CHAPTER 2

REVIEW OF LITERATURE

The name arsenic is derived from the Greek word arsenikon, meaning yellow pigmented mineral. Arsenic compounds have been in use since ancient times. The extraction of the element from arsenic compound was first reported by Albertus Magnus in 1250 A.D (Bentley and Chasteen, 2002). Arsenic ranks 20th in earth’s crust, 14th in sea water and 12th in the human body (Woolson, 1975). It exhibits metallic as well as non-metallic physical characteristics and corresponding chemical properties. Hence, it is referred to as a ‘metalloid’ (Mandal and Suzuki, 2002). It is also one of the oldest human poisons known to mankind.

2.1. Properties of Arsenic

Arsenic is a metalloid with the atomic number 33, atomic weight 74.9216, symbol As and placed in the Group V and 3rd period of the periodic table of elements together with nitrogen, phosphorus, antimony and bismuth. Arsenic is a redox-sensitive element, meaning that it can change its form through reduction (gain of an electron) or oxidation (loss of an electron). Its occurrence, distribution, mobility, and forms rely on the interplay of several geochemical factors, such as pH conditions, reduction-oxidation reactions, distribution of other ionic species, aquatic chemistry and microbial activity (Shih, 2005).

2.1.1. Environmental chemistry of arsenic

The principal natural reservoirs of arsenic are rocks. Release and mobilization of arsenic from these sources constitute the availability of this element in soil, water and air in various forms. As a result, arsenic is ubiquitous in our environment, and
humans are always and unavoidably exposed to this toxic metalloid. Under normal ecological conditions, the level of arsenic bioavailability is not a threat for human health. Soils may contain arsenic levels between 0.1 and 40 ppm (Yan-Chu, 1994; WHO, 1981), if the underlying bedrock is not disturbed or redistributed by natural or pedogenic processes (Yan-Chu, 1994). Further, concentrations of arsenic in the environment may be elevated due to certain other anthropological activities resulting in significant increase in the human exposure to arsenic (Yan-Chu, 1994; WHO, 1981; Azcue and Nriagu, 1994; Tamaki and Frankenberger, 1992; Bhumba, 1994; Goering, 1999). The solubility, stability and cellular toxicity of various forms of arsenic are widely different. Thus, studies in the chemical form of arsenic especially the two inorganic arsenic species, arsenate (As$^{5-}$) and arsenite (As$^{3-}$), their transformation, persistence and bioavailability are pertinent in the understanding of levels of human exposure to arsenic.

Chemistry of inorganic arsenic in aquatic environment, especially with variable pH and oxygen availability, is unusually complex. The important feature is that in highly aerated condition arsenate salts are dissociated in all four arsenic acid (As$^{5-}$) species (Ferguson and Gavis, 1972), $H_2AsO_4$, $H_2AsO_4^{4-}$, $HAsO_4^{3-}$ and $AsO_4^{3-}$. However, in mild reducing condition, arsenous (As$^{3-}$) acid species, $H_3AsO_3$, $H_2AsO_3^{4-}$ and $HAsO_3^{3-}$ may be stable. Arsenic acid (As$^{5-}$) is the least toxic of the inorganic forms and arsenous acid (As$^{3-}$) is more toxic in vivo than arsenic acid and also more inhibitory in vitro (Cervantes, 1994; Knowles and Benson, 1983). The stability and predominance of different arsenic species in the aquatic environment at different $\rhoH$ ranges is shown in Table 1 (Gupta and Chen, 1978).

Table 1: Inorganic arsenic species in ground water

<table>
<thead>
<tr>
<th>$\rhoH$</th>
<th>0-9</th>
<th>10-12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>As (III)</td>
<td>Arsenous acid $H_2AsO_3$</td>
<td>Arsenous acid $H_2AsO_3^{4-}$</td>
<td>Arsenous acid $HAsO_3^{3-}$</td>
<td>Arsenous acid $AsO_4^{3-}$</td>
</tr>
<tr>
<td>$\rhoH$</td>
<td>0-2</td>
<td>3-6</td>
<td>7-11</td>
<td>12-14</td>
</tr>
<tr>
<td>As (V)</td>
<td>Arsenic acid $H_2AsO_4$</td>
<td>Arsenic acid $H_2AsO_4^{4-}$</td>
<td>Arsenic acid $HAsO_4^{3-}$</td>
<td>Arsenic acid $AsO_4^{3-}$</td>
</tr>
</tbody>
</table>
2.2. Sources of arsenic

A large number of diverse chemical and biological reactions, viz. oxidation, reduction, adsorption, precipitation, methylation and volatilization participate actively in the cycling of this toxic element. These reactions control the availability of arsenic, and hence, arsenic concentrations effectively exposed to humans are governed more by arsenic speciation than by the total amount of arsenic. Soluble arsenic occurs in two primary forms: inorganic and organic. Inorganic arsenic can occur in the environment in several forms and valencies, but in natural waters, and thus in drinking-water, it is mostly found as trivalent arsenite (As (III)) or pentavalent arsenate (As (V)). The toxicity and mobility of arsenic varies with its valency state and chemical form. As (III) is generally more toxic to humans and four to ten times more soluble in water than As (V) (USEPA, 1997; USOSHA, 2001).

2.2. Sources of Arsenic

Arsenic is the twentieth most abundant element in the earth’s crust. Although arsenic exists in both organic and inorganic forms, the inorganic forms are more prevalent in water and are considered more toxic. Arsenic is introduced into the aquatic environment from both natural and man-made sources. The Earth’s crust is an abundant natural source of arsenic. It is present in more than 200 different minerals, the most common of which is called arsenopyrite. About one-third of the arsenic in the Earth’s atmosphere is of natural origin. Figure 3 represents the different sources of human exposure to arsenic. There are mainly two sources of arsenic viz., natural sources and anthropogenic sources. In nature, arsenic is distributed in a variety of minerals, commonly as arsenide of iron, iron, copper, lead, silver and gold or as sulfide minerals, for example arsenopyrite (USEPA, 2006). Now a day, almost all the world’s arsenic obtained as a byproduct of the smelting of copper, lead, cobalt and gold ores. The geochemical cycling of arsenic in the environment is through interaction of natural water with bedrock, sediments and soils, together with local atmospheric deposition (ATSDR, 2007). Volcanic action is the most important natural source (Fowler, 1983; Friberg et al., 1986).
Anthropogenic processes such as industrial activities are great sources of arsenic emissions. Arsenic based compounds have been used in pesticides, herbicides, insecticides, fungicides, rodenticides, algaecides, dye-stuff, dipping agent for sheep and vine killer (Hughes, 2002). Other anthropogenic activities resulting high arsenic level in the environment are mining, smelting and ore benefaction (USEPA, 2006).

2.2.1. Source of Drinking Water in Assam

Inorganic arsenic of geological origin is found in groundwater used as drinking water in several parts of the India. Ground water has played and will continue to play a key role in meeting the water needs of the North East. Rural peoples of this region mainly consumed water from tube wells, ponds & rivers. At present, the primary source of drinking water in rural areas are the private domestic tube wells, tapping water from shallow aquifers (50-200 ft). Drinking water was also supplied from underground by pipelines to distant places. Assam has the highest ground water potential among the north eastern states, but only 12.83 percent of ground water potential is currently utilized. The area referred to as Barak Valley includes the districts of Cachar, Hailakandi and Karimganj in the southern part of the state of Assam in North East India. The major sources of drinking water in the rural areas of Barak Valley comprise dug-wells, ponds and tanks, streams and springs, and hand-pumps that extract groundwater. Table 5, 6 and 7 represents arsenic concentration in ground water of Cachar, Karimganj and Hailakandi district respectively.

2.2.2. Routes of arsenic exposure

Arsenic is a ubiquitous micro pollutant. It is naturally found in the atmospheric air at concentration levels of about 0.4 to 30 ng m⁻³, in food at concentration levels of about 0.4 to 120 mg kg⁻¹ and in water at concentration levels from undetectable to even upto 50 mg L⁻¹ (ATSDR, 2007). Approximate environmental concentration levels and human exposure through air, food and water to arsenic is given in Table 2 (ATSDR, 2007). Inorganic arsenic, especially Arsenic III is more toxic for human than
organic arsenic. For this reason, arsenic exposure through water is more serious than that through food.

Table 2: Approximate environmental concentrations of arsenic

<table>
<thead>
<tr>
<th>Medium</th>
<th>Daily intake</th>
<th>Concentration (As)</th>
<th>Daily Exposure</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>20 m³</td>
<td>0.4-30 ng m⁻³</td>
<td>0.01-0.6 µg</td>
<td>May be higher in industrial area</td>
</tr>
<tr>
<td>Food</td>
<td>1 kg</td>
<td>0.4-120 µg kg⁻¹</td>
<td>0.4-120 µg</td>
<td>75% inorganic and 25% organic</td>
</tr>
<tr>
<td>Water</td>
<td>2 L</td>
<td>1-2 µg L⁻¹</td>
<td>2-4 µg</td>
<td>Mainly inorganic</td>
</tr>
</tbody>
</table>

2.2.3. Recommended limit of arsenic in ground water

Arsenic in ground water flows through arsenite rich bed rocks. Arsenic concentration in natural water varies widely depending upon geothermal sources, reductive desorption and oxidizing desorption at high pH and pyrite oxidation (Smedley and Kinniburgh, 2002). Arsenic concentration in river water is normally low (Otles and Cagindi, 2010). Sea water normally shows a relatively constant arsenic content of 1.5 mg L⁻¹. The concentration of arsenic in ground water ranges from less than 0.5 to 5000 mg L⁻¹ with a background concentration of less than 10 mg L⁻¹ (Smedley and Kinniburgh, 2002).

Because of the proven and widespread negative health effects on humans, in 1993, the WHO lowered the health-based provisional guideline for a “safe” limit for arsenic concentration in drinking water from 50 µg/L to 10 µg/L (i.e. from 0.05 mg/l to 0.01 mg/l). WHO retained this provisional guideline level in the latest edition of its standards (WHO 1993, 2004). The value of 10 µg/ was set as realistic limit taking into account practical problems associated with arsenic removal to lower levels. The WHO provisional guideline of 10µg/L has been adopted as a national standard by most countries, including Japan, Jordan, Laos, Mongolia, Namibia, Syria and the USA, and by the European Union (EU). Implementation of the new WHO guideline value of
10μg/L is not currently feasible for a number of countries strongly affected by the arsenic problem, including Bangladesh and India, which retain the 50μg/L limit.

2.3. Incidences of Arsenic Contamination

2.3.1. Global incidences of arsenic contamination

The delayed health effects of exposure to arsenic, the lack of common definitions and local awareness, as well as, poor reporting in affected areas are major problems in determining the extent of arsenic in ground water. There are many countries in the world where arsenic in ground water above permissible limit (WHO, 1996). The first case of arsenicosis was reported in Chile in 1962. In India, arsenic contamination was first discovered as late as 1982. By then 6.97 million people were estimated to be exposed to high arsenic content in drinking water. At present approximately 3,00,000 people are likely to be suffering from various stages of arsenicosis (Chakraborti et al., 2002; Mukherjee et al., 2006). Several countries in the world having arsenic problem in drinking water have shown in Figure 1.

![Figure 1: Global threat of arsenic contamination (Courtesy: Dr.Deepankar Chakrovorty, Jadavpur University, Kolkata)](image-url)
2.3. Incidences of arsenic contamination

Arsenic is highly soluble and mobile in water (WHO, 2004). Groundwater contamination with arsenic is consequently widespread. Arsenic concentrations above accepted standards for drinking water have been demonstrated in many countries on all continents and this should therefore be regarded as a global issue. Arsenic has been reported in groundwater in the following countries, among others presented in Table 3.

Table 3: List of countries where arsenic in ground water has been reported

<table>
<thead>
<tr>
<th>Region</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>Bangladesh, Cambodia, China (including provinces of Taiwan and Inner Mongolia), India, Iran, Japan, Myanmar, Nepal, Pakistan, Thailand, Vietnam</td>
</tr>
<tr>
<td>Americas</td>
<td>Alaska, Argentina, Chile, Dominica, El Salvador, Honduras, Mexico, Nicaragua, Peru, United States of America</td>
</tr>
<tr>
<td>Europe</td>
<td>Austria, Croatia, Finland, France, Germany, Greece, Hungary, Italy, Romania, Russia, Serbia, United Kingdom</td>
</tr>
<tr>
<td>Africa</td>
<td>Ghana, South Africa, Zimbabwe</td>
</tr>
<tr>
<td>Pacific</td>
<td>Australia, New Zealand</td>
</tr>
</tbody>
</table>

2.3.2. Incidences of arsenic contamination in India

In India, since ground water arsenic contamination first surfaced from West Bengal in early eighties, a number of other states namely Assam, Bihar, Chhattisgarh, Jharkhand, Manipur in flood plain of the Brahmaputra and Uttar Pradesh in flood plain of the Ganga River have chronically been exposed to arsenic contaminated hand tube wells water with arsenic levels above the permissible limit of 10 µg/L (Figure 2).

Many more North-Eastern Hill states in the flood plains are also suspected to have the possibility of arsenic in ground water. From Assam two districts Dhemaji and Karimganj have been surveyed for ground water arsenic contamination and both are found arsenic contaminated. The problem of ground water contamination in West Bengal was first reported in December, 1983. Over 6 million people live in areas of West Bengal, India, where ground water sources are contaminated with naturally occurring arsenic (Smith et al., 2002).
2.4. Arsenic exposure assessment in laboratory

**Human biological sample analysis as a tool to detect arsenic contamination**

Exposure to inorganic arsenic mainly occurs via drinking water, however because of potential changing of water sources during time, there is no consensus over the best route for arsenic. Arsenic persists longer in hair and nails, which can, therefore, be used as indicators of past exposure. The concentration of arsenic, along a hair may be used to estimate the timing of an exposure. The most reliable biomarker of arsenic exposure is the measure of arsenic content in urine, hair and nails, which reflects a cumulative exposure to arsenic over a long period (Agahian, 1990). Arsenic content in hair and nails can estimate the arsenic exposure over the past 6–12 months. Because arsenic accumulates in hair and nails and has limited mobility once incorporated into keratin, their analysis for arsenic content is used as an index of longer-term (several months) exposure to inorganic arsenic (Koons and Peters, 1994; Takagi et al., 1988).
Buccal micronucleus cytome (BMCyt) assay in exfoliated buccal epithelial cells

BMCyt assay in epithelial cells has shown to be a sensitive method for monitoring genetic damage in human populations (Sarto et al., 1990; Karahalil et al., 1999; Majer et al., 2001). Exfoliated epithelial cells have traditionally been used for cancer screening and biomonitoring of genotoxic effects in humans (Guzman et al., 2003). The key advantage of the MN assay is the relative ease of scoring and the statistical power obtained from scoring larger numbers of cells than are typically used for metaphase analysis (Fenech, 1999). Micronuclei study has been widely acknowledged as an effective biomarker in genotoxic studies and a sense of studies conducted in different laboratories across the world suggest the association of the frequency of MN in target or surrogate tissues and cancer development. Analysis of buccal epithelial cells by BMCyt assay are presented in Table 23. Figure 10 represents the photomicrographs of micronucleus and other nuclear abnormalities scored in the BMCyt assay.

Cell viability by trypan blue dye exclusion method

The Trypan blue exclusion test is a rapid method to assess cell viability in response to environmental insults. It is simple and inexpensive. Trypan blue is a vital stain used to selectively colour dead cells blue. Live cells with intact cell membrane are not coloured. Since cells are very selective in the compounds that pass through the membrane, in a viable cell trypan blue is not absorbed; however, it traverses the membrane in a dead cell. Hence, dead cells are shown as a distinctive blue colour under a microscope. Trypan blue dye exclusion test is used to assess cytotoxicity.

Comet Assay in vitro and in vivo

A number of techniques for detecting DNA damage, as opposed to the biological effects (e.g., micronuclei, mutations, structural chromosomal aberrations) that result from DNA damage, have been used to identify substances with genotoxic activity. Compared with other genotoxicity assays, the advantages of the technique
include: (1) its demonstrated sensitivity for detecting low levels of DNA damage; (2) the requirement for small numbers of cells per sample; (3) flexibility; (4) low costs; (5) ease of application; (6) the ability to conduct studies using relatively small amounts of a test substance; and (7) the relatively short time period (a few days) needed to complete an experiment. During the last decade, this assay has developed into a basic tool for use by investigators interested in research areas ranging from human and environmental biomonitoring to DNA repair processes to genetic toxicology. Attractive uses of this assay in genetic toxicology include: (1) as a potentially high-throughput screening assay; (2) in mechanistic studies to distinguish between genotoxicity versus cytotoxicity induced chromosomal damage; (3) in mechanistic in vivo studies to distinguish between genotoxic versus non-genotoxic carcinogens; and (4) potentially, as part of a battery of in vitro/in vivo assays used for regulatory submissions.

**Chromosomal aberration assay**

Study of chromosomal aberration in cells has now been recognized as a biological tool to identify genotoxic agents and to evaluate the extent of damage. Chromosomes are studied directly by observing and counting aberrations in metaphases. This approach provides the most detailed analysis and opportunity of detecting both numerical and structural changes in chromosomes in exposed individuals.

**Micronucleus assay**

Micronucleus (MN) assay is a well established, reliable and sensitive assay to evaluate the genotoxicity of chemical agents. Micronuclei are acentric fragments of a chromosome or whole chromosomes that are left behind during mitotic (anaphase) cellular division and appear in the cytoplasm of inter phase cells as small additional nuclei.
Sperm head abnormality assay

This assay is one of the quickest, simplest and least expensive method for identifying mutagens and carcinogens. Sperm with abnormal shapes would contain abnormal genetic material according to Wyrobek and Bruce (1978); Otubanjo and Mosuro (2001). Large reduction in sperm number or motility or large increase in sperm with abnormal shapes are associated with reduced fertility.

Total sperm count

The sperm count used as an indicator of male fertility. Total sperm count as part of a fertility evaluation includes the total density or count and the motile density. The motile density is perhaps the most important part of the semen analysis, as it reports the total number of sperm thought capable of progressing from the site of sperm deposition to the site of fertilization. The value is essential in both allowing a determination regarding whether or not a semen analysis is “normal”, as well as in providing prognostic information should advanced reproductive medical assistance be required.

Lipid peroxidation assay

Lipid peroxidation has been established as a major mechanism of cellular injury in many biological systems of plants and animals. Extensive toxicological investigations have now established that increase in lipid peroxidation, actually denotes cytotoxicity and cellular dysfunction. MDA can be found in most biological samples including foodstuffs, serum, plasma, tissues and urine as a result of lipid peroxidation. MDA concentration formed as a result of lipid peroxidation is important for the purpose of estimating oxidative stress effects on lipids.

Glutathione assay

Reduced Glutathione (GSH) is a tripeptide that contains a free thiol group. GSH is a major tissue antioxidant that provides reducing equivalents for the glutathione
2.5. Metabolism of arsenic

The metabolism of arsenic has an important role in its toxic effects. Many, but not all, mammalian species methylate inorganic arsenic (Vahter, 1994). There is also variation between species and among human populations in the rate and extent of methylation of inorganic arsenic (Vahter, 1999, 2000). Inorganic arsenic is metabolized by a sequential process involving a two electron reduction of pentavalent arsenic to trivalent arsenic, followed by oxidative methylation to pentavalent organic arsenic (reviewed in Thomas et al., 2001). The reduction can occur nonenzymatically in the presence of a thiol such as glutathione (GSH) (Delnomdedieu et al., 1994b; Scottet al., 1993). However, human liver arsenate (Radabaugh and Aposhian, 2000) and MMA' (Zakharyan et al., 2001) reductases have been partially purified and the latter enzyme appears to be a glutathione-S-transferase (omega). The methylation of arsenic is enzymatic, requiring S-adenosylmethionine (SAM) and a methyl transferase. The predominant metabolite of inorganic arsenic, dimethyl arsenic acid ((CH3)2AsO(OH)), is rapidly excreted by most mammals. Trimethylarsine oxide (TMAO, (CH3)3AsO)) is the final product in this scheme, but is found in very low amounts in urine, if at all, after exposure to inorganic arsenic. For many years, monomethylarsonous acid (MMAIII) and dimethylarsinous acid (DMAIII) have been
proposed intermediates in the metabolism of arsenic. Recently, MMA_{III} and DMA_{III} have been detected in the urine of humans chronically exposed to inorganic arsenic in their drinking water (Aposhian et al., 2000; Del Razo et al., 2001b) and in the bile of rats administered arsenite intravenously (Gregus et al., 2000).

**Sequential process of arsenic metabolism**

\[
\text{As}^{V}\text{O(OH)}_3 + 2e^- \rightarrow \text{As}^{III}(\text{OH})_3 + \text{CH}_3^+ \rightarrow \text{CH}_3\text{As}^{V}\text{O(OH)}_2 + 2e^- \rightarrow \text{CH}_3\text{As}^{III}(\text{OH})_2 + \text{CH}_3^+ \rightarrow (\text{CH}_3)_2\text{As}^{V}\text{O(OH)} + 2e^- \rightarrow (\text{CH}_3)_2\text{As}^{III}\text{OH} + \text{CH}_3^+ \rightarrow (\text{CH}_3)_3\text{As}^{V}\text{O}.
\]

Biomethylation of arsenic is considered the primary detoxification mechanism, since the highly reactive species of inorganic arsenic are potentially more toxic to the living world, including humans. A large number of diverse species of yeast, fungi, algae, plants and animals were found to transform inorganic arsenic compounds to the methyl derivatives (Maeda, 1994; Cullen et al., 1989; Aposhian, 1997 and Styblo et al., 1997). The organoarsenic species, mostly of methyl derivatives such as arsenosugars, arsenobetaine, arsenuchole and arsenolipids (Knowles and Benson, 1983), are widespread in aquatic organisms, including shrimps, lobsters, fish, sea weeds, and in many species of marine animals (Edmonds et al., 1981, 1987). It is unlikely that consumption of organoarsenic compounds in those organisms constitutes a hazard from arsenic poisoning (Furguson and Gavis, 1972). However, ingestion of certain seafood was shown to elevate the urinary arsenic content beyond that expected for the current maximum contaminant level (Buchet et al., 1994).

In higher organisms, inorganic arsenic is methylated to monomethylarsonic acid (MMA) and finally to dimethyl arsinic acid (DMA) by the methyl donor, S-adenosyl-methionine (SAM) (Buchet and Lauwerys, 1985), catalysed by methyltransferases in the presence of glutathione (Styblo and Thomas, 1997). The trivalent arsenicals are preferred substrates for methylation reactions and hence the reduction of arsenic from pentavalent to trivalent may be a critical step in the control
of the rate of metabolism of arsenic (Styblo et al., 1995). The DNA methyl transferases require SAM and as a result arsenic exposure may cause DNA hypomethylation due to continuous methyl depletion. On the other hand, DNA hypomethylation occurs concurrently with malignant transformation and in the presence of depressed levels of SAM. Thus arsenic-induced hypomethylation facilitates aberrant gene expression that results in carcinogenesis (Goering et al., 1999). It is apparent that chronic arsenic exposure had influenced adversely the methylation of MMA to DMA, though the actual physiological cause of reduced rate of methylation.

2.6. Toxicity of Arsenic

The toxicity of arsenic depends on its chemical state. Arsenic can occur in the environment in several oxidation states (-3,0,+3 and +5) but in natural water mostly found in organic form as oxyanions of trivalent arsenite (AS III) or pentavalent arsenic (AS V) (Smedley and Kinniburgh, 2002). Inorganic arsenic in its trivalent form is more toxic than pentavalent arsenic. The toxicity of arsenic also depends on the exposure dose, frequency and duration, the biological species, age and gender, as well as on individual susceptibilities, genetic and nutritional factors (Tchounwou 2003; Tchounwou 2001). Inorganic arsenic compounds in which arsenic is present in trivalent form are known to be the most toxic. The acute toxicity of a number of arsenic compounds is given in Table 4 (Chappell et al., 1999). Toxicity is expressed as the number of milligrams of the compound per kilogram of body weight that will result within a few days in the death of half of those who ingest it in a single dose. This concentration is known as LD₅₀ (Table 4).

Table 4: Acute toxicity for different arsenic compounds

<table>
<thead>
<tr>
<th>Arsenic form</th>
<th>Oral LD₅₀ (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium arsenite</td>
<td>15-40</td>
</tr>
<tr>
<td>Arsenic Trioxide</td>
<td>34</td>
</tr>
<tr>
<td>Calcium arsenite</td>
<td>20-800</td>
</tr>
<tr>
<td>Arsenobetane</td>
<td>&gt; 10,000</td>
</tr>
</tbody>
</table>
Exposure to such high levels of acute arsenic poisoning is very unlikely. However, long-term exposure to very low arsenic concentrations in drinking water is also a health hazard. Numerous references review the effect of long-term exposure to arsenic on people’s health (NRC, 2000; WHO, 2001; Ahmed, 2003; UNICEF, 2006). Figure 3 represents the various modes of arsenic toxicity.

Toxicity of arsenic categorized in to Acute toxicity and Chronic toxicity. The acute toxicity of arsenic is related to its chemical form and oxidation state. Acute toxicity of trivalent arsenic is greater than pentavalent arsenic. For example, in the mouse, the oral LD_{50} of arsenic trioxide is more than 36-fold lower than that of MMA^V. In the human adult, the lethal range of inorganic arsenic is estimated at a dose of 1–3 mg As/kg (Ellenhorn, 1997). For many years it was believed that the acute toxicity of inorganic arsenic was greater than organic arsenic and hence, the methylation of inorganic arsenic was a detoxication reaction. This Dogma was held because DMA^V, the primary excreted metabolite of inorganic arsenic, is less acutely toxic than inorganic arsenic. Recently Petrick et al. (2001) reported that MMA^{III} has a lower LD_{50} than arsenite in the hamster. The greater acute toxicity of the methylated trivalent intermediates of arsenic suggests that the methylation of arsenic is not solely a detoxication mechanism. One of the hallmarks of chronic toxicity in humans from oral exposure to arsenic are skin lesions, which are characterized by hyperpigmentation, hyperkeratosis, and hypopigmentation (Yeh et al., 1968; Cebrian et al., 1983). Human health effects of chronic arsenic toxicity are designated by the term arsenicosis which was first coined by our group (Guha et al., 1988b) and later used by (WHO, 2005) to imply a chronic disease caused by prolonged exposure in humans to arsenic. There is a wide variation of occurrence of symptoms in an arsenic exposed population.
2.6. Toxicity of arsenic

Figure 3: Sources of human exposure to arsenic and various modes of arsenic toxicity

2.6.1. Mechanism of arsenic toxicity

Many heavy metals (including As, Cd, Pb and Hg) have affinity for sulphhydryl bonds and can alter protein structure, leading to disruptions of metabolic processes (Gochfeld, 1997). The binding with sulphhydryl groups by arsenite compounds has the potential to influence a wide range of metabolic activities including cellular glucose uptake, gluconeogenesis, fatty acid oxidation and production of glutathione. This broad metabolic toxicity can produce confusing symptoms (Young, 2000). For instance, arsenic poisoning can produce thiamine deficiency symptoms in humans, because it prevents the transformation of thiamine into acetyl-coenzyme A and succinyl-coenzyme A (Dyro, 2005).

Specific functional groups within enzymes, receptors or coenzymes, such as thiols or vicinal sulphhydryls, have a major role in the activity of these molecules. Trivalent arsenicals readily react in vitro with thiol-containing molecules such as GSH
and cysteine (Scott et al., 1993; Delnomdedieu et al., 1994b). Binding of MMA\textsuperscript{III} and DMA\textsuperscript{III} to protein \textit{in vitro} occurs to a greater extent than with the pentavalent organic forms (Styblo et al., 1995). Arsenite has a higher affinity for dithiols than monothiols, as shown by the highly favored transfer of arsenite from a (GSH); arsenic complex to the dithiol 2, 3-dimercaptosuccinic acid (Delnomdedieu et al., 1993). The binding of trivalent arsenic to critical thiol groups may inhibit important biochemical events which could lead to toxicity. However, binding of arsenite at nonessential sites in proteins may be a detoxication mechanism (Aposhian, 1989). Pyruvate dehydrogenase (PDH) is a multisubunit complex that requires the cofactor lipoic acid, a dithiol, for enzymatic activity. Arsenite inhibits PDH (Peters, 1955; Szinicz and Forth, 1988; Hu et al., 1998), perhaps by binding to the lipoic acid moiety. Petrick et al. (2001) has shown that MMA\textsuperscript{III} is a more potent inhibitor of PDH than arsenite. PDH oxidizes pyruvate to acetylCoA, a precursor to intermediates of the citric acid cycle. The citric acid cycle degrades the intermediates, and this provides reducing equivalents to the electron transport system for ATP production. Inhibition of PDH may ultimately lead to decreased production of ATP. Also, intermediates of the citric acid cycle can be used in gluconeogenesis. Inhibition of PDH may explain in part the depletion of carbohydrates observed in rats administered arsenite (Szinicz and Forth, 1988; Reichl et al., 1988).

Methylated trivalent arsenicals such as MMA\textsuperscript{III} are potent inhibitors of GSH reductase (Styblo et al., 1997) and thioredoxin reductase (Lin et al., 1999). The inhibition may be due to the interaction of trivalent arsenic with critical thiol groups in these molecules. The activity of the methylated trivalent arsenicals is greater than arsenite, MMA\textsuperscript{V}, and DMA\textsuperscript{V}. Inhibition of these enzymes may alter cellular redox status and eventually lead to cytotoxicity.

2.7. Genotoxicity of Arsenic

A compound is considered to be genotoxic if it induces gene damage in concentrations which are not cytotoxic or associated with a low degree of cytotoxicity;
i.e., genotoxicity is elicited also in subcytotoxic dose ranges. Genotoxic effects of arsenic have been implicated in the carcinogenic outcomes (NRC, 1999). The genotoxic potential of arsenic has been extensively explored, primarily with cultured cells. Arsenic can be considered as genotoxic *in vitro* and *in vivo*, in man and in animals. It had been speculated recently that arsenic may cause genotoxicity via a sublinear dose-response relationship (Rudel *et al.*, 1996). This would have consequences on a putative classification of arsenic as ‘threshold’ carcinogen.

### 2.7.1. *In vitro* genotoxicity

Inorganic arsenic e.g., sodium arsenite induces large deletion (multilocus) mutations in hamster-human hybrid cells (Hei *et al.*, 1998). Chromosomal aberrations, DNA-protein crosslinks and sister chromatid exchanges are observed in hamster embryo cells (Rossman *et al.*, 1980; Lee *et al.*, 1985; Kochhar *et al.*, 1996) and human lymphocytes (Larramendy *et al.*, 1981; Jha *et al.*, 1992; Wiencke and Yager, 1991; Rasmussen and Menzel, 1997) and fibroblasts (Okui and Fujiiwara, 1986; Jha *et al.*, 1992; Dong and Luo, 1993) after exposure to inorganic arsenic. Arsenic significantly induced MNi in isolated human peripheral lymphocytes as well as when using whole blood after cytokinesis-block through cytochalasin B (Schaumlöffel and Gebel, 1998). With respect to sister chromatid exchange (SCE) induction, significantly elevated SCE frequencies were shown after sodium arsenite treatment in experiments with human peripheral lymphocytes (Gebel *et al.*, 1997; Rasmussen and Menzel, 1997).

### 2.7.2. *In vivo* genotoxicity

Administration of arsenite to mice results in a linear dose-dependent increase in micronucleated polychromatic erythrocytes (Deknudt *et al.*, 1986; Tinwell *et al.*, 1991). Micronuclei induced by arsenite are observed only in somatic cells. Also observed in bone marrow cells of mice administered arsenite are chromosomal aberrations including chromatid gaps and breaks and chromosomal rearrangements (Das *et al.*, 1993; Roy
Choudhury et al., 1996). Crude garlic extract administered before exposure to arsenite reduces its clastogenic effect (Das et al., 1993; Roy Choudhury et al., 1996).

2.7.3. Oxidative stress

Oxidative stress arises when reactive oxygen species are generated that can react with cellular constituents such as thiols and lipids. Depletion of GSH by oxidants, for example, may alter the redox status of the cell and present a stressful and toxic situation. Arsenic appears to induce oxidative stress both in vivo and in vitro. Arsenic induces heat shock or stress proteins in cultured human cells (Keyse and Tyrrell, 1989) and in vivo (Brown and Rush, 1984). Reactive oxygen species are detected in human-hamster hybrid cells within 5 min after exposure to arsenite (Liu et al., 2001). Oxidative damage in the lungs of mice is observed after exposure to DMAV (Yamanaka et al., 1991). Trivalent organic arslenicals inhibit GSH reductase (Styblo et al., 1997) and thioredoxin reductase (Lin et al., 1999).

2.8. Arsenic and Carcinogenicity

Inorganic arsenic is classified by the International Agency for Research on Cancer (IARC, 1980, 1987) and the US Environmental Protection Agency (EPA, 1988) as a known human carcinogen. Cancer has developed in individuals exposed to arsenic through medical treatment with Fowler’s solution (potassium arsenite) (Fierz, 1965), occupational exposure via inhalation at copper smelters (Lee-Feldstein, 1983, 1986) or naturally contaminated drinking water (Tseng et al., 1968; Cebrian et al., 1983). Tumors that develop after inhalation of arsenic are observed primarily in the lung (Lee-Feldstein, 1983, 1986), where as they are initially observed in the skin after oral exposure to arsenic (Tseng et al., 1968; Cebrian et al., 1983). Tumor sites include bladder, liver, and kidney (Smith et al., 1992; Bates et al., 1995).

Although it is clear that arsenic is a human carcinogen, and there are some positive results in animal studies, the mechanism of action is unknown. Many different
mechanisms of action have been proposed and several have recently been evaluated by experts in the field (ERG, 1997; NRC, 1999, 2001). Some proposed mechanisms include genotoxicity, cell proliferation, altered DNA repair and DNA methylated oxidative stress, co-carcinogenesis and tumor promotion. Defining a mechanism of action for arsenic carcinogenicity has been difficult for several reasons. Some of these include the several negative results of a carcinogenic effect of inorganic arsenic in a standard animal bioassay, the lack of evidence that arsenic is a point mutagen, the carcinogenic and promoting effects of DMA³, the toxic effects of the trivalent methylated forms of arsenic, and the myriad effects of arsenic on cell signaling.

2.9. Effects of Arsenic and Other Genotoxic Components

2.9.1. Effects of Arsenic

The first visible symptoms caused by exposure to low arsenic concentrations in drinking water are abnormal black-brown skin pigmentation known as melanosis and hardening of palms and soles known as keratosis. If the arsenic intake continues, skin depigmentation develops resulting in white spots that looks like raindrops (leukomelanosis). In a clinical study conducted in West Bengal on a population exposed to high levels of arsenic in drinking water, 94% had such “raindrop” pigmentation (Guha et al., 1998b). Palms and soles further thicken and painful cracks emerge. These symptoms are described as hyperkeratosis and can lead on to skin cancer (WHO, 2001).

Skin Pigmentation and keratosis are the specific skin lesions characteristic of chronic arsenic toxicity. Leucomelanosis appears to occur in an arsenuosis patient following stoppage of drinking arsenic contaminated water for some duration (Saha, 1984; Guha et al., 1998a; NRC, 1999). Symptoms of chronic lung disease and chronic bronchitis were present in 89 (57%) out of 156 cases of chronic arsenic toxicity caused by drinking arsenic contaminated water in West Bengal (Sarkar et al., 1998). Many
investigators variously reported symptoms like dyspepsia, nausea, diarrhoea, anorexia and abdominal pain in cases of chronic arsenic toxicity (Guha et al., 1988; Borgono et al., 1977; Zaldívar, 1974; Cebrian et al., 1983; Ahmad et al., 1997). Many workers have reported cases of liver damage, liver cirrhosis following treatment of patients with arsenic as Fowler’s solution (Morris et al., 1974; Rosenberg, 1974; Szuler et al., 1979; Nevens et al., 1990). Black foot disease, (BFD) a form of peripheral vascular disease, has been reported to be one of the important complication of chronic arsenic toxicity in Taiwan (Yu et al., 1984). Ingested inorganic arsenic has been related to an increased incidence of cardiovascular disease, especially ischaemic heart disease and has been reviewed extensively (NRC, 1999; Engel and Smith, 1994; WHO, 1981; Chen et al., 1997). Depression, sleep disorders, headache were reported in arsenicosis people showing features of neuropathy in West Bengal (Guha et al., 1998b). Significantly increased prevalence of diabetes mellitus was reported due to drinking arsenic contaminated water among subjects with keratosis reported from Bangladesh (Rahman et al., 1998). Epidemiological studies in several countries involving population with high long-term exposure to arsenic found increased risks for liver and kidney cancer (IARC, 2004).

### 2.9.2. Effects of other genotoxic components exposure in arsenic contaminated area

The main threats to human health from heavy metals are associated with exposure to cadmium, fluoride and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years.

Cadmium occurs naturally in ores together with zinc, lead and copper. Inhalation of cadmium fumes or particles can be life threatening, and although acute pulmonary effects and deaths are uncommon, sporadic cases still occur (Seidal et al.,
1993; Barbee et al., 1999). Cadmium exposure may cause kidney damage (Jarup et al., 1998). Animal experiments have suggested that cadmium may be a risk factor for cardiovascular disease (Jarup et al., 1998) and also related to different types of cancer (IARC, 1993; Kolonel, 1976).

The general population is exposed to lead from air and food in roughly equal proportions. The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system (WHO, 1995; Elliot et al., 1999). Recent research has shown that long-term low-level lead exposure in children may also lead to diminished intellectual capacity (Lidsky et al., 2003). Previous study revealed an increase in the number of structural as well as numerical chromosome and chromatid aberrations induced by arsenic and fluoride, indicating their genotoxicity. This induced genotoxicity is probably mediated by induction of oxidative stress and depletion of glutathione (Matsau et al., 1999). Genotoxic effects of arsenic and fluoride on human blood cultures of the Indian population have been reported by Nair et al., 2004. Treatment of mice with sodium fluoride increased the number of cell death, chromatid breaks (aberrant cells) compared with control (Chattopadhyay et al., 2011). Fluoride is an environmental contaminant, major sources of exposure to which are drinking water, food, dental products and pesticides. Excessive consumption of fluoride causes fluorosis, as low, progressive degenerative disorder which is known to affect predominantly the skeletal systems, teeth and also the structure and function of skeletal muscles (Kaul and Shusheela, 1974) with characteristic muscle weakness, pains in joints and fatigue with non-ulcer dyspepsia, polyurea, etc. (Susheela and Bhatnagar, 2002).