

## 5. Summary of Data Reported and Evaluation

### 5.1 Exposure data

Exposure of high levels of arsenic in drinking-water has been recognized for many decades in some regions of the world, notably in China, Taiwan (China) and some countries in Central and South America. More recently, it has been discovered that a number of other regions have drinking-water that is highly contaminated with arsenic. In most of these regions, the drinking-water source is groundwater, naturally contaminated from arsenic-rich geological formations. The primary regions where high concentrations of arsenic have been measured in drinking-water include large areas of Bangladesh, China and West Bengal (India) and smaller areas of Argentina, Australia, Chile, Mexico, Taiwan (China), the USA and Viet Nam. In some areas of Japan, Mexico, Thailand and other countries, mining, smelting and other industrial activities have contributed to elevated concentrations of arsenic in local water sources.

Levels of arsenic in affected areas may range from tens to hundreds or even thousands of micrograms per litre, whereas in unaffected areas levels are typically only a few micrograms per litre. The WHO guideline recommends that levels of arsenic in drinking-water should not exceed 10 µg/L. Arsenic occurs in drinking-water primarily as arsenate (As<sup>V</sup>), although in reducing environments significant concentrations of arsenite (As<sup>III</sup>) have also been reported. Trace amounts of methylated arsenic species are typically found in drinking-water, and higher levels are found in biological systems. Inorganic arsenic (arsenate plus arsenite) is the predominant form of arsenic in drinking-water.

In many areas where contamination of drinking-water by arsenic has been reported, current exposures have been reduced by various interventions.

## 5.2 Human carcinogenicity data

In previous monographs, the evidence of carcinogenicity to humans of exposure to arsenic and arsenic compounds, such as medical treatment with Fowler's solution and inhalation exposure of mining and smelting workers, was evaluated as *sufficient*.

Informative epidemiological studies of cancer in relation to arsenic in drinking-water include ecological studies and fewer case-control and cohort studies. For most other known human carcinogens, the major source of causal evidence derives from case-control and cohort studies, with little evidence from ecological studies. In contrast, for arsenic in drinking-water, ecological studies provide important information for causal inference. The reasons include large exposure contrasts and limited population migration. As a consequence of widespread exposure to local or regional water sources, ecological measurements provide a strong indication of individual exposure. Moreover, in the case of arsenic, the ecological estimates of relative risk are often so high that potential confounding with known causal factors cannot explain the results. Hence, in the reviews that follow, ecological studies are presented in detail.

### *Urinary bladder cancer*

The Working Group evaluated ecological studies in Taiwan (China), Chile, Argentina and Australia, cohort studies from Taiwan, Japan and the USA and case-control studies in Taiwan, the USA and Finland.

There is extensive evidence of increased risks for urinary bladder cancer associated with arsenic in drinking-water. All studies that involved populations with high long-term exposures found substantial increases in the risk for bladder cancer. Key evidence derives from ecological studies in Taiwan and Chile. In Taiwan, the evidence is supported by case-control studies and cohort studies within the exposed communities that demonstrate evidence of dose-response relationships with levels of arsenic in drinking-water. The evidence of increased mortality from bladder cancer in Chile comes from a large population with exposure to arsenic in all major cities and towns of the contaminated region.

There is also evidence of increased risks for bladder cancer from a small cohort study in Japan of persons drinking from wells that had been highly contaminated with arsenic wastes from a factory and an ecological study from Argentina with moderate exposure to arsenic in well-water. Two case-control studies that investigate low exposure to arsenic found increased risks with increasing exposure in one or more subgroups.

Considered overall, the findings cannot be attributed to chance or confounding, and they are consistent, with strong associations found in populations with high exposure. There is evidence of dose-response relationships within exposed populations.

### *Lung cancer*

The Working Group evaluated ecological studies using mortality data in Taiwan (China), Chile, Argentina and Australia, cohort studies in Taiwan, Japan and the USA and case-control studies in Taiwan and Chile.

Increased risk for lung cancer was consistently observed in ecological, case-control and cohort studies in Taiwan, Japan, Chile and Argentina. Evidence for a dose-response relationship between arsenic in drinking-water and risk for lung cancer was also observed in ecological studies in Taiwan and Argentina, in cohort studies in south-western and north-eastern Taiwan and Japan and in case-control studies in south-western Taiwan and Chile. The potential confounding effect of cigarette smoking was ruled out by direct and indirect evidence in studies from Taiwan and Chile.

Considered overall, the findings cannot be attributed to chance or confounding, are consistent and demonstrate strong associations in populations with high exposure. There is evidence of a dose-response relationship.

#### *Skin cancer*

The Working Group evaluated ecological studies from Taiwan (China), Mexico, Chile and the USA, cohort studies from Taiwan and a case-control study from the USA.

The recognition that arsenic was potentially carcinogenic arose from occurrences of skin cancer after ingestion of medicinal arsenic, arsenical pesticide residues and arsenic-contaminated drinking-water. Skin cancer is a commonly observed malignancy related to contamination of drinking-water with arsenic. The characteristic arsenic-associated skin tumours include keratinocytic malignancies (non-melanoma skin cancers), in particular squamous-cell carcinomas, including Bowen disease, and multiple basal-cell carcinomas.

Ecological studies, largely from the south-west of Taiwan, indicate substantially elevated incidence of, prevalence of and mortality rates for skin cancer associated with drinking-water highly contaminated with arsenic, with evidence of a dose-response relationship. Findings in ecological studies were substantiated in two cohort studies in the region of Taiwan that is endemic for arsenic. Increased mortality from skin cancer was found in Chile. A high prevalence of skin lesions, including skin cancers, was found in rural regions of Mexico. An excess risk for skin cancer was observed in a case-control study in the USA conducted in an area with lower concentrations of arsenic in drinking-water. A cohort study from the south-west of Taiwan reported that differences in the levels of serum  $\beta$ -carotene and urinary arsenic metabolites may modify the risk for arsenic-induced skin cancers.

#### *Liver cancer*

The Working Group evaluated ecological studies using mortality data in Taiwan (China), Chile, Argentina and Australia, cohort studies in Taiwan, Japan and the USA and a case-control study in Taiwan of liver cancer cases identified from death certificates.

Increased mortality from liver cancer was observed in the ecological studies involving a large population with high exposure to arsenic in Taiwan. Evidence for a dose-response relationship between arsenic in drinking-water and liver cancer mortality was observed in both ecological and case-control studies in Taiwan. Increased risks were also found in small cohort studies in Taiwan and Japan. Findings on mortality from liver cancer observed in ecological studies in Chile are inconsistent.

The interpretation of these findings is limited by the small number of liver cancer cases, questionable accuracy of the diagnosis of liver cancer on death certificates and potential confounding or modifying effects of chronic hepatitis virus infection or other factors.

### *Kidney cancer*

The Working Group evaluated ecological studies in Taiwan (China), Chile, Argentina and Australia, and cohort studies from Taiwan and the USA.

All studies that involved populations with high long-term exposures to arsenic found increased risks for kidney cancer. Key evidence comes from ecological studies in Taiwan and Chile. In Taiwan, the evidence is supplemented by a small cohort study of patients with Blackfoot disease. The evidence of increased mortality from kidney cancer in Chile comes from a large population with exposure to arsenic in all major cities and towns of the region. There is also evidence of increased risk for kidney cancer in populations in Argentina with moderate exposure to arsenic in well-water.

Relative risk estimates for kidney cancer were generally lower than those for urinary bladder cancer, and no studies have reported dose–response relationships on the basis of individual exposure assessment.

### *Other cancers*

The Working Group evaluated ecological studies from Taiwan (China), Chile and the USA, cohort studies from Japan and the USA and one case–control study each from Canada and the USA.

Excess mortality from prostate cancer was found in south-west Taiwan. Inconsistent findings were reported for other cancer sites.

## **5.3 Animal carcinogenicity data**

Dimethylarsinic acid was tested for carcinogenicity by administration in drinking-water in mice and rats. It was also tested in two-stage initiation–promotion studies in mice and rats. Complete carcinogenicity was observed in the urinary bladder of rats and lungs of mice. Dimethylarsinic acid exerted its carcinogenic effect on spontaneous development of tumours in p53<sup>+/-</sup> and p53<sup>+/+</sup> mice. Dimethylarsinic acid is a tumour promoter in the skin and lung of mice, and in the liver, urinary bladder, kidney and thyroid gland of rats.

After perinatal treatment, arsenic trioxide induced lung adenomas in mice and, after intratracheal instillation to hamsters, it induced lung adenomas in two of three studies. Calcium arsenate administered to hamsters by intratracheal instillation induced lung adenomas. Sodium arsenate induced tumours at various organ sites in metallothionein knockout mice. Transplacental exposure of mice to sodium arsenite induced liver and lung carcinomas, ovarian tumours (benign and malignant) and adrenal cortical adenomas. Sodium arsenite promoted skin carcinogenesis in mice. Arsenic trisulfide was negative for carcinogenicity when tested in hamsters by intratracheal instillation.

## 5.4 Other relevant data

Arsenic in drinking-water is well absorbed in the gastrointestinal tract. The trivalent species of arsenic are formed *in vivo* after exposure to pentavalent arsenic. Arsenic is metabolized by a series of reductions and oxidations and by methylation reactions. Methylated trivalent arsenic is more toxic and less genotoxic than trivalent inorganic arsenic; in contrast, methylated pentavalent arsenic is less toxic and less genotoxic than pentavalent inorganic arsenic. There is a large variation in metabolism between animal species, population groups and individuals. Both inorganic arsenic and its methylated metabolites are excreted in urine.

Acute effects due to ingestion of arsenic are characterized by severe vomiting and diarrhoea with features of shock, muscle cramps and cardiac abnormalities. Subacute exposures affect primarily the respiratory, gastrointestinal, cardiovascular, nervous and haematopoietic systems.

Most reports of chronic arsenic toxicity focus on skin manifestations such as pigmentation, with depigmentation affecting trunks and limbs and keratosis affecting hands and feet. Chronic lung disease, peripheral neuropathy, hepatomegaly and peripheral vascular disease have frequently been reported in cases of chronic exposure to arsenic. Exposure to arsenic has been associated with an increased risk for diabetes mellitus. Other systemic manifestations include cardiovascular effects, abdominal pain, anorexia, nausea, diarrhoea, cerebrovascular disease, non-pitting oedema of hands, feet or legs, anaemia and generalized weakness. In a study in Taiwan (China), significantly higher mortality from cardiovascular and peripheral vascular disease was reported among patients with Blackfoot disease compared with the general population of Taiwan or unaffected residents in endemic areas of Blackfoot disease.

The acute toxicity of trivalent arsenic is greater than that of the pentavalent form. The 50% lethal dose of arsenic trioxide in mice by the oral route varies from 15 to 48 mg/kg bw, whereas the acute lethal dose in humans varies from 1 to 3 mg/kg bw. In chronic toxicity studies, arsenic inhibits mitochondrial respiration and induces apoptosis accompanied by a loss of the mitochondrial transmembrane potential. Metallothionein is thought to have a protective effect against the toxicity of arsenic.

Arsenic can modify the urinary excretion of porphyrins in animals and humans. It also interferes with the activities of several enzymes of the haeme biosynthetic pathway. The major abnormalities in urinary porphyrin excretion in chronically exposed humans are (a) significant reductions in coproporphyrin III excretion, resulting in a decrease in the ratio of coproporphyrinogen oxidase III to coproporphyrinogen oxidase I and (b) significant increases in uroporphyrin excretion.

Exposure to arsenite or arsenate results in generation of reduced oxygen species in laboratory animals and human cells. Exposure to arsenicals either *in vivo* or *in vitro* in a variety of model systems causes induction of a number of major stress-protein families such as heat-shock proteins. Recent studies in animals demonstrated altered gene expression following acute treatment with arsenic that included DNA repair genes, acti-

vation of transcription factors, such as activator protein 1, and an increase in pro-inflammatory cytokines. All of these events could play a role in the toxicity of arsenic.

Few studies have been conducted on the immunotoxicity of arsenic. All arsenic compounds evaluated in mouse spleen cells suppressed plaque-forming cell responses to sheep erythrocytes and proliferative response to mitogens. Furthermore, arsenic impaired stimulation and proliferation of human lymphocytes *in vitro*. Recent studies suggest that apoptosis may be an important mechanism for arsenic-induced immunosuppression.

Experimental animal studies have demonstrated the developmental toxicity of trivalent and pentavalent arsenic, monomethylarsonic acid and dimethylarsinic acid. Limited human data suggest that exposure to high concentrations of arsenic in drinking-water during pregnancy may increase fetal and neonatal mortality.

The genotoxicity of arsenic is due largely to the trivalent arsenicals. In humans, arsenic is a chromosomal mutagen (an agent that induces mutations involving more than one gene, typically large deletions or rearrangements). Arsenic appears to have limited ability to induce point mutations. Elevated frequencies of micronuclei, chromosomal aberrations and aneuploidy were detected in the peripheral lymphocytes or urothelial cells, or both, of people exposed to elevated levels of arsenic. *In vitro*, arsenic was not a point mutagen in bacteria. In mammalian cells, arsenic caused various types of chromosomal mutations and aneuploidy. In combination with many genotoxic agents, including ultraviolet light, arsenic was a synergistic co-mutagen. *In vitro*, arsenite was genotoxic at micromolar concentrations. Arsenate was approximately one order of magnitude less genotoxic than arsenite, dimethylarsinic acid and monomethylarsonic acid induced genotoxicity at millimolar concentrations.

Methylarsenous acid and dimethylarsinous acid are intermediary metabolites in the methylation of arsenic. Their genotoxicity has not been fully established, but recent results implicate a major role for these metabolites and reduced (reactive) oxygen species in the induction of urinary bladder cancer in rats.

## 5.5 Evaluation

There is *sufficient evidence* in humans that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin.

There is *sufficient evidence* in experimental animals for the carcinogenicity of dimethylarsinic acid.

There is *limited evidence* in experimental animals for the carcinogenicity of sodium arsenite, calcium arsenate and arsenic trioxide.

There is *inadequate evidence* in experimental animals for the carcinogenicity of sodium arsenate and arsenic trisulfide.

Taken together, the studies on inorganic arsenic provide *limited evidence* for carcinogenicity in experimental animals.

### Overall evaluation

Arsenic in drinking-water is *carcinogenic to humans (Group 1)*.