in terms of (1) the total amino acid identity and similarity, (2) the presence of TAT signal sequence in the large subunit ArxA, (3) the large catalytic subunit ArxA, which contains a (4Fe-4S) rather than a (3Fe-4S) cluster, and (4) the small subunit ArxB, which has four (4Fe-4S) clusters in contrast to the single (2Fe-2S) center present in the Rieske subunit AoxA. Apart from this the genetic organization of the *A. ehrlichii arx* operon is different from that of the *aox* operon since (1) *arxA* encodes the large subunit upstream from *arxB* corresponding to the small subunit and (2) it encodes also a putative (4Fe-4S) containing protein ArxB′, an anchoring subunit ArxC, and a chaperone-like ArxD of the TorD family [120]. This operon is only expressed under anaerobic conditions in the presence of As(III) [139]. Furthermore, the phylogenetic relatedness of this arsenite oxidase is more

closely related to ArrAB respiratory arsenate reductases than to AoxAB arsenite oxidases [139] and has been proposed to be an ancestral form of the ArrAB respiratory arsenate reductases and AoxAB arsenite oxidases [48].

27.2.7 Arsenic Biomethylation

Biomethylation of As is a well-known biological process, which is reported in many bacteria, archaea, fungi, algae, plants, animals, and humans [140–143]. Recently, As biomethylation and biovolatilization have also been observed in a model protozoan *Tetrahymena thermophila* [144]. These organisms methylate As to different extents and produce volatile and non-volatile As species. The leading volatile species are mono-, di-, and trimethylarsine (MMA, DMA, and TMA), while methylarsonate and dimethylarsinate are the main non-volatile compounds. Although methylation was considered as a detoxification process, recent studies show that some methylated species such as mono- and dimethylarsenite can be as, or even more toxic than, inorganic forms of arsenic [47,145]. Biotransformation of As(III) to TMA(III) was first documented in fungi in 1890 [19]. The *arsM* gene encoding the As(lll) *S*-adenosylmethionine methyltransferase is prevalent and has been detected in 125 bacteria and 16 archaeans [146]. Subsequently, genome sequencing homologues of *arsM* were identified in many fungi. The ArsM from *Rhodopseudomonas palustris* was shown to confer As(III) resistance when expressed in an As-sensitive strain of *Escherichia coli* [146].

The presence of methylated As species has been reported for numerous photosynthetic organisms, including cyanobacteria, algae and plants. When the marine green alga *Chlorella salina* was exposed to 1 mM As(V) or As(III), the amount of MAs(V) and DMA(V) was 9–12% of total intracellular As [147]. A more recent study on the short-term metabolic processes of As(V) in a freshwater green alga, *Chlamydomonas reinhardtii*, showed that As(III), As(V), and MAs(V) concentrations in the cells increased from 30 min to 1 h, and then leveled off [148]. DMA(V) and oxo-arsenosugar-glycerol levels also increased continuously for 24 h. These results indicated that *C. reinhardtii* can rapidly biotransform As(V) into oxo-arsenosugar-glycerol and then oxo-arsenosugar-phosphate. The reduction of As(V) to As(III) and methylation of As(III) to DMA(V) are coupled in this process, and MAs(V) was observed to be a possible intermediate. This is different from *Rhodopseudomonas palustris*, in which no monomethylated species were observed. When the marine brown alga *Fucus serratus* was treated with different concentrations of As(V) for up to 19 weeks, As(V), As(III), MAs(V), DMA(V), and four arsenosugars were detected [149]. The proportion of As species found in *F. serratus* depended on the As(V) concentration that it was exposed to. When the As(V) concentration was at 100 mg As(V)/L, the main product was As(III). At 20 mg As(V)/L, DMA(V) and arsenosugars were the main As metabolites. These results suggest that, at higher As(V) concentrations, the As(III) methylation process becomes saturated, leading to the accumulation of As(III) and subsequent toxicity [149]. However, at this point, it is not known whether *F. serratus* actually has a gene encoding an ArsM. *Fucus serratus* is often intimately associated with specific fungi and it is possible that these fungi carry out the methylation reaction [150]. Thermophilic algae have also been shown to methylate and volatilize As. *Cyanidioschyzon* sp. isolate 5508, isolated from thermal wells in

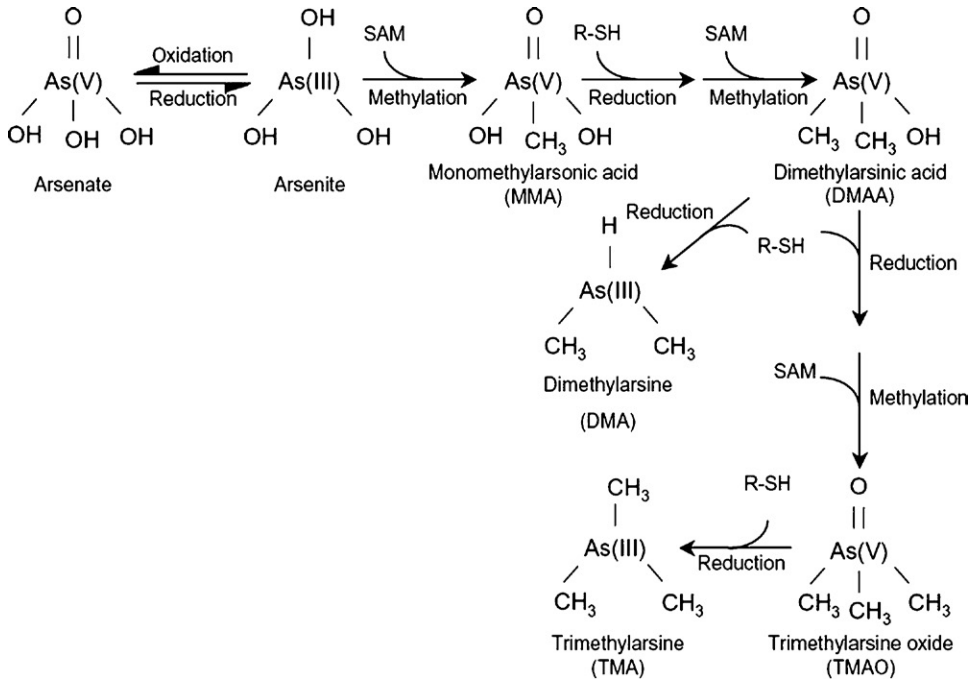

FIGURE 27–3 Biomethylation pathway in prokaryotes (SAM, *S*-adenosylmethionine; R-SH, thiol-containing compounds) [43].

Yellowstone National Park, is capable of oxidizing As(III) to As(V), reducing As(V) to As(III), and then methylating As(III) to form TMAO and DMAs(V) [146]. Two As methyltransferase genes from *Cyanidioschyzon* sp. isolate 5508, CmarsM7 and CmarsM8, were expressed in an As(III)-hypersensitive strain of *E. coli* and conferred resistance to As(III).

Cyanobacteria are ubiquitous in environments ranging from soil and water to deserts. Due to their rapid growth rates and ability to adapt to environmental changes, they are often key players in toxic aquatic environments. Methylated As species have been found in various cyanobacteria and algae. Arsenic biotransformation and volatilization has also been observed in three cyanobacteria under laboratory conditions [144]. *Microcystis* sp. PCC7806, *Nostoc* sp. PCC7120, and *Synechocystis* sp. PCC6803 accumulated large amounts of As when treated with 10 mM sodium As(III). Methylated species of As(III) were detected following exposure to higher As(III) concentrations for 14 days. Volatile arsenicals were detected when the cyanobacteria were treated with As(V) for 6 weeks. Two ArsM homologues have been cloned and purified. The enzymes were shown to methylate As(III) *in vitro* with TMA(III) as the end product [144].

27.2.7.1 Methylation Pathway

The methylation pathway is a multistep process with initial As(V) reduction followed by a cycle of oxidative methylation and reduction of the organoarsenical intermediates [151] (Figure 27–3). The pathway was proposed by Challenger [152] in 1945, based on work on the fungus *Scopulariopsis*

brevicaulis [151,152]. This addition results in the formation of different compounds including methyl arsenite, dimethyl arsenate, dimethyl arsenite, trimethyl arsine oxide (TMAsO), glutathione, and other thiol-containing compounds. *S*-adenosylmethionine is required as the source of methyl groups [19] in anaerobic bacteria. An arsenite *S*-adenosylmethionine methyltransferase, catalyzing transfer of methyl groups from *S*-adenosylmethionine to As(III) with glutathione as electron donor, has been characterized in *Rhodopseudomonas palustris* [146,153].

Microorganisms are thought to play a key role in regenerating As(V) by demethylation of methylated arsenic species to use them as carbon and energy sources. *Alcaligenes*, *Pseudomonas*, and *Mycobacterium* are able to demethylate mono- and dimethyl arsenic compounds [19], and *Pseudomonas* sp. has been shown to use DMA as carbon source [154]. Recently, a two-step demethylation of mono methylarsonic acid (MMA) to As(III) mediated by two distinct microorganisms was observed in MMA-contaminated soil samples. This process involved a reduction of MMA to methylarsonous acid by *Burkholderia* sp. MR1 followed by a demethylation to As(III) by *Streptomyces* sp. MR1 [155]. However, the mechanism of demethylation is still unknown.

27.2.8 Morphological, Physiological, and Biochemical Responses

In addition to these well-characterized resistance determinants, microbes respond at morphological, physiological, and biochemical levels when challenged with As. One of the most important microbes, cyanobacteria, has high tolerance ability to As concentrations and is capable of decontaminating As from liquid culture through intracellular accumulation and promoting volatization loss. In the case of *Anabaena* sp. PCC7120, As treatment within 48–72 h produced filament fragmentation, thickening, enlargement and vacuolation of cells, transformation of bluish green cells into yellow brown cells, and dense pigmentation at one side of the cell. One of the most interesting observations was the appearance of akinete-like structures in *Anabaena* PCC7120, never reported before [156]. With the exception of respiration, PSI, PSII, whole chain, ^{14}C uptake, NADPH and ATP contents, and nitrogenase activity were found inhibited on As treatment.

Bioaccumulation of As in *Phormidium* [157], and antioxidative enzymes of *Anabaena doliolum* [158], *Phormidium laminosum* [157] and the role of arenic resistance genes (ars operon) in *Synechocystis* [78] are also known. Proteomic and biochemical analyses revealed the role of phytochelatin, GST, phytochelatin synthase, arsenate reductase, and arsenite efflux genes *asr1102* and *alr1097* in As sequestration and shielding of the *Anabaena* sp. PCC7120 from As toxicity. These analyses further demonstrated that the *ars* genes play a central role in detoxification and survival of *Anabaena* under As stress. The proposed hypothetical model explains the interaction of metabolic proteins associated with the survival of *Anabaena* sp. PCC7120 under As stress [156] (Figure 27–4).

27.3 Arsenic Toxicity and Tolerance *in planta*

Plants play a major role in the entry of metals and metalloids into the food chain [159]. It is therefore worthwhile to understand the mechanisms by which they take up and metabolize As from the soil, sequester it within the plant, and detoxify it. In order to reduce the As uptake through

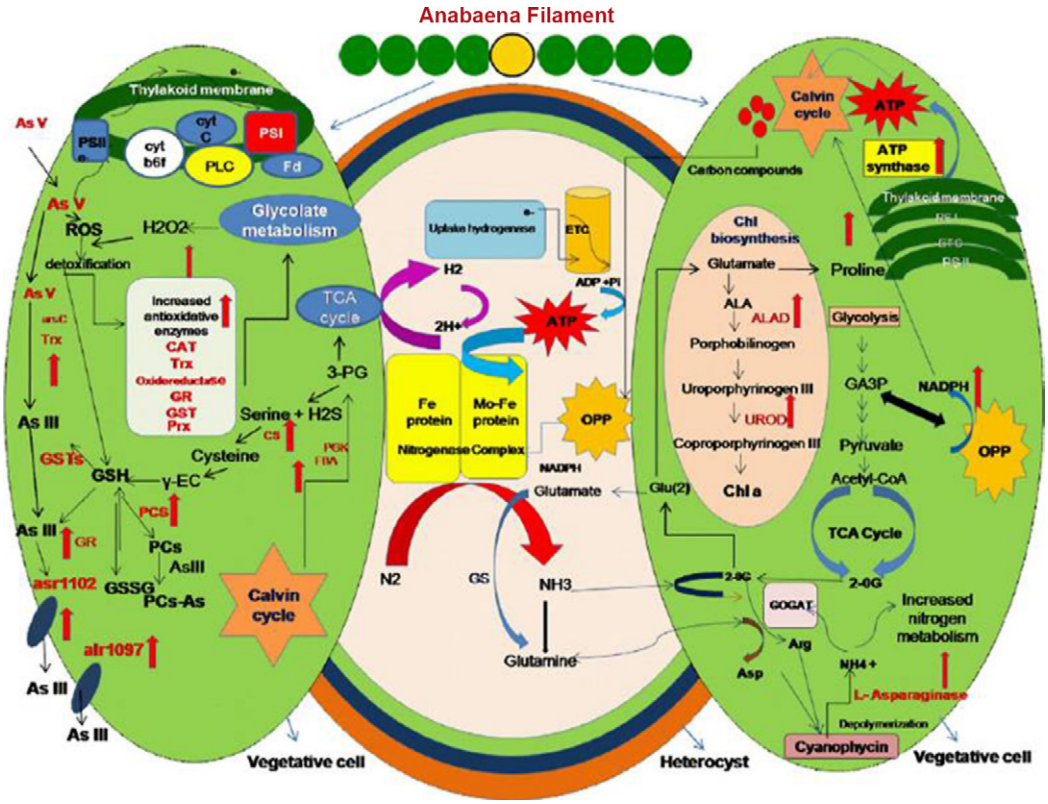

FIGURE 27–4 Hypothetical mechanistic model depicting the survival strategy of *Anabaena* sp. PCC7120 under As stress. Upward arrows indicate enhanced enzyme activity and protein expression. Figure modified after Pandey et al. [156].

consumption of contaminated plant foods, a better understanding of the mechanisms of As uptake, metabolism, and identification of the transporters responsible for arsenite detoxification by plants is a prerequisite. A potential outcome of this would be the generation of As-resistant plants for (1) phytoremediation and (2) safe cropping [37,160]. For remediation and restoration of As-contaminated sites, a diverse group of tolerant/resistant plants capable of growing at high As concentrations and able to (hyper)accumulate As in harvestable biomass are needed. In contrast, for safe cropping in areas where land and/or groundwater are contaminated, As-resistant plants that prevent accumulation in the harvested plant product are required. There is a considerable literature on the mechanisms pertaining to As uptake, accumulation, and detoxification in plants; the section below summarizes in brief the work done so far.

27.3.1 Arsenic Uptake and Efflux

The most common forms of As in soil solution available for plant uptake are arsenate, arsenite, MMA, and DMA. Their uptake mechanisms are described below.

27.3.1.1 Arsenate Uptake

Arsenate [As(V)] is the main As species in aerobic soils. It has a strong affinity for iron oxides/hydroxides in soil; thus the concentrations of arsenate in soil solutions are usually low. Arsenate and phosphate share the same transport pathway in higher plants; the Pi transporter (PHT) proteins [161–165] have a higher affinity for phosphate than for arsenate [166]. Therefore, As(V) and Pi compete for uptake through the same transport systems in As hyperaccumulators, [33,167] As-tolerant non-hyperaccumulators [164,168], as well as As-sensitive non-accumulators [169,170]. Under low Pi conditions, As(V) may outcompete Pi for entry into the plant, amplifying Pi deprivation symptoms. Conversely, Pi fertilization can protect plants, including the hyperaccumulator *P. vittata*, from As(V) toxicity [167]. Increasing or decreasing the rate of Pi and As uptake by increasing or decreasing PHT protein amount or activity at the plasma membrane through genetic means can also increase or decrease, respectively, the toxicity of As(V) [165,171–173]. A number of phosphate transporters have been characterized in plants [174,175]. There are over 100 phosphate transporters in the phosphate transporter 1 (Pht1) family, most of which are strongly expressed in roots and are likely to be involved in phosphate uptake from the external medium [175].

In *Arabidopsis thaliana*, two phosphate transporters, Pht1;1 and Pht1;4, play a significant role in phosphate acquisition from both low and high phosphorus (P) conditions [171]. The *A. thaliana* double mutant *pht1;1Δ4Δ* was much more resistant to arsenate than the wild type, indicating that Pht1;1 and Pht1;4 mediate arsenate uptake [171]. In the *A. thaliana* mutant defective in phosphate transporter traffic facilitator 1 (PHF1), the trafficking of the Pht1;1 protein from the endoplasmic reticulum to the plasma membrane is impaired [172]. This mutant was much more resistant to arsenate than the wild type, further supporting a role of Pht1;1 in arsenate uptake. Recently, Catarecha et al. [173] identified an arsenate-tolerant mutant of *A. thaliana*, *pht1;1-3*, which harbors a semidominant allele coding for the high affinity phosphate transporter PHT1;1. Rather intriguingly, the *pht1;1-3* mutant displays the dual phenotypes of decreased arsenate uptake in the short term and increased As accumulation over a longer period of growth. As the wild-type plants suffered from severe As toxicity, it is perhaps not surprising that their As accumulation capacity was curtailed compared with the mutant. Once inside the plant cell, As(V) can probably move easily from one cellular compartment to another, crossing internal membranes through various Pi transporters. For example, As(V) has been demonstrated to be a co-substrate for three mitochondrial dicarboxylate transporters, proteins localized to the inner mitochondrial membrane and responsible for dicarboxylate exchange with co-substrates such as Pi, between the cytosol and the organelle matrix [176]. As(V) can be found in the xylem, considerably loaded into the xylem vessels by PHT proteins [37,165,173,177]. However, roots of As non-hyperaccumulator have the ability to strongly retain As.

In *Arabidopsis*, only about 3% of the As taken up by the root was found to be translocated to the shoot [178]. Similar results have been found for other plants [179]. Following uptake of arsenate by roots, some of the arsenate is lost from the cells via efflux to the external medium [32]; this is analogous to the situation for phosphate, which can also be lost via efflux especially under high P conditions [180]. The mechanism of arsenate efflux is not known, but may be similar to that of phosphate efflux presumably via anion channels [180]. Xu et al. [32] showed

that arsenate added to the aerated nutrient solution was rapidly converted to arsenite by the roots of tomato and rice. Microbes living in the nutrient solution or root exudates contributed little to arsenate reduction to arsenite. This is surprising because arsenate is expected to be stable in the aerobic environment. Phosphate inhibits arsenate uptake and the subsequent production of arsenite in the external medium, suggesting that the arsenite is extruded by root cells following arsenate reduction inside the cells. Indeed, efflux of both arsenate and arsenite was observed when tomato roots preloaded with arsenate were transferred to an As-free medium. Furthermore, the protonophore carbonylcyanide m-chlorophenylhydrazone (CCCP) inhibited the efflux of arsenite, suggesting that the efflux is linked to the proton gradient across the plasma membranes, or is energy dependent.

27.3.1.2 Arsenite Uptake

As in microorganisms and mammalian tissues [181], arsenite enters plant root cells through nodulin 26-like intrinsic proteins (NIPs) [182–184]. These proteins belong to the aquaporin family of major intrinsic proteins. In rice roots, the OsNIP2;1/OsLsi1 silicon transporter has been implicated as the main As(III) uptake protein, while As(III) efflux from rice root cells to the xylem takes place through the OsLsi2 silicon transporter [184]. Other types of proteins may facilitate the transport of As(III) into cells. In As hyperaccumulating species, such as *P. vittata*, As is not immobilized in the roots, but rapidly transported as As(III) through the xylem to the fronds [185–187]. In the fronds, As(III) is sequestered as free As(III) in the vacuole [185,186], where it accumulates to extremely high levels [188]. It has been shown that PvACR3 is involved in the vacuolar sequestration of As(III) [189]. This protein is a homologue of the yeast ScACR3-p protein, a plasma membrane protein responsible for the efflux of As(III) from the yeast cell. In *P. vittata*, the PvACR3 protein still acts to give rise to efflux of As(III) from the cytosol, but instead of delivering the As(III) to the outside of the cell, PvACR3 resides on the vacuolar membrane and transports the As(III) into the vacuole. Single-copy *ACR3* genes are found in moss, lycophytes, ferns, and gymnosperms, but not in angiosperms, which may help explain the lack of As hyperaccumulators among the angiosperms [189].

27.3.1.3 Uptake of Methylated Arsenic

Like As(III), the protonated, uncharged forms of the methylated As species MMA(V) and DMA(V) enter rice roots at least in part through the aquaporin channel OsLsi1 [190]. However, the rate of uptake for MMA(V) and DMA(V) is much slower than that of As(III) or As(V) [191–193]. In contrast, the mobility within the plant of MMA(V) and DMA(V) appears to be substantially greater than that of As(III) or As(V) [190,192,194–197]. While OsLsi1 has been implicated in the uptake of both MMA(V) and DMA(V), OsLsi2 does not seem to be involved in their efflux [190]. In animal systems, MMA(III) is also transported through aquaporins; however, it is not yet known whether OsLsi1, OsLsi2, or AsIII transporters are able to transport MMA(III). Arsenic can also be metabolized by various organisms to form arsenocholine, arsenobetaine, and arseno-sugars. These compounds have been detected in some terrestrial plants. However, it has not been demonstrated that these compounds can be produced by the plant, or whether they are simply taken up in those forms from the soil. The effects of these compounds on plant metabolism are largely unknown.

27.3.1.4 Long-Distance Transport

In most plant species analyzed, arsenite dominates in the xylem sap, suggesting that it is the main form loaded into the xylem [188]. This is also true when arsenate is supplied to plant roots, and is consistent with the fact that roots have a high capacity for arsenate reduction. There is no evidence that arsenite in the xylem sap is complexed with thiol compounds [30,31]. In fact, complexation with thiols decreases arsenite mobility from roots to shoots. Rice loads arsenite into the xylem more efficiently than does wheat or barley or any other crop plants [198]. *P. vittata* has an exceedingly efficient system to load arsenite into the xylem [187], but the underpinning mechanism has not been elucidated. Little is known about phloem transport of As, such as the form of As transported and the transporters involved in phloem loading and unloading. In a recent study using rice panicles excised below the flag leaf node, Carey et al. [196] found that DMA was transported to the immature grain approximately 30 times more efficiently than arsenite. When the phloem flow was disrupted by stem girdling, transport of arsenite into the grain was decreased by 10-fold, but that of DMA by only 50%. These results suggest that arsenite is delivered to rice grain mainly through the phloem, whereas both phloem and xylem pathways make an equal contribution to the transport of DMA to grain. Further evidence from a synchrotron μ-XRF study indicates that arsenite accumulates in the ovular vascular trace of the grain, whereas DMA permeates into the outer layer of the endosperm.

27.3.2 Arsenic Toxicity to Plants

27.3.2.1 Impact on Morphology, Growth, and Productivity

Exposure of plants to toxic levels of As triggers a wide range of morphological, physiological, and metabolic alterations [199–202]. The typical symptoms shown by As-stressed plants are interveinal necrosis [203,204], whitish chlorosis, and wilting [201,202]. Arsenic inhibits root extension and proliferation [205]. Most plants possess mechanisms to retain much of their As burden in the root. However, a genotype-dependent proportion of the As is translocated to the shoot and other tissues of the plant. Once in the shoot, As can severely inhibit plant growth by slowing or arresting the expansion and biomass accumulation, as well as compromising plant reproductive capacity through infertility, lack of germination, and loss of yield and fruit production [206]. Carbonell-Barrachina et al. [199] reported that As-exposed bean plants showed average reductions of 50% and 84%, respectively, in foliar mass (leaf dry weight) and fruit yield (fruit dry weight) as compared to controls. Similarly, Shaibur et al. [201] reported that in sorghum (*Sorghum bicolor*), shoot and root dry matter yield were repressed by higher As levels. A decrease in growth of both the vegetative and root system of tomato plants was also noticed at higher As concentrations [207].

Straight head disease is a physiological disorder of rice (*Oryza sativa* L.), characterized by sterility of the rice florets/spikelets leading to reduced grain yield. It was observed that BRRI dhan 29, a popular Bangladeshi rice strain, when grown in soils spiked with As showed the severity of the disorder in a dose-dependent manner [204,208]. Low As burden causes the number of nitrogen-fixing root nodules to be repressed in soybean [209]. One of the many interesting paradoxes related to As toxicity is that plant growth is stimulated at low As concentrations [206,207,210–212]. The fact that this phenomenon occurs under axenic

conditions in cultured plants, such as *Arabidopsis thaliana* [213], indicates that the trait is not based on As disrupting plant–biotic interactions. Instead, it results either from a direct interaction of As with plant metabolism, or from an interaction of As with plant nutrients. While the mechanism is not fully understood, it has been suggested that the growth benefit arises from As stimulation of Pi uptake [167]. It has been shown that in the presence of P fertilization, the negative effects of As on growth and productivity are ameliorated [168,214–216]. Liu et al. [217] reported a significant decline in root biomass in wheat seedlings and grain yield with increase in As(III) and As(V) concentrations for all six varieties of *Triticum aestivum* studied. However, in contrast, plants supplemented with P showed only a slight or moderate decrease in grain yield. In tune with this, in winter wheat As caused a slight reduction in both root and shoot biomass. P supply, in contrast, markedly increased shoot dry weights with a slight reduction in root dry weights [218]. A decrease in plant biomass with increasing As concentration in irrigation water has also been reported by Pigna et al. [216], and this reduction was less severe in added P conditions.

27.3.2.2 Physiological and Biochemical Responses

At sufficiently high concentrations, As interferes with critical metabolic processes, which can lead to plant death. Arsenic damages the chloroplast membrane and disorganizes photosynthetic function [219]. A significant decrease in pigment synthesis, CO_2 fixation, and activity of photosystem II (PSII) was observed in oats growing under As stress [219]. Singh et al. [203] reported that As exposure did not significantly reduce the concentrations of chlorophyll a and b, and total chlorophyll in *P. vittata* regardless of the exposure time; however, it reduced those in *P. ensiformis* after 10-d exposure, proving *P. vittata* to be resistant to As. Milivojevic et al. [220] reported that the changes of maximum quantum yield of PSII (ϕ) and photochemical quenching of fluorescence q_p under the highest As concentrations point to the changes of the redox stable state of the physiological quenching of fluorescence, which was not significantly modified by As application. Cellular membranes get damaged in plants exposed to As, causing electrolyte leakage [203], often accompanied by an increase in malondialdehyde contents. Arsenic exposure induces antioxidant defense mechanisms. The synthesis of ascorbate, the γ-Glu-Cys-glytripeptide GSH, and the GSH oligomer ((γ-Glu-Cys)*n*-Gly) phytochelatin increases throughout the plant, but more so in the roots [202,203,215,221–223], while anthocyanin accumulates in leaves [173,224]. Plant transpiration intensity may be reduced [219].

27.3.2.3 Proteomic Responses of Plants to As Stress

Proteomic studies on As-induced alterations are still few and limited to maize roots [225], maize shoots [226], rice roots [227] rice leaves [228], a pseudometallophyte *Agrostis tenuis* [229], hyperaccumulator species *Pteris vittata* fronds and roots [230,231], and to the moderate As tolerant/accumulator *Artemisia annua* [224]. Expression patterns of maize (*Zea mays*) root and leaf proteins in response to As stress were described for the first time by Requejo and Tena [225,226]. It was found that seven of the 11 identified proteins were involved in cellular homeostasis for redox perturbation. This suggested that oxidative stress is a major process underlying As toxicity in plants. To figure out the precise mechanisms underlying the

toxicity of As, a comparative proteomic analysis of rice roots in combination with physiological and biochemical analyses was done by Ahsan et al. [227]. The comparative proteomic analyses identified 23 differentially expressed proteins in rice roots, including those predicted as *S*-adenosylmethionine synthetase (SAMS), cysteine synthase (CS), and novel proteins including tyrosine-specific protein phosphatase protein, and omega domain containing GST. These differentially expressed proteins are functionally involved in cell signaling, stress and detoxification, defense and development, and protein biosynthesis. Ahsan et al. [228] also reported the first proteome map of rice leaves under As stress along with physiological and biochemical responses. The increased activity of several proteins associated with energy metabolism, such as NADP-dependent malic enzyme, NAD-dependent formate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase suggest that an increased amount of energy is required to adapt to As stress. However, the down-regulation of RuBisCO and chloroplast 29 kDa ribonucleoproteins might be the possible causes of the decreased photosynthesis rate under As stress. Contrary to this finding, Duquesnoy et al. [229] identified a set of *Agrostis tenuis* leaf proteins differentially expressed in response to As exposure including a major functionally homogeneous group of enzymes including oxygen-evolving enhancer protein, RuBisCO small and large subunits, RuBisCO activase, and ATPsynthase involved in the Calvin or Krebs cycle. Bona et al. [230] also demonstrated the protein expression profile of *P. vittata* fronds in plants inoculated with one of the two arbuscular mycorrhizal (AM) fungi (*Gigaspora mosseae* or *Gigaspora margarita*) with and without As treatment. Recently, Rai et al. [224] demonstrated that proteins of photosynthesis and carbon metabolism play a key role in augmenting As tolerance to *A. annua*. The results also suggested that ABC-transporter like protein may be involved in As sequestration into vacuoles as the final detoxification step.

27.3.3 Mechanism of As Toxicity

The results from a number of hydroponic experiments have always been a matter of debate; while they agree that As phytotoxicity depends on the chemical species supplied to the plant, they disagree on the identity of the most phytotoxic form of As [193,194,199]. Hydroponic experiments are considered better than soil studies in detecting metal–plant interaction because they eliminate the complex and confounding phytoavailability issues that arise from differences in the mobility of various As species through the diverse growth substrates and thus provide a clear insight into the potency of externally supplied As on plant growth. The studies generally agree with the hydroponic survey of 46 different plant species [192] that the uptake of As by plants has the order As(III) > As(V) > MMA(V) > DMA(V), while translocation from the roots to the rest of the plant has the order DMA(V) > MMA(V) > As(V) ≥ As(III). However, no As form appears to be consistently most phytotoxic. In two *Spartina* species, where the order of uptake was As(III) > As(V) ≈ MMA(V) > DMA(V), the order of phytotoxicity was DMA(V) ≈ MMA(V) > As(III) ≈ As(V) [199]. This would suggest that DMA(V), with lowest uptake and high phytotoxicity, exerted maximum toxic effects within the plant. In contrast, the uptake order in rice was As(III) > MMA(V) > As(V) > DMA(V), similar to the order of phytotoxicity, which was MMA(V) > As(III) > As(V) = DMA(V) [194]. Finally, the order for

phytotoxicity in maize, a species with the typical order for uptake [192], was As(V) > As(III) > DMA(V) [193]. The inconsistent order of phytotoxicity of the various As species could be an indication that As has interacted differently with the available nutrients, or that the phytotoxic form of As is plant species dependent. Alternatively, the apparent inconsistency of the above results may be due to an incomplete understanding of the relative importance of the mechanism of As toxicity. After all, the mechanism through which As causes phytotoxicity has not been definitively identified and the exact species of As being the primary cause of toxicity is unknown. In this regard, the finding of MMA(III) in plants [179,232,233] is particularly intriguing. This As species is up to 18 times more cytotoxic to animal cells than As(III) [234,235]. The phytotoxicity of MMA(III) has not been tested.

Another issue that needs to be included when considering the toxicology of As is that some forms of As may be under-represented or not addressed in terms of As speciation in plants. In a study on sunflower, 15–20% of the total tissue As was not recovered from the plant tissue [31]. In beet root, As was nearly quantitatively extractable from tissues with low As burden, but in tissues with higher As burdens, up to 75% of the As was not extracted [236]. Perhaps the speciation of this missing As, or indeed the molecules to which it may be bound, will give us important insights into the underlying mechanism of As toxicity. The above arguments indicate that there is a need of fuller understanding of the action of As at cellular level, although the basic mechanism of As toxicity is conserved from microbes to the plants. The structural similarities of As(V) and Pi allow this form of the metalloid to substitute for Pi in biochemical reactions, potentially disrupting vital cellular processes. On the other hand, As(III) is highly reactive toward thiol groups, as are MMA(III) and DMA(III). The binding of As(III) to the thiol groups of proteins or enzyme cofactors may alter or inhibit their activity, also exposing cellular processes to risk. Oxidative stress brought about by the inevitable production of ROS during As exposure has recently gained favor as it is a driver of As toxicity in plants. However, which cellular processes are most sensitive to As toxicity and which As species pose the greatest threats to plant cell health remains unclear.

27.3.3.1 Phosphate Replacement

Substitution of Pi by As(V) has been demonstrated in numerous biochemical reactions, and any reaction with Pi or a Pi-ester as a substrate is a potential target for As(V) disruption [237–240]. Potential As(V)-sensitive reactions would include those central to cellular metabolism, information storage and retrieval, and cellular signaling (i.e., protein phosphorylation/dephosphorylation). When As(V) comes into contact with the surface of a cell of a plant root, the Pi transporter may be the first enzyme where As(V) may compete with Pi as observed in numerous monocots and dicots, and As-hyperaccumulators and non-hyperaccumulators [33,161,164,167,168,170]. Since Pi competes with As(V) for uptake, As(V) toxicity is lower under high Pi conditions. On the other hand, As(V) may outcompete Pi for uptake under low Pi conditions, exacerbating Pi deprivation [167]. The toxicant can also pass through the *Arabidopsis* AtPHT4 family of Pi transporters localized to the plastid and Golgi [241]. Relatively few enzymes use Pi as a substrate due to the reversible nature of most Pi-liberating reactions. Therefore, few enzymes are expected to use As(V) directly as a substrate [242]. Perhaps the

predominant Pi-requiring reaction is the phosphorylation of ADP to ATP by the F1F0-type ATP synthases found in the mitochondrial inner membrane and the thylakoid membrane. The mitochondrial enzyme uses As(V) in a reaction that produces ADP-As(V) [240]. The *K*m and *V*max of this reaction are remarkably similar for both Pi and As(V) [243], demonstrating that at least some enzymes are capable of recognizing and reacting equally well with As(V) and Pi. Other Pi-dependent enzymes capable of using As(V) include the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Like the ATP synthase reaction, the GAPDH reaction, where As(V) replaces Pi, has remarkably similar kinetic constants to those of the Pi-dependent reaction [237]. Aspartate-β-semialdehyde dehydrogenase has a critical role in the biosynthesis of essential amino acids in plants, catalyzing the reversible reductive dephosphorylation of β-aspartyl phosphate to L-aspartate-β-semialdehyde. This enzyme, too, is able to use As(V) nearly as efficiently as Pi, judged from the *K*m and *K*cat values [244].

While much of the above information comes from non-plant systems, there is little reason to believe that the behavior of homologous plant enzymes would be substantially different. The As(V)-esters produced by these and other reactions are highly unstable in water and undergo spontaneous hydrolysis [242,245]. The hydrolytic products are generally free of As(V) and the corresponding carbon compound. The instability of As(V)-esters is highlighted by the rates of hydrolysis for glucose-6-As(V) and ADP-As(V), which are at least 105-fold greater than for the corresponding Pi-ester [243]. As a consequence of the instability of As-esters, the enzymatic reactions that produce them are essentially irreversible. The products simply do not stay around long enough to allow the reverse reaction to proceed at an appreciable rate. The unstable nature of the As(V)-esters also creates futile reaction cycles around enzymes that use free As(V) to produce As(V)-esters. The As(V) and ADP released by the auto hydrolysis of ADP-As(V) produced by mitochondrial and plastid ATP synthases can be used in subsequent reactions. Such futile cycling uncouples respiratory electron transport in the mitochondrial inner membrane and photosynthetic electron transport in chloroplast thylakoid membranes from ATP synthesis [240,246–249]. This collapse of ATP production has potentially grave consequences for the energy status of the cell. While As(V)-esters are unstable, enzymes can utilize them when they are available. Hexokinase can use ADP-As(V) to arsenolate glucose to glucose-6-As(V). This product, in turn, is a substrate for glucose-6-phosphate dehydrogenase [240].

27.3.3.2 Binding Thiol Groups

The mode of action of As(III) differs substantially from that of As(V). As(III) is a thiol reactive compound that can bind up to three sulfhydryl groups [250]. This allows As(III) to act as a cross-linking agent by binding up to three monothiol molecules, such as the antioxidant GSH. Alternatively, it could bind to a single molecule of a polythiol compound, such as PC, the Cys-rich polymerization product of GSH. As(III) can also bind to thiol-containing proteins and cofactors. Dihydrolipoamide, which in plants is a cofactor associated with the mitochondrial and plastid pyruvate dehydrogenase complexes (mtPDC, ptPDC), the 2-oxo glutarate dehydrogenase complex (OGDC), the glydecar-boxylase complex (GDC), and the branched-chain 2-oxoacid decarboxylase complex (BCOADC), has been long thought to be an important cellular

target for As(III) binding [251,252]. The stability of As(III) complexes increases with the number of bonds formed. The half-life of an As(III)-monothiol peptide complex is about 1–2 s. The half-life increases to about 1.3 and 155 min when two or three intramolecular thiols are bound [250]. The binding of As(III) to dithiols is enhanced when the sulfhydryl groups are in close proximity to one another [253], but the optimal spacing for trithiols is unknown. The binding of As(III) to proteins can have profound effects on their folding [254,255]. In various systems, proteins that are known to bind As(III) include transcription factors, signal transduction proteins, proteolytic proteins, metabolic enzymes, redox regulatory enzymes, and structural proteins. Among the 35,386 predicted translation products from the *Arabidopsis* genome sequence (TAIR 10 release), there are 64,335 dithiols with optimal spacing for As(III) binding [250] on 23,578 proteins. About one-third of these dithiols, residing on 11,559 proteins, form part of a trithiol that may be optimally spaced for As(III) binding, assuming that the optimal sulfhydryl spacing for As(III) binding to trithiol groups is symmetrical with the dithiol spacing (CX0–14CX0–14C). This analysis ignores the potential for intramolecular cross-links, but raises two intriguing questions: What types of proteins in *Arabidopsis* are among the 2123 proteins lacking a Cys residue? Are there evolutionary pressures for these proteins, in particular the lack of ability to interact with As(III)? One conclusion to be drawn from these values is that As(III) has the capacity to interact with a large proportion of any cellular proteome and it will be a large task to identify which proteins among the As(III) targets are most critical to cell survival. While As(III) is an inhibitor of many enzymes, the recent finding of methylated forms of As(III) in plant cells [31,179,232,233] has important implications in this respect. Half maximal inhibition of pyruvate dehydrogenase was found to occur at about 115 μM As(III), while two- to six-fold fewer methylated As(III) derivatives were needed for similar inhibition [256]. Compared to As(III), MMA(III) is a more potent inhibitor of other enzymes including GSH reductase [257] and thioredoxin reductase [258]. MMA(III) and DMA(III) forms were also more potent inhibitors of zinc-finger protein activity than As(III), again highlighting the necessity to critically evaluate the ability of plants to methylate inorganic As into more toxic forms or reduce methylated As(V) compounds to their As(III) counterparts.

The binding of As(III) to thiols is the basis for the main detoxification pathway for both As(V) and As(III) and explains the retention by roots of up to 90% of the As taken up by a plant [178,192,259]. As(III) is either taken directly into the roots from the soil solution or rapidly produced within the root by the efficient reduction of As(V). As(III) then combines rapidly with sulfhydryl-rich protective molecules like GSH and PC [30,221,260]. The preference of As(III) to bind polythiols favors the formation of As(III)–PC3 complexes [31]. However, a variety of As(III) conjugates can exist in a plant, with 14 different complexes being isolated from sunflower [31]. The As(III)–PC conjugates can then be transported from the cytosol into the vacuole. In *Arabidopsis*, this transport is via the ABC transporters MRP1/ABCC1 and MRP2/ABCC2 [261]. Homologs of *Arabidopsis* ABCC1 and ABCC2 are found throughout the plant kingdom [177]. A homologue of ABCC2 was among several ABC transporters to be up-regulated at the transcript level in response to As in rice [262,263]. Thus, in many plants, As(III) present in root cells is rapidly complexed to PC and sequestered in the vacuole, restricting the transport of As from the root [177,260] and preventing its interaction with cellular metabolism. The impact

of As on cellular metabolism in many plants, then, depends on how efficiently As(III) can be neutralized by thiol binding and sequestration in the vacuole. Interestingly, this is not the case in hyperaccumulating species such as *P. vittata*, where there is little PC binding of As(III) in the roots [264]. Instead, As(III) taken into the plant cell from the soil solution or produced by the reduction of As(V) is rapidly translocated to the xylem and fronds for sequestration as free As(III) in the vacuole [185,187,190]. This transport of As(III) through the plant body raises the question of how *P. vittata* avoids the toxic effects of As(III) binding to enzymatic thiols.

27.4 Mechanisms of As Tolerance and Detoxification

Plant tolerance of any heavy metal/metalloid is governed by a network of physiological and molecular mechanisms, and understanding of these mechanisms and their genetic basis is an important aspect of developing plants for phytoremediation [265,266]. Different plant species may have evolved different mechanisms to tolerate excess arsenic, and even within plant species more than one mechanism could be in operation. Physiological, biochemical, and molecular approaches continue to be employed to identify the underlying mechanisms of As accumulation, tolerance, and adaptive mechanisms to cope with As stress. Resistance to As toxicity can be achieved by "avoidance" when plants are able to restrict As uptake, or by "tolerance" when plants manage to survive in the presence of high As concentrations. Avoidance involves reducing the entry of As in the cell by biosorption to cell walls, reduced uptake, or increased efflux. However, in the second situation, plants detoxify the metals through intracellular chelation, via the synthesis of amino acids, metabolites, organic acids, GSH, or HM-binding ligands such as metallothioneins and phytochelatins, compartmentation within vacuoles, and up-regulation of the antioxidant defense. There are relatively few species of plants that are naturally As tolerant. Among these are *Pteris vittata* and other members of the Pteridaceae that hyperaccumulate As [34,113,186,188,267]. The growth of these plants is not compromised during As accumulation. In contrast to As non-hyperaccumulating plants, hyperaccumulators tend not to restrict As to the roots, instead allowing transfer of the toxicant immediately to the shoots. This is likely to be an important aspect of the hyperaccumulation phenotype. The mechanisms by which these plants are able to hyperaccumulate As are being elucidated, but it is not yet clear as how they avoid As toxicity.

27.4.1 Arsenate Reduction

It has been observed that when plants are supplied As(V), typically more than 90% of the As in the roots and the shoots was found to be in the form of As(III) [29,30,32]. Thus, As(V) is readily reduced to As(III) by plants. This reduction is accepted as the first step in the major As detoxification pathways found in plants [30,221]. The reduction of As(V) to As(III) occurs both enzymatically and non-enzymatically. In the non-enzymatic pathway, two molecules of GSH are able to reduce As(V) to As(III). The oxidation of GSH is via the formation of a disulfide bond, producing a GSH dimer (GSSG) which can be rapidly recycled to two GSH molecules by GSH reductase [268]. While As(V) reduction can occur non-enzymatically, the enzymatic

rate is much higher [269]. As(V) can be directly reduced to As(III) by arsenate reductase (ACR), an enzyme first isolated from bacteria and yeast [89]. Based on homology between the yeast ACR gene *ScAcr2* and several homologous sequences from plants, an *HlAsr* cDNA was cloned from As(V)-hypertolerant *Holcus lanatus* and found to encode an enzyme with ACR activity [270]. The homologous proteins from *Arabidopsis* (AtAsr/AtACR2), *P. vittata* (PvACR2), and rice (OsACR2.1 and OsACR2.2) also have ACR activity [29,113,271]. The plant ACR2 protein is related to the CDC25 cell cycle dual specificity tyrosine phosphatases. Interestingly, AtACR2 has phosphatase activity, while the PvACR2 enzyme, like the yeast ScAcr2p protein, does not. Also like ScAcr2p, the plant ACR2 enzyme uses GSH and grx as electron sources [113,271], suggesting that the catalytic cycle involves the formation of a mixed disulfide between GSH and ACR2 that is resolved by grx [89]. The *Arabidopsis* As(V) reductase activity has an As(V)-inducible component that has been attributed to *AtACR2*, as well as a constitutive component that is not diminished in *AtACR2* T-DNA insertion lines [270]. Moreover, As(III) has been stated to remain the predominant form of As present in *AtACR2* T-DNA insertion lines supplied with As(V) [188].

Together, these results indicate that *Arabidopsis*, and thus in all likelihood other plants, possesses enzymes in addition to ACR2 that have As(V) reductase activity. Multiple enzymes from other systems have been shown to exhibit As(V) reductase activity. These include GAPDH, polynucleotide phosphorylase, purine nucleoside phosphorylase, glycogenphosphorylase, and the mitochondrial F1F0 ATP synthase [272–274]. Each of these enzymes can incorporate As(V) instead of Pi into biological molecules, forming an arseno ester that would readily undergo hydrolysis. In the presence of a suitable thiol group, for example GSH, the hydrolysis can result in the reduction of As(V) to As(III). It is not known if the analogous plant enzymes can also reduce As(V) in the presence of thiols. However, one form of the plant GAPDH is known to interact with GSH [275], suggesting it as a candidate ACR. Moreover, acytosolic triose-phosphate isomerase (cTPI) from *P. vittata* has also been shown to have ACR activity [276]. Because the TPI reaction does not involve the transfer of a Pi group, the mechanism by which Pvc TPI promotes the production of As(III) is unclear. However, like the enzymes mentioned above, the plant TPI interacts with GSH [276]. The number of enzymes that could misincorporate As(V) for Pi, and therefore have the capacity to form arseno esters, is large, providing many opportunities for the enzymatic reduction of As(V) to As(III). However, it is not known whether these enzymes affect the redox status of As *in vivo*.

27.4.2 Complexation and Sequestration of As

There is strong evidence that complexation of arsenite by PCs is an important mechanism of As detoxification, and hence tolerance, in As-non-hyperaccumulating plants. Arsenite has a high affinity to the sulfhydryl (–SH) groups of peptides such as GSH and phytochelatins. *In vitro* studies showed that GSH and arsenite form a (GS)3-arsenite complex with cysteinyl sulfhydryl as the arsenite binding site [277]. The complex is stable in the pH range from 1.5 to 7.0–7.5, but dissociates at higher pH. The tripeptide glutathione (Glu-Cys-Gly) is synthesized by gamma-glutamyl cysteine synthetase (g-ECS) and glutathione synthetase (GS). GSH can bind to several

metals and metalloids and is also a key metabolite in cellular redox balance. Increasing GSH synthesis is considered a means of increasing metal(loid) binding capacity as well as a way to increase cellular defense against oxidative stress. Since glutathione is the precursor of phytochelatin, overexpression of g-ECS or GS usually leads to higher rates of phytochelatin accumulation under metal exposure. It has been suggested that phytochelatin production, resulting in oxidized glutathione depletion, could itself cause oxidative stress [278,279].

In non-resistant plants, As(V) influx is so high that detoxification by phytochelatin complexation cannot be achieved, leading to considerable oxidative stress, which is not observed in resistant plants [280,281]. Phytochelatins are a family of cysteine-rich thiol-reactive peptides, which play important roles in processing many thiol-reactive toxicants [222]. Phytochelatins are induced by a range of metals and metalloids including Cu, As, and Cd [282]. It has been demonstrated that arsenic-sulfhydryl (–SH) complexes can be transported into the vacuoles by ycf1p transporters and confer As(III) resistance in yeast [87], and it has been suggested that higher plants may have a similar mechanism in As(III) resistance [283]. Raab et al. [31] have shown that As preferentially binds to PC3, forming the As–PC3 complex, rather than to PC2 and oxidized glutathione. The As–PC3 complex is the dominant complex formed in the As-tolerant *H. lanatus*. In contrast, PC2 was the predominant species induced by As in *Raufolia serpentine* [221], *Silene vulgaris* [284], and in the As-tolerant clone *H. lanatus* [204,285]. In the As-tolerant *C. striatus*, PC4 was the major species [168]. However, in the As-sensitive clone of *H. lanatus*, PC3 was dominant and PC4 remained at a low concentration [285]. Chickpea plants were capable of forming both phytochelatins and desglycyl peptides of phytochelatins in response to As, whereas PC3 and the corresponding desglycyl peptides showed a preference for As complexes [286]. Roots of As-exposed *Helianthus annuus* contained up to 14 different As species, including the complex of As(III) with two (c-Glu-Cys) 2-Gly molecules (As(III)–(PC2)2), the newly identified monomethylarsonic phytochelatins-2 or (c-Glu-Cys)2-Gly CH3As(MA(III)–PC2), and at least eight not yet identified species. The complex of As(III) with(c-Glu-Cys)3-Gly(As(III)–PC3) and the complex of As(III) with glutathione and (c-Glu-Cys)2-Gly(Gs-As(III)–PC2) were present in all samples taken from plants exposed to As. No As–phytochelatin complexes were found in sap, in contrast to roots, stems, and leaves, which is unequivocal evidence that As–phytochelatin complexes were not involved in the translocation of As from root to leaves of *H. annuus* [31].

Leguminous plants synthesize homo-phytochelatins in addition to phytochelatins during As stress, and many studies point to the essential role of phytochelatins in both constitutive and adaptive tolerances to As. The results presented by Schulz et al. [287] provided evidence for the phytochelatins in the detoxification of As in six non-hyperaccumulating plant species, *Agropyron repens*, *Glechoma hederacea*, *Leonurus marrubiastrum*, *Lolium perenne*, *Urtica dioica*, and *Zea mays*, in a pot experiment with high phosphate treatment. Raised concentrations of total phytochelatins were measured in plant species with a range of sensitivities to As at equivalent levels of As exposure, determined as the inhibition of root biomass. In addition, the production of phytochelatins as a function of accumulated As was studied. Long-term phytochelatin synthesis (over a 5-week period) was positively, but non-linearly, correlated with As, suggesting that probably not all As is bound by phytochelatins. In the more tolerant grasses,

A. repens and L. perrenne, it was chiefly the dithiol phytochelatins that were measured. In contrast, the dominant phytochelatin species in the less tolerant plants *V. dicica*, *G. hederacea*, *L. marrubiastrum*, and *Z. mays* were phytochelatins, while PC2 and PC3 were detected as well [287]. Wojasa et al. [288] determined overexpression of two phytochelatin synthase genes, *AtPCS1* and *CePCS*, in *Nicotiana tabacum* with respect to As tolerance and accumulation, and observed how responses relate to non-protein thiol metabolism. The expression of both genes resulted in increased As tolerance, with CePCS plants most tolerant. At the less toxic 50 lM As(V), AtPCS1, and CePCS transformants accumulated more As in roots and leaves than wild type. An increase in phytochelatin production and the level of PC2 species were detected in leaves of AtPCS1 and CePCS plants, which might explain their enhanced As accumulation and tolerance. In contrast, at the highly toxic 200 lM As(V), several disturbances in thiol metabolism of phytochelatin synthase overexpressing plants were found. The increase in As tolerance and accumulation due to *AtPCS1* and *CePCS* overexpression, observed at the As(V) concentrations similar to those found in As-contaminated soils, make these genes promising candidates for plant engineering for phytoremediation

27.4.3 Antioxidative Defense System

Despite being a non-redox active metalloid, As promotes generation of ROS, which causes oxidative stress and damage to lipid, protein, and DNA [289,290]. To combat these effects, enzymatic, e.g., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR), and non-enzymatic antioxidants, e.g., GSH, and carotenoids, are mobilized to quench ROS. SOD catalyzes conversion of the active superoxide radicals to hydrogen peroxide [223]. SOD activity in plants varies quite widely with As treatment. In some plants, like *Zea mays*, As-sensitive clones of *H. lanatus*, and the As-hyperaccumulator *P. vittata*, the enzyme is induced by low As exposure, and either stays at the same or decreased level in activity at higher As levels [278,291]. In *Arabidopsis*, genes encoding the three classes of SOD (FeSOD, MnSOD, Cu/ZnSOD) responded to As(V) differentially at the transcript level [292]. Transcripts for genes encoding a chloroplastic and a cytosolic Cu/ZnSOD were induced more than two-fold by As(V) exposure, while transcripts for an FeSOD were down-regulated about five-fold [292]. The conversion of H_2O_2 into water is brought about by CAT and GPX in peroxisomes and cytoplasm. Nevertheless, this conversion may also be done by APX in the ascorbate–glutathione cycle. Glutathione reductase is another important enzymatic component of the antioxidative defense system, which helps in maintaining a high GSH/GSSG ratio [291] of non-enzymatic antioxidants that include GSH, PC, ascorbate, carotenoids, and anthocyanin. These antioxidants generally accumulate during As exposure [168,221,261,270,278]. The production of these molecules requires metabolic acclimations, including the diversion of carbon, nitrogen, sulfur, and metabolic energy from normal growth and development. GSH and ascorbate are fairly unique among the non-enzymatic antioxidants as they can form a redox cycle. The ROS produced during As treatment typically induces an increase in the oxidation state of the redox active pools of GSH and ascorbate in favor of GSSG dimers and dehydroascorbate over the more reduced GSH and

hydroascorbate [203]. This shift in redox state arises at two levels. Superoxide and the hydroxyl radical can directly oxidize both GSH and ascorbate. In this way, GSH and ascorbate act as nucleophilic scavengers.

Alternatively, H_2O_2 can oxidize GSH and ascorbate through the action of specific peroxidases, or in the case of GSH, also through the action of GRXs and GSH-S-transferases (GST). Like SOD and catalase, GST, GRX, and/or peroxidase transcript or protein abundance, or enzymatic activity, often increase in response to As exposure [219,227,262,263,292]. As an example, in rice, at least 10 GST genes are up-regulated in response to AsV exposure, while no more than two GST genes are down-regulated [262,263]. Changes in GST gene expression do not seem to have as pronounced a role in the As(III) response, as fewer transcripts changed in abundance [263], potentially highlighting the differential effects of the two inorganic As forms on cellular metabolism. It would be worthwhile to address this by asking: Why are there changes in the isoforms of the various enzymes that are expressed? What are the energetic and metabolic costs and benefits of such shifts? The second component of the two-component H_2O_2 neutralizing system is made up of monodehydroascorbate reductase, dehydroascorbate reductase, and GSH reductase. Together, these enzymes efficiently recycle oxidized GSH and ascorbate to allow further cycles of H_2O_2 reduction. The reduction of H_2O_2 through the interdependent ascorbate–GSH cycle requires reducing power in the form of NAD(P)H, diverting this energy from other metabolic processes. The enzymes involved in the recycling of oxidized GSH and ascorbate are also often induced upon exposure of plants to As [223,227]. Thus, the interdependent ascorbate–GSH cycle, if established, has an important role in maintaining ROS balance in plants [268]. The reliance of the ascorbate–GSH cycle on the diversion of carbon to ascorbate biosynthesis, plus the diversion of carbon, nitrogen, and sulfur in the form of Glu, Cys, and Gly to support the biosynthesis of GSH and PC, requires modeling of metabolism to focus on the production of the precursors for these compounds. The oligomerization of GSH to produce PC is also induced during As exposure [203,221,223]. Increased PC synthase in *Arabidopsis*, *Brassica juncea*, and tobacco conferred increased As tolerance on these plants [222,288,293]. However, at higher levels of As, thiol metabolism was disrupted in some overexpressing lines [288].

27.4.4 Osmolyte Accumulation

Besides activating antioxidative defense machinery, plants tend to accumulate certain metabolites of low molecular weight known as compatible solutes. They allow plants to acclimate to unfavorable environments for continued survival and growth. These differ among plant species and include phytopolyhydroxylated sugar alcohols, amino acids and their derivatives, tertiary sulfonium compounds, and quaternary ammonium compounds [294]. Proline accumulates heavily in several plants under stress, protecting plants from damage caused by reactive oxygen species. It plays important roles in osmoregulation, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity, and scavenging of free radicals [295].

N,N,N-Trimethylglycine (hereafter glycine betaine) occurs naturally in a wide variety of plants, animals, and microorganisms [296]. Numerous experiments *in vitro* have indicated

that glycine betaine acts as an osmoprotectant by stabilizing both the quaternary structure of proteins and the highly ordered structure of membranes against the adverse effects of salinity and temperature [297]. In photosynthetic systems, glycine betaine provides protection to ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) and the oxygen evolving photosystem (II) complex from salt-induced inactivation and dissociation into subunits [298]. In plants, polyamines are related to various kinds of environmental stresses including osmotic stress, salt stress, acid stress, heavy metals like As, and UV radiation [299]. Polyamines have also been suggested to function as metal chelators and protect plants against metal-induced oxidative stress. The soluble protein content in the plant cells is an important indicator of their physiological state. The As toxicity in germinating rice seeds causes suppression in the activities of starch hydrolyzing enzymes α-amylase, β-amylase, and starch phosphorylase in endosperms while it leads to an increase in the ability of acid invertase in endosperms as well as embryo axes [300]. Recently, Rai et al. [202] demonstrated an increased production of artemisinin (secondary metabolite) as part of a defense strategy adopted by *Artemisia annua* against As toxicity.

27.4.5 Mycorrhization in Crop Plants: the Prospects of Arbuscular Mycorrhizae Symbiosis in Regulation of Plant Defense Response to Arsenic Toxicity

There is a considerable literature regarding symbiotic association between mycorrhizal fungi and plants where this symbiosis benefits host plants under high As conditions [204,301]. Symbiosis with mycorrhizal fungi was found to confer As resistance on the host plants. This effect may be produced by several possible mechanisms. First, mycorrhizal colonization may suppress the high affinity phosphate transport system in plant roots, leading to reduced uptake of arsenate [302]. Second, mycorrhizal fungi may enhance As resistance in the host plants by effluxing As to the external medium. This mode of action was clearly demonstrated by Sharples et al. [303], who reported that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* from an As-contaminated site developed arsenate resistance by extruding arsenite into the medium following arsenate uptake, a mechanism that is common to bacteria and fungi. Third, by enhancing P acquisition and improving the P nutrition of the host plant, mycorrhizal fungi improve plant growth, causing a dilution of As concentrations in plant tissues [217,304], or increasing cytoplasmic inorganic P. Mycorrhizal plants often have a higher P/As concentration ratio, which is likely to benefit the plant through enhanced tolerance to As. Finally, there is circumstantial evidence that mycorrhizal fungi may restrict As translocation from roots to shoots [302,304–306], although how this control is exerted is unclear and requires further investigation. Ultra et al. [305,306] reported the presence of methylated As species in the rhizosphere of sunflower (*H. annuus*) plants when it was inoculated with the arbuscular mycorrhizal fungus *Glomus aggregatum*. DMA was found at low but detectable concentrations in the rhizosphere soil but not in the bulk soil, nor in the rhizosphere soil of uninoculated plants. Mycorrhizal fungi may be able to mediate As biomethylation and release some of the methylated As in the rhizosphere. Although several studies have shown enhanced plant tolerance to As resulting from inoculation with arbuscular mycorrhizal fungi [302,304], elucidation of the effects of

arbuscular mycorrhizal fungi on uptake and translocation of As and direct evidence showing that mycorrhizal hyphae are involved in the detoxification of As are still lacking.

27.5 As Hyperaccumulation and Phytoextraction

In plants, the term "hyperaccumulation" reflects the following attributes of the plants: (1) enhanced As(V) uptake, (2) decreased As(III)–thiol complexation and As(III) efflux to external medium, and (3) enhanced xylem translocation of As(III) and vascular sequestration in fronds [188]. However, while considering a plant as an As hyperaccumulator or non-accumulator, one has to take into consideration the bioaccumulation and translocation factors [34]. Bioaccumulation factor is defined as the ratio of metal concentration in plant biomass to that in the soil, and translocation factor is defined as the ratio of metal concentration in the shoots to that in the roots [307]. Therefore, an As hyperaccumulator plant should have a bioaccumulation factor of more than 1 and a translocation factor of more than 1, as well as total accumulation of more than 1000 mg/kg As in plant biomass. Since the first discovery of As hyperaccumulation in *P. vittata* [34], a total of 12 As hyperaccumulators have been identified, all of them from within the *Pteris* genus. Not all of the *Pteris* species are As hyperaccumulators; thus, it would be interesting to investigate the phylogenetic relationship between the hyperaccumulating and non-hyperaccumulating fern species. Arsenic hyperaccumulation by *Pteris* species is a constitutive trait, with plants originating from As-contaminated and As-non-contaminated environments showing broadly similar hyperaccumulating abilities [308,309].

27.5.1 Mechanisms of As Hyperaccumulation

The As hyperaccumulation phenotype is a result of a combination of several physiological processes, although little is known about the molecular mechanisms. Hypertolerance is found in all naturally evolved hyperaccumulators; As hyperaccumulators have a much higher degree of tolerance than the non-hyperaccumulator species within the *Pteris* genus [310,311]. Compared with the non-hyperaccumulator *Pteris ensiformis*, *P. vittata* possesses a higher antioxidant capacity and also maintains a lower concentration of reactive oxygen species [203,312]. By contrast to non-hyperaccumulators, which rely on PC complexation for As detoxification and tolerance, very little of the As accumulated in the roots and fronds of *P. vittata* and *P. cretica* (≈1–3% of the total As) is complexed with PCs [264,313]. The majority of As (60–90% of the total As) in the fronds of *Pteris* species is inorganic arsenite [185–187,314–316], which appears to be stored in the vacuoles [185,186]. It may be extremely costly for plants to evolve PC-dependent As hyperaccumulation, because at the 1:3 stoichiometric ratio of As:S, the amount of S required to complex 2500 mg As/kg in the fronds of *P. vittata* would have exceeded the total amount of S typically accumulated by the plants [308]. Vacuolar sequestration of arsenite is therefore the key mechanism of As detoxification in the hyperaccumulator ferns. How this is achieved, and especially the identity of the vacuolar transporters responsible for arsenite transport across the tonoplast, deserves further investigation. Arsenate is likely to be the main form of As taken up by the hyperaccumulator ferns as they grow on aerobic soils, and the

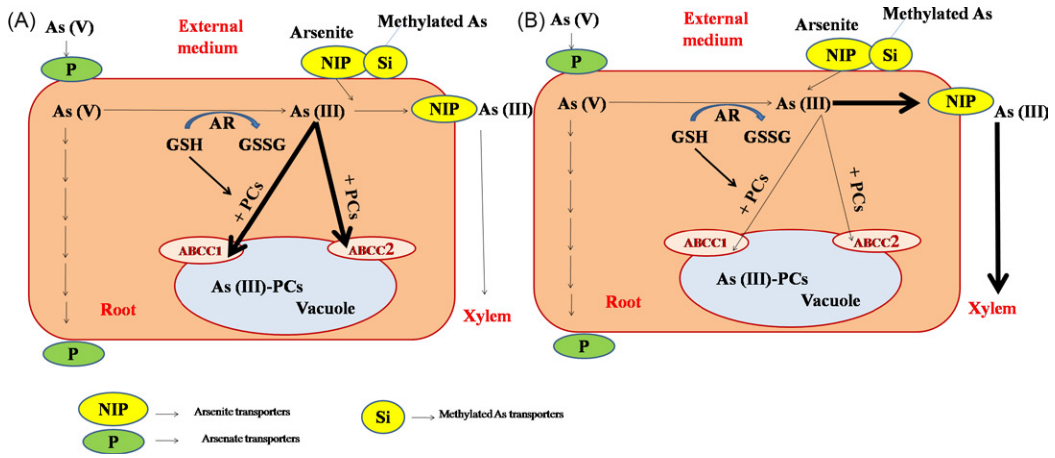

FIGURE 27–5 Schematic representation of arsenic (As) uptake and metabolism in roots of non-hyperaccumulators (A) and hyperaccumulators (B). Line thickness relates to flux rate. ABCC, ATP-binding transporters; AR, arsenate reductase; GSSG, oxidized glutathione; GSH, glutathione; PCs, phytochelatins.

uptake is via the phosphate transport system as in non-hyperaccumulator plants [317] (Figure 27–5). Compared with non-hyperaccumulator ferns, *P. vittata* and *P. cretica* have a higher arsenate influx [310,317] and a lower *K*m, indicating higher affinity of the transporter for arsenate [317]. The most striking difference lies in the efficiency of root to shoot translocation, exemplified by the large ratios of shoot to root As concentrations in the As hyperaccumulators [307,308]. The ratio of the As concentration in the xylem sap of *P. vittata* to that in the nutrient solution was about two orders of magnitude higher than that in the non-hyperaccumulators. Su et al. [187] showed that the majority (93–98%) of the As in the xylem sap of *P. vittata* was in the form of arsenite, regardless of whether the plant was treated with arsenate or arsenite.

Roots or rhizoids of *P. vittata* are likely to be the main location of arsenate reduction, with arsenite being preferentially loaded into the xylem. This is consistent with the findings of Duan et al. [269], who found the activity of the glutathione-dependent arsenate reduction only in the roots of this hyperaccumulator. Although the gene encoding an arsenate reductase has been cloned from *P. vittata* gametophytes [113], its *in planta* role and its contribution to overall arsenate reduction have not been ascertained. Also, hyperaccumulators differ from non-hyperaccumulators in that there is minimal efflux of arsenite from the roots of *P. vittata* to the external medium [187]. This, together with little PC complexation of arsenite in *P. vittata* roots [264], may explain the highly efficient xylem transfer in hyperaccumulators.

27.6 Summary Points

- Arsenic is a notorious poison that was probably more abundant on the early Earth's surface than today. Therefore, primordial organisms evolved systems to cope with it, which still exist in prokaryotes. Not only do they resist this with a wide range of mechanisms

(precipitation inside or outside the cell, extrusion or transformation), but strikingly, some of them take advantage of this toxic compound by using it as an electron donor [As(III)] or acceptor [As(V)] for energetics purposes.

- Biooxidation and bioreduction of arsenic play a key role in the mobility of this metalloid based on the different solubilities of As(III) and As(V), As(III) being more soluble than As(V). On the one hand, reduction of As(V) could lead to huge environmental pollution problems since As(III) is more toxic than As(V); on the other hand, As(III) oxidation is considered as a bioremediation process since it leads to the production of As(V) that readily adsorbs to mineral or organic substances and precipitates.
- Environmental factors influence As speciation in soil and its availability to plants. Flooding of paddy fields leads to mobilization of arsenite. Methylated As may be present in soil as a result of microbial and/or algal biomethylation, or from past uses of methylated As pesticides.
- Arsenate is taken up by plant roots through phosphate transporters, whereas the uptake of undissociated arsenite and methylated As is mediated, at least partly, by NIP aquaporin channels. Rice is efficient at As assimilation owing to arsenite mobilization in flooded paddy soil and arsenite uptake sharing the highly expressed Si pathway. Arsenic accumulation in rice grain represents a potential health risk to humans.
- Arsenate is readily reduced to arsenite, which is detoxified by complexation with thiol-rich peptides and sequestrated in the vacuoles in As non-hyperaccumulating plants.
- Excessive accumulation of As in rice can be mitigated through agronomic and crop breeding strategies. Genetic modification may be employed to engineer plants more tolerant to As, or with reduced uptake for improved food safety.

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28

Arsenic Contents and Its Biotransformation in the Marine Environment

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CHAPTER OUTLINE

28.1 Introduction

Arsenic has been known as the king of poisons and the poison of kings, many cases of its use as a poison being reported in the 1900s. The suspected cause of death of the well–known French ruler Napoleon Bonaparte is one such example of arsenic poisoning [1]. It has recently become one of the major environmental toxicants due to its increased application in wood preservatives, insecticides, fungicides, fertilizers, and semiconductors. The mining and burning of fossil fuels have broadened the global cycle of arsenic, which has led to the accumulation of large amounts of arsenic in the environment. Natural sources such as volcanoes, marine hydrothermal fluids, and microorganisms also contribute to arsenic pollution. Arsenic from these many natural and anthropogenic sources is transported into the marine environment through atmospheric depositions and riverine inputs. Coastal areas and estuaries of many countries

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including Bangladesh, Australia, Korea, Japan, China, Malaysia, India, the UK, and the USA have reported elevated arsenic pollution.

The arsenic biogeochemical cycle in marine environment involves several physicochemical (such as oxidation/reduction, precipitation/solubilization, and adsorption/desorption) as well as biological processes, in which marine organisms play a key role [2]. The toxic effects of arsenic are related to its speciation; thus, the examination of factors affecting the speciation of arsenic has become an important issue [3]. Biological (marine bacteria, phytoplankton, algae, and other marine organisms) factors perform a significant contribution to arsenic speciation and the biogeochemical cycle of arsenic in the marine environment.

Humans may be exposed to arsenic via contaminated drinking water and food, especially seafood [4], which is generally contaminated with higher concentrations of arsenic than found in other foods [5]. Countries like Japan, China, and Korea obtain their food from marine sources, which constitute a major part of their diet [6,7]. In view of the suspected human carcinogenicity of arsenite, the United States Environmental Protection Agency (USEPA) has set a human health criterion for total dissolved arsenic in sea water (0.0175 g/L) for the consumption of fish products [8]. Millions of people worldwide are at risk due to long-term arsenic-contaminated groundwater consumption [9], which has become a global public health problem. Inorganic arsenite has been classified by the International Agency for Research on Cancer as a human carcinogen [8]. Acute toxicity symptoms of inorganic arsenic in humans include cardiovascular disturbances, gastrointestinal disorders, and kidney and liver failure, whereas chronic exposure may lead to hyperkeratosis, skin pigmentations, and cancer of skin, lung, bladder, liver, and kidney [10]. The toxicity of arsenic is strongly related to the chemical species in which it is present in seafood. The organic forms of arsenic contribute more to the major portion of total arsenic in seafood than do inorganic forms. Inorganic arsenic contributes less than 1 to 4% of total arsenic in seafood [11]. The majority of organic forms of arsenic are non-toxic or moderately toxic only at elevated exposure. Recent studies on the trivalent oxidation state of methylated arsenicals suggest that this state has greater toxicity than the corresponding pentavalent methylated arsenicals and inorganic arsenite [12].

This chapter summarizes arsenic (As) concentrations and the biogeochemical cycle of As in global marine and biological systems (marine animals, bacteria, phytoplankton, etc.), and its transformation and association with the risk of arsenic toxicity to seafood consumers.

28.2 Arsenic Concentration in Sea Water

Arsenic is the 14th most abundant element in sea water [13], and its average concentration ranges between 1 and 2 μg/L in unpolluted sea water [14]. Natural sources included volcanic eruption, hot springs, atmospheric deposition through low temperature volatilization, sea spray, riverine input, geysers, and marine hydrothermal fluids from active oceanic ridges, which play a significant role in arsenic contamination in marine waters [15–17]. Average arsenic concentrations in marine waters are shown in Table 28–1. Generally, the concentration of arsenic exhibits little variation in open oceans, while estuarine water shows increased variation as a result of varying riverine inputs, salinity or redox gradients [32]. In the

Table 28–1 Arsenic Concentrations in Marine Water

Marine Water	Arsenic Contents Range (average)	References
Pacific Ocean		
Northwest Pacific	(1.6 μg/L)	[18]
Central north Pacific gyre	(1.3 μg/L)	[18]
West Pacific equatorial region	(1.4 μg/L)	[18]
Southwest Pacific	(1.17 μg/L)	[19]
Atlantic Ocean		
North Atlantic	(1.18 ± 0.09 μg/L)	[20]
Eastern North Atlantic		
Surface	(1.04 ± 0.01 μg/L)	[21]
Bottom	(1.6 ± 0.003 μg/L)	
West Atlantic	0.6–1.7 μg/L (1.22 ± 0.16 μg/L)	[22]
Indian Ocean		
East Indian	(0.9 μg/L)	[19]
North Indian	(0.8 μg/L)	[19]
Antarctic Ocean	(1.1 μg/L)	[19]
Southern Ocean		
Surface	(1.7 ± 0.06 μg/L)	[23]
Deep	(1.8 ± 0.04 μg/L)	
Yellow Sea	0.6–1.6 μg/L (1.1 μg/L)	[15]
East China Sea	0.6–1.6 μg/L (1.2 μg/L)	[15]
Southern Tasman Sea	(1.4 μg/L)	[18]
China Sea	(0.6 μg/L)	[19]
Galway Bay, Ireland	(1.7 μg/L)	[17]
Coasts, Malaysia	0.7–1.8 μg/L (1.0 μg/L)	[24]
Southeast Coasts, Spain	0.5–3.7 μg/L (1.5 μg/L)	[25]
Coastal Nakaminato, Japan	(3.1 μg/L)	[26]
Gwangyang Bay coast, Republic of Korea	Below detection limit—27.9 μg/L	[27]
Southern coast, Australia	1.1–1.6 μg/L (1.3 μg/L)	[28]
Rhône estuary, France	1.3–3.7 μg/L	[29]
Scheldt estuary, Belgium	1.8–4.9 μg/L	[30]
Krka estuary, Yugoslavia	1.8 μg/L	[31]

uncontaminated Krka estuary in Croatia, a linear increase in total arsenic was observed with increasing salinity ranging from 0.13 μg/L in fresh water to 1.8 μg/L in sea water [31]. Usually, its concentration is high when riverine inputs are affected by industrial or mining effluents. There is only a small contribution made by human activities to arsenic loads in open oceans, but estuaries and coastal waters may receive arsenic-contaminated discharges from the land sources. Arsenic concentrations reported in the Rhone estuary in France ranged between 1.3 and 3.7 μg/L [29], while a slightly elevated level of 2.7–8.8 μg/L was observed in the Tamar estuary in southwest England [33]. Total dissolved arsenic in the Scheldt estuary, Belgium, was

between 1.8 and 4.9 μg/L [30]. The Yellow Sea and East China Sea were strongly affected by input from freshwater discharge of the adjacent Changjiang (Yangtze) River in the west and the Kuroshio intrusion in the east, which had average total dissolved inorganic arsenic concentrations in samples, collected in 2002, of 1.12 μg/L and 1.21 μg/L in the Yellow Sea and East China Sea, respectively [15]. On the other hand, these concentrations are relatively lower than in other reported polluted sites, where small amounts of arsenic in estuarine waters enter the oceans. Physical processes, like adsorption of dissolved inorganic arsenic to iron oxides and flocculation of particulates at the fresh/saline water interface, have important consequences on increases in pH and salinity. This has led to the transfer of arsenic to estuary sediments and restricted arsenic flux to the oceans [14,34].

In continental shelf waters, the dissolved arsenic concentration of surface water was found to vary seasonally due to natural cycling between sediments and the overlying water column [35]. The lowest concentration is present in the spring when dissolved arsenate is scavenged by phytoplankton blooms and suspended particles. Arsenic concentration is increased in autumn due to remobilization of arsenic from sediments [8]. Total inorganic arsenic is depleted in surface waters of the open oceans [21,36]. Total inorganic arsenic concentration in surface water of the Atlantic Ocean ranges between 0.94 ± 0.02 and 1.07 ± 0.02 μg/L, collected from three different sampling stations. Relatively higher and constant concentrations are being reported in deep water (between 2300 and 4400 m) and ranges between 1.33 ± 0.03 and 1.56 ± 0.04 μg/L [21]. A similar type of vertical distribution pattern of arsenic was observed in the North Pacific Ocean [36]. A good correlation usually exists between concentrations of arsenate and phosphate in ocean water, suggesting that the vertical distribution of arsenic in the ocean is controlled by biological activity. Another correlation suggests that arsenate concentration minima often coincide with photosynthetic maxima evidenced by high concentrations of chlorophyll *a* [34].

Average arsenic concentrations in the surface sea water of the Northwest Pacific Ocean, central Pacific gyre, Western Pacific equatorial region, and Southwest Pacific Ocean are 1.6 μg/L, 1.3 μg/L, 1.4 μg/L, and 1.2 μg/L, respectively [18,19]. The east and north Indian Ocean showed average arsenic concentrations of 0.9 μg/L and 0.8 μg/L, respectively [19], whereas the Antarctic Ocean and Southern Ocean had 1.1 μg/L and 1.7 ± 0.1 μg/L, respectively, in surface sea water [19,23]. Average arsenic concentrations in clear surface water of North Atlantic, eastern North Atlantic, and western Atlantic Ocean were found to be 1.2 ± 0.1 μg/L, 1.0 ± 0.05 μg/L, and 1.22 ± 0.4 μg/L, respectively [20–22], while a slightly elevated level was reported in deep water of the eastern North Atlantic of 1.56 ± 0.03 μg/L and South Atlantic, 1.4 ± 0.1 μg/L [21]. Average concentration of total arsenic in the open coastal Pacific Ocean was around 1.0 μg/L [37] and 1.8 ± 0.04 μg/L in the North Pacific [36], and in the deep Pacific and Atlantic Oceans the value lay between 1.0 and 1.8 μg/L [34]. Average arsenic concentration reported for the southeast coast of Spain was 1.5 μg/L [25], 1.3 μg/L for the southern coast of Australia [28], and 1.0 μg/L for the coasts of Malaysia [24]. Gwangyang Bay, Republic of Korea, showed elevated total arsenic concentrations in coastal sea water of below the detection limit (27.9 μg/L) [27], whereas Galway Bay, Ireland, showed 1.7 μg/L of arsenic [17]. Average arsenic concentration reported in the southern Tasman Sea was 1.4 μg/L [18], while

in the China Sea it was 0.63 µg/L [19]. The level of arsenic for the Pacific coast near Onagawa (Miyagi, Japan) was lower, 0.6 µg/L compared to Nakaminato (Ibaraki, Japan), which was around 3.1 µg/L [26]. The concentration readings for arsenic in marine water were more consistent than those for fresh waters [8,32].

Arsenic is one of the few trace elements whose concentration in sea water was found to be higher than in river water, which may be due to the flux of arsenic from hydrothermal systems [16]. Marine hydrothermal fluid has played an important role in arsenic cycling in marine environments which emerged at the seafloor near mid-ocean ridges or in back-arc basins and island arc settings. Due to water rock interaction at elevated temperatures and potentially magmatic degassing, considerable amounts of dissolved arsenic has leached out from hydrothermal fluid systems. Fluid from mid-ocean ridge hydrothermal systems such as the East Pacific Rise had reported levels of around 80.5 µg/L arsenic while at the Mid-Atlantic Ridge, the highest reported level was around 24.0 µg/L. A highly elevated arsenic concentration of 1386.0 µg/L was reported for back-arc basin settings and of 5850.0 µg/L for island arc shallow water settings [16].

28.3 Arsenic Concentration in Marine Sediments

Marine sediments comprise significant sinks of contaminants and represent potential sources of pollution to the marine environment. The total arsenic influx into oceans was estimated at 246,110 metric tons/year. Of this total, 178,900 metric tons was sediment suspended arsenic, 62,900 metric tons was dissolved arsenic, and 4310 metric tons was derived from the atmosphere per year [32]. Total arsenic concentrations in uncontaminated near-shore marine and estuarine sediments were between 5 and 15 µg/g dry weight, while, deep-sea sediments contained an average arsenic concentration of approximately 40 µg/g [8]. Sediments from estuaries and coasts receiving drainage from industries and metal-mining areas contain significantly elevated arsenic concentrations, which threaten the aquatic biota and have been of significant environmental concern [8,38]. The coastal sediments of Gwangyang Bay, Republic of Korea, have total arsenic concentrations between 6.89 and 355 µg/g, which are higher near the mining area [27].

Arsenate is the most dominant arsenic species present in oxidized marine sediments, whereas arsenite is the dominant species in reduced sediment layers. Iron and manganese (oxy)-hydroxide, either free or coated with mineral clays and organic substances, are the major sinks for arsenic entering into marine water. Such substances scavenge dissolved arsenic from the water column and transfer them into the sediments [39]. The affinity of pelagic clays for adsorption of arsenite was three times higher than that for arsenate. Most of the arsenite that was adsorbed by iron oxides in oxidized marine sediments was rapidly oxidized to arsenate. The bacterial species present in aerobic sediments have the ability to oxidize arsenite to arsenate [8]. Arsenic concentrations in the East China Sea sediments ranged from 1.70 to 22.1 µg/g with an average of 11.5 µg/g (Table 28–2). The distribution pattern of arsenic in the East China Sea sediments was mainly controlled by clay and total organic contents, with higher arsenic concentration near the Changjiang estuary and Hangzhou Bay mouth. This indicates that

Table 28–2 Arsenic Contents in Marine Sediments

Marine Sediment	Arsenic Contents Range (average)	References
East China Sea	1.70–22.1 μg/g (11.5 μg/g)	[40]
French Mediterranean Estaque port		
Surface (0–10 cm)	194 μg/g and 107 μg/g	[41]
Depth (10–20 cm)	199 μg/g and 220 μg/g	
French Mediterranean Saint Mandrier port		
Surface (0–10 cm)	10 μg/g	[41]
Depth (10–20 cm)	12.1 μg/g	
Southeastern Baltic Sea	1.1–19.0 μg/g (3.4 μg/g)	[42]
Chemical dumpsite Liepaja in Gotland	18–29 μg/g	[43]
Chemical dumpsite Skagerrak	9–200 μg/g (25 μg/g)	[43]
Chemical dumpsite Skagerrak	75–480 μg/g	[44]
Western North Sea	<0.15–135 μg/g	[39]
Cape Howe and Sugarloaf Point, east coast, Australia	2–180 μg/g	[45]
Baltimore Harbor, USA	25.0–41.1 μg/g	[46]

fine-grained sediments and riverine inputs are the main factors controlling arsenic distribution [40]. In contrast, when environmental conditions change (redox potential), arsenic associated with sediments is released to the overlying water column, threatening the marine biota [14]. It was also suggested that marine sediments as the secondary source of arsenic may affect marine ecology. In coastal and estuarine sediments redox changes occur more often at the time of seasonal changes, predominantly in spring and autumn [8]. Marine sediments are also an important source of methylated forms of arsenic to the overlying water column. Several species of aerobic and anaerobic sediment bacteria are able to accumulate arsenate and arsenite and convert them to methylated arsenic [47–49]. The methylated arsenic has been released by the bacteria into the sediment pore water, from which they may be mixed up into the overlying water column.

As shown in Table 28–2, sediment samples collected from the French Mediterranean port of Estaque showed arsenic contamination in surface (0–10 cm) and depth (10–20 cm) sediments of around 107 μg/g and 199 μg/g, respectively, collected during the cold season (March 2009). On the other hand, the levels were 194 μg/g and 220 μg/g, respectively, when collected during the hot season (September 2009). Another less polluted French Mediterranean port, Saint Mandrier, showed arsenic contamination in surface (0–10 cm) and depth (10–20 cm) sediments of around 10 μg/g and 12.1 μg/g, respectively [41]. Arsenic mobility studies in both these ports suggested that the exchangeable fraction represents 30% of total arsenic [41]. Sediment samples collected during the year 1992 from Baltimore Harbor, USA, showed arsenic concentrations between 25.0 and 41.1 μg/g [46]. Garnaga et al. [42] reported an average 9.7 μg/g of arsenic in sediment samples collected near the chemical munitions dumpsite in the Lithuanian economic zone of the Baltic Sea. Sediments in the Baltic Sea and North Sea were relatively high compared to arsenic concentrations reported from the chemical munitions

dumpsite in the Lithuanian economic zone [42]. Studies on chemical munitions dumpsites in Gotland (Liepaja dumpsite) reported arsenic levels in the sediments to be between 18 and 28 μg/g [43]. The highest arsenic concentrations up to 480 μg/g were found in the sediments from the chemical munitions dumpsites in Skagerrak [44]. The concentrations of arsenic ranged from <0.15 to 135 μg/g in the western North Sea and Humber estuary [39].

Oxygen plays a significant role in arsenic flux from sediments to the water column [46]. In anoxic conditions, the mobility of arsenic from sediments to the water column increases many fold more than that in hypoxic and oxic conditions. Marine sediments collected from an area between Sugarloaf Point and Cape Howe on the east coast of Australia showed arsenic concentration ranges of between 2 and 180 μg/g [45].

28.4 Arsenic Speciation in Marine Ecosystems

Arsenic sublimates at 613°C, melts at 817°C, and has a density of 5.27 g/cm^3 [50]. Arsenic (As) is present in the form of various chemical species in the marine environment (Figure 28–1) and its toxicity depends on its chemical form. Due to its complex chemistry, behavior, and ecotoxicological effects on marine ecosystems, it is necessary to understand the nature of these As species. Arsenic occurs in the marine environment in four oxidation states: As^{+5}, As^{+3}, As^0, and As^{-3}. The two highest oxidation states are the most common biologically important redox states and are trivalent inorganic arsenic [arsenite; As(III)], the reduced form and pentavalent inorganic arsenic [arsenate; As(V)], and the oxidized form. On the other hand, the elemental form is very rare. As^{-3} is found only in highly reduced environments where electropotential (Eh) values are extremely low [8]. In natural conditions, both arsenite and arsenate are subjected to chemically and biologically mediated oxidation, reduction, methylation, and other reactions. This is the major reason for the availability of various species of arsenic in the marine ecosystem. Both arsenate and arsenite are pH dependent and interconvertible; thus at physiological pH, arsenate dominates as $H_2AsO_4^{-1}$ ($2.5 < pH < 7$) and $HAsO_4^{-2}$ ($7 < pH < 12$), while arsenite dominates as H_3AsO_3 ($pH < 9.3$) [51]. Arsenate is a more predominant and thermodynamically stable form in oxygenated surface marine waters [34], while arsenite is more common in anaerobic reduced environments. Pentavalent arsenate has chemical properties similar to those of phosphate, and thus it competes with phosphate in cellular reactions and inhibits oxidative phosphorylation, the key reaction of energy metabolism in metazoans, including humans, and is the most toxic form to algae [52]. However, trivalent arsenite has a strong predilection to form bonds with functional groups such as the thiolates of cysteine residues and the imidazolium nitrogens of histidine. Thus, it binds readily to many enzymes, including those involved in respiration [52]. Arsenite is reported to be as much as 60 times more toxic than arsenate to humans and to marine organisms [8].

Organic forms of arsenic are also present in the marine environment, often found in association with biological systems. These include methylated arsenicals such as monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)), which are common arsenic metabolites that exist in most environmental compartments, and others like monomethylarsonous acid (MMA(III)), dimethylarsinous acid (DMA(III)), dimethylarsinoyl acetate,

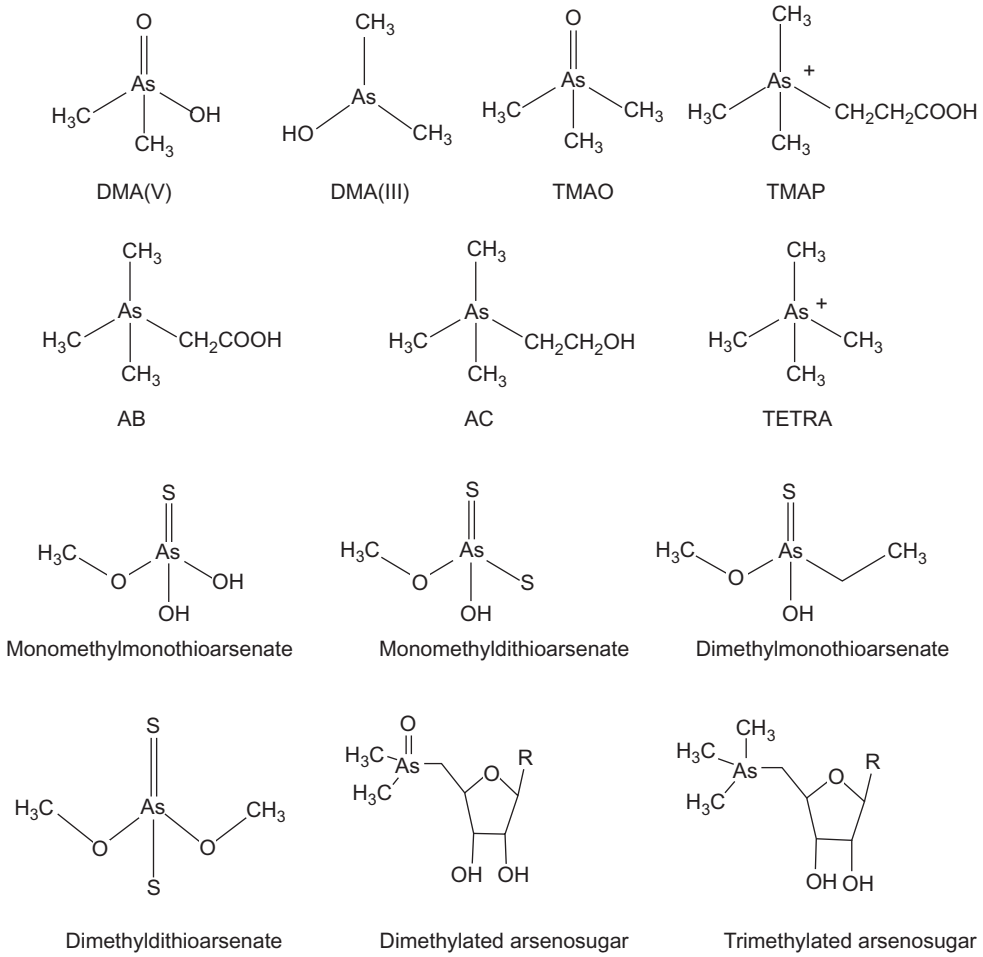

FIGURE 28–1 Arsenic species commonly found in marine ecosystems. DMA(V)—dimethylarsinous acid, DMA(III)—dimethylarsinous acid, TMAO—trimethylarsine oxide, TMAP—trimethylarsoniopropionate, AB—arsenobetaine, AC—arsenocholine, TETRA—tetramethylarsonium ion.

dimethylarsinoyl ethanol, arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium ion (TETRA), arsenosugars (AsS) [53,54], and methylthioarsenic species, which are common forms in marine ecosystems. Arsenic is methylated by marine bacteria, algae, and phytoplankton, but neither form is considered to be as toxic as inorganic arsenate and arsenite compounds [55]. In the marine environment, the most predominant arsenic forms are inorganic [As(V) and As(III)] arsenic, which contribute around 80% of total arsenic, while methylated and other organic forms contribute around 20% of total arsenic [17]. The contribution of each arsenic species in surface water of east Indian Ocean is 52% for As(V), 27% for As(III), and 21% for methylated arsenic. Similar ratios were observed in surface water of the Northwest Pacific Ocean, which were 63% for As(V), 22% for As(III), and 15% for methylated arsenic [19], although seawater temperature,

nutrient availability, and biological activities affect the concentrations of various arsenic species in sea water. The rate of release of methylated arsenicals by the macroalga *Ascophyllum nodosum* is influenced by temperature changes, with a two-fold increase noted, on increasing the temperature to between 5 and 15°C, in the rate of release of monomethylarsenic by algae; the rate of release of dimethylarsenic is increased by more than six-fold [56]. The concentration of methylated arsenic is increased with increasing water temperature and nutrient availability in Pacific surface sea water suggesting that the biological activity of the phytoplankton community in surface waters is responsible for the abundance of dimethylarsinic acid and monomethylarsonic acid [18]. Dimethylarsenic was found to be the dominant organic form of arsenic in sea water at all temperatures. In the process of detoxification, some of the marine algae and certain marine animals convert absorbed arsenate from sea water to arsenosugars [57]. Arsenosugar was first identified in 1981 from brown kelp *Ecklonia radiata* [58]. A total 15 arsenosugars were identified from certain algae and marine animals [54,59,60]. Marine algae accumulated arsenic chiefly as arsenosugars, which comprised the largest group of naturally occurring arsenicals [61].

Another important and virtually ubiquitous organoarsenical in marine animals is arsenobetaine. It generally represents 50 to >95% of the total arsenic in tissues of marine crustaceans, elasmobranchs, teleost fish, polychaetes, and gastropods [62–64]. Environmental factors like elevated inorganic arsenic concentrations through the hydrothermal fluids and sediments and food (bacterial mat) cause an unusual speciation like tetramethylarsonium ion and trimethylarsoniopropionate, which were observed in gut and muscle of *Cyclope neritea* (a gastropod) from Paleochori Bay. The control gastropod from Ria do Alvor, Portugal, has almost exclusively arsenobetaine [63]. Recently, new classes of arsenic species, thioarsenate and thio-methylated arsenic, have been reported in geothermal waters and marine organisms, respectively. Thiomethylated species are thought to be biologically formed in the presence of sulfur-rich anaerobic environments [65]. Presently, more than 50 additional arsenic species have been reported in marine organisms. Their effects in terms of toxicity to humans, however, remain unclear [4].

28.5 Arsenic Cycle in the Marine Environment

Arsenic is a ubiquitous and uniformly distributed element in unpolluted marine environments. Although open-ocean systems are relatively constant, the possibility of anthropogenic inputs of arsenic may result in the higher arsenic concentration in coastal areas and estuaries. Additionally, natural inputs like riverine discharges, atmospheric depositions, and volcanic eruptions are significant contributors of arsenic. As shown in Figure 28–2, arsenic enters the marine environment mostly in dissolved inorganic forms [66]; however, the methylated arsenicals exist in comparatively low concentrations [19,20]. In the east Indian Ocean and Northwest Pacific Ocean, 89% and 85% of arsenic was found in the inorganic form, while methylated arsenic accounted for 21% and 15%, respectively [19]. Among the inorganic forms, arsenate is the most stable and predominant form in oxic euphotic zones, a surface water rich in oxygen and light as compared to deep water in marine environments [19,66]. The similarities in the chemical properties between arsenate and macro-nutrient phosphate are largely responsible for the production of reduced and methylated arsenic by marine phytoplankton, bacteria, and algae.

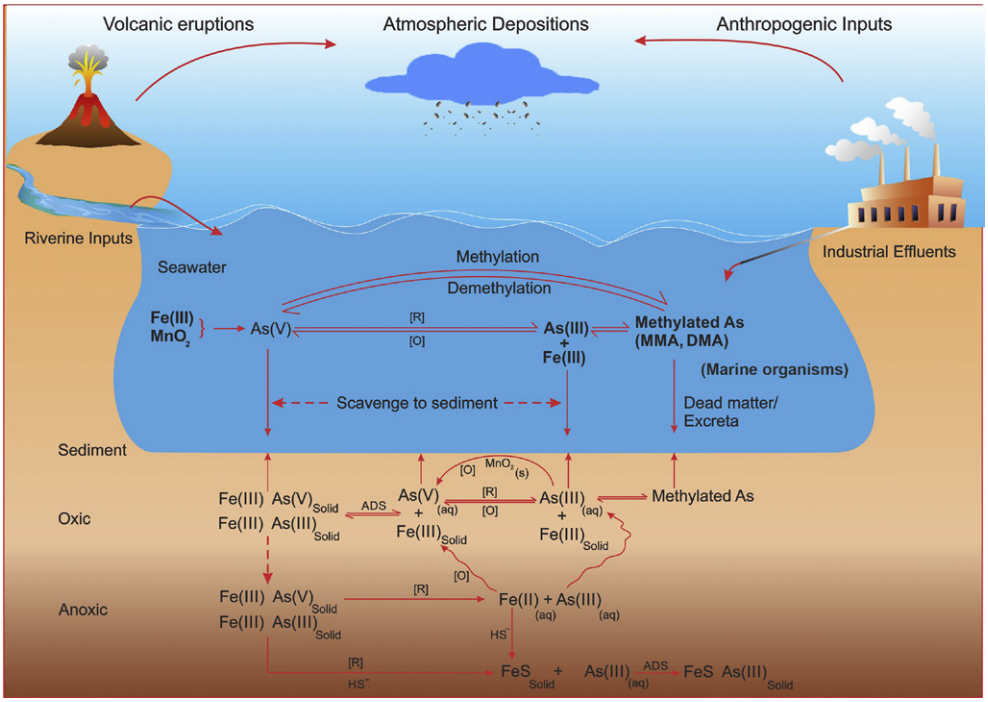

FIGURE 28–2 Arsenic cycle in the marine environment. [O]—oxidized, [R]—reduced, ADS—adsorbed. *Modified from* [15].

Arsenite has reciprocal correlation with phosphate [8], which suggests that the low phosphate concentration in the environment facilitates arsenate uptake by biological systems, which ultimately convert it to the reduced and methylated forms for their survival. Thus, phosphate concentration in sea water appears to regulate the reduction and methylation of arsenic in the marine ecosystem. The reduced and methylated forms of arsenic are produced during the process of detoxification and released back in the water column. Arsenite is a product of detoxification by bioreduction in well-oxygenated waters, which decreases rapidly below the thermocline as a thermodynamically unstable species. Its coexistence with arsenate in deep water is kinetically supported due to its relatively low oxidation rate. The methylated arsenicals produced by phytoplankton uptake are seen to be chemically stable, at least for a few months [67].

Two microorganisms, named *Apiotrichum humicola* and *Scopulariopsis brevicaulis* in laboratory experiments, are capable of forming arsenite, monomethylarsonate, dimethylarsinate, and trimethylarsine oxide when supplemented with arsenate-containing growth medium [68]. Numerous studies have described that the inorganic arsenic from sea water is converted and concentrated in the organic forms of arsenicals by bacteria [48], phytoplankton [59], and algae [69]. They are further metabolized through the food chain and accumulated as arsenobetaine in marine animals [19]. Arsenobetaine (trimethyl(carboxymethyl)arsonium zwitterion) is the

main arsenic species in marine animals, while arsenoribosides are mainly present in seaweeds. Arsenobetaine is considered the final metabolite of arsenic in the marine food chain and accumulates in marine animals to greater or lesser extents [57,70]. The other forms of organoarsenicals, like arsenocholine, arsinylribosides, tetramethylarsonium ion, phosphatidylarsenocholine, MMA, and DMA, are reported in the tissues of various marine animals [71]. The majority of organoarsenicals ingested in successive food chains by higher tropical levels are rapidly excreted. The remaining small amount is incorporated within organisms, and exported in the higher fishes or deposited as dead material in marine sediments. Organoarsenicals, associated with biological systems and/or freely available in sea water, are degraded to complete the arsenic cycle in the marine ecosystem (inorganic arsenic to inorganic arsenic via formation of organic arsenic) [72]. Finally, arsenic-containing dead material and undissolved biogenic particles sink into the marine sediments, where they biodegraded with inorganic arsenic or lower forms of organoarsenicals being released back into the water column [66,73,74]. Decomposition occurs at the sediment level as well as at the euphotic seawater level. Bacterial isolates identified as members of the *Vibrio/Aeromonas* group have the capacity to decompose arsenobetaine to dimethylarsinic acid under aerobic conditions [75]. The laboratory experiments suggest that sedimentary microorganisms have the capacity to convert higher (complex) forms of methylated arsenicals to their lower forms under aerobic conditions and even convert them into a simpler and aerobically stable form, i.e., arsenate [76,77].

Co-precipitation of inorganic arsenic with hydrated heavy metal oxides may occur, and surface active suspended particulate matter may scavenge dissolved arsenic from the water column. Adsorption of arsenic onto iron and/or manganese oxides offers the major sink of arsenic in marine sediments. In the oxidized layer of the sediments, much of the arsenic is associated (co-precipitated or adsorbed) with the hydrous iron and manganese oxide fraction or is present as $Fe_3(AsO_4)_2$ [8]. Sedimented organic matter may also have the capacity to bind arsenic [78]. Sediment–water exchange has a significant impact on the arsenic biogeochemical cycle. Sedimentary diagenesis liberates arsenic to pore water, and thus the arsenic concentration in these interstitial waters reflects solid-phase arsenic levels, although the arsenic content always remains a minor fraction of total sedimentary levels [14].

The major mechanism for arsenic liberation into pore waters is the dissolution of hydrous oxide phases to which the metalloid is adsorbed. Under reducing conditions, Fe(III) and Mn(IV) are converted into their soluble reduced forms Fe(II) and Mn(II), respectively, which release adsorbed arsenic into the pore water [8]. In anoxic sediments, arsenate is reduced to arsenite, and is released upward into interstitial waters or reacts with sulfur if available in abundance. In most marine sediments, most of the arsenic reacts with sulfides to form realgar (AsS), arsenopyrite (FeAsS), or orpiment (As_2S_3) [8]. These sulfides have a low solubility and mobility [8]. Marine sediments might also be an important source of MMA and DMA to the overlying water column. Several species of aerobic and anaerobic sediment bacteria are shown to bioaccumulate arsenate and arsenite and convert them to MMA and DMA.

The concentration of dissolved arsenic in surface waters is highly influenced by natural exchange between sediments and the overlying water column. Overall, arsenic has been categorized as a bio-intermediate trace element; it shows relative surface minimum, mid–depth

maximum, and minor deep-water enrichment [17]. Only a minor portion of the arsenic is incorporated into biological systems of the arsenic cycle and 20% of the dissolved arsenate pool enters the arsenic cycle annually [67].

28.6 Role of Marine Biological Systems in Arsenic Biotransformation

28.6.1 Phytoplankton

Phytoplankton, the most common primary producer in the marine food chain, take up arsenate from the surrounding sea water and reduces it to arsenite [79], which is readily oxidized to arsenate in oxic marine waters on excretion [59]. This constant reduction of arsenate is mainly responsible for the measurable quantities of arsenite in the water column in spite of the thermodynamic instability of arsenite. The marine environment rich in phytoplankton exhibits a high percentage of arsenite and methylated forms of arsenic such as MMA and DMA. Phytoplankton are primarily responsible for the production of reduced and methylated species of arsenic [67]. Within the phytoplankton cell, the concentration of inorganic arsenic and methylated arsenic species shows great variation. This variation greatly depends on phosphate availability, arsenic concentration in surrounding sea water, temperature, and seasonal variations, and also the phytoplankton species because of their different biotransformation efficiencies.

Marine phytoplankton not only takes up arsenate from the surrounding waters but also incorporates arsenate into an array of carbohydrate compounds and biosynthesizes organic forms of arsenicals [62]. The exact nature of this organically bound arsenic by phytoplankton remains unclear; a number of arsenolipids like arsenocholine, trimethylarsoniumlactate, and arsenobetaine have been isolated [67]. Most of the organoarsenicals in marine phytoplankton are arsenosugars, which are also the precursors of the metabolic pathway to arsenobetaine and arsenocholine [80,81]. In the biotransformation pathway, phytoplankton actively uptakes arsenate using the phosphate transport system due to its structural similarity with phosphate, leading to its toxicity in the cell [82]. The excessive arsenate has to be transported out, but due to its poor mobility, the cell first converts it into a more mobile form, i.e., arsenite. Therefore, phytoplankton reduces As(V) to As(III), which can be excreted from the cell or enter into oxidative methylation to produce pentavalent methylated arsenic species (MMA(V) and DMA(V)) via intermediate trivalent methylated arsenic species (MMA(III) and DMA(III)) [83].

A few researchers believe that the process of arsenic biotransformation to its different forms is due to the detoxification mechanism of phytoplankton [84]; however, this theory has been disputed because the trivalent methylated species (DMA(III) and MMA(III)) are more toxic than As(III) [12,85]. The order of toxicity (high to low) of various arsenic species to organisms is trivalent methyl arsenicals > arsenite > arsenate > pentavalent methyl arsenicals [12]. The trivalent methylated arsenicals are structurally different from their pentavalent counterpart and are also more reactive and carcinogenic [86]. It has been reported that As(III) and MMA(III) act through the same mechanism to produce toxicity to organisms [87,88]. However, MMA(III) creates a more stable binary complex in the enzyme active site than As(III). The

mode of action of DMA(III) toxicity is more complicated and still remains unresolved [89]. The methylated arsenicals produced by phytoplankton uptake appear to be chemically stable, at least for a few months [67].

28.6.2 Marine Bacteria

Microbe–mediated arsenic metabolism has played a major role in the ecology of arsenic, which affects the parameters of mobility and bioavailability, as well as toxicity with in the marine environment. Bacteria implicated in the processes of biotransformation readily metabolize arsenic and participate in various metabolic functions including detoxification, anaerobic respiration, methylation, and assimilation. Marine bacteria have the ability to transform inorganic arsenic species to various methylated and/or complex organic forms of arsenic [47], and also have the capacity to decompose complex organoarsenicals into inorganic arsenic [75]. To survive with arsenic compounds in their environment, microbes have developed several metabolic pathways mediated by their genetic makeup and their products [88]. The genes involved in arsenic biotransformation are *aox/aos* genes (arsenite oxidation, recently named *aio* genes), *arr* genes (anaerobic arsenate respiration), and *ars* genes (reduction and methylation). The schematic model of arsenic biotransformation in bacterial cells is summarized in Figure 28–3 [3]. Since arsenate and arsenite may act as analogues of phosphate and glycerol, respectively, they enter the microbial cells via phosphate (Pst/Pit) transporters and glyceroporin (Glp) membrane proteins, respectively [88]. Cellular arsenite uptake was also observed via hexose transporters [90] or glucose permease GLUT1 [91]. The intracellular arsenite serves as an inducer to bind the regulatory protein ArsR (encoded by *arsR* gene) leading to its conformational changes and removal of previously bound regulatory protein to the operator site

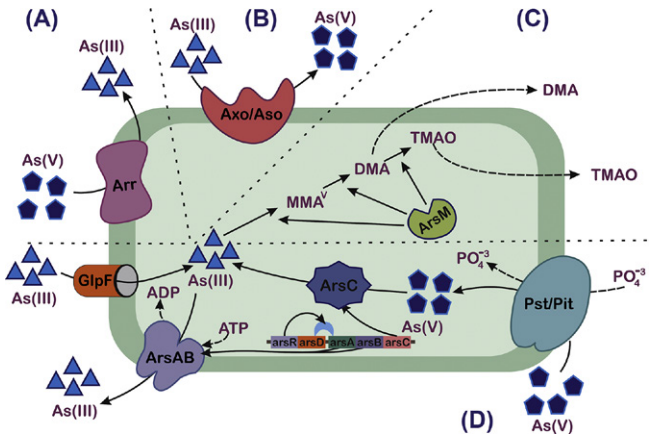

FIGURE 28–3 Arsenic biotransformation in prokaryotic cells. (A) Respiratory arsenate reductase (Arr) is involved in the reduction of As(V). (B) Arsenite oxidase (Aso/Aox) is responsible for oxidation of As(III). (C) S-adenosylmethyltransferase (ArsM) is responsible for methylation of As(III) to produce methylated arsenicals as the end product. (D) Genes (*arsRDABC*) and proteins involved in the uptake of As(III) and As(V) (GlpF and Pst/Pit transporter), reduction of As(V) (ArsC), extrusion of As(III) (ArsAB), regulation (ArsRD) by arsenic-resistant organisms. *Modified from* [4].

of the *ars* operon (Figure 28–3). The removal of the ArsR regulatory protein leads to the transcription of *ars* genes. The best characterized mechanism of arsenic detoxification in bacteria involves the reduction of arsenate to arsenite, mediated by arsenate reductase encoded by the *arsC* gene. Arsenite is further extruded by a membrane-associated efflux pump encoded by the *arsB* gene or sequestered in the intracellular compartments. Other genes like *arsD* and *arsA* (encoded for ATPase) are found along with *arsC* and *arsB* in the majority of prokaryotes [92]. The arsenic tolerance mechanism is conferred by the *ars* operon, which is located either on plasmid or chromosome in different bacterial species. Bacteria from the genera *Halomonas* and *Acinetobacter* isolated from Mandovi and Zuari estuarine water systems in Goan territory, India, have been shown to harbor *arsA*, *arsB*, and *arsC* genes on their plasmid [93]. Arsenite is formed by reduction of arsenate, either excreted from the cell or methylated to form various methylated arsenicals mediated by ArsM (S-adenosylmethyltransferase) encoded by the *arsM* gene, which are further pumped out of the cell by an unidentified transporter.

Thioarsenicals, structural analogues of oxyarsenicals in which sulfur replaces oxygen, are formed by exposure of oxyarsenicals to hydrogen sulfide [94]. Arsenic species reported to form thio species include MMA, DMA, arsenosugars, dimethylarsinoyl ethanol, and dimethylarsinoyl acetate. These species are thought to be formed in the presence of hydrogen sulfide during anaerobic degradation of seaweed [65]. Studies have shown that anaerobic microflora from human feces or mouse cecum and gastrointestinal tracts of marine organisms may convert DMA(V) into thiolated arsenic compounds such as dimethylthioarsenate and trimethylarsine sulfide [95–97]. Marine sediments and hydrothermal waters are rich in sulfur, where the reducing conditions and relatively high pH may favor the formation of thiomethylated arsenicals by anaerobic sedimentary bacteria. So far none of the bacterial species in the marine environment have been identified for their ability to synthesize thiomethylated arsenicals, either from inorganic arsenic or from methylated forms.

Oxidation of arsenite to arsenate mediated by arsenite oxidase, encoded by *aox/aos/aoi* genes, was observed in bacteria and archaeans as the detoxification mechanism. The toxicity of arsenic depends on its oxidation state, while arsenite is 100 times more toxic than arsenate in most biological systems [98]. *Acinetobacter junii* SeaH-As6s and *Marinobacter* sp. SeaH-As6w, isolated from coastal sediment and sea water, respectively, from Gwangyang Bay, Republic of Korea, were found to oxidize arsenite to arsenate.

Apart from the operon-mediated detoxification mechanism, *Bacillus* sp. strain XZM002 has shown an additional arsenic tolerance mechanism by changing its shape to reduce its surface area to survive in the presence of high arsenic concentrations in its environment. The initial rod shape was altered to oval and then to circular form with gradual increase in extracellular arsenic concentration. The circular form will have the least surface area for arsenic uptake and thus will exhibit less toxicity [99].

Marine bacteria have the main role in the biogeochemical arsenic cycle of decomposing complex organoarsenicals into their simple organic forms or even into inorganic arsenic forms [75,100]. Two bacterial strains of the group *Vibrio/Aeromonas* were isolated from coastal sediments, and efficiently decomposed arsenobetaine in aerobic conditions to dimethylarsinic acid. The addition of sediment itself in the media, as the source

of arsenobetaine-decomposing microorganisms, has shown the decomposition pattern of arsenobetaine to trimethylarsine to inorganic arsenic [75]. The oxidation and demethylation of methylated arsenicals by bacteria has been studied in marine waters [101].

Arsenic mobility in the environment was significantly enhanced by bacterial cell-mediated arsenate reduction. Two different pathways for arsenate reduction were observed in microorganisms encoded by *ars* and *arr* systems. The arsenate reductase encoded by *ars* genes encodes cytoplasmic arsenate reductase associated with a detoxification mechanism, whereas membrane/periplasmic arsenate reductase encoded by *arr* genes were associated with cellular respiration [102–104].

28.6.3 Algae

Marine algae are an important primary producer in the marine food chain, and affect the biogeochemical cycle of arsenic in the marine ecosystem. Marine algae have the capacity to accumulate 1000 times more arsenic than its concentration in the surrounding sea water [105]. This may contribute to the trophic transfer of arsenic to higher levels of the marine food chain and thus may pose a threat to human health [79]. Much of the organoarsenicals present in the tissues of marine animals are derived directly or indirectly from consumption of marine algae [8]. Zooxanthellae (algae), symbiotically present in clam mantle tissues, accumulate arsenate from the sea water and convert it to organic forms such as arsenoribosides and arsenotaurine, and pass the arsenic to the host [106]. In marine algae, inorganic arsenic accounts for between 1 and 50% of total arsenic and the remainder is present in different organoarsenical forms.

Biotransformation of arsenic in algal cells is initiated with the uptake of inorganic arsenic forms. Usually, the cellular uptake of arsenate competes with phosphate but few marine algae show the phosphate-independent cellular uptake of arsenate, suggesting the existence of more than one arsenate uptake mechanism in algae [107]. Following uptake, algae reduce arsenate to arsenite, and then subsequent methylations lead to the formation of methylated forms such as MMA, DMA, TMA, and various arsenoribosides. This biotransformation and rapid excretion of methylated arsenic MMA and DMA suggest methylation as the potent arsenic detoxification mechanism present in algae [34]. Methylation of arsenic involves an enzyme-mediated transfer of the methyl group via methylcobalamine or S-adenosyl methionine. The reduction of DMA with adenosyltmethionine produces dimethylarsinyladenosine, which may lead to further glycosidation to form dimethylarsinoribosides. Reductive methylation of this intermediate might lead to the formation of trimethylarsonioribosides [8].

Marine algae are found to be the principal producer of arsenosugars. Nearly 15 different arsenosugars were identified in marine algae containing pentavalent arsenic bound to two methyl groups [108]. The different classes of algal species have shown different forms of arsenoribosides. The brown macroalgae have shown the accumulation of more sulfonate (SO_3-ribose) and sulfate (OSO_3-ribose) arsenoribosides, whereas glycerol (OH-ribose) and phosphate (PO_4-ribose) arsenoribosides were dominant in red and green macroalgae [109]. The brown alga (*Ecklonia radiate*) from unpolluted waters of Western Australia accumulates inorganic arsenate from sea water and converts it to a variety of arsenosugars [110].

Arsenate accumulation capacities of seaweeds (*Fucus spiralis* and *Ascophyllum nodosum*) were four times higher than for arsenite accumulation [111]. This suggested more toxic arsenite is excreted rapidly after the reduction mediated by membrane-associated transporter proteins. Recently, the role of metalloproteins (phytochelatins) in the detoxification of arsenic has been reported in marine microalgae (*Phaeodactylum tricornutum*) [112]. Phytochelatins (PCs), intracellular cysteine-rich metal-binding thiols containing polypeptide, are key metalloproteins involved in heavy metal homeostasis and detoxification in the cells of microorganisms. The synthesized PCs build a complex with arsenite (As(III)–PC) and are sequestered into vacuoles through the activity of ATP binding cassette (ABC) transporters, being finally excreted from the cells [113].

28.6.4 Marine Animals

Most marine animals have limited ability to accumulate arsenate from surrounding sea water. Despite this limited ability, concentrations of total arsenic vary widely in tissues of marine animals [8]. Total arsenic in whole and muscle tissues of marine organisms worldwide ranges from below the detection limit to more than 2500 μg/g dry weight [8]. Natural concentrations of total arsenic in the tissues of marine animals, other than marine mammals, often are less than 10 ppm. The geometric mean As concentration ranges from 0.25 μg/g dry weight in marine animal tissues [8]. The highest concentrations of arsenic have been reported in tissues of marine animals that feed primarily on phytoplankton or macroalgae, The taxa of which include planktonic crustaceans, bivalve mollusks, herbivorous snails, and some polychaeta worms [8]. Oysters (*Crassostrea virginica*) from coastal waters of the US Gulf of Mexico contain 4.1 to 39 μg/g of total arsenic [114]. Marine organisms from arsenic-contaminated environments generally contain higher concentrations of arsenic than do organisms from uncontaminated sites [8]. Arsenic found in marine animals in inorganic or organic forms (monomethyl arsenic, dimethyl arsenic, arsenosugars, and arsenocholine) account for only a minor fraction of total arsenic.

The main arsenic compound found virtually in all marine animals is arsenobetaine [115], which is a relatively stable quaternary arsenium compound and considered non-toxic. Arsenobetaine is the most common form of arsenic observed in the majority of marine fishes and is also known as “fish arsenic” [79]. It is rapidly excreted without bio transformation in marine animals. The concentration of arsenobetaine in marine animals usually represents 50 to 95% of the total arsenic in tissues of marine crustaceans, polychaeta, elasmobranchs and teleost fish and 10 to more than 95% of the total arsenic in sponges, coelenterates, mollusks, and echinoderms [8,63].

The tetramethylarsonium ion is also commonly found in marine animals, particularly in bivalve mollusks. High percentages of tetramethylarsonium ion were observed in gut (16.2%) and tissue (23.5%) of *Cyclope neritea* (gastropod) inhabiting arsenic-rich marine shallow water hydrothermal system off Milos island, Greece [63]. Other forms of arsenic such as trimethylarsine oxide and arsenocholine were also noted in marine animals, generally as minor arsenic constituents. The occurrence and distribution of arsenic species in a particular animal depends on its habitat, food preference, physiology, and arsenic species exposure [63,64].

Arsenosugars were found in a few herbivorous marine animals where the source was almost certainly the marine algae as their feed [115].

The origin and biosynthesis of arsenobetaine are controversial. Basically, four pathways have been proposed for arsenobetaine biosynthesis. In the first two pathways, dimethylated arsenosugars are converted into arsenobetaine through dimethylarsinoyl ethanol and dimethylarsinoyl acetate or arsenocholine [10]. In several marine animals and marine algae, dimethylarsinoyl ethanol and dimethylarsinoyl acetate were observed but at low levels. The existence of these two forms supports the concept that a pathway exists from dimethylated arsenosugars to arsenobetaine. In the third pathway, arsenobetaine is converted from trimethylated sugar [10]. Despite the natural occurrence of trimethylated arsenosugars in marine organisms, their very low concentrations do not account for the presence of arsenobetaine at a high concentration in marine animals [10,57]. However, a comparatively high concentration of trimethylated arsenosugar was reported in *Haliotis rubra* (abalone) [116], which suggests that trimethyl arsenosugar might contribute to the synthesis of arsenobetaine in this marine animal. In the fourth pathway, it is postulated that arsenobetaine is synthesized from dimethylarsinous acid and 2-oxo acids, glycoxylate, and pyruvate [10].

The high level of arsenobetaine in marine animals is related to the salinity of sea water. Organisms are known to utilize various osmolytes, low molecular weight osmotically active solutes, to adapt to osmotic stress [10]. Glycine betaine is the nitrous analogue of arsenobetaine [117] and behaves as an osmolyte in marine animals [118]. Once the arsenobetaine is synthesized in the marine food chain, it is taken up by the cells by using the uptake mechanism for glycine betaine [117]. Arsenobetaine was not found in *Corbicula japonica* (bivalve), which lives in the low salinity estuary [119]. It is suggested that arsenobetaine was not required as an osmolyte in a low salinity environment. It has been reported that arsenobetaine levels increased in blue mussels that had been maintained at high salinity [120]. The arsenobetaine levels decreased in the gill but not in other tissues when the blue mussels that had been maintained at high salinity were transferred to low salinity sea water. This suggested that arsenobetaine behaved as an osmolyte in the blue mussel and that the gill responded sooner to osmotic changes than did other tissues [10]. Although controversial, the study of retention capacity of arsenobetaine in seawater- and freshwater-adapted Atlantic salmon found no significant difference between seawater and freshwater groups, whereas the arsenobetaine level in the muscle of seawater-adapted wild salmon was found to be 10-fold higher than that of a freshwater-adapted wild salmon [121]. This study has suggested that high arsenobetaine levels in seawater-adapted wild salmon might be due to the arsenobetaine level in the diet of wild salmon rather than an adaptation to salinity [10]. Bioaccumulation of arsenobetaine in marine animals is still controversial.

28.7 Arsenic in Seafood and Its Toxicity

Arsenic has been documented as a global toxin that affects human health and shown to promote several types of cancers such as skin, urinary bladder, liver, and lung, as well as several

non–cancer diseases such as diabetes mellitus, hypertension, and cardiovascular and cerebrovascular diseases [122]. Humans can be exposed principally to arsenic by drinking contaminated water and food, especially seafood [4], which generally contain higher concentrations of arsenic than do other food [5]. The concentration of arsenic in marine organisms ranges between 5 and 100 μg/g dry mass, while terrestrial foods levels of arsenic range between 0.05 and 0.4 μg/g dry mass [4]. Around 90% of dietary arsenic in the US diet comes from seafood and saltwater finfish [123]. A few countries like Japan, China, and Korea rely on food from marine sources as a major part of their diet [6,7].

Arsenic toxicity is strongly related to the chemical species in which it is present in seafood. Organic forms of arsenic contribute more seafood total arsenic than do inorganic forms, which contribute less than 1 to 4% of the total arsenic [11]. Arsenobetaine, the most widespread and abundant organoarsenic compound found in marine animals, accounts for more than 80% of the total arsenic content [5]. Other organic arsenicals found in seafood include methylated arsenic such as monomethylarsonic acid, dimethylarsinic acid, trimethyl arsine oxide, tetramethylarsonium ion, arsenosugars, and arsenocholine [11]. Inorganic arsenic is considered the most toxic and is classified as a human carcinogen by oral and inhalation routes [5], while methylated arsenicals are considered as moderately toxic. Other organoarsenicals like arsenobetaine, arsenocholine, and arsenosugars have shown no toxicity.

Following uptake of arsenic-containing seafood, arsenic is easily absorbed by the human gastrointestinal tract and transported to the blood. Absorption with regard to dose was more than 95% in cases of arsenobetaine and arsenocholine, while 50% in the case of arsenite [7]. In the human body, inorganic arsenic species is converted to methylated arsenic via sequential reduction (arsenate to arsenite) and oxidative methylation. The reduction is carried out by glutathione, cysteine, and dithiothretol and methyl groups provided by S–adenosyl methionine. The monomethyl, dimethyl, and trimethyl species thus formed are generally less reactive with tissue components and more readily excreted in urine than the original inorganic species [11]. Metabolism of arsenic species in the human body after the ingestion of the seafood *Anemonia sulcata* showed that around 95% of total arsenic in cooked anemones was bioaccessible and 85% of the amount ingested was eliminated from the body via urine in 90 hours. Arsenobetaine followed by dimethylarsinate was the major compound found in both cooked anemonia and human urine after 10 hours of ingestion of seafood, while dimethylarsinate was most abundant in human serum 30 minutes after ingestion, followed by arsenobetaine [7].

As discussed earlier, arsenobetaine represents the most predominant and rapidly excreted form of arsenic in seafood. With respect to potential toxicity, arsenobetaine is non-mutagenic, non-cytotoxic, and non-immunotoxic and has no transforming activity in mammalian cells [11]. Acute oral LD_{50} of arsenobetaine in mice was higher than 10 μg/g [124]. Arsenocholine, a metabolic precursor of arsenobetaine in marine animals, was reported at only minor levels in seafood [11]. Although studies of its toxicity were limited it was regarded as essentially a non-toxic compound [125]. Like arsenobetaine it is non-toxic and non-mutagenic. The acute oral LD_{50} in mice was 6.5 g/kg, whereas the acute intravenous LD_{50} was 187 μg/g [124]. Recent attention has focused on the oxidation state of methylated arsenicals excreted in the urine of seafood eaters. Trivalent methylated arsenic compounds such as methylarsonous acid and

dimethylarsinous acid have greater toxicity than the corresponding pentavalent methylated arsenic compounds and also inorganic arsenic [11,12]. It was found that arsenite and methylarsonous acid act through the same mechanism to produce toxicity. However, methylarsonous acid created a more stable binary complex in the enzyme-active site than did arsenite. The mode of action of dimethylarsinous acid toxicity is more complicated and mostly unresolved [113]. However, trivalent methyl arsenic has not been documented in urine of seafood eaters until now. The reason for this failure may include low concentrations in urine, instrument detection limitations, or the unstable nature of trivalent methylated arsenic forms [11].

Trimethyl arsine oxide (TMAO) has been considered non–toxic and identified in some species of marine animals as a minor arsenic species, although rarely detected except at trace levels [11,34]. Levels were higher in stored, frozen fish than in fresh fish, probably secondary to postmortem breakdown, but dietary intake of TMAO was probably extremely low [11,126]. In *in vitro* studies, TMAO caused no cell growth inhibition, did not induce sister chromatid exchange, and caused only rare clastogenic effects at concentrations up to 10 mg/cm^3 [11].

Tetramethyl arsonium ion is normally a minor species in marine animals such as finfish and fish, but might be a major species in some mollusks [11,34]. It was seen that levels of TETRA may be enhanced by freezing or dry cooking (grilling, roasting, and baking) at temperatures >160°C, especially in charred meat, likely due to the thermal decarboxylation of arsenobetaine [11]. Due to the limited quantity of TETRA in consumed fish, acute poisoning by TETRA was unlikely [11]. *In vitro* studies showed that high concentrations of TETRA inhibit cell growth [127]. In marine algae, a total of 15 different arsenosugars have been identified; almost all consist of pentavalent arsenic bound to two methyl groups [108]. Of these, there are four principal arsenosugars primarily found in marine algae. Their principal dietary source is seaweed, a common component of the Chinese diet [6]. Other sources include oyster, mussels, crab, fish, and clam [6]. In mammalian cells, arsenosugar was not cytotoxic at micromolar levels [11].

However, the above–mentioned arsenic compounds were not distributed uniformly throughout the tissues of marine animals. For example, in finfish, inorganic compounds and DMA were found almost exclusively in the viscera, while the arsenic content of muscles was almost all arsenobetaine [54]. Differences in tissue distribution between muscles and viscera have been documented in a variety of fish, carnivorous gastropods, and crustaceans [63,71]. It has been suggested that the degree of arsenic toxicity is also dependent on the particular type of ingested tissue. The risk of ingested seafood arsenic therefore depends on the nature and quantity of arsenic species present in the particular food and the metabolites formed as a consequence of expected metabolic activities.

Based on the consideration of anticipated dose and anticipated metabolism, it is likely that seafood arsenic does not contribute significantly to arsenic-associated carcinogenicity [11]. The most abundant form of arsenic generally reported in marine animals was arsenobetaine, a compound that is non-toxic and excreted without transformation. The amounts of TETRA produced by dry cooking of arsenobetaine-containing fish were not likely to achieve toxic levels. Similarly, the levels of inorganic arsenic and methylated arsenic found in seafood are sufficiently low to mitigate concerns about their possible toxic effect in seafood. On the other hand, DMA and arsenosugars in seafood have posed at least theoretical risks [11]. In general, the absence of arsenic

toxicity reported in humans and other mammals after consumption of large amounts of seaweed and seafood [108] has supported the lack of acute toxicity. However, because the ingestion of seafood might lead to the generation of metabolites involved in arsenic–induced carcinogenesis, it is worthwhile considering the potential role of dietary seafood in long-term cancer risk.

28.8 Future Directions

Arsenic concentrations found in unpolluted marine environments do not represent a significant hazard to marine organisms and their consumers. However, the coastal areas affected by industrial and mining inputs have elevated levels of arsenic, which are much higher than the USEPA human health (fish consumption) water quality criteria for total arsenic in sea water [8]. Therefore, removal of arsenic from industrial effluents has become a necessity to keep the marine ecosystem safe. The strategy generally applied to minimize such threats is to immobilize bioavailable arsenic as the metals cannot be degraded like organic pollutants [128]. Physicochemical technologies are sometimes not suitable for larger volumes and have some drawbacks such as high cost and generation of secondary contaminants. Recently, low-cost environment–friendly technologies have stimulated interest in studies in the bioremediation of metal [129]. B-acteria play a significant role in bioremediation [128–130]. More than 50 arsenic species have been identified in marine organisms [4], and it is expected that more will be identified as analytical techniques advance. The methylation processes for inorganic arsenic like MMA(III) and DMA(III) have recently been reported to be highly toxic, which are to be investigated in relation to toxicity in higher animals including humans. Future legislation on permissible levels of arsenic in seafood must include data on the types of arsenic species present and their toxicological properties.

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