IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

Volume 84

Some Drinking Water Disinfectants and Contaminants including Arsenic

Monograph 1 : Arsenic in Drinking Water

4.2. Toxic effects

DN Guha Mazumder

Summary of Arsenic Toxicity

Acute effects due to ingestion of arsenic (As) are characterized by severe vomiting and diarrhoea with features of shock, muscle cramps and cardiac abnormality. However, subacute effects mainly involve the respiratory, gastrointestinal, cardiovascular, nervous and hemopoietic systems.

Most reports of chronic As toxicity focus attention on skin manifestation like pigmentation (diffuse, spotty, blochy), de-pigmentation (spotty) affecting trunks and limbs and keratosis (diffuse/nodular) affecting hands and feet. Chronic lung disease, (chronic cough, chronic bronchitis) peripheral neuropathy, hepatomegaly (Non cirrhotic portal fibrosis) and peripheral vascular disease (with/without gangrene of the finger/toes) are frequently reported by many investigators in cases of arsenicosis. Other systemic manifestations, reported by some workers are cardiovascular manifestation (Ischaemic heart disease, hypertension), abdominal pain, anorexia, nausea or diarrhea, cerebrovascular disease, non pitting edema of hand/feet/leg, diabetes mellitus, anaemia and generalized weakness.

Skin cancer is the most common cancer found to be associated with chronic As toxicity. In one study more than 70% of skin cancer patients were associated with keratosis while 90% had pigmentation. In another study a significantly higher mortality from cardiovascular, peripheral vascular disease and cancer of bladder, skin and lung and liver was reported among Black foot disease (BFD) patients compared to the general population in Taiwan or residents in BFD endemic areas.

Acute toxicity, of trivalent As is greater than the pentavalent form. The LD_{50} of As trioxide for mouse by oral rout varies from 15-48 mg/kg while the acute lethal dose in humans varies from 1-3 mg/kg.

In chronic toxicity As inhibits cellular respiration at the level of mitochondria. Disruption of oxidative phosphorylation and concomitant decrease in cellular levels of ATP are important in causing cellular injury and cell death, because ultrastructural morphometric alternation of mitochondria and alteration of cellular respiratory function are closehy correlated. Recent study highlights that arsenites induces apoptosis accompanied by a loss of the mitochondria transmembrane potential.

Arsenic can modify the urinary excretion of porphyrins in animals and humans. Arsenic also interferes with the activities of several enzymes of the heme biosynthesis pathway, such as aminolevulinate synthase (ALA-S), porphobilinogen deaminase (PBGD), uroporphyrinogen III synthase (Uro III S), uroporphyrinogen decarboxylase (URO-D), coproporphyrinogen oxidase (COPRO-O), ferrochelatase and heme oxygenase (H-O). The major abnormalities in urinary pophyrin excretion in chronically exposed humans are a) significant reductions in coproporphyrin III excretion resulting in decrease in COPRO III/COPRO I ratio and b) significant increase in uroporphyrin excretion.

Oxidative stress is considered as an important mechanism in As induced carcinogenesis. The exposure of arsenite, as trioxide or arsenate have been shown to results in generation of ractive oxygen species (ROS) in laboratory animals and human cells. Recent study in animals demonstrated altered gene expression by acute As treatment which included, stress related components, DNA damage and repair responsive genes, activation of transcription factors such as AP-1, complex and increase in proinflammatory cytokines. All these events could play an integral role in response to acute arsenic toxicity.

Exposure to arsenical either in vitro or in vivo in a variety of model systems has been shown to cause induction of a number of major stress protein families such as heat shock

proteins (Hsp). Among them are members with low molecular weight such as metallothionin (MT) and ubiquitin (UB) as well as ones with masses of 27, 32, 60, 70, 90 and 110 Kda. MT is thought to have a protective effect against As toxicity.

There are few studies on the immunotoxicity of As. All the As compounds were reported to suppress plaque forming cell response in spleen cells of mice to sheep eythocytes and the proliferative response to mitogens (PHA). Further As was found to impair stimulation and proliferation of human lymphocytes in vitro. Recent study suggested that apoptosis may be an important mechanism for As induced immunosuppression.

4.2. Toxicity due to ingestion of arsenic in drinking water.

A. Human toxicity

1. Acute and sub acute toxicity:

Acute effects caused by the ingestion of inorganic arsenic (As) compounds, mainly arsenic (III) oxide, are well documented in the literature. The major lesion is profound gastrointestinal damage, resulting in severe vomiting and diarrhea, often with blood tinged stools. Other acute symptoms and signs include muscular cramps, facial edema, and cardiac abnormalities shock can develop rapidly as a result of dehydration. Sub acute effects mainly involve the respiratory, gastrointestinal, cardiovascular, nervous and haematopoietic systems (WHO 1981).

2. Chronic arsenic toxicity.

Most of the reports of chronic As exposure in man, 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 As in drinking water, medications or occupational and environmental exposure, show that chronic As exposure adversely affect multiorgan system. The clinical appearance of the non carcinonatous manifestations of As intoxication in man is insidious in onset and is dependent on the magnitude of the dose and the time course of its exposure.

Cutaneous manifestations:

The specific cutaneous lesions of chronic As toxicity are characterized by pigmentation and keratosis. These have been reported from different regions of the world which included, Argentina, Chile, China, Japan, Mexico, India (West Bengal), and Bangladesh, where the content of As in drinking-water was elevated (Zaldivar 1974, Borgono et al., 1977; Cebrian et al., 1983; Saha 1984; Chakraborty and Saha 1987; Guha Mazumder et al., 1988, 1992, 1998a, Ahmad et al., 1997, 1999, Milton and Rahman, 1999, Guo et al, 2001). The magnitude of As dose and the time frame of exposure necessary to induce the hyperpigmentation and hyperkeratosis characteristic of chronic As intoxication have undergone limited investigation. Among the population exposed to As in drinking water in the Antofagasta region of Chile, cases of cutaneous arsenicosis, including both hyperpigmentation and hyperkeratosis, have been described in children as young as 2 years of age (Rosenberg 1974). In the cohort of 40,421 inhabitants of southeastern Taiwan investigated by Tseng and colleagues, the youngest subjects found to have hyperpigmentation and hyperkeratosis, were reported to be in the ages 3 and 4, respectively, in an early report (Tseng et al. 1968).

The hyperpigmentation of chronic As poisoning commonly appears in a finely freckled, "raindrop" pattern that is particularly pronounced on the trunk and extremities distributed bilaterally symmetrically. But that might also involve mucous membranes such as undersurface of tongue or buccal mucosa (Yeh 1973; Tay 1974; Saha 1984, 1995, Guha Mazumder 1988, 1992, 1998a, Milton and Rahman 1999, Ahmad et al 1999).

Arsenical hyperkeratosis appears predominantly on the palms and the plantar aspect of the feet, although involvement of the dorsum of the extremities and the trunk have also been described. Occasional lesions might be larger and have a nodular or horny appearance. In severe cases, the hands and soles present with diffuse verrucous lesions. Cracks and fissures may be severe in the soles (Sommers and McManus 1953, Black 1967, Tseng et al 1968, Yeh 1973, Zaldivar 1974, Tay 1974, Borgono et al, 1977, Cebrian 1983, Saha 1984, Guha Mazumder et al, 1988, 1998(a), Ahmad et al 1997).

Histological examination of the lesions typically reveals hyperkeratosis with or without parakeratosis, acanthosis, and enlargement of the rete ridges. In some cases, there might be evidence of cellular atypia, mitotic figure, in large vacuolated epidermal cells (Tay 1974). Yeh (1973) classified arsenical keratosis into two types: a benign type A, (further subgrouped into those with no cell atypia and those with mild cellular atypia); and a malignant type B. Lesions of Bowen's disease (intraepithelial carcinoma, or carcinoma in situ), basal-cell carcinoma, or squamous-cell carcinoma. Skin cancer might arise in the hyperkeratotic areas or might appear on nonkeratotic areas of the trunk, extremities, or hand (Sommers and McManus 1953; Yeh 1973). Compared to control cases there was gradual increase in the value of proliferate activity in the cells of cancer and dysplastic skin biopsy samples as evidenced by increase in AgNOR count (Paul et al 2000).

Guha Mazumder et al (1998b) carried out the first population survey with individual As exposure data on 7683 participants in West Bengal to ascertain the prevalence of keratoses and hyperpigmentation. As content of their current water source ranged up to 3.4 mg/L. Though 80% of participants consumed water with As level < 0.5 mg/L. Out of 4093 female and 3590 male participants, 48 and 108 people had keratotic lesions and 127 and 234 people had pigmentation respectively. Clear exposure-response relationships were found for water-arsenic levels and the prevalence of these arsenic-induced skin effects. Males were more affected than females. 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. However, the survey examined only the participants' primary current drinking-water source. Similar cross-sectional study was conduced in Bangladesh by Tondel et al (1999). They interviewed and examined 1,481 subjects ≥ 30 years of age. A total of 430 subjects had skin lesions. Individual exposure assessment could only be estimated by present levels. Arsenic water concentrations ranged from 0.01 to 2.04 mg/L and the crude overall prevalence rate for skin lesions was 29/100. This study also showed a higher prevalence rate of arsenic skin lesions in males than females, with clear dose-response relationship.

Haque et al (in press) recently completed a nested case-control study of the previous study (Guha Mazumder et al 1998b) to examine the dose-response relationship between low As concentration (< 0.5 mg/L) in drinking water and As induced skin lesions using detailed exposure assessment. The exposure assessment incorporates arsenic concentration data from current and past water sources used in households and work sites. A subset of 158 participants had complete water histories (69 cases and 89 controls). No cases currently with a skin lesion had peak arsenic water concentrations less than 0.1 mg/L. There were 8 cases who currently had skin lesions, and who had ingested peak arsenic concentrations between 0.1-0.19 mg/L. These 8 cases comprised 4 males (ages 31 to 75 years), and 4 females (ages 21 to 66 years). All 8 cases had hyperpigmentation, while 4 also had keratoses. One male case was exposed to 0.115 mg/L, while the others had ingested known peak concentrations above 0.15 mg/L. Of the 49 cases who currently had skin lesions, and for whom samples were collected from all known sources, 31 cases were confirmed by photograph, including the case who was exposed to a peak of 115 μg/L.

Skin cancers are frequently associated with hyperkeratotic lesion (Yeh, 1973). Hyperkeratosis occurs more commonly and earlier in an As exposed population than does skin cancer. A dose response analysis of hyperkeratotic lesions may therefore allow one to observe potential carcinogenic response at lower exposures than has been done with skin cancer. Necessary information for the risk assessor to estimate dose-response would be the length and intensity of exposure and the prevalence (or incidence if possible) of hyperkeratosis by exposure and age (H. Gibbs in North et al 1997).

Respiratory disease.

The possible role of chronic As ingestion in the genesis of nonmalignant pulmonary disease has been suggested in a few case series describing medical problems among individuals chronically exposed to increased concentrations of As in drinking water. Among a total study cohort of 180 residents of Antofagasta, Chile, exposed to drinking water containing As at 0.8 mg/L, 38.8% of 144 subjects with "abnormal skin

pigmentation" complained of chronic cough, compared with 3.1% of 36 subjects with normal skin (Borgono et al. 1977). In autopsies of five children from the Antofagasta region with an antecedent history of cutaneous arsenicosis and postmortem findings of extensive (nonpulmonary) vascular disease, two of the subjects were noted to have chronic bronchitis, slight bronchiectasis and slight diffuse interstitial fibrosis of lung (Rosenberg 1974). Symptoms of chronic lung disease were present in 89 (57%) out of 156 cases of chronic arsenic toxicity caused by drinking As contaminated water in West Bengal (Guha Mazumder et al 1998a). Lung function tests carried out on 17 patients showed features of restrictive lung disease in 9 (53%) and combined obstructive and restrictive lung disease in 7 (41%) cases.

To investigate the relationship of non-malignant respiratory disease with ingested As, Guha Mazumder et al (2000) have analyzed data from the cross-sectional survey of 7,683 participants who were clinically examined and interviewed, and the As content in their current primary drinking water source was measured. Because there were few smokers, analyses were confined to nonsmokers (N=6,864 participants). Study subjects included those who had As associated skin lesion such as hyperpigmentation and hyperkeratosis, and who were also highly exposed at the time of the survey (As water concentration ≥ 0.5 mg/L). Individuals with normal skin and low As water concentration (<0.05 mg/L) were used as the referent group. In participants with skin lesions, the age adjusted prevalence odds ratio (POR) estimates for cough, crepitations and shortness of breath for females were 7.8, 9.6 and 23.2 and for males 5, 6.9 and 3.7 respectively.

Results of chronic As toxicity on respiratory system was studied in Bangladesh by Milton et al (2001). They studied 218 individuals (94 exposed to AS: 136 to 1 mg/L and 124 control cases), mostly non smokers. The overall crude prevalence (or risk) among the exposed subjects for chronic cough and chronic bronchitis was three times the prevalence in control population. They also reported that females were more affected than males.

Occurrence of chronic respiratory disease in the form of chronic cough or chronic bronchitis due to chronic ingestion of As through drinking water has also been reported

by many other investigators (Chakraborty and Saha 1987, Hotta 1989, Kilburn 1997, Chowdhury et al 1997, Ma et al 1999, Ahmad et al 1999).

Effect on gastrointestinal system.

Chronic As toxicity has been reported to produce various gastrointestinal (GI) symptoms. Hotta 1989 reported incidence of gastrointestinal impairment in 76% of subjects exposed to environmental As exposure at Torku, Japan. The symptoms were not serious in most of the patients as they had possibly been afflicted in the initial stage of disease. Gastroenteritis was reported in a study 1447 cases of chronic arsenicosis caused by drinking As contaminated water (0.05-1.8 mg/L) in the Inner Mongolian Autonomous region of China (Ma et al, 1999). Out of 248 patients suffering form chronic arsenicosis following drinking As contaminated water (0.05 – 14.2 mg/L) in West Bengal, India, GI symptoms characterized by dyspepsia was present in 60 out of 156 (38.4%) of cases. studied (Guha Mazumder et al 1998a). Many investigatiors variously reported symptoms like nausea, diarrhoea, anorexia and abdominal pain in cases of chronic arsenic toxicity (Rosenberg 1974, Zaldivar 1974, Borgono et al 1977, Cebrian et al 1983, Guha Mazumder et al 1988, Ahmad et al 1997). However, in an epidemiological study carried out in the affected population there was no difference in the incidence of pain abdomen among people drinking As contaminated water and control population (27.84% vs 31.81%) (Guha Mazumder et al 2001).

Effect on liver.

Exposure to inorganic As compounds has been associated with the development of chronic pathological changes in the liver. Several authors have reported cases of liver damage following treatment with As in the trivalent inorganic form (Morris et al., 1974; Szuler et al., 1979; Cowlishaw et al 1979; Nevens et al. 1990). A common finding in these reports was portal hypertension without signs of liver cirrhosis. All patients had been on the As medication, mostly Fowler's solution for several years. Typical cutaneous signs of long-term As exposure were also observed in some of the patients. There have

also been case reports on liver cirrhosis following medication with inorganic As compounds (Franklin et al., 1950, Rosenberg 1974).

Datta et al (1979) reported portal hypertension associated with periportal fibrosis in nine patients who were found to have high As level in their liver in Chandigarh, India. Two of those patients had been found to be drinking As contaminated water (0.549 and 0.360 mg/L). Guha Mazumder et al (1988) reported from West Bengal hepatomegaly in 62 out of 67 members of families who drank As contaminated water (0.2-2 mg/L) while only six of 96 people from the same area who took safe water. Thirteen As exposed patients who had hepatimegaly were further investigated in the hospital. All showed various degree 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 salme) 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 Bromosulphathlion retention test of three patients were abnormal. The As level in liver tissue (estimated by Neutron Activation Analysis) was found to be elevated in 10 out of those 13 cases (As levels 0.5 to 6 mg/kg control 0.10 ± 0.04 mg/kg). Santra et al (1999) subsequently reported hepatomegaly in 190 out of 248 case of chronic arsenicosis investigated in the same hospital. 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.

Liver enlargement has variously been reported in cases of chronic As toxicity due to drinking of As contaminated water by other workers (Saha 1984, Chakraborty and Saha 1987, Ma et al 1999, Ahmad et al 1997, 1999).

Chronic cardiovascular effects.

Arsenic has been well documented as one of the major risk factors for blackfoot disease, 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, Black foot disease (BFD) can be divided into two reaction groups, arteriosclerosis obliterans and thromboangitis obliterans. The prevalence of BFD has been reported to be 8.9 per 1000 among 40,421 inhabitants studied by Tseng et al (1968) at Taiwan. The villages surveyed were arbitrarily, divided according to the As content in the well water in to low (below 0.3 mg/L), mid (0.3-0.6 mg/L) and high (above 0.6 mg/L) group. The prevalence of BFD revealed a clear cut ascendency gradient from low to mid to high group for both sexes and the three different age groups studied (Tseng 1977). Ahtereogenecity and carcinogenecity of high arsenic artisan well water was examined by Chen et al (1988). The lifetable method used to analyze cancer mortality of 789 BFD patients followed for 15 years showed a significantly higher mortality from cardiovascular, peripheral vascular disease and cacners of bladder, skin, lung and liver among BFD patients as compared with general population in Taiwan or residnets in the BFD endemic area. That humic substance play an etiologic role in BFD has not been substantiated by epidemiologic or animals studies. A causal role for As in the induction of BFD offers the best explanation for the observations in Taiwan (Engel et al 1994).

Comparable peripheral vascular disorders with varying degrees of severity including Raynaud's syndrome and acrocyanosis have also been reported among people drinking As contaminated water by Rosenberg 1974, Zaldivar 1974, Borgono et al 1977, Tseng et al 1996, Guha Mazumder et al., 1998a, Ma et al 1999, Ahmad et al 1999). It need to be emphasized that there are differences in the prevalence of peripheral vascular disease causing gangrene and amputation among different population exposed to As, the incidence being high in Taiwan, while low in Chile, India and Bangladesh while there is no report from Maxico and Argentina (Engel et al 1994).

A recent epidemiological study has reported an increased prevalence of hypertension among residents in the endemic area of blackfoot disease and a dose-response relationship between ingested inorganic As and prevalence of hypertension (Chen et al.,

1995). The investigators studied a total of 382 men and 516 women residing in As hyperendemic areas in Taiwan. They observed 1.5 fold increase in age and sex adjusted prevalence of hypertension compared with residents in non endemic areas. The higher the cumulative As exposure the higher was the prevalence of hypertension. The dose response relation remained significant after adjustment for age, sex, diabetes mellitus, proteinuria, body mass index and serum triglyceride level. Increased hypertension prevalence was also observed in 6.2% among patients affected with As-induced skin lesions (144) compared to none in those without skin lesion (36) in Antafagesta, Chile by Borgno et al 1977. Rahman et al (1999) conducted cross sectional evaluation of blood pressure on 1595 people in Bangladesh and demonstrated association of average and cumulative As exposure in drinking water with a risk of hypotension .

Mortality rates from ischemic heart disease (ISHD) among residents in 60 villages of the area in Taiwan with endemic arsenicasis from 1973 through 1986 were analyzed by Chen et al (1996) to examine their association with arsenic concentration in drinking water. Based on 1355915 person years and 217 ISHD deaths, the cumulative ISHD 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 $\geq$ 0.6 mg/L. A cohort of 263 patients affected with BFD and 2293 non-BFD residents in the endemic area of arseniasis 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 ISHD 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 $\geq$ 20.0 mg/L years compared with 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.

Ingested inorganic As has been related to an increased incidence of cardiovascular disease, especially ischemic heart disease and has been reviewed extensively (WHO, 1981; Engel et al., 1994, Chen et al, 1997; NRC 1999, NRC 2001).

Effect on Nervous system.

Abnormal electromyographic (EMG) fuidings suggestive mostly of sensory neuropathy was reported in 10 out of 32 subjects exposed to drinking As contaminated well water (range 0.06 to 1.4 mg/L) in Canada (Hindmarsh et al 1977). Paresthesia was present in 74 out of 156 patients of chronic arsenicosis due to drinking of As contaminated water (0.5 – 14.2 mg/L) in West Bengal, India. Objective evaluation of neuronal involvement, done in 29 patients, showed abnormal EMG in 10 (30.8%) and altered nerve conduction velocity and EMG in 11 (38%) cases (Guha Mazumder et al, 1997). Evidence of paraesthesia/peripheral neuropathy due to chronic exposure of As through drinking water has also been reported by many other workers (Cebrian et al 1983, Saha 1984, Hotta 1989, Kilburn 1997, Ma et al 1999, Ahmad et al 1997, 1999).

The relationship between the prevalence of cerbrovascular disease and ingestion of inorganic As in drinking water was reported by Chiou et al (1997) 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 homevisit personlal interviews and ascertained by review of hosptial medical records according to the World Health Organization 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. Arsenic concentration in the well water of each household was determined by hydride generation and atomic absorption spectrometry. A significant dose-response relationship was observed between arsenic concentration in well water and prevalence of cerebrovascular disease after adjustement for age, sex, hypertension, diabetes mellitus, cigarette smoking and alcohol consumption. The biological gradient was even 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, 0.001 to 0.05, 0.051 to 0.299, and > 0.3 mg/L. Increased incidence of cerebrovascular

disease in chronic arsenicosis cases have also been reported by others (Hotta 1989, Chen 1997, Ma et al 1999).

Kilburn (1997) reported occurrence of peripheral neuritis, sleep disturbances, weakness and cognitive and memory impairment in residents of Byan College Station, Texas exposed to As from air and water from As trioxide used to produce defoliants from an Atochem plant. Siripitayakunkit et al (1999) reported retardation of intelligence among 529 children (6-9 yrs) living in Thailand having chronic exposure of As from the environment.

Diabetes Mellitus :

To examine the association between ingested inorganic As and prevalence of diabetes mellitus, Lai et al (1994) studied 891 adults residing in villages in Southern Taiwan. The status of diabetes mellitus was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. They 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 by a multiple logistic regression analysis giving a multivariate adjusted odds ratio of 6.61 and 10.05, respectively, for those who has a cumulative arsenic exposure of 0.1-15.0 and greater than 15.0 ppm year compared with those who were unexposed.

Rahman et al (1998) form Bangladesh reported a significantly increased prevalence of diabetes mellitus due to drinking arsenic contaminated water among subjects with keratosis compared with subjects who did not have keratosis. A significnat trend in risk between an approximate, time weighted arsenic expsoure and the prevalence of diabtes mellitus strenghened the possibility of a causal association. However, the lack of a comprehensive, systematic long-term sampling of the water supplies in the study area is a limiation 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.

Other effects:

Generalised weakness and fatigue have been reported in chronically As exposed people following drinking As contaminated water by Zaldivar 1974, Saha 1984, Guha Mazumder et al 1988, 1992, 1998, 2001, Kilburn 1997). Conjunctival congestion and non pitting edema of the legs and hands have also been reported in patients of chronic As toxicity in West Bengal and Bangaldesh (Guha Mazumder et al 1998, Chowdhury et al 1997, Ahmad et al 1997, 1999).

Except anaemia no hematological abnormality has been described in cases of chronic As toxicity due to drinking As contaminated by Guha Mauzmder et al (1988, 1998, 1999). Hematological consequences of subacute and chronic As toxicity has been extensively reviewed in NRC (1999) report. But except anaemia no other abnormality has been consistently found to be reported.

B.   Arsenic Toxicity – Biochemical (Human and Experiential)

Mitochondrial damage:

Disruption of oxidative phosphorylation and concomitant decrease in cellular levels of ATP are thought to be important central events in the onset of cellular injury and cell death, because alterations in mitochondrial structure, disruption of mitochondrial respiratory function are closely correlated.

Hepatic phosphate resonances were evaluated by Chen et al (1986) in vivo by ^{31}p nuclear magnetic resonance (^{31}P-NMR) following a single intravenous dose of sodium arsenite (10 mg/kg) in rats weighing 350-450 g. Acute in vivo administration of arsenite rapidly decreased intracellular pools of all ATP phosphate with concomitant increases in

inorganic phosphate and phosphomonoesters. In the phosphodiester resonance region, glycerolphosphorylcholine was also increased. The data suggest that liver cannot compensate for the rapid loss of NAD-linked substrate oxidation via other metabolic pathways, such as glycolsis for the production of ATP.

Arsenic fed to laboratory animals is known to accumulate in the mitochondria which has been related to the swelling of this subcellular organelle in a number of tissues, especially the liver. It has been suggested that the effects of arsenic on mitochondiral pyruvate utilization results from arsenic binding to the lipoic acid and dithiol moieties of the PDH complex. The initial step in the mitochondrial metabolism of pyruvate, which is catalyzed by this multienzyme complex, involves the formation of acetyl-CoA and the generation of CO_2 and NADH. This complex is composed of three enzymes: pyruvate decarboxylase (pyruvate dehydrogenase), dehydrolipoate transacetylase, and dihydrolipoate dehydrogenase. It is the second two enzymes which involve active dithiol moieties. The first enzyme of the complex is regulated by inactivation and activation reactions, which are controlled by a phosphorylation/dephosphorylation mechanism. Thus, phosphorylation and the concomitant inactivation of PDH is catalyzed by a MgATP-requiring kinase, and dephosphorylation and concomitant reactivation is catalyzed by a Mg- and Ca-requiring phosphatase. In order to examine whether or not this phosphorylation/dephosphorylation mechanism was a site of action for arsenic, PDH levels, before and after in vitro activation, were measured in tissue from arsenic-fed animals by Schiller et al (1977).

The adult male Charles River CD rats used in this experiment were given deionized drinking water containing 0, 20, 40, and 85 ppm arsenic as sodium arsenate (As^{+5}) for 3 and 6 weeks. Assay of Pyruvate Dehydrogenase was carried out in the liver tissue obtained from the animals. After 3 weeks, the effects of arsenic at the highest dose level were pronounced on the basal activity (before activation), with inhibition up to 48% of the conrol values. The total PDH (after activation) was inhibited by 14, 15, and 28% of the control values at 20, 40 and 85 ppm As^{+5}, respectively. A similar pattern of inhibition of PDH was observed at 6 weeks, although the inhibition was lower at the highest dose.

Monomethylarsonous acid (MMA^{III}) was proposed an intermediate in the methylation of inorganic arsenic. MMA^{III}, like arsenite, contains arsenic in the +3 oxidation state. This important arsenic species has recently been identified and quantitated in the livers of male Golden Syrian hamsters following ip injection of radioactive arsenate. Metabolism and toxicity studies demonstrate that MMA^{III} is in fact a stable toxic metabolite of inorganic arsenic and not a transient intermediate. Such data also show that MMA^{III} is present in appeciable concentrations in mammals, including the human, following exposure to inorganic arsenic. Toxicity of MMA^{III}, however, has not earlier been demonstrated in vivo nor has its mechanism of toxicity been established in the intact animal (Petrick et al 2001).

Petrick et al (2001) have compard the in vivo toxicities of MMA^{III} and arsenite in hamsters. Male Golden Syrian hamsters 11-12 weeks old and weighted 100-130 g were injected ip MMA^{III} oxide or sodium arsenite. Six animals were used for each dose. When MMA^{III} or sodium arsenite was administered intraperitoneally to hamsters, the LD$_{50s}$ were found to be 29.3 and 112.0 µmol/kg of body wt, respectively. Further, inhibition of hamster kidney or purified porcine heart pyruvate dehydrogenase (PDH) activity by MMA^{III} or arsenite was determined. To inhibit hamster kidney PDH activity by 50%, the concentrations (mean±SD) of MMA^{III} as methylarsine oxide, MMA^{III} as diiodomethylarsine, and arsenite were 59.9±6.5, 62.0±1.8, and 115.7±2.3 µM, respectively. To inhibit activity of purfied porcine heart PDH activity by 50%, the concentrations (mean ±SE) of MMA^{III} as methylarsine oxide and arsenite were 17.6±4.1 and 106.1±19.8 µM, respectively. These data demonstrate that MMA^{III} is more toxic than inorganic arsenite.

Fowler et al (1977) carried out investigations to delineate the subcellular manifestations of arsenic toxicity following chronic exposure using combined ultrastructural and biochemical techniques. 72 male Charles River CD rats were divided into four groups of 18 each and fed a casein-based purified diet. These groups were given access to deionized drinking water containing 0, 20, 40, or 85 ppm arsenic as sodium arsenate

(As^{+5}) respectively for 6 weeks. At the end of this period, the animals were killed in groups of three each and the livers removed. Mitochondrial respiration studies were conducted. In situ swelling of liver mitochondria was the most prominent ultrastructural change observed. Mitochondrial respiration studies indicated decreased state 3 respiration and respiratory control ratios (RCR) for pyruvate/malate but not succinate mediated respiration. Specific activity of monoamine oxidase which is localized on the outer mitochondrial membrane showed increases of up to 150% of control and cytochrome-C oxidase which is localized on the inner mitochondrial membrane showed increase in specific activity of 150-200%. Activity of malate dehydrogenase which is localized in the mitochondrial matrix remained unchanged at any dose level. These studies indicate that decreased mitochondrial respiration is only one aspect of arsenic toxicity to this organelle. Marked arsenic-mediated perturbation of important enzyme systems localized in mitochondria which participate in the control of respiration and other normal mitochondrial functions are also important manifestations of cellular dysfunction.

A positive quantitative in vivo correlation between Mitochondrial structure and function and their alteration following administration of sodium arsenate have further been demonstrated by Fowler et al (1979). Male Charles River CD rats in a series of replicate experiments were divided into two groups and fed a casein-based semipurified diet for 6 weeks while being given access to deionized drinking water containing 0 or 40 ppm of arsenic as sodium arsenate. At the end of the exposure period, the animals were killed by decapitation. Ultrastructural morphometric and biochemical studies were conducted on hepatic mitochondria. Morphometric analysis disclosed an over-all 1.2-fold incrase in the relative mitochondrial volume density and 1.4-fold increase in the surface density of the inner mitochondrial membrane of asenate-exposed rats. These structural changes were associated with a 1.5-fold increase in C-leucine incorporation into all mitochondrial proteins, which was primarily associated with the acid insoluble membranous fraction. Mitochondria from arsenate-treated rats showed a marked disruption of normal conformational behavior with depression of nicotinamide adenine dinucleotide (NAD)-linked substrate oxidation and a resulting in vivo increase in the mitochondrial (NAD) to

(NADH) ratio. Observed changes in mitochondrial membranes from arsenate exposure also resulted in 1.5 to 2-fold increases in the specific activities of the membrane marker enzymes monoamine oxidase, cytocharome oxidase, and Mg2+ ATPase. Acitivity of malate dehydrogenase, which is localized in the mitochondrial matrix, was unchanged.

Brown et al (1976) demonstrated alteration of normal ultrastructure and respiratory ability of proximal renal tubules following adminstration of arsenate. Male sprague-Dawley rats weighing 70-150 g were divided into four groups. All groups were fed laboratory chow. The control groups received deionized water while the experimental groups received 40, 85 or 125 ppm arsenic as sodium arsenate (Na2 HasO4 – 7H2O) in deionized water (n=28, 11, 7 and 10 in each group respectively). After 6 wk, the rats were killed by decapitation in pairs comprised of one experimental matched with one control rat by weight. Kidneys of these animals were quickly excised and capsule removed. Combined oxygen electrode and electron microscopic studies were conducted on kidneys. Decreased state 3 respiration and respiratory control ratios were observed in kidneys of rats given the 85 and 125 ppm dose levels. Ultrastructural alterations, which consisted of swollen mitochondria and increased numbers of dense autophagic lysosome-like bodies, were confined to proximal tubule cells of these same animals.

The general mechanisms underlying apoptosis are a subject of intense debate. Recently, Kroemer et al (1995) have forwarded the hypothesis that the mitochondrial permeability transition (PT^2) pore, a polyprotein complex formed in the contact site between the inner and the outer mitochondrial membranes, has a key role in the control of apoptosis. Larochette et al (1999) evaluated the possibility whether arsenic compounds act on mitochondria to induce apoptosis. U937 cells transfected with a SFFV neo vector containing the human bcl-2-gene or the neomycin resistance gene (Neo) only or 2B4 11-T cell hybridoma cells were cultrued in RPM1 1640 medium supplemented with 10% FCS, L-glutamine, Hepes, and antibiotics. Cells ($1\text{-}5 \times 10^5$ ml^{-1}), as well as variable doses of sodium arsenite, sodium arsenate, phenylarsine oxide (PAO), p-arsanilic acid (PAA), 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195; all from Sigma, St. Louis, MO) and the caspase inhibitors N-benzy-loxycarbonyl-Val-

Ala-Asp. Fluoromethylketone (Z-VAD fmk, 100 µM), t-butyloxycarbonyl-Asp.fluoromethylketone (Boc-D.fmk) and the cathepsin inhibitor N-benzyloxycarbonyl-Phe-Ala.fluoromethylketone (Z-FA.fmk, 100 µM) were used.

The investigators observed that arsenite induces apoptosis accompained by a loss of the mitochondrial transmembrane potential ($\Delta\Psi m$). Inhibition of caspases prevents the arsenite-induced nuclear DNA loss, but has no effect on the dissipation and cytolysis induced by arsenite. In contrast, Bcl-2 expression induced by gene transfer prevents all hallmarks of arsenite-induced cell death, including the collapse. PK11195, a ligand of the mitochondrial benzodiazepine receptor, neutralizes this Bcl-2 effect. Mitochondria are required in a cell-free system to mediate arsenite-induced nuclear apoptosis. Arsenite causes the release of an apoptosis-inducing factor (AIF) from the mitochondrial intermembrance space. This effect is prevented by the permeability transition (PT) pore inhibitor cyclosporin A, as well as by Bcl-2, which is known to function as an endogenous PT pore antagonist. Arsenite also opens the purified, reconstituted PT pore in vitro in a cyclosporin A- and Bcl-2-inhibitible fashion. Altogether these data suggest that arsenite can induce apoptosis via a direct effect on the mitochondrial PT pore.

Urinary Porphyrins and Heme Biosynthetic Enzyme Activities and Arsenic

Arsenic can modify the urinary excretion of porphyrins in animals and humans. Arsenic also interferes with the activities of several enzymes of the heme biosynthesis pathway, such as aminolevulinate synthase (ALA-S), porphobilinogen deaminase (PBGD), uroporphyrinogen III synthase (Uro III S), uroporphyrinogen decarboxylase (URO-D), coproporphyrinogen oxidase (COPRO-O), ferrochelatase and heme oxygenase (H-O) (Garcia Vargas and Hernandez-Zavala, 1996).

Rodents exposed for six weeks to sodium arsenate in drinking water showed a substantial increase in the urinary excretion of porphyrins, with the excretion of uroporphyrin exceeding that of coproporphyrin (Woods and Fowler, 1978). Male Sprague − Dawley rats (CD strain) (150-200 g) or male C57 Black mice (20-30 g), were randomly divided

into four groups of 12 animals each and were given access to laboratory chow and deionized drinking water containing 0, 20, 40 or 85 ppm of arsenic as sodium arsenate (As^{5+}) for up to 6 weeks. Controls were run concurrently with arsenic-treated animals throughout the investigation. Livers of animals were homogenized and mitochondria and microsomal fractions were then prepared. Continuous prolonged exposure to sodium arsenate at 20, 40, or 85 ppm in the drinking water resulted in depression of hepatic δ aminolevulinic acid (ALA) synthetase and heme synthetase, the first and last enzymes in heme biosynthesis, respectively, in both rats and mice. ALA synthetase was maximally depressed to approximately 80% of control values at 40 ppm in both species, whereas heme synthetase activity was maximally decreased to 63 and 75% of control at 85 ppm in rats and mice, respectively. Uroporphyrinogen I synthetase, the third enzyme in heme biosynthesis, was increased at all doses in the mouse, whereas ALA dehydratase, the second heme biosynthetic pathway enzyme, was unaltered in either species. Concomitantly, urinary uroporphyrin concentraions were elevated by as much as 12 times, and coproporphyrin by as much as nine times, the control values in the rat. Similar patterns of elevated porphyrin excretion were seen in the mouse. In contrast, no changes were observed in the activities of cytochrome oxidase or cytochrome P-450, 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.

Cebrian et al (1988) demonstrated that sodium arsenite is a potent inducer of heme oxygenase, which is the ratelimiting enzyme of heme degradation. Male Wistar albino rats, University of Surrey strain weighing from 180 to 210 g) were fasted for 24 hours before treatment and until killed. Animals received 0.1 ml of sodium chloride, 0.9% (w/v) or As salts by subcutaneous injection. The doses of AsIII were 12.5, 25, 50, 75 and 100 µmol/kg and those of AsV were 25, 50, 100, 150 and 200 µmol/kg. Animals were killed by cervical dislocation 16 hours after injection. In the subchronic study, animals were exposed to AsIII in the drinking water at a concentration of 50 mg/L for periods of 5, 10, 20 or 30 days, and food was withheld for 24 hours before sacrifice. The livers were

excised and perfused and homogenized. Tyrptophan pyrrolase activity, 5-Aminolevulinate synthease activity and heme oxygenase activity were measured. Cytochromes P-450 and b5 contents were also measured. Acute arsenic (As) administration produced in rat liver a decrease in the heme satruation of trypotphan pyrrolase (TP), accompanied by dose-related increased in 5-aminolevulinate synthetase (ALAS) and heme oxygenase (HO) activities, along with a corresponding decrease in cytochrome P-450 (P-450) concentration. The relationship between heme synthesis and degradation was altered as a result of As treatment. The magnitude of these effects was related to the oxidation state of arsenic, sodium arsenite (AsIII) being more potent than sodium arsenate (AsV). These results support the contention that the heme saturation of TP is sensitive to treatments that modify liver heme concentration. The increase in HO activity produced by As appears to be mediated by a mechanism largely or entirely independent of heme. The main effects of continuous exposure to AsIII were an initial decrease in the heme saturation of TP, which remained constant during the period of treatment, and an initial increase in ALAS activity, which after ten days of exposure dropped somewhat but remained above control values. No significant effects on HO or P-450 concentration were observed. These results were interpreted as indicative that a new balance between heme synthesis and degradation had been reached and that an adaptive response to the subchronic effects of AsIII was taking place.

Garcia Vargas et al (1994) studied altered urinary porphyrin excretion in a human population chronically exposed to arsenic in Mexico. A detailed study of the urinary excretion pattern of porphyrins in humans chronically exposed to As via drinking water was performed using high performance liquid chromatography (HPLC). Thirty-six individuals (15 men and 21 women) were selected from a town which had 0.400 mg/L of As in drinking water. The control group consisted of thirty-one individuals (13 men and 18 women) whose As concentration in drinking water was 0.020 mg L. The major abnormalities in the urinary porphyrin excretion pattern observed in arsenic exposed individuals were (a) significant reductions in coproporphyrin III excretion resulting in decreases in the COPRO III/COPRO I ratio, and (b) significant increases in uroporphyrin excretion. Both alterations were responsible for the decrease in the COPRO/URO ratio.

No porphyrinogenic response was found in individuals with urinary As concentrations below 0.1 mg of As/g of creatinine. However, as arsenic concentrations exceeded this value, the excretion of porphyrins (except coproporphyrin III) increased proportionally. The prevalence of clinical signs of arsenicism showed a direct relationship to both As concentration in urine and time-weighted exposure to As. A direct relationship between time-weighted exposure and alterations in urinary porphyrin excretion ratios was also observed. The alterations found are compatible with a lower uroporphyrinogen decarboxylase activity in arsenic exposed individuals.

Arsenic and Oxidative stress:

The mechanisms by which arsenic may induce cancer have not been fully understood. Besides various mechanisms that have been proposed, oxidative stress is a relatively new theory for arsenic-induced carcinogenesis. That exposure of arsenite, arsenic torioxide or arsenate result in the generation of reactive oxygen species (ROS) in laboratory animals or in cultured animal and human cells has been reported by many investigators [Wang et al (1996), Hei et al (1998), Chen et al (1998), Lynn et al (2000), Ahmad et al (2000), Liu et al (2001), Chouchane and Snow (2001)] The topic has been reviewed by Thomas et al, 2001, Ercal et al, 2001 and Del Razo et al 2001.

Ahmad et al (1999) investigated the biochemical effects of DMA expsoure in B6C3F1 mice using six biochemical parameters - DNA damage, the reduced and oxidized glutathione (GSH, GSSG) content, cytochrome P-450 content, ornithine decarboxylase (ODC) activity in liver and/or lung, and alanine aminotransferase activity (ALT) in serum. Adult female mice B6C3F1 mice (25-30 g body weight) were randomly placed into four treatment groups. Four or five control mice were run on each of 5 experimental days. The total number of mice run on each experimental day was either ten or 12. The control group of mice received distilled water alone. 720 mg/kg of DMA was given by oral gavage at one of three times (2h, 15h or at both 21 and 4 h) before sacrifice. Significant ($P<0.05$) decrease in liver GSH and GSSG consents (15-37%) were observed.

Some evidence of DMA induced hepatic DNA damage (at the $P < 0.10$ level only) was observed. Pulmonary and hepatic ODC (orithim decarbosylase) activities were reduced (19-59%) by DMA treatment.

Ishinishi N et al (1980) studied the effect of chronic toxicity of arsenic trioxide in rats with special reference to the liver damages. Four groups of male adult Wistar rats were given per os distilled water containing 0, 0.125, 12.5 or 62.5 ppm arsenic as As_2O_2 respectively, for 7 months and there after they were given distilled water without adding of As_2O_2 for 4 months. Despite of no difference of growth in rats among 4 groups, chronic exposure to As_2O_2 induced not only liver injuries but also dose-dependent proliferation of the bile duct with chronic angitis. The liver injuries were characterized by degenerative changes in hepatocytes such as cloudy swelling, disordered trabeculae or irregularity of hepatocyte tracts and spotty coagulative necrosis with infiltration of round cells. Sarin et al demonstrated (1999) hepatic fibrogenesis using chronic arsenic ingestion studies in a murine model. Swiss albino mice (360), 5-7 weeks old, with a mean weight of 25 g, were equally divided into different groups to daily arsenic feed. A significant increase in the hepatic protein and collagen was seen compared with controls, hepatic 4-hydroxyproline levels, indicative of fibrogenesis, were increased 4-14 folds with different dosages of arsenic compared to the controls.

Effects on levels of glutathione and some related enzymes in tissues after an acute arsenic exposure in rats were studied by Maity and Chatterjee 2001. In this study male rats of Wistar strain, maintained on either 18% or 6% protein (casein) diet, received an acute i.p. exposure to sodium arsenite (As3+) at its LD50 dose (15.86 mg/kg body weight). One hour after the arsenic exposure, glutathione (GSH) concentration was significantly depleted and lipid peroxidation was increased. Acute arsenic exposure significantly increased the glutathione peroxidase (GPx) activity in liver. The glutathione-S-transferase (GST) activity signficantly decreased in liver of the 18% protein-fed animals. No signicant change in glutathione reductase (GR) or glucose-6-phosphate dehydrogenase (G6PDH) activity was observed. In the present investigation, liver as a whole seems to be more affected in terms of GSH level and GST activity.

A variety of genes related to base excision repair and oxidative stress are coordinately up-regulated by nM concentrations of iAs (which are equivalent to low ppb levels). ROS induced by low levels of iAsIII or iAsV increase the transcription of the activator protein 1 (AP-1) and the nuclear factor-kB (NF-kB). This results in cell signaling binding of transcription factors to DNA, stimulation of cell proliferation and up-regulation of gene expression including mdm2 protein, which is a key regulator of the critical anticancer gene p53. Other genes related to cellular redox control, such as glutathione reductase and glutathione peroxidase, are also up-regulated in parallel with the DNA repair genes. In contrast, high levels of iAs inhibit the activation of NF-kB, inhibit cell prliferation, and induce apoptosis. Apoptosis is triggered by the generation of H_2O_2 through the activation of flavoprotein dpendent superoxid-producing enzymes (i.e., NADPH oxidase) and involve an early decrease of the peroxide potential in the mitochondiral membrane. This event probably acts as a mediator to induce apoptosis through the release of cytochrome c to the cytosol and the activation of caspase 3, leading to DNA fragmentation (Reviewed by Del Razo et al 2001).

Study of oxidative stress in humans:

8-Hydroxy-2-deoxyguanosine (8-OhdG) is generated by hydroxyl radical (Kasai and Nishimuar, 1984), singlet oxygen (Devasagyayam et al, 1991), or direct electron transfer, which dose not involve the participation of any ROS (Kasai et al, 1992), 8-OhdG is considered to be one of the main oxidative base damage to DNA, and may cause mutation (G:C to T:A) at DNA replication (Shibutani et al, 1991). Currently, 8-OhdG is widely accepted as a sensitive marker of oxidative DNA damage and oxidative stress.

Matsui et al (1999) examined whether neoplastic and precancerous skin lesions of arsenic-related individuals are oxidatively stressed using 8-OhdG as a marker. Samples of arsenic keratosis, arsenic-induced Bowen's diseasae and arsenic-induced Bowen's carcinoma arising in non-sun-exposed or less sun-exposed areas were obtained from 28 individuals (age 26-83) from eitehr Taiwan, Thailand, or Japan living in areas where

chronic arsenicism was endemic. These patients revealed clinical featrues characteristic of arsenicism such as cutaneous melanosis, keratosis of palms and soles (arsenic keratosis) and multiple Bowen's disease and internal malignancy.

The investigators studied the presence of 8-hydroxy-2-deoxyguanosine by immunohistochemistry using N45.1 monoclonal antibody in the 28 cases of arsenic related skin neoplasm and arsenic keratosis as well as in 11 cases of arsenic unrelated Bowen's disease. The frequency of 8-hydroxy-2-deoxyguanosine positive cases was significantly higher in arsenic-related skin neoplasms (22 of 28; 78%) than in arsenic-unrelated Bowen's disease (one of 11; 9%) ($p < 0.001$ by χ^2 test). 8-Hydroxy-2-deoxyguanosine was also detected in normal tissue adjacent to the arsenic-related Bowen's disease lesion. Furthermore, arsenic was detected by neutron activation analysis in the deparaffined skin tumor samples of arsenic related disease (four of five, 80%), whereas arsenic was not detected in control samples. The results strongly suggest the involvement of reactive oxygen species in arsenic-induced human skin cancer.

Arsenic and stress proteins:

Exposure to arsenicals either in vitro or in vivo in a variety of model systems has been shown to cause the induction of a number of the major stress protein families such as heat shock proteins (Hsp). Among them are members with low molecular weight, such as metallotionein (MT) and ubiquitin (UB) as well as ones with massess of 27, 32, 60, 70, 90 and 110 kDa. In most of the cases, the induction of stress proteins depends on the capacity of the arsenical to reach the target, its valence, and the type of exposure, arsenite being the biggest inducer of most Hsp in several organs and systems. Hsp induction is a rapid dose-dependent response (1-8h) to the acute exposure to arsenite. Thus, the stress response appears to be useful to monitor the sublethal toxicity resulting from a single exposure to arsenite. The capacity of arsenicals to modulate the expression and/or accumulation of stress proteins bas been studied by Caltabiano et al (1986), Keyse and Tyrell (1989) Wijk et al (1993), Wu and Welsh (1996), Wijeweera et al (2001) and has been reviewed by Bernstam & Nriagu (2000) and Del Razo et al 2001.

Metallothionein (MT) is a low molecular weight, cysteine rich, metal binding protein. MT has been proposed to play an important role in the homeostasis of essential metals, in the detoxication of heavy metals, and in the scavenging of free radicals. Moreover, MT is a small protein easily induced by heavy metals, hormones, acute stress, and a variety of chemicals. The cysteine contents and the capacity of these MT isoforms to bind metals are similar (Sato and Bremerner 1993, NRC 1999).

The induction of MT is observed following oral administration, the doses of organic arsenicals (MMA and DMA) required for MT induction are one order higher than those of inorganic arsenicals (As(III) and As(V) (Maitani et al 1987). Because of its high sulfhydryl content, MT has also been suggested to react with free radicals and electrophiles (Klaassen and Cagen 1981). MT can serve as a sacrificial scavenger for hydroxyl radicals in vitro (Thornalley and Vasak 1985). MT is induced by oxidative stress-producing chemicals (Bauman et al 1993) and has been shown to protect against oxidative damage (Sato and Bremner 1993).

The effect of various arsenic forms on the tissue concentration of MT was determined in Mice by Kreppel et al 1993. Male CF-1 mice (25-30 g) were injected sc with either 55, 70, 85, 100, 115, 130 or 145 µmol/kg As(III); 165, 210, 255, 300, 345, 390, or 435 µmol/kg As(V); 100, 500, 1250, 2000, 2750, 3500, 4250, 5000, 5750, 6500 or 7250 µmol/kg MMAA; 2750, 3500, 4250, 5000, 5750, 6500, 7250, 8000, 8750, 9500, or 10250 µmol/kg DMAA. Controls were injected with an equal volume (0.1 ml/10 g body wt) of saline. MT content in hepatic cytosol was quantitated by the Cd-hemoglobin assay. As(III) was found to be a potent hepatic MT inducer in that a 30-fold increase in MT was observed at the dose of 85 µmol/kg. In comparison, it took 3,50, and 120 fold higher molar amounts of As(V), MMAA and DMAA, respectively to produce a similar effect. MMAA produces the largest increase in hepatic MT (80-fold), followed by As(III) (30-fold), As(V) (25-fold) and DMAA (10-fold). However, none of the arsenicals induced MT in mouse primary hepatocyte culture. Both MT-I and MT-II were coordinately induced by as(III), As(V) and MMAA. MT induction by As(III) was further characterized

following sc administration of arsenite (85 µmol/kg). Hepatic MT induction peaked at 24 hr, and in addition to the liver, As(III) also increased MT in kidney, spleen, stomach, intestine, heart, and lung. MT-I mRNA increased 24-, 52-, and 11-fold at 3, 6, and 15 hr after As(III) administration. This induction profile is similar to that observed after Zn or Cd exposure. This study showed that arsenicals are effective inducers of MT in vivo and their potency and efficacy are dependent on the chemical form of arsenic. As(III) is a potent hepatic MT inducer for both MT-I and MT-II and this effect is associated with an increase in MT mRNA.

Stress related gene expression in mice treated with inorganic arsenic has been studied by Liu et al 2001. Adult male 129/Sv mice aged 6-8 weeks were used in this study. Mice were injected sc in the dorsal thoracic midline with 100 µmol/kg (As(III), 300 µmol/Kg As(V), or the same volume of saline (10 ml/kg). To examine stress-related gene expression, livers were removed 3 h after arsenic injection for RNA and protein extraction. The Atlas Mouse Stress/Toxicology array revealed that the expression of genes related to stress, DNA damage, and metabolism was altered by acute arsenic treatment. Expression of heme oxygenase 1 (HO-1), a hallmark for arsenic-induced stress, was increased 10-fold, along with increases in heat shock protein-60 (HSP60), DNA damage inducible protein GADD45, and the DNA excision repair protein ERCC1. Down regulation of certain cytochrome P450 enzymes occurred with arsenic treatment. Multiprobe Rnase protection assay revealed the activation of the c-Jun/AP-1 transcription complex after arsenic treatments. Western blot analysis further confirmed the enhanced production of arsenic-induced stress proteins such as HO-1, HSP70, HSP90, metallothionein, the metalresponsive transcription factor MTF-1, nuclear factor kappa B and c-Jun/AP-1. Increases in caspase-1 and cytokines such as tumor necrosis factor-$\propto$ (TNF-$\propto$) and macrophage inflammatory protein-2 were also evident. The results of this study profiled gene expression patterns in mice treated with inorganic arsenicals. The altered gene expressions by acute arsenic treatments include stress related components, DNA damage and repair-responsive genes, activation of transcription factors such as the AP-1 complex and increased in proinflammatory cytokines. All these events could play an integral role in response to acute arsenic toxicity.

SP expression is under a complex regulatory mechanism which requires the integration of multiple signal pathways. The interrelationships among stress signaling, cell death and oncogenesis after As exposure need further research (Del Razo et al 2001).

Nutrient deficiency and arsenic toxicity:

Methylation is considered to be the detoxification mechanism for arsenic. Accumulation of inorganic arsenic as a consequence of saturation or inhibition of the methylation process may result in increased toxicity.

A subchronic animals bioassay was carried out by Okoji et al (1999) to establish a maximum tolerated dose for short-term arsenic exposure in C57BL/6 mice on methyl deficient diets. Ninety young-adult male C57BL/6 mice approximately 8 weeks old were placed randomly into one of six treatment groups and pretreated with either the methyl sufficient or methyl-deficient diet for a period of 21 days. Following the pretreatment period, animals received arsenic as sodium arsenite in drinking water for the remainder of the study. Mice were administered arsenic via drinking water: 0, 2.6, 4.3, 9.5 or 14.6 mg sodium arsenic/kg/day. Dosing continued 7 days a week for 130 days. Deaths of 3 of the control animals (methyl-sufficient/no arsneic) at Day 111 of the study did not appear to be compound-related. The death of a single animal in the high-dose gorup did appear to be treatment-dependent. A dose related reduction was observed for liver weight. Mild to severe fatty infiltration was observed in the livers of methyl-deficient/arsenic treated animals. Severe liver damage was noted in 2 animals form the 2.6 and 4.3 mg/kg/d groups. In addition, hypertrophy/hyperplasia of the bladder was found in 43/60 mice treated with sodium arsenite. The hyperplasia and hypertrophy of the bladder epithelium may be in reponse to toxic insult or it may be indicative of alterations in the expression or control of cell-cycle genes. There was evidence for hypertrophy/hyperplasia in the test animal groups with no evidence for these changes in controls. The number of animals with hypertrophy/hyperplasia doubled betwene the 2.6 and 4.3 mg/kg/day gorups. Based on these results the author conclude that 2.6 mg/kg/day is a reasonable maximum

tolerated dose. The no observed effect level (NOEL) and no observed adverse effect level (NOAEL) of this study could not be determined as the lowest dose administered produced detrimental effects.

Arsenic and Immunotoxicity:

Although there are many studies which evaluated the immunological effects of environmental toxic substances such as lead, cadmium and mercury, only a few studies on arsenic have been reported.

Yoshida et al (1986) reported immunological effects of arsenic compounds on mouse spleen cells in vitro. Spleens from male C57BL/6N mice were removed asceptically. Sterile viable spleen cells were cultured with 20 µl/ml of arsenic solution (sodium arsenite, sodium arsenate and DMMA). Arsenic concentration in the solution were 1, 5, 10, 50, 100 and 500 mg/ml) for control 0.9% NaCl was added. For plaque forming cell response, spleen cells 2.53 x 10^5 cells/ml were cultured in triplicated and incubated with 8 x 10^6 steep erythrocytes. The number of direct (IgM) plaque forming cells (PFC) were enumerated. Spleen cells (2.5 x 10^6 cells/ml) were also cultered for 48 hrs with or without mitogens PHA (Phytohaemagglitine P). The three kinds of arsenic compounds : sodium arsenite, sodium arsenate and dimethyl arsenic acid, at high doses, suppressed the plaque-forming cell response to sheep erythrocytes and the proliferative response to mitogens, whereas at low doses they enhanced both responses. And each of arsenic compounds differs in strengths at which the modulation effects on both responses were exerted. The strength was comparable to general toxicity of arsenic compounds.

To ascertain genetic damage, a pilot study on arsenic exposed groups was carried out by Ostrosky-Wegman et al (1994) to detect lymphocyte proliferation kineties and genotixc effect in arsenic exposed people. The exposed group consisted of 11 individuals (9 females and 2 males) from Santa Ana, Coahuila, where the drinking water contained 0.39 mg/l of arsenic, 98% of it in its pentavalent form and the rest trivalent. The non-exposed group (13 individuals, 11 females and 2 males) was chosen from Nuevo Leon, Coahuila,

where As level in the drinking water ranged from 0.019 to 0.026 mg/l during 1987-1989 but during sampling was performed, the levels rose to 0.060 mg/l due to new piping linking several towns of the area. Venous samples were taken, and were rapidly processed to start the lymphocyte cultures and the HGPRT (hypoxanthine guanine-phosphoribosyltransferase) locus assay. The analysis of CA (Chromosomal aberrations) and SCE (Sister-chromatid exchanges) was performed in 100 consecutive first-division metaphases and in 30 consecutive second-division metaphases, respectively, all with 46 centromeres. The proportion of first, second, third and subsequent metaphases was determined in 100 consecutive mitoses, to carry out the study on proliferation kinetics. Mutagenicity and the HGPRT locus and genotoxicity in the B. subtilis rec assay were also carried out with urine samples. The highly exposed group excreted greater amounts of As, neverthelesss the rec assay showed negative results. An interesting finding is that the cell-cycle kinetics exhibited a significant difference between the groups studied, the average generation time (AGT) was longer in the highly exposed group. The percentages of chromosomal absserrations and the frequencies of sister-chromatid exchanges were similar in both populations, although complex aberrations were more frequent in the highly exposed groups, which also showed a higher average variation frequency in the HGPRT assay, but the two latter observations were not statistically significant. The lag in lymphocyte proliferation could mean an impairment of the immune response due to arsenic exposure.

Since inhibition of lymphocyte proliferation has been used to identify agents which depress the cellular immune response, Gonsebatt et al (1992) investigated in vitro the effect of arsenic on human lymphocyte stimulation and proliferation using arsenic concentrations similar to those found in blood. When human lymphocytes collected form healthy donors (2 males, 2 females) were exposed to arsenite and arsenate (10^{-7}, 10^{-8} and 10^{-9} M) during culture and harvested after 24 h, a dose-related inhibition of proliferation was observed. Cultures were also treated with 10^{-7} M of arsenite and arsenate for 2, 6 and 24 h at the beginning of the cultrues in the presence or absence of phytohemaggluatiin (PHA). Inhibition of stimulation and proliferation was directly related to the length of treatment. The results show that, at the concentrations tested, arsentie and arsenate

impaired lymphocyte stimulation and proliferation and confirm the fact that chronic arsenic exposure can affect the proliferation of whole blood lymphocytes.

A human monitoring study was subsequently carried out by Gonsebatt et al (1994) to explore the effect on lymphocyte proliferation of chronic exposure to arsenic (As) via drinking water. Blood and urine samples were taken from volunteers from a town where levels of As in the drinking water averaged 412 µg/l, and from a matched group of individuals, with similar socio-economic status, that drank water with average As level of 37.2 µg/l. Exposure was assessed by questionnaires and by determining the levels of As in urine and water samples. The evaluation of the peripheral blood lymphocyte proliferation was done at different culture times using labelling (LI), mototic (MI) and replication indexes (RI) as endpoints. No significant differences were seen for either LI or MI, except for MI in 72 h cultures and in LI in males and females with skin lesions vs those without lesions. Significant differences in RI were seen for exposed females but not for males. Correlations between LI and MI showed that progression form the initial S- to M- phase is altered in exposed individuals. The results obtained corroborate the slower cell kinetics found previously in the pilot study by Ostrosky-Wegman et al, 1991.

From the preceding reports it appear that inorganic arsenic is immunotoxic, but the mechanism of immune suppression is not clear. Harrison and McCoy (2001) showed that trivalent arsenic inhibits enzymatic activity of the lysosomal protease cathepsin L (cathL) in the murine antigen-presenting B cell line TA3. CathL plays an important role in antigen prcessing, the mechanism by which antigen-presenting cells cleave foreign protein antigens to peptides for stimulating a T cell reponses. Deficient proteolysis may lead to diminished immune reponses. They demonstrated that arsenite suppressed enzymatic activity within TA3 cells after 4 h exposure without affecting cell viability. Kinetic analyses reveled that the chemical was a reversible, partially noncompletitive inhibitor of CathL with a Ki of 120 µM. However, an 18 h arsenite exposure triggered massive cell death at concetrations that were substantially lower than those required for enzymatic inhibition. Morphological analysis and annexin V staining showed that arsenite-exposed TA3 cells underwent apoptosis within 18 h and early stages of apoptosis

began by 4 h. There findings suggest that apoptosis may be an important mechanism for arsenic-induced immunosuppression.

References :

AHMAD S, KITCHIN KT, CULLEN WR. (2000). Arsenic species that cause release of iron from ferritin and generation of activated oxygen. *Arch Biochem Biophys*. 15: 195-202.

AHMAD S, ANDERSON WL & KITICHIN KT. (1999). Dimethylarsinic acid effects on DNA damage and oxidative stress related biochemical parameters in B6C3F1 mice. *Cancer Letters*. 139: 129-135.

AHMAD SA, BANDARANAYAKE D, KHAN AW, HADI SA, UDDEIN G AND HALIM MA (1997). Arsenic contamination in ground water and arsenicosis in Bangladesh. *Interntl. J of Env. Heath Res*. 7:271-276.

BAUMAN JW, LIU J & KIAASSEN C (1993). Production of metallothionein and heat shock proteins in Response to Metals. *Fundmental & Applied Toxicology* 21:15-22.

BERNSTAM L, & NRIAGU J (2000). Molecular aspects of Arsenic stress. *J Toxicol Environ Health* 3:293-322.

BLACK, M.D. (1967) Prolonged ingestion of arsenic. *Pharm. J.* **(Dec. 9):**593-597.

BORGONO, J.M., VICENT, P., VENTURINO, H. & INFANTE A. (1977) Arsenic in the drinking water of the city of Antofagasta: Epidemiological and clinical study before and after the installation of the treatment plant. *Environ. Health Perspect.* **19:** 103-105.

BROWN MM, RHYNE BC, GOYER RA. (1976). Intracellular effects of chronic arsenic administration on renal proximal tubule cells. *J Toxicology and Environmental Health*. **1:** 505-514.

CALTABIANO M, KOESTLER TP, POSTE G & GREIG RG (1986). Induction of 32- and 34-kDa stress proteins by sodium arsenite, Heavy metals, and thiol-reactive agents. *J Biological Chemistry* 261:13381-13386.

CEBRIAN, M.E., ALBORES, A., AGUILAR, M.,& BLAKELY, E. (1983). Chronic arsenic poisoning in the north of Mexico. *Hum. Toxicol*. 2:121-133.

CEBRIAN ME, ALBORES A, CONNELLEY JC & BRIDGES JW. (1988). Assessment of Arsenic Effects on Cytosolic Heme Status Using Tryptophan Pyrrolase as an Index. *J Biochem Toxicol*. **3:** 77-86.

CHAKRABORTY, A.K. & SAHA, K.C. (1987) Arsenical dermatosis from tubewell water in West Bengal. *Indian J. Med. Res.* 85: 326-334.

CHEN B, BURT CT, GOERING PL, FOWLER BA, LONDON RE. (1986) In vivo 31P nuclear magnetic resonance studies of arsenite induced changes in hepatic phosphate levels. *Biochem Biophys Res Commun*. 139(1):228-34.

CHEN, C.J., WU, M.M., LEE, S.S., WANG, J.D., CHENG, S.H., & WU, H.Y. (1988). Atherogenicity and carcinogenicity of high arsenic artesian well water. multiple risk factors and related malignant neoplasms of Blackfoot disease. *Arteriosclerosis* **8:**452-460.

CHEN CJ, LIN LJ, HSUEH YM ET AL. (1994). Ischemic heart disease induced by ingested inorganic arsenic. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Science and Technology Letter, Northwood. 83-90.

CHEN C-J, HSUEH Y-M. LAI M-S, SHYU M-P, CHEN S-Y, WU M-M, KUO T-L & TAI T-Y (1995) Increased prevalence of hypertension and long-term arsenic exposure. *Hyperension*, 25: 53-60.

CHEN, C.-J., CHIOU, H.-Y., HUANG, W.-I. CHEN, S.-Y., HSUEH, Y.-M., TSENG, C.-H., LIN, L.-J., SHYU, M.-P. & LAI, M.-S. (1997). Systemic non-carcinogenic effects and developmental toxicity of inorganic arsenic. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Chapman & Hall, London. **11**: 124-134.

CHEN JC, CHIOU HY, CHIANG MH, LIN LJ, TAI TY. (1996). Dose response relationship between Ischemic heart disease mortality and long term arsenic exposure. *Arteriosclerosis*. 16: 504-510.

CHEN YC, LIN-SHIAU SY, LIN JK. (1998). Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. *J Cell Physiol*. 177: 324-33.

CHIOU H-Y, HUANG W-I, SU C-L, CHANG S-F, HSU Y-H & CHEN C-J (1997). Dose response relationship between prevalence of cardiovascular disease and ingested inorganic arsenic. *Stroke* 28:1717-1723.

CHOUCHANE S, & SNOW ET. (2001). In Vitro Effect of Arsenical Compounds on Glutathione-Related Enzymes. *Chem. Res. Toxicol*. 14: 517-522.

CHOWDHURY TR, MANDAL BK SAMANTA G, ET AL. (1997). Arsenic in groundwater in six districts of West Bengal, India: the biggest arsenic calamity in the world: the status report up to August, 1995. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Chapman & Hall, London. **9**: 93-111.

CHOWDHURY UK, BISWAS BK, CHOWDHURY TR, SAMANTA G, MANDAL BK, BASU GC, CHANDA CR, LODH D, SAHA KC, MUKHERJEE SK, ROY S, KABIR S, QUAMRUZZAMAN Q & CHAKRABORTI D. (2000). Ground water arsenic contamination in Bangladesh and West Bengal, India. Environ Health Perspect. 108:393-397.

COWLISHAW JL, POLLARD EJ, COWEN AE & POWELL LW. (1979). Liver Disease Associated with Chronic Arsenic Ingestion. *Aust N.Z.J. Med*. 310-313.

DATTA DV, MITRA SK, CHHUTTANI PN AND CHAKRAVARTI RN. (1979) Chronic oral arsenic intoxication as a possible aetiological factor in idiopathic portal hypertension (non-cirrhotic portal fibrosis) in India. *Gut* 20:378-384.

DEL RAZO LM, QUINTANILLA-VEGA B, BRAMBILA-COLOMBRES E, CALDERON-ARANDA ES, MANNO M, ALBORES A. (2001). Stress proteins induced by arsenic. *Toxicol Appl Pharmacol*. 1177: 132-48.

DEVASAGAYAM TPA, STEENKEN S, OBENDORF MSW, SCHULZ WA & SIES H. (1991). Formation of 8-Hydroxy(deoxy) guanosine and Generation of Strand Breaks at Guanine Residues in DNA by Singlet Oxygen. *Biochemistry*. 30: 6283-6289.

ENGEL R & SMITH A (1994) Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 countries in the United States. *Arch Environ Health,* 49: 418-427.

ERCAL N, GURER-OURER H, & AYKIN-BURNS N. (2001). Toxic metals and oxidative stress part I : Mechanisms involved in Metal induced oxidative damage. *Current Topics in Medicinal Chemistry*. 6: 529-539.

FOWLER BA, WOODS JS AND SHILLER CM. (1977).   Ultrastructural and Biochemical Effects of Prolonged Oral Arsenic Exposure on Liver Mitochondria of Rats. *Environ. Health Perspect*. 19:197-204.

FOWLER BA, WOODS JS, SCHILLER CM. (1979). Studies of Hepatic Mitochondrial Structure and Function. *Laboratory Investigation*. 41:313-320.

FRANKLIN M, BEAN WB, AND HARDIN RC. (1950) Fowler's solution as an etiologic agent in cirrhosis. *Am. J. Med. Sci*. 589-596.

GONSEBATT ME, VEGA L, MONTERO R, GARCIA-VARGAS G, DEL RAZO LM, ALBORES A, CEBRIAN ME & OSTROSKY-WEGMAN P. (1994). Lymphocyte replicating ability in individuals exposed to arsenic via drinking water. *Mut Res* 313:293-299.

GONSEBATT ME, VEGA L, HERRERA LA MONTERO R, ROJAS E, CEBRIAN ME, & OSTROSKY-WEGMAN P. (1992). Inorganic arsenic effects on human lymphocyte stimulation and proliferation. *Mut Res* 283:91-95.

GUHA MAZUMDER, D.N., CHAKRABORTY, A.K. GHOSE A.ET AL. (1988) Chronic arsenic toxicity from drinking tubewell water in rural West Bengal. *Bull. Wld Health Org.,* **66:** 499-506.

GUHA MAZUMDER, D.N., DAS GUPTA, J., CHAKRABORTY, A.K., CHATTERJEE, A., DAS, D., 7 CHAKRABORTY, D. (1992). Environmental pollution and chronic arsenicosis in South Calcutta. *Bull. Wld Health Org.* 70(4): 481-485.

GUHA MAZUMDER DN, DAS GUPTA J, SANTRA ET AL. (1997). Non-cancer effects of chronic arsenicosis with special reference to liver damage. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Chapman & Hall, London. **10**: 112-124.

GUHA MAZUMDER , D.N., DAS GUPTA, J., SANTRA, A., PAL, A., GHOSH, A. SARMAR, S. (1998a) Chronic Arsenic Toxicity in West Bengal – The Worst Calamity in the World. *J. Indian Med. Assocn.* Vol. 96, no. 1, pp4-7 & 18.

GUHA MAZUMDER, D.N., HAQUE, R., GHOSH, N., DE, B.K., SANTRA, A., CHAKRABORTY, D., & SMITH, A.H. (1998b). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *Int. J. Epidemiol,* 27:871-877.

GUHA MAZUMDER DN, DE BK, SANTRA A ET AL. (1999) Chronic Arsenic Toxicity Epidemiology, Natural history and Treatment. *Clinical Toxicology*. 335 – 347.

GUHA MAZUMDER DN, HAQUE R, GHOSH N. ET AL. (2000) Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. *International J Epidemiology* 29:1047-1052.

GUHA MAZUMDER DN, GHOSH N, DE BK, SANTRA A ET AL. (2001). Epidemiological study on various non carcinomatous manifestations of chronic arsenic toxicity in a district of West Bengal. *Arsenic Exposure and health effects IV*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Elsevier Science, Oxford. 153-164.

GUO X, FUJINO Y, KANEKO S, WU KEGONG, XIA Y, & YOSHIMURA T. (2001). Arsenic contamination of ground water and prevalence of arsenical dermatosis in the

Hetao plain area, Inner Mongolia, China. *Molecular & Cellular Biochemsitry* 222:137-140.

GARCIA-VARGAS G & HERNANDEZ-ZAVALA A. (1996) . Urinary porphyrins and Heme Biosynthetic Enzyme activities measured by HPLC in arsenic toxicity. *Biomedical Chromatography*. **10:** 278-284.

GARCIA-VARGAS G, DEL RAZO LM, CEBRIAN ME, ALBORES A, OSTROSKY-WEGMAN P, MONTERO R, GONSEBATT ME, LIM CK & MATTEIS FD. (1994) Altered urinary porphyrin excretion in a Human population chronically exposed to arsenic in Mexico. *Human & Experimental Toxicology*. **13:** 839-847.

HARRISION MT, MCCOY KL. (2001). Immunosuppression by arsenic : a comparision of cathepsin L inhibitoin and apoptosis. *Interal. Immuno* 1:647-656.

HAQUE R, GUHA MAZUMDER DB, SAMANTA S, GHOSH N, KALMAN D, SMITH MM, MITRA S, SANTRA A, LAHIRI S, DAS S, DE BK & SMITH AH. (Inpress). The dose response relations between arsenic concentrations in drinking water and skin lesions in West Bengal, India. *Epidemiology*.

HEI TK, LIU SX & WALDREN C (1998). Mutagenicity of arsenic in mammalian cells : Role of reactive oxygen species. *Proc. Natl. Acad. Sci. USA* 95: 8103-07.

HOTTA, N. (1989). Clinical aspects of chronic arsenic poisoning due to environmental and occupational pollution in and around a small refining spot [in Japanese]. *Nippon Taishitsugaku Zasshi* [Jpn. J. Const. Med.] **53(1/2):**49-70.

HUGHES MF. (2002). Arsenic toxicity and potential mechanisms of action. *Toxicol Lett*. 133(1):1-16.

ISHINISHI N, TOMITA M & HISANAGA A. (1980). Study on chronic toxicity of arsenic trioxide in rats with special reference to the liver damages. *Fukuoka Acta Med*. 71:27-40.

KASAI H & NISHIMURA S. (1984). Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Research*. 12: 2137-2145.

KATO K, YAMANAKA K, NAKANO M, HASEGAWA A & OKADA S (2000). 72-kDa stress protein (Hsp72) Induced by Adminstration of Dimethylarsinic acid to Mice accumulates in Alveolar Flat Cells of lung a target organ for Arsenic carcinogenesis. *Biol. Pharm. Bull* 23:1212-1215.

KASAI H & YAZUMI Z. (1992). Photosenstitized Formation of 7,8-Dihydro-8-oxo-2-deoxyguanosine (8-Hydroxy-2-deoxyguanosine) in DNA by Riboflavin: A None Singlet Oxygen Mediated Reaction. *J Am Chem Soc*. 114:9692-9694.

KEYSE SM & TYRRELL R (1989). Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation , hydrogen peroxide, and sodium arsenite. *Proc. Natl. Acad. Sci*, USA 86:99-103.

KILBURN KH. (1997). Neurobehavioral impairment from long-term residential arsenic exposure. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Chapman & Hall, London. **14**: 159-177.

KREPPEL H, BAUMANJW, LIU J, MCKIM JM & KLAASSEN C (1993). Induction of Metallothionein by arsenicals in Mice. *Fundmental & Applied Toxicology* 20:184-189.

KROEMER G, PETIT P, ZAMZAMI N, VAYSSIERE JL & MIGNOTTE B. (1995) The biochemistry of programmed cell death. *FASEB*. **9:** 1277-1287.

LAI M-S, HSUEH Y-M, CHEN C-J, SHYU M-P, CHEN S-Y, KUO T-L, WU M-M, & TAI T-Y (1994) Ingested inorganic arsenic and prevalence of diabetes mellitus. *Amer J Epidemiol*, 139: 484-492.

LAROCHETTE N, DECAUDIN D, JACOTOT E, BRENNER C, MARZO I, SUSIN SA, ZAMZAMI N, ZIE Z, REED J & KROEMER G. (1999). Arsenite induces apoptosis via a Direct effect on the mitochondrial permeability transition pore. *Experimental Cell Research*. **249:** 413-421.

LIU J, KADIISKA MB, LIU Y, LU T, QU W, WAALKES MP. (2001). Stress-related gene expression in mice treated with inorganic arsneicals. *Toxicol Sci*. 61: 314-20.

LIU SX, ATHAR M, LIPPAI I, WALDREN C & HEI TK. (2001). Induction of Oxyradicals by arsenic : Implication for mechanism of genotoxicity. *Proc. Natl. Acad. Sci. USA*. 98: 1643-1648.

LIU J, LIU Y, GOYER A, ACHANZAR W & WAALKES MP (2000). Metallothionein-I/II Null Mice are more sensitive than Wild-type mice to the hepatotoxic and nephrotoxic effects of chronic oral or injected inorganic arsenicals. *Toxicological Sciences* 55:460-467.

LYNN S, GURR JR, LAI HT, JAN KY. (2000). NADH Oxidase Activation Is Involved in Arsenite-Induced Oxidative DNA Damage in Human Vascular Smooth Muscle Cells. *Circ Res*. 86: 514-519.

MA HZ, XIA YJ, WU KG ET AL. (1999). Human Exposure to Arsenic and Health Effects in Bayingnormen, Inner Mongolia. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Elsevier Science, London. 127-131.

GUHA MAZUMDER DN, DAS GUPTA J, SANTRA ET AL. (1997). Non-cancer effects of chronic arsenicosis with special reference to liver damage. *Arsenic Exposure*

and health effects, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Chapman & Hall, London. **10**: 112-124.

MATSUI M, NISHIGORI C, TOYOKUNI S, TAKADA J, AKABOSHI M, ISHIKAWA M, IMAMURA S & MIYACHI Y. (2002). The role of oxidative DNA damage in human arsenic carcinogenesis : detection of 8-hydroxy-2-deoxyguanosine in arsenic-related bowen's disease. *J Invest Dermatol* 113: 26-31.

MAITY S & CHATTERJEE AK (2001). Effects on levels on glutathione and some related enzymes in tissues after an acute arsenic exposure in rats and their relationship to dietary protein deficiency. *Arch Toxicol*. 75: 531-537.

MAITANI T, SAITO N, ABE M, UCHIYAMA S & SAITO Y (1987). Chemical form dependent induction of hepatic zincthionein by arsenic administration and effect of co-administreed selenium in mice. *Toxicology Letter*. 39:63-70.

MILTON AH, HASAN Z, RAHMAN A AND RAHMAN M (2001). Chronic arsenic poisoning and respiratory effects in Bangladesh, *J Occup Health* 43:136-140.

MILTON AH & RAHMAN M (1999). Environmental pollution and skin Involvement pattern of chronic arsenicosis in Bangladesh. *J Occup Health* 41:207-208.

MORRIS JS, SCHMID M, NEWMAN S. ET AL. (1974) Arsenic and Non cirrhotic portal hypertension. *Gastroenterology* 66:86-94.

NEVENS F, VAN STEENBERGEN W, SCIOT R, DESMET V AND GROOTE JD. (1990) Arsenic and non-cirrhotic portal hypertension – A report of eight cases. *J Hepatology* 11:80-85.

NORTH, D.W., GIBB, H.J. & ABERNATHY, C.O. (1997) Arsenic: past, present and future considerations. in Arsenic: Exposure and Health Effects, C.O. abernathy, R.L. Calderon, and W.R. Chappell, eds. London: Chapman & Hall. Pp. 406-423.

NRC (NATIOANL RESEARCH COUNCIL) (1999). Arsenic in drinking water, National Academic Press, Washington DC l177-191.

NRC (NATIOANL RESEARCH COUNCIL) (2001). Arsenic in drinking water, National Academic Press, Washington DC 25-27.

OSTROSKY-WEGMAN P, GONSEBATT ME, MONTERO R, VEGA L, BARBA H, ESPINOSA J, PALAO A, CORTINAS C, GARCIA-VARGAS G, DEL RAZO LM & CEBRIAN M (1991). Lymphocyte proliferation kinetics and gentotoxic findings in a pilot sutdy on individuals chronically exposed to arsenic in Mexico. *Muta Rese* 250:477-482.

OKOJI RS, LEININGER J, FROINES J (1999). Subchronic toxicity study of sodium arsenite in Methyl-Deficinet male C57BL/6 Mice. Eds. W.R. Chappell, C.O. Abernathy and R.L. Calderon 225-231.

PAUL PC, CHATTOPADHYAYA A, DUTTA SK, MAZUMDER DN, SANTRA A. (2000) Histopathology of skin lesions in chronic arsenic toxicity – grading of changes and study of proliferative markers. *Ind J Pathol Microbiol*. 43(3):257-64.

PETRICK JS, JAGADISH B, MASH EA AND APOSHIAN HV. (2001). Monomethylarsonous acid (MMA[III]) and arsenite: LD50 in Hamsters and In vitro inhibition of Pyruvate Dehydrogenase. *Chem Res Toxicol* 14:651-656.

RAHMAN M, TONDEL M, AHMAD SA & AXELSON O (1998) Diabetes mellitus associated with arsenic exposure in Bangladesh. *Am J Epidemiol*, 148: 198-203.

ROSENBERG. H.G. (1974). Systemic arterial disease and chronic arsenicism in infants. *Arch. Pathol*. 97(6):360-365.

SAHA KC (1984) Melanokeratosis from arsenic contaminated tube well water. *Ind J Dermat*. 29:37-46.

SAHA, K.C. (1995). Chronic arsenical dermatosed from tube-well water in West Bengal during 1983-87. *Ind. J. Dermatol*. 40 :1-12.

SAHA KC & CHAKRABORTI D. (2001). Seventeen years experience of arsenicosis in West Bengal, India in arsenic exposure and Health Effect IV, Ed. Chappell WR, Abernathy CO and Calderon RL. Elsevier Science Ltd, Oxford. 387-396.

SATO M & BREMNER I (1993). Oxygen free radicals and metallothionein. *Radical Biology & Medicine* 14:325-337.

SCHILLER C.M., FOWLER B.A., WOODS JS. (1977). Effects of Arsenic on Pyruvate Dehydrogenase Activation. Environ. *Health Perspect*. 19:205-207.

SANTRA A, MAITY A, DAS S, LAHIRI S, CHARKABORTY SK & GUHA MAZUMDER DN. (2000). Hepatic Damage Caused by Chronic Arsenic Toxicity in Experimental Animals. *Clinical Toxicology*. 38: 395-405.

SANTRA A, DAS GUPTA J, DE BK, ROY B, GUHA MAZUMDER DN (1999). Hepatic manifestations in chronic arsenic toxicity. *Ind J Gastroenterology*., 18: 152-155.

SARIN SK, SHARMA G, BANERJEE S, KATHAYAT R & MALHOTRA V. (1999). Hepatic fibrogenesis using chronic arsenic ingestion : Studies in a murine model. *Ind. J Experimental Biology*. 37: 147-151.

SHIBUTANI S, TAKESHITA M & GROLLMAN AP. (1991). Insertion of specific bases during DNA synthesis past the oxidation damaged base 8-oxodG. *Letter to Nature*. 349: 431-434.

SIRIPITAYAKUNKIT U, VISUDHIPHAN P, PRADIPASEN M ET AL. (1999). Association between Chronic Arsenic Exposure and Children's Intelligence in Thailand. *Arsenic Exposure and health effects*, Eds. C.O. Abernathy, R.L. Calderon and W.R. Chappell, Elsevier Science, London. 141-149.

SOMMERS, S.C. & McMANUS, R.G. (1953). Multiple arsenical cancers of skin and internal organs. *Cancer* 6: 347-359.

SZULER, I.M., WILLIAMS, C.N. HINDMARSH, J.T. & PARK-DINESOY H. (1979). Massive variceal hemorrhage secondary to presinusoidal portal hypertension due to arsenic poisoning. *Can. Med. Assoc. J*. 120:168-171.

NRC (NATIOANL RESEARCH COUNCIL) (1999). Arsenic in drinking water, National Academic Press, Washington DC l177-191.

TAY, C.H. (1974). Cutancous manifestations of arsenic poisoning due to certain Chinese herbal medicine. *Australas. J. Dermatol.* 15(3): 121-131.

THORNALLEY PJ & VASAK M (1985). Possible role for metallothionein in protection against radiation induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochemica et Biophysica Acta*. 827:36-44.

THOMAS DJ, STYBLO M, LIN S. (2001). The cellular metabolism and systemic toxicity of arsenic. *Toxicol Appl Pharmacol*. 15: 127-44.

TSAI SM, WANG TN, KO YC. (1999). Mortality for certain disease in areas with high levls of arsenic in drinking water. *Arch Environ Health*. 54: 186-193.

TSENG, W.P. (1977). Effects and dose-response relationships of skin cancer and blackfoot disease with arsenic. Environ. *Health Perspect*. 19: 109-119.

TSENG, W.P. CHU HM, HOW, S.W., FONG, J.M., LIN, C.S. & YEH, S. (1968). Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst*. 40:453-463.

TONDEL M, RAHMAN M, MAGNUSON A, CHOWDHURY OA, FARUQUEE MH & AHMAD SA (1999). The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. *Environ. Health Perspect.* 107: 727-729.

TSUDA T, BABAZONO A, YAMAMOTO E, KURUMATANI N, MINO Y, OGAWA T, KISHI Y & AOYAMA H (1995) Ingested arsenic and internal cancer: a historial cohort study followed for 33 years. *Amer J Epidemiol*, 141: 198-209.

WANG TS, KUO CF, JAN KY & HUANG H (1996). Arsenite induces apoptosis in Chinese hmster ovary cells by generation of reactive oxygen species. *J Cell Physiol*. 169: 256-68.

WHO (1981). Environmental Health Criteria 18; Arsenic Geneva p 82.
WOODS JS & FOWLER BA. (1978). Altered regulation of mammaliam hepatic Heme Biosynthesis and Urinary Porphyrin Excretion during Prolonged Exposure to Sodium Arsenate. *Toxicology & Applied Pharmacology*. **43:** 361-371.

WIJK RV, WELTERS M, SOUREN JEM, OVELGONNE H & WIEGANT FAC (1993). Serum Stimulated cell cycle progression and stress protein synthesis in C3H10T1/2 Fibroblasts Treated With Sodium Arsenite. *J Cellular Physiology* 155:265-272.

WIJEWEERA JB, GANDOLFI AJ, PARRISH A & LANTZ CR (2001). Sodium Arsenic Enhances AP-1 and NFkB DNA Binding and Induces Strees Protein Expression in Precision Cut Rat Lung Slices. *Toxicological Sciences* 61:283-294.

WU W & WELSH MJ (1996). Expression of the 25-kDa heat-shock protein (HSP27) correlates with resistance to the toxicity of Cadmium Chloride, Mercuric Chloride, cis Phatinum(II) Diammine dichloride, or Sodium Arsenite in Mouse Embryonic Stem Cells Transfected with Sense or Antisense HSP27 cDNA. Toxicology & Applied Pharmacology 141:330-339.

YAMANAKA K, HASEGAWA A, SAWAMURA R, OKADA S. (1991). Cellular response to oxidative damage in lung induced by the adminsitration of dimethylarsinic acid, a major metabolite of inorganic arsenics, in mice. *Toxicol Appl Pharmacol*. 108: 205-13.

YAMANAKA K, HASEGAWA A, SAWAMURA R, & OKADA S. (1989). Dimethylated arsenics induce dNA strand breaks in lung VIA the production of active oxygen in mice. *Biochemcial & Biophysical Research Comunications*. 165: 43-50.

YEH, S. (1973). Skin cancer in chronic arsenicism. Hum. Pathol. 4: 469-485.

YOSHIDA T, SHIMAMURA T, & SHIGETA S (1986). Immunological effects of arsenic compounds on mouce spleen cells in vitro. *Tokai J Exp Clin Med*, 11(5):353-359.

ZALDIVAR, R. (1974). Arsenic contamination of drinking water and food-stuffs causing endemic chronic poisoning. *Beitr. Pathol*. 151:384-400.