

## REVIEW

# Arsenic Exposure and its Health Effects and Risk of Cancer in Developing Countries: Micronutrients as Host Defence

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### Abstract

Arsenic (As) is a ubiquitous metalloid found in several forms in food and the environment, such as the soil, air and water. The predominant form is inorganic arsenic in drinking water, which is both highly toxic and carcinogenic and rapidly bioavailable. As is currently one of the most important environmental global contaminants and toxicants, particularly in the developing countries. For decades, very large populations have been and are currently still exposed to inorganic As through geogenically contaminated drinking water. An increased incidence of disease mediated by this toxicant is the consequence of long-term exposure. In humans, chronic ingestion of inorganic arsenic (>500mg/L As) has been associated with cardiovascular, nervous, hepatic and renal diseases and diabetes mellitus as well as cancer of the skin, bladder, lung, liver and prostate. Contrary to the earlier view that methylated compounds are innocuous, the methylated metabolites are now recognized to be both toxic and carcinogenic, possibly due to genotoxicity, inhibition of antioxidative enzyme functions, or other mechanisms. As inhibits indirectly sulphhydryl containing enzymes and interferes with cellular metabolism. Effects involve such phenomena as cytotoxicity, genotoxicity and inhibition of enzymes with antioxidant function. These are all related to nutritional factors directly or indirectly. Nutritional studies both in experimental and epidemiological studies provide convincing evidence that nutritional intervention, including chemoprevention, offers a pragmatic approach to mitigate the health effects of arsenic exposure, particularly cancer, in the relatively resource-poor developing countries. Nutritional intervention, especially with micronutrients, many of which are antioxidants and share the same pathway with As, appears a host defence against the health effects of arsenic contamination in developing countries and should be embraced as it is pragmatic and inexpensive.

**Key Words:** Arsenic - contamination - nutrition - micronutrients - health - malignancies - carcinogenicity

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### Introduction

Arsenic (As) is a naturally occurring metalloid that is present in food, soil and water. The heavy metal is a recognized toxicant and carcinogen present in industrial settings and in the environment (Shi et al., 2004). As is currently one of the highest priority hazardous substances globally (NRC, 1999). Different types of As are recognized to exist in the environment and in the human body; inorganic and organic arsenic. Millions of people are currently exposed all over the world to this ubiquitous toxicant at exposure levels leading to long-term toxicity, particularly cancer (Basu et al., 2001; Gebel, 2000).

Exposure to As is a more serious problem in developing countries where, due to inadequate municipal potable water supply, many populations resort to sinking tube wells which are unfortunately contaminated by geogenic arsenic. Chronic ingestion of inorganic As contaminated drinking water is considered the major source for the risk to human health. In humans, the chronic

ingestion of inorganic arsenic (>500m/day As) has been associated with cardiovascular, nervous, hepatic and renal diseases and diabetes mellitus as well as cancer of the skin, urinary bladder, lung, liver and prostate (Gebel, 2000).

Epidemiological studies indicate that populations exposed to high levels of arsenic are susceptible to develop liver, bladder, skin and lung cancer (Cheu et al., 2001; Smith et al., 1998; Steinmaus et al 2000; Tseng et al., 1968; Bates et al., 1992; Schwartz, 1997; Blot and Fraumeni, 1995; Bates et al., 1995). In addition to its carcinogenic effects, As exposure has been suggested to play a role in black foot disease (a form of peripheral vascular disease) (Tseng et al., 1996), type II diabetes mellitus (Tseng et al., 2000) and cardiovascular disease (Engel et al., 1994).

Cancer of the liver, common in developing countries is an important member of the various types of cancer associated with chronic arsenic ingestion based on epidemiological evidence (IARC, 1987). These health

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effects of exposure or ingestion of arsenic are of major public health significance as over 200 million people, (the population of the whole of Africa thirty years ago) largely in developing countries, are exposed to As in drinking water. This contamination is currently greatest in China, Bangladesh and India where over 60% of drinking water may be affected.

Despite these major health implications of As, its contribution or potential contribution to serious health problems in developing countries is insufficiently recognized. The primary purpose of this review is to focus on the health or potential (occult) health effects of arsenic contamination in developing countries, examining the public health significance, pragmatic methods of prevention of associated toxicity including carcinogenicity, and priorities within an economic framework in which costs, cost-effectiveness and cost benefit analysis will also be highlighted.

## History of Arsenic Exposure

After the Second World War there was a marked departure from inorganic chemicals as pesticides into the use of carbon-based or organic pesticides; a few of the old materials however persisted (Carson, 2002). Chief among these is As, which is still the basic ingredient in a variety of weed and insect killers (Carson 2002) which are of particular relevance to developing countries as an attempt to enhance food production to feed the teeming population, thus accentuating the risk of exposure.

Furthermore, As is a highly toxic mineral occurring widely in association with the ores of various metals (many developing countries have a significant proportion of their economy dependent on mineral exploitation and mining), and in small amounts in volcanoes, in the sea and in spring water, a major source of water for many rural populations in developing countries. The relations of As to man are varied and historic. The tasteless nature of As compounds makes them more dangerous toxicants. This property has been exploited to make As a favourite agent of homicide from long before the time of ancient poisoners to the present (Carson, 2002). As is one of the constituents in English chimney soot and along with certain aromatic hydrocarbons is considered responsible for the carcinogenic action of the soot which was first recognized about two centuries ago by the English physician, Percival Pott (Pott 1775). The economic situations in most developing countries imply the use of low technology based domestic and industrial processes, many of which are associated with increased generation of soot.

## Association between Arsenic and Cancer in Humans and Animals

Cancer is perhaps the most feared of the consequences of exposure to environmental contaminants. The association between As and cancer in men and animals is historic. Huerper (Huerper, 1957), gives a fascinating account of the consequences of exposure to As. Reichenstein, a city in Silesia, for almost a thousand years had been the site of mining for gold and silver ores and

for several hundred years for As ores. As the centuries progressed, As wastes accumulated in the vicinity of the mine shafts and were swept down from the mountains. Underground water also became contaminated and As entered the drinking water. For several centuries a significant segment of the population suffered from the disease known then as Reichstein disease, a chronic form of arsenicism with accompanying disorders of the liver, skin, the gastrointestinal and nervous systems (Hutchinson, 1895). Malignant tumors were also commonly found in Reichstein disease. Reichstein disease is now mainly of historic significance; improved modern water supplies has largely eliminated As from water supplies. This is largely only true in developed countries. As still constitutes a significant contaminant in water supplies in many developing countries (NRC, 1999, Bates et al., 1995). In Cordoba province in Argentina, for instance, chronic As poisoning accompanied by arsenical skin cancers is endemic because of the contamination of drinking water derived from rock weathering containing arsenic. The eradicated Reichstein disease can be easily recreated or simulated as well as the endemic arsenicism in Cordoba by protracted use of arsenical insecticide which is common in developing countries in an attempt to eradicate disease and improve agricultural yield to feed the teeming populations in these countries.

Though As contamination largely focuses only on man, As contaminated environment affects also animals. An interesting report emanating from Germany in 1936 illustrates this (Carson, 2002).

Radiation from certain rocks or As washed out of soil or rocks to contaminate food or water supplies have been associated with malignancy. The history of cancer is long but our recognition of the etiologic agents causing it has been slowly evolving. The first recognition of the involvement of environmental agents in the carcinogenic process was first described by Pott (1775). He observed that the scrotal cancer so common among chimney sweeps must be caused by the soot that accumulated on their bodies. Though he could not defend his hypothesis in the strict scientific manner, modern research methods have now isolated the harmful constituents in soot and vindicated Pott. For over a century after Pott's classical observation, very little progress was made on the involvement of chemicals in the human environment and their relationship to cancer by repeated skin contact, inhalation or swallowing. However, it had been observed that skin cancer was prevalent among workers exposed to As fumes in wales (Carson, 2002). It was also observed that workers in the cobalt mines in Saxony and in the uranium mines at Joachimsthal in Bohemia were susceptible to a pathology of the lungs, later identified as cancer (Carson, 2002). These events were, however, pre-industrial, before the explosion of industries, the industrial revolution, a phase that most developing countries appear to be in today, which involves the use of As and its compounds.

A common use of As is as an insecticide. One of the earliest pesticides associated with cancer is As, found in sodium arsenite as a weed killer, and in calcium arsenate and various other compounds as insecticides. In the district

of Freiberg, Saxony, smelters for silver and lead poured As fumes into the air which was blown out, polluting the surrounding countryside and settling down upon the vegetation. Horses, cows, goats and pigs, which fed on this vegetation, showed signs and symptoms of As poisoning, such as loss of hair and thickening of the skin (Huerper, 1957). Wild animals, such as deer inhabiting nearby forests, were also observed to exhibit abnormal pigment spots and precancerous warts (Carson, 2002; Huerper, 1957).

Further evaluation indicated that one had a definitely cancerous lesion. Both domestic and wild animals were also affected by arsenical enteritis, gastric ulcers, and cirrhosis of the liver. Furthermore, sheep kept near the smelters developed cancers of the nasal sinus; and at their death As was found in the brain, liver and tumors (Carson, 2002; Huerper, 1957). Heavy mortality among insects, especially among bees was also recorded. Rainfalls washed arsenical dust from the leaves of vegetation and carried it along into the water of brooks and pools; a great many fish died. Apart from the food chain effect of As the effect on direct agricultural production, including animal husbandry, a major economic activity in many developing countries, is obvious. Detailed studies, including nutritional investigations conducted in Taiwan, showed an increased rise of blackfoot disease in Taiwan (Tseng et al., 1996; Valenzuela et al., 2005). Additionally, the combination of low intake of micronutrients and chronic exposure to As in drinking water has been considered to lead to greater susceptibility to carcinogenesis in Taiwan (Cheu et al., 2001; Tseng et al., 1968).

## Metabolism of Arsenics

In most mammals, including humans the biomethylation of inorganic As is an essential step for its elimination. This leads to the synthesis of organic arsenicals. Among the most recognized arsenical metabolites are monomethyl arsenic acid (MMA) and dimethylarsinic acid (DMA). DMA is formed through alternating reduction of pentavalent arsenate to trivalent arsenite and addition of methyl groups through the action of methyl transferase enzymes, reactions that require S-adenosine methionine. The metabolites are subsequently eliminated through the urine. Cullen et al, (1984) have proposed a sequence for methylation of inorganic As, though the complete sequence of events is still incompletely elucidated.

$i\text{AS(V)} \succ i\text{AS(III)} \succ \text{MMA(V)} \succ \text{MMA(III)} \succ \text{DMA(V)} \succ \text{DMA(III)}$

The methylation of arsenic was initially considered a detoxification process. Recent studies have, however, shown that some of these metabolites can also be carcinogenic (Gebel, 2001). A number of factors have been identified in different mammalian species, including humans, which affect methylation (Bertolero et al., 1981; Buchet et al., 1980; Tam et al., 1997; Vahter, 1981) These include gender, age, ethnicity, dose, route and form of administration, pregnancy, nutritional status and genetic

polymorphism (Vahter, 2001). Available evidence suggests that arsenic biotransformation involves both bioinactivation and detoxification steps (Vahter, 2001). The possibility exists that DMA plays a significant role in arsenic carcinogenicity in humans, therefore, elucidation of the mode and stage at which it exerts or commences its deleterious effect deserves attention, as this will enhance not only cancer risk assessment but also cancer prevention strategies. This has received a good deal of attention recently (Wanibuchi et al., 2004) and will be examined in greater detail subsequently.

Another major metabolite of As that is important for its toxic and carcinogenic potential is MMA. In combination with As metabolites, it has been found to promote preneoplastic lesions in the liver possibly through a mechanism of oxidative stress in experimental models (Nishikawa et al., 2001). This appears to hold more carcinogenic potential for humans who are more susceptible to the health hazards of As (Goerring et al., 1999). This is probably of greater significance in developing countries where the biologic processes for biological protection to arsenicals, such as the immune system and the antioxidant defense system, are compromised (Anetor et al., 2005)

## Studies of Arsenic Metabolites

Improved understanding of As metabolites is important because they are currently considered more potent in toxicity than inorganic As. Earlier studies on As epidemiology have concentrated on inorganic arsenite AS(III) and arsenate AS(V); experimental evidence was limited until recently (Yamanaka et al., 1989). DMA(V) has since then been consistently demonstrated to have a wide range of carcinogenic effects in F344 rats in our laboratory and others around the world. (Yamamoto et al., 1995) investigated the promotion effects of DMA(V) in a two-stage carcinogenicity test after multi-organ initiation and found that DMA(V) significantly enhanced tumor formation in the urinary bladder, kidney, liver and the thyroid gland. A study to assess the promotional activity of DMA(V) on rat liver carcinogenesis and possible mechanisms involved (Wanibuchi et al., 1997), using the medium-term bioassay (Ito test) and varying doses in drinking water for six weeks, showed that DMA(V) increased the excretion of 8-hydroxydeoxyguanosine (8-OHdG), elevated cell proliferation (PCNA) and increased the number and areas of glutathione S-transferase placental form (GST-P) positive foci. Ornithine decarboxylase (ODC) was also increased in the liver exposed to DMA(V); consistent with dose-dependent promotion of DMA(V), it also increased oxidative DNA damage and increased cell proliferation, all consistent with the carcinogenic potential of this major As metabolite.

In another experiment investigating dose-dependent promotion effects of DMA(V) on a medium-term rat urinary bladder bioassay, DMA(V) was administered at doses ranging from 2 to 100ppm in drinking water for 32 weeks after BBN initiation. Dimethylarsinic acid exerted dose-dependent promoting activity; 10ppm was the

minimum effective dose. Increase in 5-bromo-2-deoxyuridine (BrdU) labeling index was observed suggesting that enhanced turnover may play a major role in the profound promoting effect of DMA(V) (Li et al., 1998). Though most earlier studies largely examined the promotional effects of DMA(V), there are indications it may have other potentials. Previous investigators (Li et al., 1998; Chen et al., 1999) have reported that DMA(V) demonstrated promotional effects in the National Cancer Institute (NCI) - Black Reiter rat (NBR), which is more resistant to BBN urinary bladder carcinogenesis compared to other strains. Additionally, NBR rats, which lack alpha-2-globulin-synthesizing ability, demonstrated induction of preneoplastic lesions (papillary or nodular hyperplasia) of bladder in the DMA(V) treated group (Wanibuchi et al., 1996). It is noteworthy that the induction of preneoplastic lesions was significantly more increased in the DMA(V) treated group than the carcinogen BBN treated group. Furthermore, carcinomas were enhanced as well as increased in BrdU labeling index of the urinary bladder epithelial cells for the DMA(V) treated group. It is probable that apart from promotion activity, DMA(V) may also have initiation potential in some of these experiments.

These observations have a number of far reaching implications for developing countries that are at greater risk of As exposure. This may be aggravated by the known interaction between infections and chemicals in carcinogenesis (Clayson, 2001). This may arise from the inflammatory changes associated with infections, which may enhance the effect of chemicals through oxidative damage and consequently the carcinogenic process. Toxic metal exposure, though strictly controlled and rather uncommon in the developed countries, is seen quite often in developing countries (Ercal et al., 2001), putting them at greater risk of toxic metal associated pathology.

### **Mode of Arsenic Toxicity and its Health Effects in Developing Countries**

Like other heavy metals As has electron-sharing affinities that can result in formation of covalent attachment (Bondy, 1996). These attachments are mainly formed between heavy metals and sulphhydryl groups of proteins (Quig, 1998). The tripeptide, glutathione (GSH), found in mammalian tissues including humans, is present at millimolar concentrations and accounts for over 90% of the total non-protein sulfur. The physiological and pathological roles of GSH in cellular damage, including those due to As, are well recognized (Meister, 1994). Interactions of toxic metals with GSH metabolism are an essential part of the toxic response of many metals (Hultberg et al., 2001), including As.

When GSH is depleted by any metal, GSH synthesizing systems adjust to make more GSH from cysteine via the  $\gamma$ -cysteine glutamyl cycle, GSH is usually not effectively maintained, if GSH depletion continues because of chronic ingestion of As in drinking water (Quig, 1998; Meister, 1994). Several enzymes involved in antioxidant defenses may protect against this imbalance. This is most unlikely in most developing countries because

of prevailing micronutrient deficiency disorders which are endemic in these countries (Underwood and Smitasiri, 1999). Micronutrients are the source of most antioxidant agents; this puts populations in these countries at a greater disadvantage to the health effects of As.

Additionally, enzymes may become inactive due to direct binding of As to their active sites, especially if the sites contain sulphhydryl groups (Quig, 1998). Furthermore, zinc, which serves as a cofactor for many enzymes, may be replaced by As making the enzyme inactive. Zinc deficiency is prevalent in most developing countries (Gibson, 1994). Zinc interestingly is essential for vitamin A metabolism and deficiency of vitamin A predisposes to cancer and skin disease, thus potentiating the effect of As. All these in concert will put populations in developing countries at greater risk to the adverse health effects of As contamination. As previously indicated, As is associated with several diseases including diabetes (Lai et al., 1994), hypertension (Chen et al., 1995) and tumors of the skin, bladder, liver and lungs (Chen and Wang, 1990). The mechanisms by which As may induce cancer have not been fully elucidated (NRC, 1999; Modi et al., 2004). However, besides various mechanisms that have been proposed, oxidative stress is currently the theory most accepted as the major factor in arsenic-induced carcinogenesis. Arsenic-induced oxidative stress has been comprehensively reviewed by Kitchen et al (Kitchen, 2001) and Bernstam et al (Bernstam and Nriagu, 2000). However, recent reports (Cohen et al., 2006) are beginning to challenge the oxidative stress phenomenon in arsenic induced carcinogenesis as the major factor in this disorder. This is yet to be universally recognized. It remains to see how the scientific community will receive this emerging concept.

Malnutrition in developing countries implies greater vulnerability to arsenic-induced cancer and other non-communicable diseases, which are already known to be steadily on the increase in many developing countries (Parkin et al., 1993). The contribution of As to the raised disease burden in these countries is uncertain, but it is most likely to be substantial given the huge population ingesting As in drinking water and the geogenic nature of As. Various lines of evidence for arsenic-induced free radical formation have been suggested (Ercal et al., 2001).

These include:

- 1) Direct evidence for arsenic-induced free radical formation
- 2) Indirect evidence for arsenic-induced oxidative stress
- 3) Effects of arsenic on cellular antioxidant defense systems

Biomarkers of oxidative stress have been reported with arsenite treatment over the last decade 8-OHdG, a major biomarker of ROS-induced DNA damage, has been associated with As exposure. Yamanaka et al(2001) observed increased 8-OHdG in urine samples of mice gavaged with 220 mg/kg of DMA(V). Furthermore, in a long-term carcinogenesis study of rats, hepatic 8-OHdG levels were found to be raised in DMA-treated rats,

suggesting an increased rate of ROS attack on DNA (Wanibuchi et al., 1997). One possible implication of this in developing countries is greater genome instabilities, which is the precursor of proliferative disorders and may indeed be contributory to the rising incidence of malignancies in developing countries (Parkin et al., 1993; Phillip, 1985) where extensive populations in these countries consume As in drinking water.

## **Carcinogenicity and Arsenic Contamination**

As ground waters are contaminated with toxic substances and other chemicals, there is danger that not only poisonous but also, cancer producing substances are being introduced into the public water supplies. An investigation at the National Cancer Institute (NCI) in USA warned long ago that the danger of cancer hazards from the consumption of contaminated water will grow considerably within the foreseeable future (Carson, 2002; Huerper, 1957). Indeed a study in Holland in the early 1950s (a period comparable to the phase of development in most developing countries) provides support for the view that polluted water ways may carry a cancer hazard. Cities obtaining their drinking water from rivers had a higher death rate from cancer than did those whose water supply came from sources presumably less susceptible to pollution, such as wells (Carson, 2002).

As, the environmental contaminant most clearly established as causing cancer in man, has been reported in two historic cases in which polluted water supplies caused wide spread occurrence of cancer. In one case, As came from the slag heaps of mining operations, (an activity on the increase in Nigeria and other developing countries as previously indicated) (Carson, 2002; Phillip, 1985). These conditions may easily be enhanced in areas of mining activities when the As content of the earth crust is brought to the surface. The surface in such areas becomes heavily contaminated with As. The rains then carry part of the As into streams, rivers and reservoirs, as well as into the vast subterranean seas of ground water. This in turn carries with it higher carcinogenicity events in the affected communities.

## **Nutritional Status and its Effect on Arsenic Contamination and Health in Developing Countries**

Among the factors that influence cellular response and susceptibility to environmental contaminants, including As, nutrition has received increasing recognition in recent years (Chow, 2002). Although the mechanism of chronic As toxicity is not fully understood, studies of populations in Taiwan (Tseng et al., 1968), Chile (Bogono and Greiber 1971; Bogono et al., 1977) and Mexico (Cerbian et al., 1983), living in regions endemically contaminated with As (0.3-0.8mg/L) in drinking water, have manifested the well known signs of As toxicity such as hyperpigmentation, keratosis, skin cancer and black foot disease (NRC, 1999; Cheu et al., 2001; Smith et al., 1998; Tseng et al., 1968; Tseng et al., 1996).

Some other populations exposed to similar As levels

in economically advanced countries like the United States did not exhibit these health effects (Valentine et al., 1992; Southorick et al., 1983; Harrington et al., 1978). This suggests the possibility that other factors may play significant roles in the expression of chronic As toxicity, including differences in duration of exposure to As and importantly to nutritional status.

Direct studies on the effects of nutrition on As toxicity have been hindered by lack of an adequate animal model of chronic As toxicity and carcinogenicity. Nutritional status in developing countries may be particularly relevant in that populations exhibiting As toxicity have mostly been those of low economic status and also suffering from some degree of malnutrition (Gebel, 2001).

Nutritional studies in Chile revealed that food energy and total protein intakes below the recommended daily allowances (Zaldivar et al., 1978) are equivocal. The possible effect of the possible risk factor of low protein diet and health effects of As contamination in disadvantaged populations prompted Engel and Receivour (Engel And Receivour, 1993) to study low protein diet. They found that the exposed population in Taiwan had protein and methionine intakes that met recommended levels. This may however not be representative of the majority of the population in developing countries. Moreover, questions have been raised whether the cysteine and methionine levels they suggested as being required for detoxification of As toxicity are below the methylation capacity needed to detoxify extra As burden, even though those levels may be adequate for normal populations (Beck et al., 1995) Mushak and Crocetti (Mushak and Crocetti 1995) have argued theoretically that these micronutrient levels may still be adequate.

Different micronutrients have different effects on the toxicity of metals; these have also been shown to exhibit different manifestations in different regions of developing countries. Selenium deficiency has been known to be endemic in China, giving rise to disorders such as Keshan's disease and Kaschin Beck's disease. Selenium status has been demonstrated to be inversely related to the risk of hepatocellular carcinoma mortality (Sakoda et al., 2005). It is also considered that As contamination may be contributory to hepatocellular carcinoma. Selenium deficiency has been suggested to play a role in arsenic-associated health abnormalities. The role of selenium deficiencies in arsenic-induced skin cancer has also been discussed (Wagner et al., 1979). A study conducted by Valentine et al (Valentine et al., 1994) initially evaluated the differences in selenium intakes between populations in rural northern Mexico and a region in California known to be exposed to equal levels of As in drinking water but exhibiting different degrees of toxicity. This study interestingly revealed that the only difference nutritionally from the recommended daily allowances was a greater lack of vitamin A in the Mexican study. It is noteworthy that vitamin A, which plays an essential role for normal vision, growth, immune function and maintenance of epithelial cells (disorders of which increase carcinogenicity), is one of the major deficient micronutrients of current focus by the international community in the developing world (Levin et al., 1993).

These studies in humans, along with an understanding of how various forms of As exert their toxicities and carcinogenicities, how they are metabolized and detoxified or ameliorated, and other ways by which As may effect populations in developing countries may provide better insight into how nutritional status may enhance host defenses against As toxicity, and the corollary, how endemic deficiencies of protective micronutrients put the populations in the developing world at greater risk of the deleterious health effects of As toxicity.

This may be particularly important in the case of cancer in which As has been strongly implicated. Most cancers develop from a single abnormal cell through several successive rounds of mutations and natural selection. This process commences with a change in the cell's DNA sequence. DNA damage has to occur during its replication and there exist several phenotypic expressions of this alteration. The study of the biochemical and molecular alterations during cell transformation are the central theme of many research activities regarding As toxicity and carcinogenicity. These are usually regulated by enzymes and processes dependent on micronutrients or modulated by them.

### **Diet and Mechanisms of Arsenic Toxicity: Implications for Developing Countries**

Pentavalent arsenic, As(v), is chemically similar to phosphorus. Arsenate uncouples oxidative phosphorylation by substitution for phosphate in ATP synthesis (Mitchell et al., 1971; Gresser, 1981). One implication of this is reduced energy for work performance in countries that are dependent mainly on manual or semi-manual operations, which will in turn result in low productivity and poor economy and health.

That phosphate and arsenate can share the same transport mechanism has been demonstrated by decreased intestinal absorption of arsenate with phosphate infusion in the rat (Gonzalez et al., 1995), owing to the considerable abundance of phosphate in most natural foods. Theoretically, dietary phosphate could competitively displace arsenate uptake and decrease the toxicity of As from contaminated drinking water.

The use of phosphate supplement to ameliorate arsenic-induced health effects has been largely unexplored. Phosphorus, being so abundant in natural foods that deficiency states are uncommon, naturally may indeed have offered some degree of protection against As related health effects. There is need for a proper investigation of this possibility to understand the decreased risk, if any, that phosphate offers in these populations, and the converse owing to famine and starvation common in developing nations.

Another chemical similarity exists between As and selenium, which generally allows for antagonistic effects between both metalloids (Levander, 1977). Unlike the situation with phosphate no interaction exists between As and selenium at the gut level. Instead, As and selenium each enhances the biliary excretion of the other (Levander and Baumann 1966). This mechanism may be useful in controlling As ingestion. But a report in the late 1970's

suggests that As appears to abolish the anti-carcinogenic effects of selenium (Schrauzer et al., 1978), though this requires further clarification to demonstrate the benefit of the protective role of selenium against As toxicity. More recent studies tend to confirm this protective effect. Selenium is now known to alleviate As toxicity (Sakoda et al., 2005). Selenium ion also partially reverses or prevents the uncoupling of oxidative phosphorylation by arsenate (Hill, 1975). Selenium has also been reported to decrease the teratogenic toxicity of arsenate in experimental models when both selenate and arsenate salts are administered simultaneously (Ferm, 1977).

All of the above observations suggest that adequate or even extra selenium in the diet may alleviate the health effects associated with As contamination in the populations at risk in developing countries.

Further studies on arsenic-essential nutrient interactions show decreases in selenium and iron levels during the progression of black foot disease in which As has been implicated (Tseng et al., 1996; Wang et al., 1994). The interaction between As and protein-containing food substances deserves some attention concerning As related health effects. Trivalent arsenicals, such as arsenite, interact with sulphhydryl containing amino acids, peptides and proteins (Winski and Carter, 1995). Arsenite exerts its cellular toxicity by binding major sulphhydryl groups resulting in enzyme inhibition. As earlier indicated, GSH is a thiol tripeptide and antioxidant that plays a key role in many xenobiotic detoxification reactions, including As detoxification. Several studies have demonstrated that cellular toxicity is inversely related to intracellular GSH levels and that As toxicity is exacerbated by GSH depletion. But GSH level is dependent on the nutritional status of the subjects (Bray and Taylor, 1993). The sources of sulphur amino acids on which GSH is dependent understandably are low when intake of the sulfur amino acids is low in the diet. Thus, content of low sulfur containing amino acids or poor protein diets (common in developing countries) generally leads to low GSH availability, and this is associated with more pronounced arsenic-induced cellular toxicity (Baumann et al., 1988). Poor protein diet also aggravates As toxicity in another manner; low intake of sources of methyl groups, such as methionine, choline or protein, decrease As excretion (especially excretion of the major metabolite DMA(V) in urine) and enhances retention of As in tissues (Vahter et al., 1987). Diets deficient in good sources of methyl donors, such as methionine and choline, decrease S-adenosyl methionine (SAM) levels, thus enhancing intoxication processes (Shivapurkar and Poirier, 1983). Zinc is another essential micronutrient that is deficient in major developing countries, especially in Africa (Gibson, 1994). Zinc induces increased synthesis of the low molecular-weight, cysteine rich metal binding protein, metallothionein (MT), suggesting that As toxicity can be decreased by this mechanism, and thus the prevalence of zinc deficiency in these resource poor countries may predispose them to the adverse effects of As exposure. Experimental evidence, however, suggests that this mechanism may not account for the protection zinc offers against arsenic toxicity (Kreppel et al., 1994). Despite the

uncertainty of the mechanism of the tolerance to As toxicity, optimum or even above average zinc intake is associated with increased elimination of As. Thus, efforts to improve zinc nutrition in developing countries in which As contamination is a major public health concern may be a pragmatic measure to ameliorate the health problems of As contamination.

## **Oxidative Stress Associated with Arsenic Contamination**

While the modes of action are complex and multifarious, increased oxidative stress is directly or indirectly responsible for causing and/or exacerbating the adverse effects from exposure to a number of environmental agents (Chow 1991), including As. Oxidative damage, which may exacerbate many of the various pathologies already prevalent in the major developing countries, is a consequence of decreased antioxidant potential and increased oxidative stress (Chow 1979). Current hypotheses favour the view that lowering oxidative stress can have clinical benefit. More and more evidence indicates that a proper balance between oxidants and antioxidants is involved in maintaining health and longevity and that altering this balance in favour of oxidants may result in pathological responses causing functional disorders and disease.

The metabolism of As generates reactive oxygen species that may lead to the damage of major biomolecules, including DNA, proteins, lipids and other body constituents of which these are component parts, including antibodies.

As concentrations in tissues such as the liver, heart and kidneys have been positively correlated with lipid peroxidation in rats (Ramos et al., 1995). Studies in human fibroblasts (Lee and Ho, 1995) with arsenite indicate that it induces the body's antioxidant activities.

Arsenite has been reported to induce haem oxygenase, resulting in haem degradation, iron release and decreases in the cytochrome P450 biotransformation enzymes that are involved in both endogenous and xenobiotic metabolism (Albores et al., 1995). Another major antioxidant, enzyme superoxide dismutase (SOD) activity, was also increased by sodium arsenite treatment of human fibroblasts (Lee and Ho, 1995), again reiterating the role of antioxidants in combating the health disorders associated with As contamination.

The dietary antioxidants, vitamins A and E, may also alleviate As toxicity and can be manipulated as a dietary tool against As contamination. Experimental studies confirm that supplementation of vitamin E could at least in part prevent arsenite-induced death of human fibroblasts (Lee and Ho, 1994).

A pragmatic and sustainable method of deriving the benefit of the antioxidant defense system against the huge problem of As contamination in developing countries is copious dietary intake of fruit and vegetables, particularly coloured vegetables (rich in vitamin A and  $\beta$ -carotene), which have been inversely associated with the odds ratio of lung cancer in tin miners at high risk of arsenic exposure and associated risk factors (Forman et al., 1992).

Famine and starvation, which are common in many developing countries especially in Africa as a result of wars and other social strife, may aggravate the health effects of As contamination in these countries. Chronic exposure to As may result in mitochondrial changes that block coenzymes and enzymes such as lipoic acid and dehydrogenase involved in bioenergetics and ultimately hypoglycaemia. The inhibition of pyruvate dehydrogenase gluconeogenesis (Szsincz and Forth, 1988) may also contribute to this, suggesting that protein and micronutrients may be more important than carbohydrate deficiency caused by suppression of gluconeogenesis, which may aggravate As poisoning. Studies in experimental models show that starved rats were more susceptible to As(III) than rats with free access to food (Szsincz and Forth, 1988). Starvation, so common in some developing countries at risk of As contamination, may also involve other important nutritional factors.

As pointed out earlier As exposure may play a role in the development of diabetes mellitus, based on studies of the association with As in drinking water in Taiwan (Laim et al., 1994) in addition to occupational exposure to As in copper smelter workers in Sweden (Rahman and Axelson, 1995), reminiscent of what happens in smelters dotted all over many developing countries.

Nutritional modulation by way of supplements may attenuate the degree of As toxicity and carcinogenicity. Arsenite-associated bone marrow chromosomal aberrations were found to be reduced in experimental models fed crude garlic extract (Roychoudhury et al., 1996). The beneficial effect may derive from arsenite's affinity for the sulphur moieties in many of the chemical constituents of garlic extract. This garlic and the related onions, which are fortuitously common in the regions where As contamination is most prevalent, can, through appropriate health education, be employed in prophylaxis against the adverse health effects of As contamination. This experimental report has recently been corroborated by the observation of Fukushima et al (2001) that environmental compounds are likely involved in the development of many human cancers, that their elimination would prevent cancer. But this is not practical, thus it is important to search for naturally occurring or synthetic compounds that may suppress or prevent the process of carcinogenesis. As contamination appears a very appropriate candidate for chemo-preventive intervention.

This view also appears consistent with epidemiological and experimental observations that strongly correlate the dietary intake of food, vegetables and medicinal plants with reduced risk of cancer and other health problems (Wattenberg, 1990; Steinmetz and Potter, 1991; Sultana et al., 2005). Whatever the mechanisms and degree of actual success achieved with nutrients, it is clear that nutritional status may play an important role in the expression of As toxicity. The recent study of Wei et al (2005) on effects of co-administration of antioxidants and arsenicals and the inhibitory effects of antioxidants in bladder carcinogenesis again elegantly demonstrates the promise of micronutrients to pragmatically combat the serious health effects of As contamination in affected

populations.

In the past it was thought that the most important nutritional factor, at least in principle, is adequate protein intake for the detoxifying methylation reactions. With the demonstration of the carcinogenic nature of some of the major methylated compounds of As (Sultana et al., 2005; Wei et al., 2005; Wei et al., 1999; Wei et al., 2002); this is unlikely. Rather it is most probable that given the mode of toxicity of As largely through oxidative stress, it is likely that micronutrients, which are constituents of the antioxidant defense system, may be more effective as weapons against the adverse health effects of As contamination in developing countries. The fact that studies of highly exposed populations from developing countries have been employed as the basis for setting regulatory levels in other countries implies that the effect of nutritional status on As contamination occupies a central position in combating the adverse health effects of As. Further studies are, however, required to determine the specific nutrients that are more protective against As toxicity and to fully establish if correction of dietary deficiencies will be adequate to handle the magnitude of the As burden or whether megadoses will be required remains speculative. Though the recent study of Islam et al (2004) on the manipulation of air and bacteria in controlling As contamination in water is promising, its application in the poorly educated populations in developing countries may be fraught with difficulties.

## Conclusions

As contamination is undoubtedly a problem in many developing countries, although the true magnitude of the problem in some others, particularly in Africa, is incompletely elucidated. Scattered reports and experimental evaluations indicate that the burden there is also large.

Data from mechanistic studies indicate that micronutrients have a significant role in modulating the toxicity of As.

Micronutrients interact with As at various stages in the body-affecting metabolism, including absorption, distribution and excretion. One other mechanism is oxidative stress, which accompanies micronutrient deficiency disorders so common in these countries. One implication of this is that populations in developing countries who unfortunately also consume diets deficient in micronutrients will also be increasingly susceptible to the adverse health effect of As.

Evidence abounds that adequate diet can ameliorate the deleterious effects of As contamination. The studies that have evaluated the effects of nutritional status on As toxicity will also facilitate the process of risk assessment in these populations.

Additionally, the opportunity provided by the interaction of nutrition with As contamination shows clearly that this is a pragmatic approach to curtail the adverse health effects of As contamination. Consequently, it is no longer acceptable to ignore the effects of nutrition on the health of the huge population of humans exposed to As contamination. The traditional methods for

controlling toxic exposure generally are to remove the affected individuals or population from the site of the exposure or to remove the source of exposure.

In the case of As contamination, very large populations, about 200 million people globally, particularly in India and the Gunga-Meghna-Bruhmapura (GMB) of Bangladesh. Under such circumstances, it is impracticable to remove everyone from exposure or to remove the source of exposure. In situations like this, nutritional intervention as a form of chemoprevention may prove to be the best host defense against As contamination and it appears within the reach of these relatively resource poor countries. Thus, the nutritional intervention approach will go a long way to mitigate the problem of As contamination of these large populations in developing countries, which eventually affects their health and ultimately their economy - a vicious circle.

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