ARSENIC IN DRINKING-WATER

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1. Exposure Data

1.1 Chemical and physical data

Arsenic is the 20th most common element in the earth's crust, and is associated with igneous and sedimentary rocks, particularly sulfidic ores. Arsenic compounds are found in rock, soil, water and air as well as in plant and animal tissues. Although elemental arsenic is not soluble in water, arsenic salts exhibit a wide range of solubilities depending on pH and the ionic environment. Arsenic can exist in four valency states: –3, 0, +3 and +5. Under reducing conditions, the +3 valency state as arsenite (As^{III}) is the dominant form; the +5 valency state as arsenate (As^{V}) is generally the more stable form in oxygenized environments (Boyle & Jonasson, 1973; National Research Council, 1999; O'Neil, 2001; WHO, 2001).

Arsenic species identified in water are listed in Table 1. Inorganic As^{III} and As^{V} are the major arsenic species in natural water, whereas minor amounts of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) can also be present. The trivalent monomethylated (MMA^{III}) and dimethylated (DMA^{III}) arsenic species have been detected in lake water (Hasegawa *et al.*, 1994, 1999). The presence of these trivalent methylated arsenical species is possibly underestimated since only few water analyses include a solvent separation step required to identify these trivalent species independently from their respective

Table 1. Some arsenic species identified in water[a]

Name	Abbreviation	Chemical formula	CAS No.	pKa
Arsenous acid (arsenite)	As^{III}	$As(OH)_3$	13464-58-9	9.23, 12.13, 13.4
Arsenic acid (arsenate)	As^{V}	$AsO(OH)_3$	7778-39-4	2.22, 6.98, 11.53
Monomethylarsonic acid	MMA^{V}	$CH_3AsO(OH)_2$	124-58-3	4.1, 8.7
Monomethylarsonous acid	MMA^{III}	$CH_3As(OH)_2$	25400-23-1	
Dimethylarsinic acid	DMA^{V}	$(CH_3)_2AsO(OH)$	75-60-5	6.2
Dimethylarsinous acid	DMA^{III}	$(CH_3)_2AsOH$	55094-22-9	
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$	4964-14-1	

[a] From National Research Council (1999); Francesconi & Kuehnelt (2002); Le (2002)

pentavalent analogues. Other unidentified arsenic species have also been reported in seawater and fresh water, and could represent up to 20% of the total arsenic (Francesconi & Kuehnelt, 2002; Le, 2002).

1.2 Analysis

Studies of human exposure to arsenic and its consequences for human health require two different kinds of arsenic analyses depending on whether quantitative or qualitative results are required. Several methods have been developed and improved for the measurement of total arsenic, and have been widely used for the evaluation of drinking-water contamination and the resulting concentrations of arsenic in humans. On the other hand, analytical methods allowing arsenic speciation have gained increasing interest. The environmental fate and behaviour, bioavailability and toxicity of arsenic vary dramatically with the chemical form (species) in which it exists, the inorganic As^{III} and As^{V} being, for example, far more toxic than MMA and DMA. Thus selective methods that determine the relative concentration of the different arsenic species in drinking-water are required when more precise assessments of their impact on human health are needed.

Analytical methods for arsenic have been reviewed (National Research Council, 1999; WHO, 2001; Goessler & Kuehnelt, 2002).

The most commonly used methods for the analysis of arsenic and arsenic compounds in water and biological samples are described below, and their characteristics are summarized in Table 2.

1.2.1 *Preservation of samples*

Assessment of human exposure to arsenic through drinking-water relies on the analysis of arsenic in water and in biological samples. Biological markers may more accurately reflect total dose of exposure in populations exposed to low, but potentially carcinogenic levels of arsenic in drinking-water. Many tissues contain arsenic following exposure to the element, but not all represent useful biomarkers. For example, arsenic is removed from blood within a few hours and excreted through the kidneys and urine within a few days. Determination of arsenic in urine is commonly used as a measure of recent exposure. Hair and nails have been shown to provide reliable biomarkers for long-term chronic exposure to arsenic in humans (Karagas *et al.*, 1996, 2000). However, nails are preferred to hair since their contamination with arsenic from the air is negligible, whereas hair can adsorb 9–16% exogenous inorganic arsenic (Mandal *et al.*, 2003). Karagas *et al.* (2001a) found that measurements of arsenic in both toenails and water were reproducible over a 3–5-year period.

Depending on the sample studied and the type of analysis to be performed, particular caution must be taken to overcome problems related to sample contamination and stability of the arsenic species. For determining total element concentrations, the main considerations for sample collection and storage are to prevent contamination and to minimize

Table 2. Most commonly used analytical methods for arsenic and arsenic compounds in water and biological samples

Methodology	Sample analysed	Detection	Detection limit	Advantages	Disadvantages	References
Colorimetric/spectro-photometric methods	Water Urine, serum Hair, nails	Total arsenic	~ 40 µg/L	Low cost, very simple, uses a simple spectrophotometer		Kingsley & Schaffert (1951); Vogel *et al.* (1954); Dahr *et al.* (1997); Pillai *et al.* (2000); Goessler & Kuehnelt (2002)
Inductively coupled plasma–atomic emission spectrometry (ICP–AES)	Water	Total arsenic	~ 30 µg/L			SM 3120 (1999); Environmental Protection Agency (1994a); Goessler & Kuehnelt (2002)
Inductively coupled plasma–mass spectrometry (ICP–MS)	Water Nails	Total arsenic	0.1 µg/L	Analytical method approved by US EPA	Spectral and matrix inter-ference	Environmental Protection Agency (1994b); Chen *et al.*, 1999; Goessler & Kuehnelt (2002)
High resolution (HR)–ICP–MS	Water Urine Nails	Total arsenic	0.01 µg/L	Solves spectral interferences in samples with complex matrices		Gallagher *et al.* (2001); Karagas *et al.* (2001, 2002)
Instrumental neutron activation analysis (INAA)	Hair, nails Tissues	Total arsenic	~ 0.001 µg/g	Reference method for detection of arsenic		Garland *et al.* (1993); Nichols *et al.* (1993); Pan *et al.* (1993); Pazirandeh *et al.* (1998); Karagas *et al.* (2001)
Electrothermal atomization laser–excited atomic fluorescence spectrometry (ETA–LEAFS)	Serum	Total arsenic	0.065 µg/L	Requires only minimal sample volume, sample pretreatment and measurement time		Swart & Simeonsson (1999)
Graphite furnace–atomic absorption spectrometry (GF–AAS)	Water, urine Hair, nails, tissues	Total arsenic	~ 0.025 µg/g	Analytical method approved by US EPA	Pre-atomization losses, requires the use of matrix modifyers	Agahian *et al.* (1990); SM 3113 (1999); WHO (2001)

Table 2 (contd)

Methodology	Sample analysed	Detection	Detection limit	Advantages	Disadvantages	References
Hydride generation–atomic absorption spectrometry (HG–AAS)	Water Urine Hair, nails	Total arsenic and arsenic speciation	0.6–6 µg/L	Analytical method approved by US EPA		Braman & Foreback (1973); Crecelius (1978); Le *et al.* (1994a,b); Chatterjee *et al.* (1995); Lin *et al.* (1998); Ng *et al.* (1998); Wyatt *et al.* (1998a,b); Shraim *et al.* (1999, 2000); SM 3114 (1999)
Hydride generation–quartz furnace–atomic absorption spectrometry (HG–QF–AAS)	Water Tissues	Total arsenic and arsenic speciation	0.003–0.015 µg/L	Inexpensive		Environmental Protection Agency (1996c)
High-performance liquid chromatography (HPLC)–HG–AAS	Urine	Total arsenic and arsenic speciation	1–47 µg/L			Lamble & Hill (1996); Kurttio *et al.* (1998)
HPLC or solid-phase cartridge separation combined with hydride generation–atomic fluorescence spectrometry (HPLC–HG–AFS)	Water, urine	Arsenic speciation	0.05–0.8 µg/L	Rapid, inexpensive No need for sample pretreatment		Le & Ma (1997); Aposhian *et al.* (2000); Le *et al.* (2000a,b); Gong *et al.* (2001); Yalcin & Le (2001)
HPLC–ICP–MS	Water Water, urine Hair, nails	Total arsenic	0.01 µg/L 0.14–0.33 µg/L	No need for sample pretreatment	Expensive and often time-consuming Spectral and matrix interference	Shibata & Morita (1989); Londesborough *et al.* (1999); Chatterjee *et al.* (2000); Mandal *et al.* (2001); Shraim *et al.* (2001); Karagas *et al.* (2002); Mandal *et al.* (2003)

loss of trace amounts of analytes. High-density polyethylene containers are usually preferred to glass containers because they are less adsorptive for arsenic. These are precleaned with nitric acid and then rinsed with distilled water.

Groundwater sampling is carried out by allowing the well-water to flow through the pumping pipe for approximately 10 min before collection.

Traditionally, water and urine samples are acidified with sulfuric or nitric acid to reduce potential adsorption of trace elements onto the surface of the sample container and to prevent bacterial proliferation. Samples can then be kept at +4 °C or at room temperature and preferably measured within 7 days (Lin *et al.*, 1998; Rahman *et al.*, 2002). Pande *et al.* (2001) reported, however, that all the field kits they evaluated were subject to negative interference if samples were acidified with nitric acid for preservation; they showed that acidification using 5% ascorbic acid instead of nitric acid eliminates interference.

In iron-rich waters, the stability of As^{III} and As^{V} can be affected by the formation of iron precipitates (iron oxides and/or hydroxides designated by 'FeOOH'). These precipitates can form during transport to the laboratory for analysis of arsenic. Studies of laboratory reagent water containing both As^{III} and Fe^{III} indicated that, within 18 h at room temperature, the resulting FeOOH precipitates contained a mixture of As^{III} and As^{V} with near quantitative removal of aqueous arsenic. Addition of a chelating agent such as ethylenediamine tetraacetic acid (EDTA), by sequestering Fe^{III}, inhibits the formation of FeAsOH precipitates and preserves the stability of arsenic species in iron-rich waters for more than 10 days (Gallagher *et al.*, 2001).

Reliable information from speciation analysis requires that the concentration of individual species of the element be unchanged by handling and treatment of the sample. Although traditionally used for their preservation, acidification of samples is not suitable since it leads to changes in arsenic speciation.

For urine specimens, low temperature (4 °C and –20 °C) conditions are required if they are to be stored up to 2 months without substantial changes in arsenic speciation (except for MMA^{III} and DMA^{III} species). For longer storage times, the stability of arsenic species varies with the complex matrix and pH of the urine, and accurate measurement of inorganic As^{III} and As^{V} separately is more difficult since As^{V} is rapidly reduced to As^{III}. MMA^{V} and DMA^{V} are more stable (for up to 4.5 months). The trivalent arsenic species, monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}), suspected to be key metabolic intermediates in human urine, are extremely unstable. It was shown that over 90% of MMA^{III} was rapidly oxidized to MMA^{V} in urine samples when stored at +4 °C or –20 °C over a 5-month period, while DMA^{III} was completely oxidized to DMA^{V} within 1 day (Gong *et al.*, 2001). In a recent review, these authors found that the use of a complexing agent, diethylammonium diethyldithiocarbamate (DDDC), improved the stability of MMA^{III} and DMA^{III} in urine samples. In the presence of DDDC (1–10 mM), MMA^{III} was found to be stable for 4 months at –20 °C (with a recovery of 85–95%) and DMA^{III} was partially preserved. Approximately 80% of DMA^{III} remained after 3 weeks of storage and 10–24% remained after 4 months (Jiang *et al.*, 2003). The use of other additives (such as hydrochloric acid,

sodium azide, benzoic acid, benzyltrimethylammonium chloride and cetylpyridinium chloride) has no particular benefit (Feldman *et al.*, 1999; Chen *et al.*, 2002).

For arsenic speciation, well-water is usually filtered at the sampling site using a 0.45 μm filter (Lin *et al.*, 1998).

Methods for on-site separation of As^{III} and As^{V} species immediately after water-sample collection using solid disposable cartridges can be efficiently used for speciation of particulate and soluble arsenic. A measured volume of the sample is passed through the 0.45-μm membrane filter, then serially through a connected silica-based strong anion-exchange cartridge. The filter captures particulate arsenic, while the anion-exchange cartridge retains As^{V}. Arsenite is not retained and is detected in the effluent. Arsenate is subsequently eluted with 1 M hydrochloric acid (HCl) from the anion-exchange cartridge and then analysed for concentration (Le *et al.*, 2000a).

In hair and nail samples, the arsenic species are less prone to change. For analysis of total arsenic, as for speciation methods, these specimens are usually prepared according to the International Atomic Energy Agency (IAEA) procedure (Ryabukhin, 1978).

Following extensive washing to eliminate exogenous arsenic resulting from air contamination, approximately 100 mg of each hair sample are usually placed in a Teflon beaker, mixed with acetone and then washed with distilled water. Nails are treated similarly to hair following brushing. Samples are weighed prior to analysis (Lin et *al.*, 1998; Mandal *et al.*, 2003). More stringent washing procedures have also been described for complete removal of surface contamination, by incubating nails for 20 min in 1% Triton X100 before analysis (Chen *et al.*, 1999).

1.2.2 *Analytical methods for measurement of total arsenic*

Determination of total arsenic in biological samples in most cases requires the complete destruction of the organic matrix. During this process, all the organic arsenic compounds should be converted into inorganic arsenic by oxidative digestion. Acid digestion (or wet ashing) (Kingsley & Schaffert, 1951) and dry ashing (George *et al.*, 1973) are the two basic methods that have been widely employed for oxidative digestion of samples prior to analysis. A microwave-assisted digestion technique has been developed recently and is currently used as a rapid preparation for sample analysis (Le *et al.*, 1994c; Goessler & Kuehnelt, 2002). For analysis of soft biological tissues using inductively coupled plasma (ICP) techniques, a simple partial digestion in a closed vessel at low temperature and pressure is often sufficient for the sample preparation and pretreatment step (WHO, 2001).

Historically, colorimetric/spectrophotometric methods have been used to determine total arsenic concentration. Several commercial field kits have been based on these methods. At present, laboratories often prefer more sensitive methods such as atomic absorption spectrometry (AAS), neutron activation analysis (NAA), atomic emission spectrometry (AES), mass spectrometry (MS) or atomic fluorescence spectrometry (AFS).

(*a*) *Colorimetric/spectrometric methods*

These methods take advantage of the formation of volatile arsine (AsH_3) gas to separate arsenic from other possible interference with the sample matrix. The colorimetric methods are easy to use and inexpensive in terms of equipment and operator cost. They are useful for the semi-quantitative determination of high concentrations of arsenic in water.

The silver diethylthiocarbamate (AgDDTC) method is the most popular spectrophotometric method for the determination of arsenic in water. The method is based on the generation of arsine either with zinc and hydrochloric acid or sodium borohydride in acidic solutions. The arsine gas is then flushed through a solution of diethylthiocarbamate in pyridine or pyridine/chloroform. The red-coloured complex can be measured at 520 nm. Using a modification of this method, Dhar *et al.* (1997) reported a detection limit of 40 µg/L for arsenic in water samples, with a 95% confidence.

Pillai *et al.* (2000) reported a new simple and reliable spectrophotometric method to determine total arsenic in environmental and biological samples. It involves bleaching the pinkish-red dye Rhodamine-B (measured at 553 nm) by the action of iodine released from the reaction between potassium iodate and arsenic in a slightly acidic medium.

The classic Gutzeit test (Vogel, 1954) is derived from the historical Marsh test. It is based on the generation of arsine (AsH_3) from arsenic compounds by the addition of zinc granules to concentrated sulfuric acid. The arsine can be detected by its reaction on a strip of filter moistened with silver nitrate or mercuric chloride, which produces a grey or a yellow to reddish-brown spot, respectively.

Field test kits

The high concentrations of arsenic currently found in groundwater in many parts of the world pose an important challenge because of the large number of wells that must be tested. This is particularly true in Bangladesh and other Asian hot spots such as Myanmar, Nepal, Cambodia, Laos, Viet Nam and India. Although less accurate than laboratory-based methods, field kits that allow on-site semi-quantitative determination of arsenic concentrations in well-water are of vital importance, since in these countries, the current laboratory capacity cannot cover the high level of analytical needs. Field testing has several advantages. In Bangladesh and other hot climates, attempts to keep samples cool over a long period of transport to a laboratory can be difficult. With field kits, there is no need for transport, no storage and therefore no need for preservation, which in addition reduces the cost of analysis and the time required for the well owner to be informed. Field kits are also simple to use after reasonable training of technicians.

These tests, however, must be accurate and sensitive enough to assess the level of arsenic contamination.

Much concern about the reliability of field kits recently led to careful evaluations of commercially available kits (Pande *et al.*, 2001; Rahman *et al.*, 2002a,b; Environmental Protection Agency-Battelle, 2002a,b; Erickson, 2003). The original field kit widely used in Bangladesh had a stated minimal detectable concentration of 100 µg/L, which largely exceeded the maximum permissible arsenic concentration defined by WHO (10 µg/L) and

even the maximum stated by most developing countries (50 µg/L). Fortunately, the newer field test kits are more sensitive. Evaluations of these kits are summarized in Table 3.

A modification of the Gutzeit method using mercuric bromide is the basis of most commercial field kits. A test strip moistened with mercuric bromide is exposed to arsine gas derived from the sample solution, to form complex salts of arsenic and mercury. These reactions give a yellow [$H(HgBr_2)As$] to brown [$(HgBr)_3As$] to black [Hg_3As_2] stain. The intensity of the yellowish-brown colour developed on the test strip is proportional to the arsenic concentration in the sample. When the reaction is completed, the test strip is compared with a colour chart provided with the kit and allows semi-quantitative determination of total arsenic concentration.

More recent field kits include digital measurement of arsenic levels without depending on the judgement of the technician's eyes to detect the difference between colour shades of the coloured strip (Arsenator, PeCo test). The improvement in reading results in higher sensitivity and reliability (Environmental Protection Agency-Battelle, 2002a,b; Durham & Kosmus, 2003).

In addition, promising biological tools (bacterial biosensors) may lead to new kits for quantitative and qualitative measurement of arsenite and arsenate in aqueous solution (Flynn *et al.*, 2002; Stocker *et al.*, 2003).

(*b*) *Inductively coupled plasma–atomic emission spectrometry (ICP–AES)*

ICP–AES involves the use of plasma, usually argon, at temperatures between 6000 and 8000 °K as the excitation source. The analyte is introduced into the plasma as an aerosol. A typical detection limit achievable for arsenic with this technique is 30 µg/L. Because of the rather high detection limits, ICP–AES is not frequently used for the determination of arsenic in biological samples (Goessler & Kuehnelt, 2002).

In August 2002, ICP–AES was withdrawn from the US Environmental Protection Agency (US EPA)-approved analytical methods for arsenic since this technique is inadequate to meet the requirements of the new EPA standard for arsenic in drinking-water of 10 µg/L (10 ppb), effective since February 2002 (Environmental Protection Agency, 2002).

(*c*) *Inductively coupled plasma–mass spectrometry (ICP–MS)*

ICP–MS is superior to ICP–AES with respect to detection limits, multi-element capabilities and wide linear dynamic range. This technique combines the ICP as the ion source with a mass analyser. Quadrupole mass filters are the most common mass analyser; double-focusing magnetic/electrostatic sector instruments and time-of-flight mass analysers are also used (Goessler & Kuehnelt, 2002).

ICP–MS is classified among the US EPA-approved analytical methods for arsenic (Environmental Protection Agency, 2002), with a detection limit of 0.1 µg/L.

The sensitivity can be further improved by the use of hydride generation (HG) techniques leading to a more efficient sample introduction and to matrix removal. The use of a high-resolution mode with HG–ICP–MS allows a 10-fold decrease in the detection

Table 3. Evaluation of some field test kits for analysing arsenic in water

Field test kits	Kit capability	Minimum detection limit of arsenic	Detection range	Rate of false positive/false negative	Effects of interferences (sodium chloride, iron, sulfate, acidity)	Occupational hazard potential (OH)	Time required per test	Evaluation reference
Quick™ (industrial test kit, Rock Hill, USA)	Semi-quantitative	~ 5–20 µg/L	5, 10, 20, 40, 60, 100, 200, … 500 µg/L	0–4%/5–16%	ND	Safe	< 15 min	Environmental Protection Agency-Battelle (2002a)
AS75 (PeCo test kit) (Peters Engineering, Graz, Austria)	Semi-quantitative	~ 15–50 µg/L	10, 20, 30, … 100 µg/L 2.5, 5, 10, 20, … 60 µg/L	0–3%/0%	None	Safe	ND	Environmental Protection Agency-Battelle (2002b)
AAN (Asia Arsenic Network, Japan)	Semi-quantitative	~ 20 µg/L	20, 50, 100, 200,… 700 µg/L	19%/71%	Some with sulfide	Accidental escape of arsine gas may cause OH.	15 min	Pande *et al.* (2001); Rahman *et al.* (2002)
E. Merck (Germany)	Qualitative for arsenic concentration > 50 µg/L	~ 50–100 µg/L	100, 500, 1000, 1700, 3000 µg/L	21%/60%	Some with sulfide	Accidental spillage of acid and escape of arsine gas may cause OH.	30 min	Pande *et al.* (2001); Rahman *et al.* (2002)
NIPSOM (National Institute of Preventive and Social Medicine, Bangladesh)	Qualitative for arsenic concentration > 50 µg/L	~ 10–20 µg/L	10, 20, 50, 100, 200, 300 … 700 µg/L	21%/33%	Some with sulfide	Accidental spillage of acid and escape of arsine gas may cause OH.	5 min	Pande *et al.* (2001); Rahman *et al.* (2002)
AIIH-PH (All India Institute of Hygiene and Public Health, India)	Semi-quantitative	~ 50 µg/L	> 50 µg/L	25%/1%	Sulfide interference eliminated	Accidental spillage of acid and escape of arsine gas may cause OH.	30 min	Pande *et al.* (2001); Rahman *et al.* (2002)
GPL (General Pharmaceuticals Ltd, USA)	Semi-quantative	~ 10 µg/L	10, 50, 100, 200, 400, 500 … 1500 µg/L	10%/32%	ND	Accidental spillage of acid and escape of arsine gas may cause OH.	20 min	Rahman *et al.* (2002)
Aqua (Aqua Consortium, Calcutta, India)	Semi-quantitative	~ 100 µg/L	> 50 µg/L	ND	Sulfide interference eliminated	Accidental spillage of acid and escape of arsine gas may cause OH. Contact with HgBr2 paper affects fingers of the user.	15 min	Pande *et al.* (2001)

limit (0.01 μg/L) for arsenic in water samples. HG–ICP–MS can be used for biological samples such as urine and nails (Chen *et al.*, 1999; Gallagher *et al.*, 2001; Karagas *et al.*, 2001a, 2002).

(*d*) *Neutron activation analysis (NAA)*

Instrumental NAA is an accurate and sensitive means to measure arsenic. The method can analyse relatively small biological samples, and has been used efficiently to measure total arsenic in hair, nails and other tissues, with a detection limit of approximately 0.001 μg/g (Pan *et al.*, 1993; Garland *et al.*, 1996; Nichols *et al.*, 1998; Pazirandeh *et al.*, 1998; Karagas *et al.*, 2001a).

(*e*) *Electro-thermal atomization laser–excited atomic fluorescence spectrometry (ETA–LEAFS)*

ETA–LEAFS is a highly sensitive and selective method that has been developed by the combination of laser-excited atomic fluorescence spectrometry with electro-thermal atomization in graphite cup or tube furnaces. The technique provides excellent analytical performance at ultra-trace levels, with a detection limit of 0.065 μg/L for arsenic in undiluted serum. This approach allows measurements to be taken directly on the serum samples after a simple dilution step. It also minimizes the amounts of sample required and can provide multiple measurements when only limited amounts of sample are available (Swart & Simeonsson, 1999).

(*f*) *Atomic absorption spectrometry (AAS)*

AAS is one of the most common analytical procedures for measuring arsenic in both environmental and biological materials. The main methods are flame AAS (FAAS), electro-thermal AAS (ET–AAS), also referred to as graphite furnace AAS (GF–AAS), and HG–AAS.

FAAS, with a relatively high detection limit (~1 mg/L), was never seriously considered for determining arsenic in environmental and biological samples.

The principal difference among the various AAS techniques is the means and form of presentation and atomization of the sample.

In GF–AAS, a small aliquot, rather than a continuous flow of sample, is deposited in a graphite furnace in which it is completely dissolved and mineralized *in situ*. The analyte is vaporized to form volatile hybrids. Matrix modifiers, such as a mixture of palladium and magnesium, must be used to protect the analyte from premature volatilization before vaporization, and therefore loss of arsenic. GF–AAS is classified among the approved US EPA analytical methods for arsenic in water (Environmental Protection Agency, 2002). It has been used for the determination of total arsenic in water and many biological samples (Agahian *et al.*, 1990).

HG–AAS uses the hydride generation technique, which can easily be connected to various detection systems and greatly improves the detection limit of all methods. The HG

technique is based on the production of volatile arsines (by the addition of either zinc/hydrochloric acid or a sodium borohydrate/acid mixture) which are transported by an inert gas to the detection system. HG–AAS is probably the most widely used method to determine total arsenic in water (Rahman *et al.*, 2001; Chakraborti *et al.*, 2002) and various matrices (Wyatt *et al.*, 1998a; Das *et al.*, 1995). HG–AAS is also classified among the US EPA-approved analytical methods for arsenic in water (Environmental Protection Agency, 2002). Detection limits for total arsenic in water achievable by this technique are around 0.6 µg/L.

1.2.3 *Analytical methods for arsenic speciation*

The combination of high-performance separation methods with highly sensitive instrumental detection systems is necessary to determine arsenic species (arsenic speciation) at trace levels. These combinations, referred to as hyphenated techniques, have been extensively described by Goessler and Kuehnelt (2002).

Three steps are required for arsenic speciation: the extraction of arsenic from the sample, the separation of the different arsenic species and their detection/quantification. The extraction procedure should be as mild and complete as possible. A combination of various extractants is often necessary to remove all the arsenic; polar and organic solvents or water are commonly used for this purpose. In many cases (water or urine samples), extraction may not be necessary. In the next step, a combination of separation procedures is usually required because of the different chemical properties of the arsenic compounds (anionic, neutral, cationic). Selective HG and high-performance liquid chromatography (HPLC) are the most commonly used. After the different arsenic compounds have been separated, they must be detected with a suitable detector. All the methods cited in Section 1.2.2 have been used more or less successfully to identify and determine arsenic compounds. Some efficient and sensitive hyphenated methods, commonly used or recently developed, are described below and presented in Table 2.

(*a*) *AAS–derived hyphenated methods*

Hydride generation quartz furnace atomic absorption spectrometry (HG–QF–AAS) is an improved modification of GF–AAS, described by the US Environmental Protection Agency (Environmental Protection Agency, 1996c), in which the graphite furnace is replaced by a quartz furnace. The method is designed to measure both total arsenic and arsenic species in water (range, 0.01–50 µg/L) and in tissue (range, 0.01–500 µg/g dry weight for arsenic and arsenic species). The detection limits for total inorganic arsenic, As^{III} and As^{V} have been determined to be 3 ng/L and 15 ng/L for DMA and MMA, respectively, when no background element or interference is present.

Modifications of the HG–AAS method have also been described that allow the determination of arsenic species (As^{III}, As^{V}, MMA, DMA) in water and biological samples (Braman & Foreback, 1973; Crecelius, 1978; Le *et al.*, 1994a,b,c; Hasegawa *et al.*, 1994; Lin *et al.*, 1998; Ng *et al.*, 1998). These modifications, which involve trapping the arsine

species at liquid nitrogen temperature (–196 °C), allow the elution by chromatography of each compound at room temperature. Ng *et al.* (1998) described, for example, an optimized HG–cold trap–AAS procedure for the speciation of arsenic in urine, with detection limits of 0.25 µg/L, 0.325 µg/L and 0.75 µg/L for inorganic arsenic species, MMA and DMA, respectively. On the other hand, using the HG–AAS method after cold trapping and chromatographic separation, Hasegawa *et al.* (1994) were able, for the first time, to separate the trivalent MMA^{III} and DMA^{III} species from the pentavalent DMA and MMA species in natural water following solvent extraction using DDDC.

A system that can separate arsenic species using on-line HPLC prior to their on-line decomposition by microwave digestion, prereduction with L-cysteine and analysis by HG–AAS (HPLC–HG–AAS) has been developed (Lamble & Hill, 1996), and enables the full speciation of arsenobetaine, MMA, DMA, As^{III} and As^{V} in biological samples. A simple modification of the system can determine total arsenic in the sample. A comparable system was used to determine total arsenic and arsenic species in urine specimens, with detection limits of 1.0, 1.6, 1.2 and 4.7 µg/L for As^{III}, As^{V}, MMA and DMA, respectively (Kurttio *et al.*, 1998).

(*b*) *Atomic fluorescence spectrometry (AFS)-derived hyphenated techniques*

AFS is an excellent detector of arsenic compounds; it is, in addition, rather simple and inexpensive. AFS has been used to detect arsenic hybrids in the ultraviolet spectral region because of the small background emission produced by the relatively cool hydrogen diffusion flame. The use of cold vapour or HG, together with an intense light source, enables very low detection limits to be reached.

A rapid method for speciation of As^{III}, As^{V}, MMA and DMA (and also arsenobetaine) has been developed based on the rapid separation of the target arsenic species on one or two 3-cm HPLC guard columns, followed by HG–AFS (Le & Ma, 1997). This simple method provides the complete speciation of arsenic present in water and urine samples within 1.5 min with no need for treatment of the sample. Detection limits for the four arsenic species in urine samples are 0.4–0.8 µg/L.

More recently, a solid-phase extraction cartridge linked to HG–AFS was described for speciation of arsenic in water and urine, with detection limits of 0.05 µg/L in water. The disposable cartridges are inexpensive and specific for selective retention of arsenic species, and the method is suitable for routine determination of trace levels of arsenic species in drinking-water to comply with the more stringent environmental regulations (Yalcin & Le, 2001).

HPLC–HG–AFS has led to the speciation in urine of trace levels of trivalent MMA^{III} and DMA^{III} together with the other arsenic species (Gong *et al.*, 2001).

(*c*) *Inductively coupled plasma–mass spectrometry (ICP–MS)-derived hyphenated methods*

Among the detector methods, ICP–MS is certainly not the cheapest. The advantage of ICP–MS lies in its multi-element capabilities, excellent detection limits and wide linear range. Moreover, low detection limits are not restricted to the hybrid-forming arsenic compounds (Goessler & Kuehnelt, 2002).

Numerous methods have been developed for the speciation of arsenic using the separation power of HPLC combined with the sensitivity of ICP–MS detection (Shibata & Morita, 1989; Le *et al.*, 1998; Londesborough *et al.*, 1999; Chen *et al.*, 1999; Chatterjee *et al.*, 2000; Mandal *et al.*, 2001, 2003).

High-temperature (column temperature at 70 °C) HPLC–ICP–MS was used to determine 13 arsenic and selenium species in urine (Le *et al.*, 1998). The high temperature achieved an improved resolution and faster separation. The speciation of six arsenosugar metabolites in urine can be completed in 19 min at 70 °C compared with 37 min at room temperature.

Londesborough *et al.* (1999) reported an improved HPLC–ICP–MS method for the speciation of eight anionic, cationic or neutral arsenic species (As^{III}, As^{V}, MMA, DMA, arsenobetaine, arsenocholine, trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TMA)) using a single ion-exchange column, with detection limits of 0.19, 0.52, 0.29, 0.16, 0.16, 0.58, 0.6 and 0.38 µg/L, respectively. In this method, the matrix of biological samples noticeably affects the column efficiency.

High sensitivity was also obtained with the development of the HPLC–ultrasonic nebulizer high-power nitrogen-microwave–ICP–MS method, which could be particularly useful for arsenic speciation in samples with high chloride concentrations since no chloride interference ($as^{40}Ar^{35}Cl$) was observed in urine with a chloride matrix of up to 10 000 mg/L (Chatterjee *et al.*, 2000).

Using optimized HPLC–ICP–MS, Mandal *et al.* (2001) detected the trivalent MMA^{III} and DMA^{III} species for the first time in urine samples, with no prechemical treatment, with detection limits in the range of 0.14–0.33 µg/L.

In conclusion, depending on the specific need, reliable results should be obtainable provided that special care is taken in the preservation and preparation of samples and the method of analysis is chosen carefully.

1.3 Natural occurrence

Arsenic is a metalloid that occurs naturally; it is the component of more than 245 minerals. Examples of arsenic levels in some geological materials are given in Table 4. Arsenic is commonly concentrated in sulfide-bearing mineral deposits, especially those associated with gold mineralization, and it has a strong affinity for pyrite, one of the more ubiquitous minerals in the earth's crust. It is also concentrated in hydrous iron oxides. Arsenic and its compounds are mobile in the environment. Weathering of rocks converts

Table 4. Levels of arsenic in geological materials

Materials	Concentration (mg/kg)	Source
Earth crust total	1–1.8	Matschullat (2000)
Upper crust	1.5–2	Matschullat (2000)
Igneous rocks		
Basic basalt	02–113	Mandal & Suzuki (2002); Smedley & Kinniburgh (2002)
Gabbro, dolorite	0.06–28	Mandal & Suzuki (2002); Smedley & Kinniburgh (2002)
Acidic granite	0.2–13.8	Mandal & Suzuki (2002); Smedley & Kinniburgh (2002)
Sedimentary rocks		
Phosphorites	0.4–188	Smedley & Kinniburgh (2002)
Sandstones	0.6–120	WHO (1981); Mandal & Suzuki (2002)
Shale and argillite	0.3–500	Hale (1981)
Schist and phyllite	0.5–143	Hale (1981)
Carbonates	0.1–20	Matschullat (2000); Mandal & Suzuki (2002)
Coals	0.3–35 000	Smedley & Kinniburgh (2002)
Sulfide minerals		
Pyrite	100–77 000	Smedley & Kinniburgh (2002)
Pyrrhotite	5–100	Boyle & Jonasson (1973)
Chalcopyrite	10–5000	Smedley & Kinniburgh (2002)
Galena	5–10 000	Smedley & Kinniburgh (2002)
Sphalerite	5–17 000	Smedley & Kinniburgh (2002)
Marcasite	20–126 000	Smedley & Kinniburgh (2002)
Oxide minerals		
Haematite	up to 160	Smedley & Kinniburgh (2002)
Iron oxide	up to 2000	Smedley & Kinniburgh (2002)
Iron(III) oxyhydroxide	up to 76 000	Smedley & Kinniburgh (2002)
Sulfate minerals		
Jarosite	34–1000	Smedley & Kinniburgh (2002)

arsenic sulfides to arsenic trioxide, which enters the arsenic cycle as dust or by dissolution in rain, rivers or groundwater. Arsenic can also enter the food chain, causing widespread distribution throughout the plant and animal kingdoms. The occurrence and behaviour of arsenic in the environment have been extensively reviewed (Cullen & Reimer, 1989; Tamaki & Frankenberger, 1992; Matschullat, 2000; Mandal & Suzuki, 2002; Nordstrom, 2002; Smedley & Kinniburgh, 2002).

A limited range of geological environments can result in significant natural elevation of arsenic in water supplies (Nordstrom, 2002). These include: organic rich (black) shales, Holocene alluvial sediments with slow flushing rates, mineralized and mined zones (most

often gold deposits), volcanogenic sources, thermal springs, closed basins in arid-to-semi-arid climates, particularly in volcanic regions, and strongly reducing aquifers with low sulfate concentrations.

Depending on prevailing climatic and hydrological conditions, soils and sediments, surface waters, groundwaters and air can become enriched in arsenic where these geological conditions prevail.

1.3.1 *Arsenic speciation in natural materials*

Mineral forms in which arsenic is present in soils are approximately 60% arsenates and 20% sulfides and sulfosalts; the remaining 20% includes arsenides, arsenites, oxides, silicates and elemental arsenic.

These mineral forms are generally weathered to the inorganic water-soluble species, arsenate (As^{V}) and arsenite (As^{III}), with arsenate dominating under oxidized conditions and arsenite under reduced conditions (Cullen & Reimer, 1989). Under both aerobic and anaerobic conditions, micro-organisms can transform inorganic arsenic into organic forms such as MMA, DMA and volatile TMA. TMA in the air is then rapidly converted into water-soluble species, As^{V} and TMAO (Pongratz, 1998; Turpeinen *et al.*, 1999, 2002). These compounds can also be degraded by microflora. In certain materials, organic arsenic compounds naturally build up to high concentrations (Mandel & Suzuki, 2002; Smedley & Kinniburgh, 2002).

1.3.2 *Abundance and distribution of arsenic*

(*a*) *Soils and sediments*

Measurements of background arsenic levels in surface soil are all compromised by atmospheric deposition of anthropogenically derived arsenic. Anthropogenic sources to soil include use and resuspension of arsenic-based pesticides, mining, smelting, manufacturing and waste-disposal activities. Shotyk *et al.* (1996) showed that arsenic levels were 20-fold higher in surface horizons of ombrotrophic (rain-fed) peat bogs than in lower horizons. This high level was due to industrially derived inputs of arsenic. Centuries of mining activities can result in an extremely high concentration of arsenic in soils. This is the case in South-West England where arsenic concentrations in some old smelter and/or mine areas range from 24 to 161 000 mg/kg (Farago *et al.*, 1997).

Koljonen (1992) estimated a global average level of arsenic in soils of 5 mg/kg, but concentrations vary considerably among geographical regions. Arsenic concentrations in sediments in lakes, rivers and streams in the USA ranged from 0.1 to 4000 mg/kg. Levels of arsenic in a detailed survey of Finland, which has a low population density and is remote from major centres of pollution, ranged up to 60 mg/kg for the 1164 samples tested (Lahermo *et al*., 1998). Soils formed from arsenic-enriched geological substrates can have naturally higher levels than the ranges quoted. These ranges must therefore be considered as typical background levels rather than absolute ranges.

Soils formed on top of arsenic-rich bedrocks have elevated levels of this element. Colbourn *et al*. (1975) reported mean arsenic levels of 88 mg/kg (range, 24–250 mg/kg; $n = 18$) in soils formed naturally from parent material consisting of metamorphic aureole around a granitic intrusion. The Strassegg area in Gasen (Styria, Austria) has extensive arsenopyrite (FeAsS) mineralization, with the ore body running close to the surface (Geiszinger *et al*., 2002). The soils formed on top of this ore vein are enriched in arsenic, with levels ranging from 700 to 4000 mg/kg, and are used for agronomic cultivation.

Soils formed in and around ancient and modern hot springs with elevated arsenic in geothermal fluids have naturally elevated levels of arsenic due to enrichment of the parent material of the soil (Ballantyne & Moore, 1988). The ancient hot-spring system at Rhynie, north-eastern Scotland, has cherts with arsenic levels ranging from 15 to 300 mg/kg (Rice *et al*., 1995). Sinter from active hot springs in the Taupo Volcanic Zone, New Zealand, have arsenic levels ranging from below detection limits to 1646 mg/kg (McKenzie *et al*., 2001). An area of at least 10 km^2 in St Elizabeth, Jamaica, has a geochemical anomaly, whereby arsenic concentrations in soil reach 400 mg/kg (Lalor *et al*., 1999). The anomalous values may result from an ancient hot-spring environment responsible for the introduction and deposition of pyrite and arsenopyrite in the limestone bedrock, which were subsequently oxidized and weathered, leading to arsenic-rich soils.

Sediment levels of arsenic in the Waikato River, New Zealand, ranged from 7.9 to 1520 mg/kg dry wt, resulting in high levels of arsenic in sediment living biota, such as the freshwater mussel, *Hyridella menziesi* (Hickey *et al*., 1995).

In a number of delta environments in South-East Asia, deep fluvial and deltaic Pleistocene-Holocene sediments have accumulated (up to 10 km thick in Bangladesh) (Nickson *et al*., 2000). During glaciation, river levels were 100 m lower than in interglacial times, and at this time of low sea level, the sediments were flushed and oxidized, leading to iron (Fe^{III}) oxyhydroxide precipitation on sediment surfaces. These sedimentary iron oxyhydroxides scavenge arsenic, with arsenic levels reaching up to 517 mg/kg in FeOOH phases (Nickson *et al*., 2000). Under reducing conditions caused by microbial metabolism of sedimentary organic matter (present at up to 6% as C), in which sulfate levels are low, insoluble Fe^{III} is converted to soluble Fe^{II}, leading to the mobilization of arsenic from the dissolved FeOOH phase. Although traces of arsenic-rich pyrites are found in the sediments, they are present in quantities that are too small for pyrite oxidation to contribute significantly to arsenic in groundwaters.

Water percolating from hot-spring systems into the surrounding soil or sediment also causes a rise in arsenic concentrations (Langner *et al*., 2001; Koch *et al*., 1999).

The Antofagasta Region, northern Chile, is characterized by volcanism (Queirolo *et al*., 2000a). High levels of arsenic are found in soils and river sediments in this region (Caceres *et al*., 1992), and crops (maize and potato) grown on these soils have high levels of arsenic, reaching 2 mg/kg in maize (Queirolo *et al*., 2000b).

Arsenic concentrations in mineralized zones rich in arsenic are further elevated, often severely, by mineral extraction and processing (Smedley & Kinniburgh, 2002).

(*b*) *Groundwaters*

Under natural conditions, the greatest range and the highest concentrations of arsenic are found in groundwater as a result of the strong influence of the water–rock interactions and the favourable physical and geochemical conditions in aquifers for the mobilization and accumulation of arsenic. Arsenic is particularly mobile at pH values typically found in groundwater (pH, 6.5–8.5) under both oxidizing and reducing conditions.

Background concentrations of arsenic in groundwater in most countries are less than 10 µg/L and sometimes substantially lower. However, values quoted in the literature show a very wide range, from < 0.5 to 5000 µg/L. Most high levels of arsenic in groundwater are the result of natural occurrences of arsenic. Cases of arsenic pollution caused by mining are numerous but tend to be localized.

Arsenic can occur in the environment in several oxidation states (–3, 0, +3 and +5) but, in natural waters, is mostly found in inorganic forms as oxyanions of trivalent arsenite (As^{III}) or pentavalent arsenate (As^{V}). Redox potential (Eh) and pH are the most important factors controlling arsenic speciation. Under oxidizing conditions, arsenate is dominant, as the $H_2AsO_4^-$ form at low pH (less than approximately 6.9), or as the $HAsO_4^{2-}$ form at higher pH. Under reducing conditions at pH less than approximately 9.2, the uncharged arsenite species H_3AsO_3 predominates (Smedley *et al.*, 2002).

In two recent reviews, Smedley and Kinniburgh (2002) and Smedley *et al.* (2002) focused extensively on the factors that control arsenic concentration in groundwater.

In relatively pristine habitats where anthropogenic activity can be excluded as a contributor to arsenic levels in aquifers, Lahermo *et al*. (1998) found that arsenic levels in groundwaters in Finland reached up to 1040 µg/L, with a median of 0.65 µg/L (n = 472). The highest levels of arsenic were found in groundwaters from wells drilled in Precambrian bedrock.

In an extensive groundwater survey in the USA, Welch *et al*. (2000) reported that approximately half of the 30 000 samples analysed had naturally occurring arsenic levels ≤ 1 µg/L, with about 10% exceeding 10 µg/L. Geothermal water and high evaporation rates are associated with arsenic concentrations ≥ 10 µg/L in ground- and surface waters.

There are three major types of natural geological condition giving rise to high levels of arsenic in groundwaters:

(i) aquifers composed of rocks or sediments enriched with arsenic-containing minerals of geogenic origin, such as sulfide mineralization;

(ii) aquifers containing sediments coated with iron oxyhydroxide-(FeOOH) phases enriched in arsenic through hydrological action, where arsenic is mobilized into porewater by reducing conditions;

(iii) aquifers enriched in arsenic through high rates of evaporation in arid areas, leading to increased mineral concentration in groundwaters; the arsenic is mobile in such aquifers because of the high pH (> 8.5) caused by concentration of alkali and alkali earth metals in solution.

Geochemical conditions similar to the alluvial sediments in Bangladesh exist in the Red River alluvial tract in the city of Hanoi, Viet Nam, where FeOOH reduction is thought to have led to the high arsenic levels recorded in groundwaters (Berg *et al*., 2001). Smedley and Kinniburgh (2002) outline that the reducing conditions observed in Bangladesh/West Bengal and Viet Nam aquifers are similar to those in the regions of Taiwan, China, northern China and Hungary that suffer from high levels of arsenic in groundwaters.

Smedley *et al*. (2002) studied the geochemistry of arsenic in groundwaters from Quaternary loess aquifers, which were high in arsenic, in an area thought to spread over 10^6 km^2 in La Pampa province, central Argentina. Dissolved arsenic ranged from 4 to 5300 μg/L, with 73% of samples exceeding 50 μg/L. The conclusions drawn for La Pampa province may be applicable elsewhere in determining which regions are vulnerable to arsenic and related water-quality problems: "Under oxidising conditions, vulnerable aquifers potentially occur where several important criteria coincide: semi-arid climatic conditions with limited recharge where high-pH groundwater can be generated; young (Quaternary) sediments or volcanic sediments; and slow groundwater-flow conditions. Such aquifers are likely to have been poorly flushed over the geologically-short timescale since deposition and hence will have had little opportunity for removal of trace elements such as arsenic from the aquifer." Similar conditions exist in the Lagunera and Sonora regions of Mexico and in the Atacama Desert, Chile (Smedley & Kinniburgh, 2002).

(*c*) *Surface waters*

Matschullat (2000) collated measurements of arsenic in surface waters. Levels of arsenic dissolved in uncontaminated stream waters ranged from 0.1 to 1.7 μg/L, and those in seawaters were 1.5–1.7 μg/L. Concentrations in open seawater show little variation from the value of 1.5 μg/L (Smedley & Kinniburgh, 2002).

Arsenic in surface stream waters in Finland, which could be considered a pristine environment because of its low population density and remote geographical location, ranged from 0.06 to 1.6 μg/L (median, 0.36 μg/L; $n = 1157$) (Lahermo *et al*. 1998). These levels correlated well with arsenic levels in glacial till, with the highest stream water levels occurring in catchments with metamorphic, volcanic and sedimentary geologies. Levels in the more geographically remote part of Finland were lower than those in the south, which is nearer to continental Europe. Arsenic levels in Finnish water were lower than those for continental Europe, again emphasizing the pristine nature of the Finnish environment.

The Ciwidey River, West Java, drains a catchment dominated by the Quaternary volcano Patuha, which contains an acid crater lake (pH < 1) (Sriwana *et al*., 1998). Arsenic in the crater lake was recorded to be 279 μg/L, with the stream draining this lake having levels of 57 μg/L. In the tributary river of the stream, levels dropped to below 1 μg/L. In a crater lake with naturally elevated levels of arsenic, such as Lake Xolotlan in Nicaragua, mean arsenic concentrations ranged from 10.23 to 30.13 μg/L (Lacayo *et al*. 1992).

Takatsu and Uchiumi (1998) studied water from Lake Usoriko, Japan, which is acidified by hot springs. The sediments of this lake contained 1.6% by mass of arsenic, with arsenic levels in the open lake waters ranging from 10 to 450 μg/L.

Levels of arsenic in drinking-water extracted from the Waikato River, New Zealand, for the city of Hamilton averaged 32 µg/L. Arsenic concentrations appear to follow a regular seasonal variation, being approximately 10–25 µg/L higher in the summer months, and fall to 6 µg/L after water treatment (McLaren & Kim, 1995). The elevated levels of arsenic in the Waikato river are of natural origin, as its catchment is the volcanic region of the Central Plains (Hickey *et al.*, 1995).

Natural surface waters in the Antofagasta region of Chile, originating from springs, have very high levels of arsenic because of zones mineralization associated with volcanic activity (eruptions, vents, geysers and thermal springs). Surface water is used as drinking-water and to irrigate crops (Queirolo *et al*., 2000a,b). Arsenic levels reached 3000 µg/L in rivers and canals in this region, with many rivers routinely having levels over 100 µg/L.

In an area with similar volcanic activity in the Salta Province, Argentina, high levels of arsenic have been recorded in thermal springs, tap-water and river water (Vahter *et al*., 1995).

High levels of arsenic have been recorded in rivers in arid areas of Chile and Argentina where surface water is dominated by base-flow (whereby groundwater flows into the river from surrounding rock) (Caceres *et al*., 1992; Lerda & Prosperi, 1996). Caceres *et al*. (1992) found concentrations in surface water up to 22 mg/L. The high degree of evaporation that occurs in these regions concentrates the arsenic leached from weathered rocks. Such surface waters have high pH, due again to high rates of evaporation that lead to concentration of alkaline and alkaline earth cations leached from the rocks.

(*d*) *Air*

Concentrations of arsenic in ambient air in remote locations range from < 1 to 3 ng/m^3, but concentrations in cities may range up to 100 ng/m^3. Arsenic in ambient air is usually a mixture of arsenite and arsenate, with organic species being of negligible importance except in areas of arsenical pesticide application or other industrial activity (WHO, 2001). Sources of arsenic to air include use and resuspension of arsenic-based pesticides, mining, smelting, manufacturing and waste-disposal activities. Arsenic may be introduced into the atmosphere directly from these processes, or it may be derived from sediment and soil particles being entrained into the atmosphere or the production of volatile arsenic metabolites, such as arsines, from soils (Woolson, 1977; Turpeinen *et al.*, 2002). Defining what constitutes natural levels is, therefore, difficult.

(*e*) *Other*

Arsenic has been detected in rainwater at concentrations ranging from < 0.005 to 45 µg/L, with higher levels occurring in contaminated areas (WHO, 2001).

Arsenic compounds are abundant in certain seafoods at concentrations as high as several hundred milligrams per kilogram. Although marine animals contain many arsenic compounds, most species contain arsenobetaine as the major arsenical. Arsenobetaine is not metabolized by humans and is believed to have low or negligible toxicity. Inorganic

arsenic and arsenosugars can, however, be present in some marine algae, seaweeds, oysters, mussels and clams (reviewed by Francesconi & Kuehnelt, 2002).

Dimethylarsinate is often the major arsenical constituent of species of fungi. Arsenite and arsenate are also commonly found in fungi (Francesconi & Kuehnelt, 2002).

Inorganic arsenic species are dominant in the chemistry of arsenic in terrestrial plants (Francesconi & Kuehnelt, 2002) and, although less studied, the concentration of arsenic in wheat and vegetables grown in countries highly contaminated with arsenic could be relevant to human health. Most of the vegetables cultivated in the Antofagasta Region (northern Chile), which is characterized by volcanic events (eruptions, thermal springs), are found at local markets of a population of approximately 4000 people. In this region, very high arsenic contents have been reported in Socaire and Talabre (1850 μg/kg in corn and 860 μg/kg in potatoes, including potato skins, respectively), two towns situated close to the Lascar volcano (Queirolo *et al.*, 2000b). These values exceed the national standard for arsenic (500 μg/kg) by approximately 400% and 180%, respectively.

In Bangladesh, contamination of agricultural soils from long-term irrigation with arsenic-contaminated groundwater led to phyto-accumulation in food crops. Various vegetables harvested in Samta village in the Jessore district have been reported to contain high concentrations of arsenic (range, 306–489 μg/kg) (Alam *et al.*, 2003). In West Bengal (India), high arsenic contents have also been reported in many vegetables and spices, especially in the skin of most vegetables, as a result of the dependence of the agricultural system on groundwater (Roychowdhury *et al.*, 2002, 2003).

Moreover, high concentrations of arsenic have been reported in fruit, vegetables, grain and meat in regions contaminated by anthropogenic pollution; this is the case in the Moscow region (Russia), which has been shown to be contaminated by fertilizer industry plants (Zakharova *et al.*, 2002). High levels of arsenic have also been reported in plants, vegetables and cow's milk, as a consequence of heavy contamination of soils, surface and groundwaters by arsenic attributed to industrial sources (veterinary chemicals, pharmaceuticals, pesticide industries) in the area of Patancheru, Andhra Pradesh (India) (Sekhar *et al.*, 2003).

Interestingly, rare plants are able to accumulate exceedingly high concentrations of arsenic (in the order of 1% dry mass). Brake fern (*Pteris vittata*) in particular is extremely efficient at extracting arsenic from soils and translocating it into its fronds. Arsenic concentrations in fern fronds, growing in soil spiked with 1500 mg/kg arsenic, increased from 29.4 to 150 861 mg/kg within 2 weeks. Since it acts as an arsenic hyperaccumulator, brake fern could be used in the remediation of arsenic-contaminated soils (Ma *et al.*, 2001).

1.4 Human exposure

The natural and anthropogenic occurrence of arsenic in drinking-water has been recognized as a major public health issue in several regions of the world over the past two or three decades. Areas affected by arsenic span the globe, and significant exposures have been identified in Bangladesh, India, Taiwan, China, Mexico, Argentina, Chile and the

USA. Table 5 summarizes the geological characteristics of the regions of the world with naturally elevated levels of arsenic in the drinking-water.

Recent reviews have outlined the worldwide problem of arsenic in drinking-water (WHO, 2001; Mandal & Suzuki, 2002; Nordstrom, 2002; Smedley & Kinniburgh, 2002; Chakraborti *et al.*, 2003b).

1.4.1 *Exposure in Bangladesh*

In terms of the population exposed, the problem of arsenic contamination in much of southern and eastern Bangladesh is the most serious in the world, and occurs in groundwater from the alluvial and deltaic sediments that make up much of the area. In addition, it is complicated by large variability in arsenic levels at both local and regional scales.

In Bangladesh, tubewells began to be used for drinking-water in the 1970s to control the problem of gastrointestinal disease linked to contamination of shallow wells and surface waters. In the 1990s, it was discovered that the water from many of these wells was contaminated with arsenic. Since then, extensive research has been carried out to characterize the extent of the problem. Figure 1 shows the districts in Bangladesh affected by arsenic and Table 6 gives an overall picture of the database. Table 7 shows the distribution of concentrations of arsenic in hand tubewells, and Table 8 summarizes the levels of arsenic measured in biological samples.

The level of contamination with arsenic of tubewells in Bangladesh exceeded both the World Health Organization guideline of 10 µg/L and the Bangladesh permissible limit of 50 µg/L (Dhar *et al*., 1997; Smith *et al*., 2000a; Kinniburgh & Smedley, 2001: Alam *et al.*, 2002).

A survey of 27 districts in Bangladesh up to January 1997 analysed over 3000 water samples and revealed that 38% of them contained more than 50 µg/L arsenic (Dhar *et al*., 1997). In another survey examining 294 tubewells, 85 samples (29%) were contaminated by arsenic at levels above 50 µg/L (Ahmad *et al*., 1997). Between September 1996 and June 1997, all functioning wells (n = 265) in the village of Samta in the Jessore District were tested for arsenic (Biswas *et al*., 1998). Approximately 91% of the wells contained arsenic at levels higher than 50 µg/L. Furthermore, 600 people-were examined clinically, and a few hundred hair, nail and urine samples were tested using flow injection HG–AAS. The data obtained showed that 99% of urine samples and 98% of nail samples of the population studied in Samta village contained levels of arsenic above normal and 78% of hair samples above toxic levels. The arsenic problem of Bangladesh became highlighted when an international conference was held in Dhaka, Bangladesh, in 1998 (Dhaka Community Hospital Trust and School of Environmental Studies, 1998).

By March 1998, it was reported that 4196 of 9024 wells in Bangladesh tested for arsenic contained levels higher than 50 µg/L and 884 wells had levels higher than 500 µg/L (Mandal *et al*., 1999). A Rapid Action Programme (RAP) was performed by field kit in a sample of 500 villages with a total population of 469 424. Approximately 62% of the 32 651 tubewells sampled had levels of arsenic above 100 µg/L (Quamruzzaman *et al*., 1999).

Table 5. Regions of the world with naturally elevated levels of arsenic in groundwater

Country/region	Affected area (km^2)	Potentially exposed population	Arsenic concentration (μg/L)	Environmental conditions	Reference
Bangladesh	118 849	~ 3×10^7	< 0.5–2500	Hollocene alluvial/deltaic sediments; abundance of organic matter; strongly reducing, neutral pH, high alkalinity, slow groundwater flow rates	Chakraborti *et al*. (2002); Smedley & Kinniburgh (2002)
India/West Bengal	38 865	6×10^6	< 10–3200	Same as Bangladesh	Chakraborti *et al*. (2002); Smedley & Kinniburgh (2002)
Viet Nam				Pleistocene and Holocene sediments; strongly reducing conditions	Berg *et al*. (2001)
China/ Taiwan	4 000	~ 10^5	10–1820	Coastal zones, sediments, including black shales; strongly reducing, artesian conditions, some groundwaters contain humic acids	Smedley & Kinniburgh (2002)
China/ Xinjiang, Shanxi	38 000	~ 500	40–750	Holocene alluvial plain; reducing	Smedley & Kinniburgh (2002); Cao (1996)
Thailand	100	1.5×10^4	1–< 5000	Dredge quarternary alluvium; oxidation of disseminated arsenopyrite due to mining	Smedley & Kinniburgh (2002)
Mongolia/ Inner Mongolia	4 300	~ 10^5	< 1–2400	Holocene alluvial and lacustrine sediments; strongly reducing, neutral pH, high alkalinity, some groundwaters contain humic acids	Cao (1996); Smedley & Kinniburgh (2002); Sun *et al*. (2001)

Table 5 (contd)

Country/region	Affected area (km^2)	Potentially exposed population	Arsenic concentration (μg/L)	Environmental conditions	Reference
Argentina/ Chaco-Pampean Plain	10^6	2×10^6	< 1–7550	Holocene and earlier loess with rhyolitic volcanic ash; oxidizing, neutral to high pH, high alkalinity; groundwaters often saline	Nordstrom (2002); Smedley & Kinniburgh (2002)
Northern Chile/ Antofagasta	35 000	5×10^5	100–1000	Quaternary volcanogenic sediments; generally oxidizing, arid conditions, high salinity	Queirolo *et al*. (2000a); Smedley & Kinniburgh (2002)
Bolivia		5×10^4		Same as Argentina and Northern Chile	Nordstrom (2002)
Mexico	32 000	4×10^5	8–620	Volcanic sediments; oxidizing, neutral to high pH	Smedley & Kinniburgh (2002)
Germany/ Bavaria	2 500		< 10–150	Mineralized sandstone	Nordstrom (2002)
Hungary, Romania/ Danube Basin	110 000	4×10^5		Quaternary alluvial plain; reducing conditions, some high in humic acid	Smedley & Kinniburgh (2002)
Spain		$> 5 \times 10^4$	< 1–100	Mineralization; alluvial sediments	Nordstrom (2002)
Greece		1.5×10^5		Mineralization; thermal springs; mining	Nordstrom (2002)
Ghana		$< 1 \times 10^5$	< 1–175	Sulfide mineralization, particularly arsenopyrite; gold mining	Nordstrom (2002)

Table 5 (contd)

Country/region	Affected area (km^2)	Potentially exposed population	Arsenic concentration (µg/L)	Environmental conditions	Reference
Canada/Moira Lake, Ontario	100		50–3000	Mine tailing; ore mining	Smedley & Kinniburgh (2002)
Canada/British Columbia	50		0.5–580	Sulfide mineralization in volcanic rocks; neutral to high pH groundwater	Smedley & Kinniburgh (2002)
USA/Arizona	200 000		< 1300	Alluvial basins, some evaporites; oxidizing, high pH	Smedley & Kinniburgh (2002)
USA/ California	5 000		< 1–2600	Holocene and older basin-fill sediments; internally drained basin, mixed redox conditions, high salinity	Smedley & Kinniburgh (2002)
USA/Nevada	1 300		< 2600	Holocene mixed aeolian, alluvial and lacustrine sediments; mainly reducing, some high pH, some with high salinity due to evaporation	Smedlley & Kinniburgh (2002)

Figure 1. Degree of arsenic contamination in 64 districts in Bangladesh

From Chakraborti *et al.* (2002)

Table 6. Status of contamination of groundwater by arsenic in Bangladesh

	Bangladesh
Total area (km^2)	148 393
Population (millions)	120
Total number of districts	64
Total number of water samples analysed	34000
Samples containing > 10 µg/L arsenic (%)	56.35
Samples containing > 50 µg/L arsenic (%)	37.38
Number of districts affected by arsenic (> 50 µg/L)	50
Population of districts affected by arsenic (millions)	104.9
Area of districts affected by arsenic (km^2)	118 849
Number of villages affected by arsenic (arsenic in drinking-water > 50 µg/L)	2000
Number of people drinking arsenic-contaminated water > 50 µg/L (millions)	25

From Chakraborti *et al*. (2002)

Table 7. Distribution of arsenic concentrations in water samples from hand tubewells

Total no. of water samples analysed	Arsenic concentration range (µg/L)							
	< 10	10–50	51–99	100–299	300–499	500–699	700–1000	> 1000
34 000	14 991	6429	2949	5812	2174	894	479	272
	44.1%	18.9%	8.7%	17.1%	6.4%	2.6%	1.4%	0.8%

From Rahman *et al.* (2001)

In continuing surveys of 42 districts affected by arsenic in Bangladesh, Chowdhury *et al*. (2000a,b) reported the analysis of 10 991 water samples of which 59% contained arsenic levels above 50 µg/L.

Of the 34 000 drinking-water samples collected in Bangladesh up to August 2001, 272 contained ≥ 1000 µg/L arsenic (Table 6; Chakraborti *et al*., 2002). The highest concentration of arsenic measured in drinking-water in Bangladesh was 4700 µg/L. In the Chiladi village of Senbagh Thana in the Noakhali district, 100% of tubewell-water samples contained arsenic concentrations ≥ 50 µg/L, 94% contained ≥ 300 µg/L and 28% contained ≥ 1000 µg/L.

Table 8. Concentrations of arsenic in samples of hair, nails, urine (metabolites) and skin scale collected from the areas in Bangladesh affected by arsenic

Parameter	Arsenic in hair[a] (µg/kg)	Arsenic in nails[b] (µg/kg)	Arsenic in urine[c] (µg/L)	Arsenic in skin scale[d] (µg/kg)
No. of observations	4 386	4 321	1 084	705
Mean	3 390	8 570	280	5 730
Median	2 340	6 400	116	4 800
Minimum	280	260	24	600
Maximum	28 060	79 490	3 086	53 390
Standard deviation	3 330	7 630	410	9 790
% of samples having arsenic above normal	83.15	93.77	95.11	–

From Rahman *et al.* (2001)

[a] Normal levels of arsenic in hair range from 80 to 250 µg/kg; 1000 µg/kg indicates toxicity.

[b] Normal levels of arsenic in nails range from 430 to 1080 µg/kg

[c] Normal levels of arsenic in urine range from 5 to 50 µg/1.5 L (per day)

[d] Normal value for skin scale arsenic not defined

Thousands of hair, nail and urine samples from people living in villages affected by arsenic have been analysed (Table 8). Approximately 90% of children under 11 years of age living in the affected areas show levels of arsenic in hair and nails above the normal level (Rahman *et al*., 2001).

A comparative study reported analyses of arsenic species in urine samples ($n = 42$) from one affected village of Madaripur district, where the average concentration of arsenic in drinking-water was 376 µg/L, and a non-affected village ($n = 27$), where the concentration of arsenic in drinking-water is known to be below 3 µg/L (Chowdhury *et al*., 2003). The average urinary levels of arsenic of children were higher than those of adults. The ratios of MMA to inorganic arsenic and of DMA to MMA were 0.93 and 4.11 in adults and 0.74 and 8.15 in children, respectively.

Chakraborti *et al.* (1999a) reported arsenic concentrations in hand tubewells from 100 to 415 m in depth in all geographical regions in Bangladesh. The report indicated that 99% of the tubewells analysed that were deeper than 300 m had an arsenic concentration below 50 µg/L. Understanding the mechanism of arsenic release to groundwater in Bangladesh should help to provide guidance for the placement of safe new water wells (Nickson *et al.,* 1998, 2000).

1.4.2 *Exposure in India*

(*a*) *Contamination by arsenic of groundwater in northern India*

A preliminary study was reported in 1976 on arsenic in dug wells, hand pumps and spring water from Chandigarh and different villages of the Punjab, Haryana and Himachal Pradesh in northern India (Datta & Kaul, 1976). A value as high as 545 µg/L arsenic was obtained in one water sample from a hand pump. Datta (1976) further reported high arsenic content in the liver of five of nine patients with non-cirrhotic portal hypertension who had been drinking arsenic-contaminated water. To date no further information on arsenic poisoning in northern India is available.

(*b*) *Contamination by arsenic of groundwater in West Bengal*

Since 1984, extensive research in West Bengal has revealed that this region has one of the most serious problems with groundwater contamination by arsenic in wells used for drinking-water. Figure 2 shows the districts in West Bengal affected by arsenic and Table 9 gives an overall picture of the database and the extent of the problem. Table 10 shows the distribution of concentrations of arsenic in hand tubewells in areas of West Bengal, and Table 11 summarizes the levels of arsenic measured in biological samples.

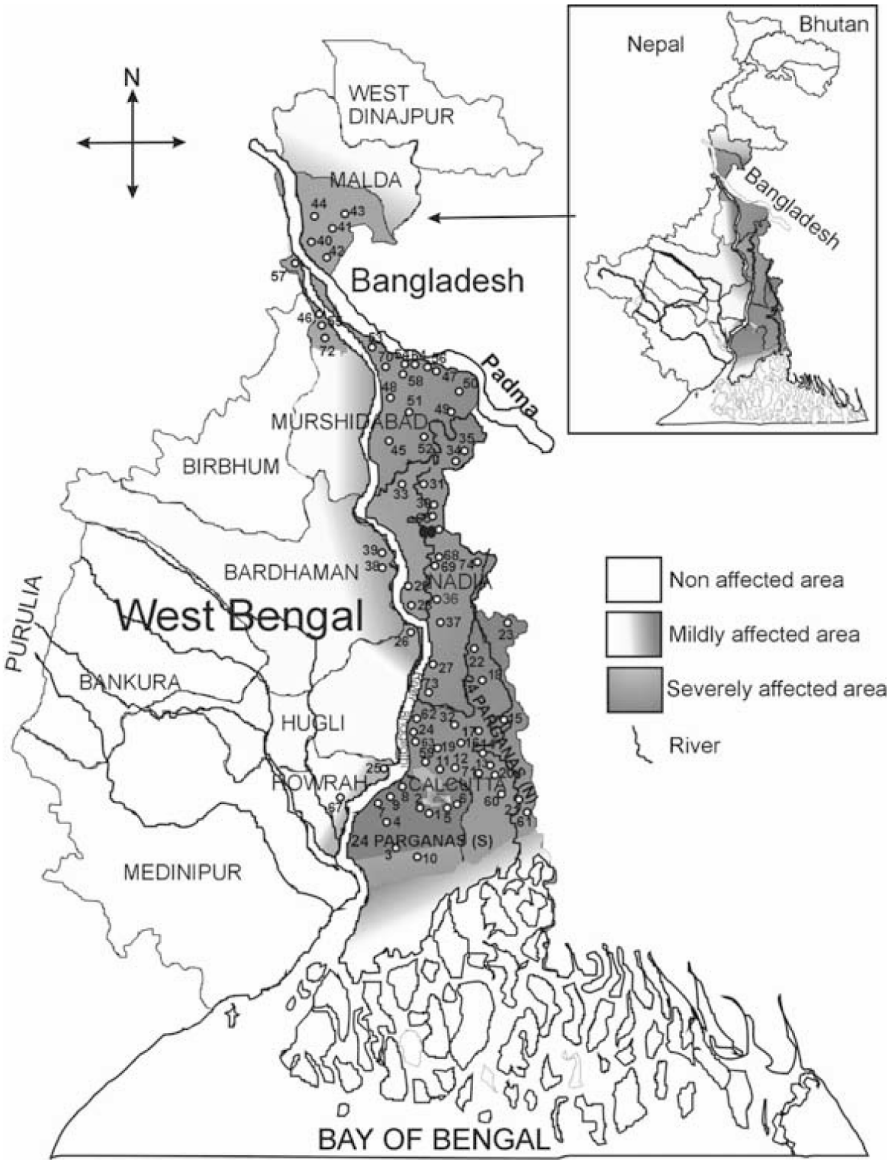
Contamination of groundwater by arsenic was first detected in the state of West Bengal, India, in 1983 (Garai *et al*., 1984). Sixteen people whose drinking-water came from two hand tubewells in one village in the district of 24-Parganas were identified as having arsenical skin lesions. Arsenic concentrations in these tubewells were 1250 and

Table 9. Status of contamination of groundwater by arsenic in West Bengal, India

	West Bengal
Total area (km^2)	89 193
Population (millions; according to 1991 Census)	68
Total number of districts	18
Total number of water samples analysed	105 000
Samples containing > 10 µg/L arsenic (%)	51
Samples containing > 50 µg/L arsenic (%)	25
Number of districts affected by arsenic (> 50 µg/L)	9
Population of districts affected by arsenic (millions)	42.7
Area of districts affected by arsenic (km^2)	38 865
Number of blocks/police stations affected by arsenic	74
Number of villages (approx.) affected by arsenic (arsenic in groundwater > 50 µg/L)	2700
Number of people drinking arsenic-contaminated water > 50 µg/L (millions)	6

From Chakraborti *et al* (2002)

Figure 2. Areas of West Bengal in which drinking-water is contaminated with arsenic



From Chakraborti *et al.* (2002)

Table 10. Concentrations of arsenic in water samples from hand tubewells in West Bengal, India

No. of water samples analysed	Arsenic concentration range (µg/L)							
	< 10	10–50	51–99	100–299	300–499	500–699	700–1000	> 1000
101 934	49 310	27 309	10 005	11 782	2354	724	334	116
	48.4%	26.8%	9.8%	11.6%	2.3%	0.7%	0.3%	0.1%

From Rahman *et al.* (2001)

Table 11. Concentrations of arsenic in samples of hair, nails, urine (metabolites) and skin scale collected from the areas in West Bengal (India) affected by arsenic

Parameters	Arsenic in hair[a] (µg/kg)	Arsenic in nails[b] (µg/kg)	Arsenic in urine[c] (µg/L)	Arsenic in skin scale[d] (µg/L)
No. of observations	7 135	7 381	9 795	165
Mean	1 480	4 560	180	6 820
Median	1 320	3 870	115	4 460
Minimum	180	380	10	1 280
Maximum	20 340	44 890	3 147	15 510
Standard deviation	1 550	3 980	268	4 750
% of samples having arsenic above normal	57	83	89	–

From Rahman *et al*. (2001)

[a] Normal levels of arsenic in hair range from 80 to 250 µg/kg; 1000 µg/kg indicates toxicity.

[b] Normal levels of arsenic in nails range from 430 to 1080 µg/kg

[c] Normal excretion of arsenic in urine ranges from 5 to 40 µg/1.5 L (per day)

[d] Normal value for skin scale arsenic not defined

700 µg/L. Saha and Poddar (1986) reported that 36 villages from 18 police stations/blocks of six districts were affected in 24-Parganas, Murshidabad, Nadia, Barddhaman, Midnapur and Maldah. Water samples from 207 hand tubewells were analysed and 105 (50.7%) showed arsenic concentrations above 50 µg/L; the highest concentration recorded was 568 µg/L. Analysis of arsenic in hair, nails and skin-scale from people in the affected villages confirmed exposure to arsenic.

In 1987, an epidemiological survey in six villages of three districts (24-Parganas, Barddhaman and Nadia) revealed 197 patients with arsenical dermatosis in 48 families

(Chakraborty & Saha, 1987). Of 71 water samples collected from tubewells of the affected villages, the concentration of arsenic in 55 (77.5%) was higher than the permissible limit (50 µg/L) for arsenic in drinking-water in India. The mean arsenic concentration in 31 water samples collected from tubewells of affected families was 640 µg/L and that in 40 water samples collected from tubewells of unaffected families was 210 µg/L. Another epidemiological investigation (Guha Mazumder *et al*., 1988) in a village in 24-Parganas also found evidence of effects of arsenic in 62 (92.5%) of 67 members of families who drank contaminated tubewell-water (level of arsenic, 200–2000 µg/L). In contrast, only six (6.25%) of 96 persons from the same area who drank water with a level of arsenic < 50 µg/L showed any effects.

In 1991, a report from the government of West Bengal (Steering Committee, Arsenic Investigation Project, 1991) concluded that water of the intermediate aquifer in areas of West Bengal was polluted with arsenic. Neither the shallow (first) nor the deep (third) aquifers had reported arsenic contamination. The sand grains in the arsenic-contaminated aquifer were generally coated with iron and material rich in arsenic.

In October 1994, a committee constituted by the government of West Bengal (Committee Constituted by Government of West Bengal, 1994) reported arsenic contamination in 41 blocks in six districts of West Bengal. The committee analysed about 1200 water samples from these six districts for arsenic and other common water-quality parameters, and the highest concentration of arsenic reported was 3200 µg/L.

The expanding database on the problem of arsenic contamination in West Bengal has been documented in a continuing series of publications. By December 1994, it was reported that 312 villages from 37 blocks/police stations in six districts in West Bengal were affected by contamination of groundwater with arsenic. From extrapolation of the data, it was predicted that more than 800 000 people were drinking arsenic-contaminated water from these districts, and based on the analysis of several thousand water samples, average arsenic concentrations in the wells sampled ranged from 193 to 737 µg/L (Das *et al.*, 1994; Chatterjee *et al*., 1995). The highest arsenic concentration of 3700 µg/L was found in a hand tubewell from a village in South 24-Parganas district. Groundwater and urine samples from affected villages were also analysed for arsenite, arsenate, MMA and DMA. Groundwater contained arsenate and arsenite in a ratio of approximately 1:1. In urine, DMA and MMA were the predominant species, together with some arsenite and arsenate. Das *et al.* (1995) reported high arsenic levels in the hair, nails, urine, skin-scale and a few liver tissues (biopsy) of people from arsenic-affected villages who had arsenical skin lesions.

Based on the analysis of 20 000 water samples from areas of West Bengal, Mandal *et al.* (1996) reported that seven districts (North 24-Parganas, South 24-Parganas, Nadia, Barddhaman, Murshidabad, Maldah, Hugli) were affected by arsenic. Approximately 45% of these samples had arsenic concentrations above 50 µg/L, and the average concentration was approximately 200 µg/L.

Groundwater contamination was reported in 985 villages from 69 police stations/ blocks in nine districts of West Bengal on the basis of analyses of 58 166 water samples.

The nine districts were Maldah, Murshidabad, Barddhaman, Hugli, Howrah, Nadia, North 24-Parganas, South 24-Parganas and Calcutta. After extrapolation of data from the water analyses and screening villagers for arsenical skin lesions, it was estimated that about 5 million people were drinking-water contaminated with levels of arsenic above 50 µg/L. The total population in the nine districts of West Bengal affected by arsenic is about 43 million (Chowdhury *et al.,* 2000a,b).

On the basis of an analysis of 101 394 hand tubewells and approximately 25 000 biological samples, and screening of 86 000 persons in affected villages of West Bengal, Rahman *et al.* (2001) reported that 2600 villages were affected by arsenic in groundwater at levels of > 50 µg/L and that approximately 6 million people drank water contaminated with arsenic at levels above 50 µg/L. Mandal *et al.* (2001) identified DMA^{III} and MMA^{III} for the first time in urine from the affected areas of West Bengal.

Roychowdhury *et al.* (2002) reported total arsenic in food composites collected from a few arsenic-affected villages in Murshidabad district, West Bengal, where arsenic-contaminated groundwater was used for agricultural irrigation. The report showed average daily dietary intake of arsenic from foodstuffs for adults and children of 180 and 96.5 µg, respectively.

Rahman *et al.* (2003) studied North 24-Parganas, one of the nine affected districts of West Bengal, for 7 years. On the basis of analyses of 48 030 water samples and 21 000 hair, nail and urine samples, and screening of 33 000 people in North 24-Parganas, it was estimated that about 2 million and 1 million people are drinking water contaminated with arsenic at levels above 10 and 50 µg/L, respectively.

(i) *Source of contamination of groundwater by arsenic in West Bengal*

When the contamination of drinking-water by arsenic was first discovered in West Bengal, tubewell strainers, pesticides, insecticides and other anthropogenic sources were first considered as possible origins of the groundwater contamination (Chakraborty & Saha, 1987). However, Das *et al.* (1994) showed that a single deep tubewell supplying water to a few villages in Maldah, one of the nine arsenic-affected districts, was drawing nearly 150 kg arsenic per year, indicating that the source of arsenic was geological. Analyses of bore-hole sediments showed high concentrations of arsenic in only a few soil layers and the arsenic therein was found to be associated with iron pyrites. Das *et al.* (1995, 1996) also confirmed analytically the existence of arsenic-rich pyrites in bore-hole sediment. It was proposed that heavy drawing of groundwater and aeration of the aquifer leads to the decomposition of arsenic-rich pyrites and consequently contamination of groundwater with arsenic. Similar conclusions were reached by Mallick and Rajagopal (1995).

Bhattacharya *et al.* (1997, 1998) reported an association between arsenic and hydrated ferric oxide (HFO) and its mobilization to the aquifer due to changes in redox conditions during the development of groundwater. Ahmed *et al.* (1998) and Nickson *et al.* (1998, 2000) also suggested that reduction of HFO resulted in the mobilization of arsenic from absorbed HFO.

(ii) *Contamination of groundwater by arsenic in the residential area of Behala-Calcutta due to industrial pollution*

In Calcutta, chronic arsenicosis was first reported by Guha Mazumder *et al.* (1992). The study of Chatterjee *et al.* (1993) on the source of arsenic and the magnitude of the contamination revealed that a chemical factory producing several chemical compounds, including the insecticide Paris-Green (copper acetoarsenite), was responsible for the contamination. This factory had been producing about 20 tonnes of Paris-Green per year for approximately 20 years. Analysis of soil surrounding the production waste-dumping ground showed very high concentrations of arsenic (as high as 10 000 µg/g). Nineteen hand tubewells, used for drinking and cooking in the immediate area, showed very high concentrations of arsenic (up to 39 000 µg/L). The concentration of arsenic in hand tubewells decreased the farther the wells were located from the dumping ground. A follow-up study in the affected areas (Chakraborti *et al.*, 1998) showed that the total average concentration of arsenic in the 19 hand tubewells sampled previously had decreased by only 10–15% from the levels observed 8 years before.

(*c*) *Contamination of groundwater by arsenic in Chhattisgarh State*

Contamination of groundwater by arsenic was reported in a few villages of Rajnandgaon district of Chhattisgarh by Chakraborti *et al.* (1999b). The present State of Chhattisgarh had been within the State of Madhya Pradesh 2 years previously. The source of arsenic in groundwater is natural and geological both for the alluvial Bengal Basin and the rocky belt of Dongargarh-Kotri zone of Rajnandgaon district. The total population of the district is 1.5 million. Except for two towns — Rajnandgaon and Khairagarh — the entire district depends on tubewells and dugwells. Water samples (n = 146) were collected from 22 villages of Chowki block, Rajnandgaon district, and levels of arsenic in groundwater were found to be above 10 µg/L in eight villages and above 50 µg/L in five villages, with the highest concentration being 880 µg/L. From 150 hair samples examined, approximately 75% of people were found to have levels of arsenic in hair above toxic levels. Pandey *et al.* (1999) also reported contamination of groundwater by arsenic in the Rajnandgaon district of Chhattisgarh. Of 390 samples analysed, 26 sites were found to be contaminated with arsenic, with the highest concentration being 1010 µg/L. The number of people at risk was estimated at 10 000. Pandey *et al.* (2002) established that the extent of the arsenic contamination in this area is even greater; about 30 000 people residing in 30 villages and towns are directly exposed to high levels of arsenic in drinking-water (up to 3050 µg/L arsenic) and more than 200 000 people are at risk. The source and mobilization process of arsenic from affected areas of Rajnandgaon district Chhattisgarh was reported by Acharyya (2002).

(*d*) *Contamination of groundwater by arsenic in Middle Ganga Plain, Bihar*

In the Middle Ganga Plain, Bihar, tubewells replaced dugwells about 20 years ago. Analyses of the arsenic content of 206 tubewells from Semria Ojha Patti (95% of the total in the village) showed that 56.8% exceeded concentrations of 50 μg/L, with 19.9% > 300 μg/L. The distribution indicated that, of the 5000 residents of Semria Ojha Patti, 18.8% used safe water (< 10 μg/L arsenic), 24.7% used water containing 10–50 μg/L arsenic, 56.8% used water containing > 50 μg/L, and 19.9% used water containing > 300 μg/L. The concentrations of arsenic in urine, hair and nail correlated significantly (r = 0.72–0.77) with concentrations in drinking-water. Of the 51 urine samples analysed, 98% had levels of arsenic above that of the normal secretion, with 47% > 500 mg/L, 33% > 1000 mg/L and 5.9% > 3000 mg/L; 57.6% of hair samples and 76.3% of nail samples were found to be above the normal range (Chakraborti *et al.*, 2003a).

(*e*) *Contamination of groundwater and surface water by arsenic in the industrial region of Patancheru, Andhra Pradesh*

Patancheru, in the Medak District of Andhra Pradesh, is one of the major industrial estates, situated 30 km from Hyderabad. The main source of arsenic has been identified as Park Trade Centre, Gaddapotharam Bulk Drug Factory, which makes veterinary drugs based on arsonic acid, as well as other sources such as the pesticide and drug intermediate industries. The solid wastes of these industries are dumped indiscriminately near Kazipally Lake, and represent a source of contamination of nearby waters and soils. Arsenic contamination was evaluated in 14 villages in this area. Very high levels of arsenic were found in the range of 80–8960 μg/L and 140–7350 μg/L in surface water and groundwater, respectively. In both surface water and groundwater, the average arsenite (As^{III}) concentration was about 20% of total arsenic (Sekhar *et al.*, 2003).

Samples of blood, urine, hair and nails from 193 inhabitants of these 14 contaminated villages were analysed. Arsenic levels in the biological samples were very high, ranging from 400 to 1400 μg/kg in blood (control, 6–10 μg/kg), from 60 to 160 μg/L in urine (control, 6–10 μg/L), from 300 to 940 μg/kg in hair (control, 10–130 μg/kg) and from 500 to 1630 μg/kg in nails (control, 120–160 μg/kg). High concentrations of arsenic were also detected in vegetables, plants and cow's milk in this area and represent a second possible source of exposure for the population (Sekhar *et al.*, 2003).

1.4.3 *Exposure in Central and South America*

In South America, the main source of exposure to arsenic has been the natural contamination of drinking-water. In this area, arsenic originates from the geological formations associated with volcanoes, affecting Chile, Bolivia, Peru and Argentina in the Andean region (Queirolo *et al*., 2000a). The largest populations affected are the Antofagasta Region in northern Chile, with approximately 400 000 exposed inhabitants, and the Córdoba Province in Argentina, with approximately 630 000 people exposed. Mexico also

has naturally occurring arsenic in drinking-water, which is best characterized in the Lagunera region, in central northern Mexico, where approximately 400 000 people are exposed. The mean levels in drinking-water for these populations range from 50 to 500 µg/L; in isolated wells, levels reach as high as 6897 µg/L arsenic. Exposure has been recorded since the beginning of the last century. Currently, most areas in these regions receive water with levels of arsenic below 50 µg/L.

Exposures to arsenic due to contaminated air, soils and water as a result of copper, gold or silver mining have been described in Mexico (Díaz-Barriga *et al*., 1993; Calderón, 1999; Mejía *et al.*, 1999), Chile (Romo-Kroger & Llona, 1993; Romo-Kroger *et al.*, 1994; Santolaya *et al*., 1995; Sancha, 1997; Flynn *et al*., 2002), Brazil (Romo-Kroeger & Llona, 1993) and Nicaragua (Cruz *et al*., 1994). The area affected in central Mexico is San Luis de Potosí. In Chile, environmental and occupational exposures to arsenic in air have been reported in the Andes Mountains in Regions II, III, V and VI and the Metropolitan Region, where five major copper mining plants are located (Ministerio de Salud, 1986; Santolaya *et al*., 1995; Ferreccio *et al*., 1996), but no secondary contamination of drinking-water.

(*a*) *Mexico*

In Mexico, most studies of arsenic in drinking-water have been conducted in the States of Durango and Coahuila, which constitute the Lagunera Region (Table 12). Del Razo *et al*. (1990) studied 128 wells from 11 counties and found arsenic contents of 8–624 µg/L; 50% of the wells had arsenic levels > 50 µg/L. They estimated that at least 400 000 people, mostly from the rural areas of the region, have been exposed to levels of arsenic > 50 µg/L. Since the 1960s, when arsenic contamination was first identified, the polluted wells have gradually been replaced and, by the end of 1989, most of the population was receiving water with arsenic levels below 20 µg/L (Cebrián *et al*., 1994). Some contamination of drinking-water by arsenic has been reported in the State of Hidalgo in the Zimapan Valley, where the exposed population has been estimated at 35 000, and levels of arsenic in the drinking-water ranged from 21 to 1070 µg/L (Armienta *et al*., 1997; Gomez-Arroyo *et al*., 1997).

In San Luis de Potosí, in central Mexico, exposure to arsenic associated with mining activities arises from drinking-water, soil and dust, and the estimated exposed population is 600 000. Mean arsenic concentrations in air have been measured at 0.48 µg/m^3 (0.36–0.88 µg/m^3) (Díaz-Barriga *et al*., 1993), and concentrations of arsenic in drinking-water vary from 9.9 to 20.9 µg/L (with some wells near the smelter having concentrations that range from 105 to 6897 µg/L). Studies of soil in San Luis de Potosí have demonstrated extremely high levels of arsenic in the vicinity of the mines (188–944 µg/g, Díaz-Barriga *et al*., 1993; 2215–2675 µg/g, Mejía *et al.*, 1999), and also in the dust of the nearby households (800–1182 µg/g, Díaz-Barriga *et al*., 1993; 1780–9950 µg/g, Mejía *et al.*, 1999). By 1991, the copper mining companies that caused the air, soil and water contamination of the area implemented dust control technologies and other measures to control soil pollution (Cebrián *et al*., 1994).

Table 12. Exposure to arsenic in drinking-water in Mexico

Location	Source of water	No. of samples studied	Year	Total arsenic in water (μg/L; range)	Reference
Sonora, Hermosillo	Wells of 29 cities	173	NR	2–305	Wyatt *et al*. (1998a)
Lagunera Region	Wells, different towns	171	1970s–1980s	7–624	Cebrían *et al*. (1983); Del Razo *et al*. (1990); García-Vargas *et al*. (1994); Gonsebatt *et al*. (1997); Hernández-Zabala *et al*. (1999)
Zimapán, Hidalgo	Aquifer, 6 different towns		(Since 1970)	21–1070	Gomez- Arroyo *et al*. (1997)
San Luis de Potosí	Tap-water, Morales and Graciano	19	NR	9.9–20.9	Díaz-Barriga *et al*. (1993)
	Wells near smelter	NR	NR	106–6897	Meíja *et al.* (1999)

NR, not reported

Levels of arsenic in urine and hair are presented in Tables 13–14. Levels of arsenic in hair were high in samples from subjects exposed to arsenic in water in Zimapán, and were twice those in samples from subjects in Mexico City, which were also above the reference value, probably due to air pollution (Armienta *et al*., 1997).

(*b*) *Argentina*

In Argentina, the main source of arsenic in drinking-water has been from wells, with concentrations ranging from 40 to > 4500 μg/L (Table 15), and arsenic was first reported in well-water in 1917 (Arguello *et al*., 1938). In 1970 and 1980, aqueducts from rivers with low levels of arsenic were built to replace the use of well-water, but some populations continued to be exposed (Hopenahyn-Rich *et al*., 1996a,b,c). The provinces with high levels of arsenic in their well-water are: Córdoba, Salta, La Pampa, Santa Fé, Tucuman, Santiago del Estero, San Luis and part of Buenos Aires. The best characterized is Córdoba, a region in central Argentina, that extends over an area of 165 000 km^2 and has a population of 2 750 000, distributed in 26 counties. In some counties of Córdoba, high levels (between 100 and 2000 μg/L) of arsenic were recorded in drinking-water during the 1930s (Hopenhayn-Rich *et al*., 1996a).

In Córdoba, Hopenhayn-Rich *et al*. (1996a) obtained data from various sources, including measurements of arsenic in drinking-water from official national health reports made in the 1930s, a survey in 1942, two studies reported in 1968 and 1985 and a water survey reported in 1973. Based on the available measurements, average exposure of the population of each town was estimated, assuming that all people drank the same concentration

Table 13. Total arsenic in human urine samples in Mexico, Argentina and Chile

Location	No. of exposed subjects studied	Year	Mean arsenic in urine	Range	Reference
Mexico					
Lagunera, Santa Ana	36 adults		489 µg/gc	109–1829 µg/gc	García-Vargas *et al*. (1994);
	35 adults		548 µg/gc	295–849 µg/gc	Del Razo *et al*. (1997);
	37 adults		848 µg/L	88–2058 µg/L	Hernández-Zavala *et al*. (1999)
San Luis de Potosí	80 children		51.6 µg/gc	18.2–186.2 µg/gc	Calderón *et al*. (2001)
	112 children		70.5 µg/gc	17.7–497.7 µg/gc	Meíja (1999)
	133 children		117.6 µg/gc	33–594 µg/gc	Díaz-Barriga *et al*. (1993)
Argentina					
Córdoba province, MJ	282		160 µg/L	60–410 µg/L	Lerda (1994)
Santa Fe, Tortugas	155		70 µg/L	10–600 µg/L	Lerda (1994)
San Antonio	11		274 µg/L	126–440 µg/L	Vahter *et al*. (1995)
Other towns	15		36 µg/L	13–89 µg/L	Vahter *et al*. (1995)
San Antonio	10 lactating women		400 µg/L	250–610 µg/L	Concha *et al*. (1998a)
	11 pregnant women		335 µg/L	116–439 µg/L	Concha *et al*. (1998c)
San Antonio and Taco	34 children	1994	382 µg/L	125–621 µg/L	Concha *et al*. (1998b)
San Antonio	23 women		344 µg/L	90–606 µg/L	
Chile					
Region I	93 general population	1984–95	45 µg/L	10–92 µg/L	Venturino (1987); Sancha (1997)
Rest of Chile	2472 general population	1984–2000	13 µg/L	5–49 µg/L	Venturino (1987); Sancha (1997); CONAMA (2000)
Antofagasta	164 general population	1968	NR	1–700 µg/L	Gonzalez (1970)
Antofagasta	262 general population	1994–2000	69 µg/L	18–99 µg/L	Sancha (1997); CONAMA (2000)
Calama	239 general population	1977–95	76 µg/L	21–124 µg/L	Borgoño *et al*. (1980); Sancha (1997)
San Pedro	265 general population	1997	611.7 µg/L	61–1893 µg/L	Hopenhayn-Rich *et al*. (1996b,c); Moore *et al*. (1997a,b)

gc, grams of creatinine; NR, not reported

Table 14. Arsenic in human hair samples in Mexico and Chile

Location (source of exposure)	Year of sample	No. of subjects	Total arsenic in sample (μg/g)		Reference
			Mean	SD (range)	
Mexico					
Zimapán	NR	120	8.5	3.56	Armienta *et al*. (1997)
Mexico City (water)		17	4.6	1.96	
San Luis de Potosí	NR				Díaz-Barriga *et al*. (1993)
(smelter (1.5 km))		75	9.9	(1.4–57.3)	
(smelter (25 km))		25	0.5	(0.2–1.2)	
Lagunera (wells)	NR	35	NR	(0–23.3)	Chávez *et al*. (1964)
Chile					
Iquique	1969	26	0.8	NR	Borgoño & Greiber (1971)
Antofagasta	1968–76	607	7.7	4.2–14.8	Gonzalez (1970); Borgoño & Greiber (1971); Sandoval & Venturino (1987)
Antofagasta	1986–92	293	0.42	0.01–3.68	Jamett *et al*. (1992); Peña *et al*. (1992)
Calama	1977	203	3.75	0–10	Borgoño *et al.* (1980)
Calama	1986–92	60	4.28	0.98–14.2	Jamett *et al*. (1992); Peña *et al*. (1992)
Chuquicamata	1986–92	60	17.19	3.03–54.77	Jamett *et al*. (1992); Peña *et al*. (1992)
Puchuncaví	1990	151	2.178	0.103–18.023	Chiang *et al*. (1990)
Valparaíso	1990	NR	0.434	0.015–1.525	Chiang *et al*. (1990)

SD, standard deviation

of arsenic. It was estimated that 273 014 people had been exposed to an average of 178 μg/L arsenic and another 406 000 people had been exposed to some arsenic (at least one measurement of 120 μg/L in water). A report available through CEPIS/PAHO (Penedo & Zigarán, 2002) described the arsenic content of 100 water samples from wells in Córdoba and confirmed Hopenhayn's estimations: they estimated that 625 861 people were exposed to arsenic, with regional averages ranging from 70 to 180 μg/L and individual well measurements from 10 to 1900 μg/L.

The Salta Province is the only area where high levels of arsenic have also been found in surface waters (Penedo & Zigarán, 2002). In the provinces of Salta and Jujui, in north-western Argentina, samples from five rivers had arsenic levels ranging from 52 to 1045 μg/L, and samples from three surging thermal springs had arsenic levels of 128–10 650 μg/L (de Sastre *et al.*, 1992). The population of San Antonio de los Cobres is the best studied in this province (Vahter *et al*., 1995; Concha *et al*., 1998a,b,c). San Antonio de los Cobres is a village in the Salta Province, 3800 m above sea level, with

Table 15. Exposure to arsenic in drinking-water in Argentina

Location	Source of drinking-water wells	No. of samples (year)	Total arsenic in water (μg/L)		Reference
			Average	Range	
Córdoba Province	Bell-Ville	NR (1917–20)	NR	1120–4500	Arguello *et al*. (1938)
Córdoba Province	Marcos Juárez	282 (NR)	130	10–660	Lerda (1994)
Córdoba Province	2 counties	118 (1942)	178	40–533	Hopenhayn-Rich *et al*. (1996a)
Córdoba Province	5 counties	67 (NR)	70–180	10–1900	Penedo & Zigarán (2002)
Santa Fe Province	Tortugas	155 (NR)	20	0–70	Lerda (1994)
Salta Province	School pipes	18 (NR)	592	4–1490	Astolfi (1971)
Salta Province	San Antonio San Antonio	2 areas (NR) 1 well	NR NR	93–440 8250–10 650	de Sastre *et al.* (1992)
	San Antonio	10 (1994)	167	117–219	Vahter *et al*. (1995); Concha *et al*. (1998a); Del Razo *et al*. (1999)

NR, not reported

approximately 5000 inhabitants (Vahter *et al*., 1995). Until recently, this population had been drinking-water from wells with arsenic contents varying from < 1 to 440 μg/L, with one well reaching 9450 μg/L on average. Arsenic levels in urine are presented in Table 13 and other biomarkers in Table 16.

There are no studies of arsenic in air in Argentina. High levels of arsenic have been found in prepared food and soups in San Antonio de los Cobres (soup, 259–427 μg/g; prepared food, 131–418 μg/g; Concha *et al*., 1998b).

(*c*) *Chile*

Northern Chile (Regions I–III) is an expanse of 250 000 km^2, of which 35 000 are quaternary volcanic rocks rich in arsenic (Queirolo *et al*., 2000a). Arsenic reaches the population through drinking-water and through contamination of air and soil, as a result of mining activities.

In Chile, the main sources of drinking-water are rivers that originate in Cordillera de los Andes and reach the Pacific Ocean. Rivers in northern Chile (Regions I and II) have high natural arsenic concentrations, particularly those from the Region of Antofagasta. Arsenic concentrations in rivers in Region II vary along its course, depending on the arsenic content of its tributaries, and range from 30 to 3310 μg/L but reach 14 250 μg/L in some hot springs (Table 17) (Alonso, 1992; Queirolo *et al*., 2000a). Exposure of the

Table 16. Arsenic in other biological samples in Salta Province, Argentina

Location	No. of subjects	Type of sample (year)	Total arsenic in sample		Reference
			Median	Range	
San Antonio de los Cobres	9 women	Breast milk	2.3 µg/kg[a]	0.83–7.6 µg/kg	Concha *et al*. (1998a)
		Blood	9.8 µg/L	4.4–19 µg/L	
		Urine	390 µg/L	250–610 µg/L	
		Blood	µg/L	µg/L	Vahter *et al*. (1995)
San Antonio	15		7.6	2.7–18.3	
Santa Rosa	5		1.5	1.1–2.0	
Olacapato	5		1.3	1.2–2.4	
Tolar Grande	5		1.3	1.0–1.3	
	Children	Blood (1994)	µg/L	µg/L	Concha *et al*. (1998b)
San Antonio and T Pozo	36		9.1	5.5–17	
Rosario de Lerma	20		0.8	0.27–1.5	
	Women				
San Antonio and T Pozo	27		9.3	2.7–18	
Rosario de Lerma	11		0.95	0.69–1.8	
San Antonio de los Cobres	Pregnant women				Concha *et al*. (1998c)
	11	Blood	11 µg/L	5.6–13 µg/L	
	11	Cord blood	9.0 µg/L	6.0–12 µg/L	
	11	Placenta	34 µg/kg	17–54 µg/kg	
	10	Maternal milk	3.0 µg/kg[a]	2.3–4.8 µg/kg	

[a] µg/kg fresh weight

Table 17. Concentration of arsenic in surface waters in Chile, Region II, 1983–86

River studied and location	Mean total arsenic in water (µg/L)
Salado River	
Tatio Hot Springs	14 250
Codelco Mine Reservoir	7 500
Before Toconce River	3 310
Toconce River Before Salado	600
Before Curti	860
Ayquina	980
Before Loa River	760
Loa River	
Before Salado	270
Yalquincha	800
Finca	910
Before San Salvador River	1 380
San Salvador Before Loa	1 270
La Posada Bridge	1 500
Quillagua	1 440
Outlet of River	1 360
Upper Loa River Basin	210–330
Gorges south of Salado river	30–60
Spring north of Salado river	190–370

From Alonso (1992)

population in this region has ranged from 40 to 860 µg/L, depending on the rivers used for its water supply; 1958–70 was the highest exposure period for the largest population (approximately 300 000) (Table 18).

Sancha (1997) estimated the total number of people exposed in 1996 to specific levels of arsenic in the drinking-water in Chile: 7 million inhabitants (53.3%) were exposed to less than 10 µg/L; 5.5 million (41.9%) were exposed to 10–30 µg/L; 450 000 inhabitants (3.4%) were exposed to 30–50 µg/L; 170 000 inhabitants (1.3%) were exposed to 50–60 µg/L; and 1500 (0.01%) were exposed to 600–800 µg/L.

There are a few studies of arsenic in general environmental air in Chile (Romo-Kroger & Llona, 1993; Romo-Kroger *et al*., 1994; Sancha, 1997; COSUDE, 2000). Sancha (1997) and COSUDE (2000) covered a large part of the country from 1994 to 1999. They found that cities not in the vicinity of copper smelting operations had arsenic levels in the air ranging from 0.001 to 0.057 µg/m^3, with a population of approximately 6 million people. The cities located 30–45 km from a copper smelter had arsenic levels in the air ranging from 0.01 to 0.14 µg/m^3 and had approximately 755 000 inhabitants. The cities in the vicinity (within 10 km) of smelters had arsenic levels in air ranging from 0.03

Table 18. Average concentration (μg/L) of arsenic in drinking-water in Regions II and I and the rest of Chile

Region	Town	Population (2002 census) living in exposed areas	1930–57	1958–70	1971–77	1978–80	1981–87	1988–94	1995–2002	Reference
II	Tocopilla and Elena	31 175	250	250	636	110	110	40	–	Ferreccio *et al*. (2000)
	Calama	136 739	150	150	287	110	110	40	– 38	Ferreccio *et al*. (2000) Sancha (1997)
	San Pedro	4 883	600	600	600	600	600	600	–	Ferreccio *et al*. (2000)
	Chiu Chiu	250	–	–	–	–	–	–	753	Smith *et al*. (2000b)
	Caspana	275	–	–	–	–	–	–	13	Smith *et al*. (2000b)
	Antofagasta and Mejillones	306 548	90	860	110	110	70	40	– 32	Ferreccio *et al*. (2000) Sancha (1997)
I	Arica-Iquique	426 351	–	–	–	–	–	–	32	Sancha (1997)
III–XIII	Rest of Chile	14 213 266	–	–	–	–	–	–	5	Sancha (1997)

Averages supplied by Empresa Servicios Sanitarios de Antofagasta for 1950–67 and Servicio de Salud Antofagasta for 1968–94
For 1995–2000, data and ranges published in studies

to 2.4 μg/m^3, with an estimated 60 000 people exposed. The rest of the country that was not sampled has a population of approximately 6 million people whose exposure is estimated to be in the lowest range of < 0.010 μg/m^3 (COSUDE, 2000).

Mean levels of arsenic in the air inside the Chuquicamata copper mine, the world's largest open copper mine, for the period 1952–91 ranged from 1.6 μg/m^3 in the administrative areas to 201.72 μg/m^3 in the smelting areas (Ferreccio *et al*., 1996). This exposure has decreased in the last decade in correlation with the implementation of new technologies in smelting to avoid arsenic contamination. Workers exposed to arsenic had urinary levels ranging from 40 to 490 μg/L in 1992; between 1987 and 1990, 32–58% of workers in exposed areas had levels of urinary arsenic above 300 μg/L.

In Region II, 1020 people were examined between 1987 and 2002; the mean total arsenic in urine was 225 μg/L, ranging from 1 to 1893 μg/L. In comparison, in Region I which has intermediate exposure to arsenic in water (40 μg/L), 91 people were examined and their urinary arsenic averaged 45.5 μg/L, ranging from 10 to 92 μg/L. In the rest of Chile (arsenic in drinking-water, < 10 μg/L), 2472 people were sampled and mean urinary arsenic was 13 μg/L, ranging from 5 to 49 μg/L (Table 13). Arsenic measured in hair from people in Chile is presented in Table 14.

(*d*) *Other*

In Nicaragua, there has been concern regarding contamination with heavy metal of Lake Asososca, which is a source of drinking-water for Managua. The level of arsenic in sediment was found to be 4.1 μg/g, and that in water ranged from 0 to 18.07 μg/L, with a mean concentration of 5.86 μg/L, well below current water standards. Higher values of 25 μg/L were found in Lake Monte Galán (Cruz *et al*., 1994). An earlier study conducted in Lake Xolotlán found arsenic levels in surface water ranging from 10.2 to 30.1 μg/L; wastewater from a thermal plant discharging into the river contained concentrations of 5295–16 700 μg/L (Lacayo *et al*., 1992).

In Brazil, concerns have been raised regarding arsenic contamination as a result of gold mining in the zone of Minas Gerais, in south-eastern Brazil. In 1998, urinary arsenic was measured in 126 schoolchildren, and a mean concentration of 25.7 μg/L (range, 2.2–106 μg/L) was found. Environmental studies in the surrounding areas found mean levels of arsenic in surface water of 30.5 μg/L (range, 0.4–350 μg/L); levels of arsenic in soils ranged from 200 to 860 mg/kg; and sediments had a mean concentration of 350 mg/kg, ranging from 22 to 3200 mg/kg (Matschullat *et al*., 2000).

1.4.4 *Exposure in South-East Asia*

There are many reports on the human exposure to arsenic in the drinking-water in South-East Asia. High concentrations of arsenic in drinking-water have been documented in China (Cao, 1996), Taiwan, China (Tseng *et al.*, 1968; Chiou *et al.*, 1997a), Thailand (Choprapawon & Rodcline, 1997), and Viet Nam (Berg *et al*., 2001). The use of artesian wells, which were later shown to have high levels of arsenic in the water, began in the

early 1920s in southern Taiwan, China (Tseng *et al.*, 1968), in the early 1950s in Inner Mongolia (Ma *et al*., 1996), in the late 1950s in north-eastern Taiwan, China (Chiou *et al.*, 1997a, 2001), in the early 1960s in Xinjiang, China (Wang, 1996), in the late 1980s in Ronbipool, Thailand (Choprapawon & Rodcline, 1997), in the early 1990s in Shanxi, China (Cao, 1996) and in the mid-1990s in Viet Nam (Berg *et al.*, 2001). There have been several reports on industry-related exposure to arsenic through drinking-water contamination from tin mining in Ronpibool, Thailand (Choprapawon & Rodcline, 1997).

Table 19 summarizes data on arsenic contamination of drinking-water in various regions of South-East Asia.

(*a*) *China*

Several geographical areas in mainland China have a high content of arsenic in the drinking-water, including Xinjiang, Inner Mongolia and Shanxi (Cao, 1996). The villages with high concentrations of arsenic in the drinking-water in Inner Mongolia are clustered in Bamen and Huhehot. Ma *et al.* (1996) reported the arsenic concentration in the water of 9733 wells in Bamen: 2465 had levels of arsenic > 50 μg/L; in five counties of Bamen, the percentage of wells with an arsenic concentration > 50 μg/L varied, ranging from 11 to 59%; more than 500 villages had at least one well with an arsenic concentration > 50 μg/L; and the level of arsenic in drinking-water from all wells from the two areas ranged from < 50 to 890 μg/L. The water from a total of 497 wells in Huhehot were tested for arsenic: 111 had an arsenic level > 50 μg/L; 48 villages had at least one well with arsenic concentration > 50 μg/L; and the level of arsenic in drinking-water ranged from < 81 to 890 μg/L (Ma *et al.*, 1996). Sun *et al.* (2001) reported a survey on the concentration of arsenic in 303 wells in a village in Inner Mongolia: 77 wells (25.4%) had a level of arsenic < 10 μg/L, 85 (28.1%) had levels of 10–49 μg/L, 131 (43.2%) had levels of 50–499 μg/L and 10 (3.3%) had levels of ≥ 500 μg/L.

In the highly contaminated area of Xinjiang, located in Tunguei, arsenic concentrations in well-water in 15 villages of the area ranged from 50 to 850 μg/L, and were mostly between 100 and 500 μg/L (Wang, 1996).

Sun *et al.* (2001) reported a survey of 2373 wells in 129 villages in the Basin of Datong and Jinzhong, Shanxi, in 1994–95. Levels of arsenic in drinking-water ranged from < 50 to 4440 μg/L and 833 wells had an arsenic concentration > 50 μg/L. The percentage of wells with an arsenic concentration > 50 μg/L in seven counties of the area varied from 6.3 to 54.7%.

(*b*) *Taiwan, China*

There are two endemic areas of arseniasis in Taiwan, China. One is located in the south-western coastal area where Blackfoot disease, a unique peripheral vascular disease associated with long-term ingestion of arsenic from artesian well-water, is endemic. There are four townships in this area: Peimen, Hsuehchia, Putai and Ichu. High levels of arsenic in artesian wells and patients with Blackfoot disease have also been documented in two neighbouring townships, Hsiayin and Yensui. Another endemic area of chronic arsenic

Table 19. Contamination of drinking-water by arsenic in various regions of South-East Asia

Country	Area/population	Sample	Level of arsenic (range; µg/L)	Source of arsenic	Reference
Taiwan (China)	South-western Blackfoot disease-endemic area (Peimen, Hsuehchia, Putai, Ichu)	13 artesian well-water	240–960	Natural	Blackwell *et al.* (1961)
		34 artesian well-water	350–1100	Natural	Chen *et al.* (1962)
		11 artesian well-water	340–896	Natural	Yeh (1963)
		97 artesian well-water	10–1100	Natural	Kuo (1968)
	North-eastern endemic area of chronic arsenic poisoning (Chuangwei, Wuchieh, Chiaohsi, Tungshan)	3901 well-water	< 0.15–3590	Natural	Chiou *et al.* (2001)
	Taiwan (314 townships)	83 656 well-water	< 10–> 1000	Natural	Lo (1975)
Thailand	Thammarat Province	Surface water	< 0.5–583 < 0.5–28.4 As^{III}	Arsenopyrite wastes	Williams *et al.* (1996)
		Shallow water	1.25–5114 < 0.5–125 As^{III}		
		Surface water	4.8–583	Mining	Choprapawon & Porapakkham (2001)
		River	541–583		
China	Inner Mongolia	497 well-water (Huhhot)	< 10–1860	Natural	Ma *et al.* (1996); Luo *et al.* (1997)
		9733 well-water (Bamen)	< 50–890	Natural	Ma *et al.* (1996)
	Xinjiang	Well-water in 15 villages (Tunguei)	50–850	Natural	Wang (1996)
	Shanxi	2373 well-water in 129 villages (Datong, Jinzhong)	< 50–4440	Natural	Sun *et al.* (2001)

Table 19 (contd)

Country	Area/population	Sample	Level of arsenic (range; µg/L)	Source of arsenic	Reference
Japan	Fukuoka	67 well-water	1–293 11–220 As^{V} 15–70 As^{III}	Natural	Kondo *et al.* (1999)
	Sendai		1–35		
	Takatsuki		3–60		
	Kumamoto		5–66		
Viet Nam	Red River Basin	68 tubewells,	1–3050 (72% > 10 µg/L)	Natural	Berg *et al.* (2001)
		8 treatment plants	11–190		

toxicity is located in the Lanyang Basin of north-eastern Taiwan, in which there are four townships: Chiaohsi, Chuangwei, Tungshan and Wuchieh (Table 19).

In the area of south-western Taiwan where Blackfoot disease is endemic, Blackwell *et al.* (1961) reported levels of arsenic of 240–960 μg/L in 13 artesian wells, Chen *et al.* (1962) reported levels ranging from 350 to 1100 μg/L in 34 artesian wells and Yeh (1963) found levels ranging from 340 to 900 μg/L in water samples from 11 wells. Kuo (1968) carried out a larger survey of 97 artesian wells in 42 villages of the six townships in the endemic area and found concentrations in well-water ranging from 10 to 1100 μg/L, with a median of 500 μg/L. In this south-western area, the arsenic concentration was higher in water from deep artesian wells than in that from shallow wells, showing a correlation coefficient of $r = 0.627$ ($p < 0.01$). Arsenate was the dominant species of arsenic in the artesian well-water.

Lo (1975) reported a nationwide survey of arsenic content in drinking-water from 83 656 wells in 314 precincts and townships. In total, 15 649 (18.7%) wells had an arsenic concentration ≥ 50 μg/L and 2224 (2.7%) had an arsenic concentration ≥ 350 μg/L. Most townships with high arsenic concentration in well-water were found to cluster in south-western and north-eastern Taiwan.

Chiou *et al.* (2001) tested the water from 3901 tubewells in 18 villages of four townships in the north-eastern endemic area of chronic arsenic toxicity by the HG–FAAS method in 1991–94. The arsenic content ranged from undetectable (< 0.15 μg/L) to 3590 μg/L.

(*c*) *Thailand*

The Ronpibool district is situated approximately 70 km south of Nakorn Sri Thammarat Province, in the southern part of Thailand, and had a total population of approximately 23 000 in 1998. A geological survey found that the potential sources of arsenic contamination in the mining areas were from high-grade arsenopyrite waste piles in bedrock mining localities, sub-ore grade waste-rock piles, sulfide-rich wastes from ore-dressing plants, disseminated sulfide waste from small-scale prospecting and floatation activities and alluvial tin workings (Choprapawon & Porapakkham, 2001).

In 1994, a collaborative study was initiated to establish the distribution and geo-chemical form of arsenic in surface drainage and aquifer systems in the area. Surface waters were sampled at 26 stations and groundwater samples were collected from 23 shallow wells and 13 deep boreholes. Concentrations of arsenic in samples of surface water ranged from < 0.5 (limit of detection) to 583 μg/L and As^{III} levels ranged from < 0.5 to 28.4 μg/L. Concentrations in shallow groundwater samples ranged from 1.25 to 5114 μg/L and As^{III} levels ranged from < 0.5 to 125 μg/L. Concentrations in deep borehole samples ranged from 1.25 to 1032 μg/L and As^{III} levels ranged from < 0.5 to 53.6 μg/L (Williams *et al*., 1996).

In another study, significant concentrations of arsenite (As^{III}) were detected in several of the water samples with the highest levels of arsenic (28.4, 25.6 and 24.9 μg/L),

although arsenate (As^V) remained the dominant species (more than 92% of the total) (Choprapawon & Porapakkham, 2001).

(*d*) *Viet Nam*

Berg *et al*. (2001) reported arsenic contamination of the Red River alluvial tract in the city of Hanoi, Viet Nam, and in the surrounding rural districts. Because of naturally occurring organic matter in the sediments, the groundwaters are anoxic and rich in iron. In rural groundwater samples from private small-scale tubewells, contamination levels ranged from 1 to 3050 μg/L, with an average concentration of 159 μg/L arsenic. In a highly affected rural area, the groundwater that is used directly as drinking-water had an average concentration of 430 μg/L. Analysis of raw groundwater pumped from the lower aquifer for the Hanoi water supply yielded arsenic levels of 240–320 μg/L in three of eight treatment plants and 37–82 μg/L in another five plants. Aeration and sand filtration that are applied in the treatment plants for the removal of iron lowered the arsenic content to levels of 25–91 μg/L, but 50% remained above 50 μg/L. The high arsenic concentrations found in tubewells (48% above 50 μg/L and 20% above 150 μg/L) indicate that several million people consuming untreated groundwater might be at a considerable risk for chronic arsenic poisoning.

(*e*) *Japan*

In March 1994, high concentrations of arsenic (> 10 μg/L) were detected in 29 of 67 well-water samples in the southern region of the Fukuoka Prefecture, Japan. The range of arsenic concentrations was 1–293 μg/L: As^V ranged from 11 to 220 μg/L; As^{III} ranged from 15 to 70 μg/L; and MMA and DMA were both < 1 μg/L. The maximum concentration was lower than the figures recorded in Taiwan, China, and India, but higher than those reported in Sendai (range, 1–35 μg/L), Takatsuki (range, 3–60 μg/L) and Kumamoto (range, 5–66 μg/L), Japan (Kondo *et al*., 1999).

Arsenic concentrations in water from 34 wells in the Niigata Prefecture were measured between 1955 and 1959 as part of a historical cohort study using the Gutzeit method, and ranged from non-detectable to 3000 μg/L: six wells had a non-detectable concentration; 17 wells contained < 1000 μg/L; and 11 wells contained ≥ 1000 μg/L. All wells with arsenic concentrations > 100 μg/L were located within a distance of 500 m from a factory that produced arsenic trisulfide (Tsuda *et al*., 1995).

(*f*) *Other*

In a recent United Nations Economic and Social Commission for Asia and the Pacific–United Nations International Children's Emergency Fund–World Health Organization (UNESCAP-UNICEF-WHO, 2001) expert group meeting, contamination of groundwater by arsenic was also reported from other countries including Lao People's Democratic Republic, Cambodia, Myanmar and Pakistan. It has also been reported from Nepal (Tandukar *et al.*, 2001; Shreshta *et al*., 2002).

1.4.5 *Exposure in other countries*

Exposure in other countries is summarized in Table 20.

(*a*) *Africa (Egypt, Ghana) and the Middle East (Iran)*

In a 1999 study of 100 subjects in Cairo, Egypt, arsenic was measured by HG–AAS (detection limit, 1 µg/L) in hair samples and drinking-water. Levels of arsenic in hair samples ranged from 40 to 1040 µg/kg and levels in drinking-water samples were less than 1 µg/L (Saad & Hassanien, 2001).

Concentrations of arsenic in groundwaters from two areas in Ghana — the Obuasi area in the Ashanti region and the Bolgatanga area of the Upper East region — ranged from < 1 to 64 µg/L [As^{III} range, 6–30 µg/L] and < 1 to 141 µg/L [As^{III} range, < 1–9 µg/L], respectively. Sulfide minerals such as arsenopyrite and pyrite were present in the Birimian basement rocks of both areas and these constitute the dominant sources of arsenic. Concentrations were lowest in the shallowest groundwaters, and increased at greater depths. The lateral and vertical variations in dissolved arsenic concentrations were controlled by ambient pH and redox conditions and by the relative influences of sulfide oxidation and sorption (Smedley, 1996).

Concentrations of arsenic were measured in the scalp hair of three groups of people from a village in western Iran using NAA. One group consisted of healthy subjects, the second of subjects with suspected arsenic poisoning, and the third of subjects with confirmed arsenic poisoning. The arsenic content of water sources used by the inhabitants was also measured. The average arsenic concentration in hair was 200 µg/kg in the healthy group, 4900 µg/kg in the group with suspected poisoning and 5600 µg/kg in the group with arsenic poisoning; arsenic concentrations in water samples varied between 30 µg/L and 1040 µg/L (Pazirandeh *et al*., 1998).

(*b*) *Australia*

Australia is a country rich in minerals that present a significant source of natural arsenic contamination to the environment, in addition to anthropogenic sources such as mining activities and pesticide use. In 1991, survey data showed elevated levels of arsenic in the surface water and groundwater in Victoria, particularly around gold mining areas. Concentrations of arsenic in groundwater ranged from < 1 to 300 000 µg/L (n = 109) and those in surface water ranged from < 1 to 28 300 µg/L (n = 590). In a follow-up study of the same region in the mid-1990s, arsenic concentrations ranged from 1 to 12 µg/L in groundwater samples (n = 18), from 1 to 220 µg/L in surface water samples (n = 30) and from 1 to 73 µg/L in drinking-water samples (n = 170) (Hinwood *et al*., 1998).

In an investigation of the relationship between environmental exposure to arsenic from contaminated soil and drinking-water and the incidence of cancer in the Victoria region, median arsenic concentrations in groundwater ranged from 1 to 1077 µg/L (total range, 1–300 000 µg/L; n = 22 areas) (Hinwood *et al*., 1999).

Table 20. Concentrations of arsenic (As) in drinking-water in other countries

Country	Population	Date	Sample (no.)	Levels (µg/L)	Source of arsenic	Reference
Africa						
Egypt	Cairo	1999	Tap water (5 districts)	1	NR	Saad & Hassanien (2001)
Ghana	Obuasi area	NR	Groundwater	< 1–64 (total As) 6–30 (As^{III})	Natural	Smedley (1996)
	Bolgatanga area	NR		1–141 (total As) 1–9 (As^{III})		
Middle East						
Iran	West Iran	NR	Spring water (20)	30–1040	Natural	Pazirandeh *et al.* (1998)
Australia						
Victoria	Victoria	mid-1990s	Groundwater (18) Surface water (30) Drinking-water (170)	1–12 1–220 1–73	Natural anthropogenic (mining, pesticide)	Hinwood *et al.* (1998)
			Ground- and surface water (22 geographical areas)	1–300 000 (1–1077 medians)		Hinwood *et al.* (1999)
Europe						
Finland		1993–94	Groundwater (69)	17–980		Kurttio *et al.* (1998)
		1996	Wells (72) Control population	< 0.05–64 (median, 0.14)	Natural	Kurttio *et al.* (1999)
Spain	Madrid	1998	353 water supplies Wells (< 2% of population uses wells)	74% < 10 23% 10–50 3.7% > 50	Natural	Aragones Sanz *et al.* (2001)
Romania (Transylvania)	Bihor and Arad counties	1992–95	Drinking-water	0–176	Natural	Gurzau & Gurzau (2001)
Switzerland	Grisons Canton Valais Canton	1998	Public water supplies (336) 14 000 people	< 10–170 12–50	Natural	Pfeifer & Zobrist (2002)
United Kingdom	South-west		Private supplies (3)	11–80		Farago *et al.* (1997)

Table 20 (contd)

Country	Population	Date	Sample (no.)	Levels (µg/L)	Source of arsenic	Reference
North America						
Canada	Nova Scotia	NR	Well-water (94 households)	1.5–738	Natural	Meranger *et al.* (1984)
		1981–85	Communities (121)	< 2–34		Health Canada (1992)
	Rural areas (Saskatchewan)	NR	Private wells and municipality wells (61 wells)	< 1–117	Natural	Thompson *et al.* (1999)
USA						
Western USA		NR	Rainwater and snow	< 0.002–0.59		Welch *et al.* (1988)
			Rivers	0.20–264		
			Lakes	0.38–1000		
			Seawater	0.15–6.0		
			Groundwater	130–48 000	Mining area	
				50–2750	Basin fill deposits	
				170–3400	Volcanic areas	
				80–15 000	Geothermal area	
Maine, Michigan, Minnesota, South Dakota, Oklahoma, Wisconsin			Groundwater 17 496 samples	40% > 1 5% > 20		Welch *et al.* (1999)
National Survey		NR	Surface water (189)	68 max	Natural	Chen & Edwards (1997)
			Groundwater (239)	117 max		
Arizona	Verde Valley		Groundwater (41)	10–210 µg/L		Foust *et al.* (2000)
Illinois	Groundwater	1994–2001	Deep glacial drift aquifer	> 5–83	Natural	Warner (2001)
			Shallow glacial drift aquifier	1–28		
Montana, Wyoming		1988–95	Madison River	35–370	Natural	Nimick *et al.* (1998)
		1973–95	Missouri River	2–69		
National survey	National	mid 1990s	Drinking water supplies (21 120)	6–17% > 5 1–3% > 20		Frey & Edwards (1997)
National survey	36% population of US population	1992–93	Water companies (140 utilities)	56% > 0.5 16% > 2 5% > 5	Natural	Davis *et al.* (1994)

Table 20 (contd)

Country	Population	Date	Sample (no.)	Levels (µg/L)	Source of arsenic	Reference
Missouri and Iowa		NR	Family wells (11)	34–490	Natural	Korte & Fernando (1991)
New Jersey		1977–79	Groundwater (1064) Surface water (591)	1 (median) 1160 (max.) 1 (median) 392 (max.)	Natural	Page (1981)
Ohio		NR	88 wells	0–96	Natural	Matisoff *et al.* (1982)
Alaska		1976	Well-water (59)	1–2450		Harrington *et al.* (1978)
Oregon		1968–74	Tap-water (558)	0–2150 8% > 50		Morton *et al.* (1976)
New Hampshire		1994	Drinking-water (793)	< 0.01–180		Karagas *et al*. (1998, 2002)
Utah		1978–79	Community water supplies (88)	0.5–160	Natural	Bates *et al*. (1995)
Utah		1976–97	151 drinking-water (151)	3.5–620	Natural	Lewis *et al*. (1999)
National survey	25 states		Groundwater systems Surface water systems	5.3% > 10 0.8% > 10	Natural	Environmental Protection Agency (2001)

NR, not reported

(*c*) *Europe (Finland, Romania, Spain, Switzerland, United Kingdom)*

Samples of well-water were collected in Finland between July and November 1996. The final study population (144 627 from a register-based cohort) consisted of 61 bladder cancer cases and 49 kidney cancer cases diagnosed between 1981 and 1995, as well as an age- and sex-balanced random sample of 275 subjects (reference cohort). To evaluate the validity of water sampling, two water samples were taken from each of 36 randomly selected wells at two different times (on average 31 days apart; range, 2 h–88 days). The arsenic concentrations in the original samples and field duplicates were not significantly different. The arsenic concentrations in the wells of the reference cohort ranged from < 0.05 to 64 µg/L (median, 0.14 µg/L). Five per cent of the reference cohort had arsenic concentrations > 5 µg/L and 1% (4/275) had consumed well-water containing levels of arsenic that exceed the WHO drinking-water quality guideline value of 10 µg/L (Kurttio *et al*., 1999). Locally in Finland, drinking-water from privately drilled wells contains high concentrations of arsenic up to 980 µg/L (Kurttio *et al*., 1998). The arsenic is of geological origin.

In the north-west region of Transylvania, Romania, drinking-water contains arsenic as a result of the geochemical characteristics of the land. The geographical distribution of arsenic in drinking-water in this region, sampled between 1992 and 1995, was heterogeneous, with a mixture of high (mostly in rural areas) and low concentrations in contiguous areas (range, 0–176 µg/L arsenic). Estimates indicated that about 36 000 people were exposed to concentrations of arsenic in the drinking-water ranging from 11 to 48 µg/L, and about 14 000 inhabitants were exposed to arsenic levels exceeding 50 µg/L (Gurzau & Gurzau, 2001).

In 1998 in Madrid, Spain, arsenic concentrations of more than 50 µg/L, the maximum permissible concentration for drinking-water in Spain, were detected in some drinking-water supplies from underground sources. In the initial phase, water samples from 353 Madrid water supplies were analysed. In a second phase, 6 months later, analyses were repeated on those 35 water supplies that were considered to pose a possible risk to public health. Seventy-four per cent of the water supplies studied in the initial phase had an arsenic concentration of less than 10 µg/L, 22.6% had levels of 10–50 µg/L and 3.7% had over 50 µg/L. Most of the water supplies showing arsenic levels greater than 10 µg/L were located in the same geographical area. In the second phase, 26 of the 35 water supplies were in the same range (10–50 µg/L arsenic) as in the first survey; nine had changed category, six of which had less than 10 µg/L and three had more than 50 µg/L. In Madrid, less than 2% of the population drinks water from underground sources (Aragones Sanz *et al*., 2001).

In Switzerland, areas with elevated levels of arsenic have been found primarily in the Jura mountains and in the Alps. Weathering and erosion of rocks containing arsenic releases this element into soils, sediments and natural waters. The limit for drinking-water (50 µg/L) in Switzerland is not generally exceeded but, in the cantons of Ticino, Grisons and Valais, concentrations of arsenic above 10 µg/L have been found in the drinking-

water. The canton of Grisons tested all of the 336 public water supplies in 1998. In 312 drinking-water supplies, arsenic concentrations were below 10 μg/L (93%), while 21 samples had arsenic concentrations between 10 and 50 μg/L (6%). Three samples exceeded the Swiss limit of 50 μg/L (0.9%); the maximum concentration found was 170 μg/L. Ore deposits and sediments in the canton of Valais have also been known for some time to contain arsenic. The drinking-water in this area was not tested for arsenic until 1999. Since then, it has been determined that in this canton approximately 14 000 people live in areas where the drinking-water contains between 12 and 50 μg/L (Pfeifer & Zobrist, 2002).

Although levels of arsenic in public water supplies are low, there is concern about the 20 000–30 000 private well-water supplies in South-West England, particularly those in old mining areas, which undergo limited or no treatment. From limited available data, three private supplies of those tested in Cornwall had arsenic levels above the 5-μg/L detection limit, and contained 11, 60 and 80 μg/L (Farago *et al*., 1997).

(*d*) *North America*

(i) *Canada*

Samples from 61 groundwater sources, including 25 privately owned wells and 36 wells operated by rural municipalities, in Saskatchewan, Canada, were tested for arsenic. For virtually all of the rural municipal wells, no chemical or physical water treatment was performed other than periodic chlorination, whereas approximately half of the private wells underwent some form of water treatment. The most commonly used forms of water treatment included water softening with an ion exchange device, filtration and removal of iron. Arsenic was not detected in 25 samples (10 private wells and 15 rural municipal wells) using a method with a detection limit of 1 μg/L; 34 samples (13 private wells and 21 rural municipal wells) had levels between 1 and 50 μg/L; only two wells (private) had levels greater than 50 μg/L (maximum concentration, 117 μg/L) (Thompson *et al*., 1999).

In an earlier survey of water supplies from 121 communities in Saskatchewan sampled between 1981 and 1985, arsenic levels were below 10 μg/L in 88% and below 2 μg/L in 42% of the samples taken; the maximum level recorded was 34 μg/L (Health Canada, 1992).

The concentration of total soluble inorganic arsenic (arsenate plus arsenite) was measured in duplicate water samples from the wells of 94 residents in seven communities in Halifax County, Nova Scotia, where arsenic contamination of well-water was suspected. Levels of arsenic exceeded 50 μg/L in 33–93% of wells in each of the communities; in 10% of the wells sampled, concentrations were in the range of 500 μg/L. The total measured levels ranged from 1.5 to 738.8 μg/L (Meranger *et al*., 1984).

(ii) *USA*

The occurrence of arsenic in groundwater has been reported in the USA for areas within the states of Alaska, Arizona, California, Hawaii, Idaho, Nevada, Oregon and Washington. High concentrations are generally associated with one of the following geo-

chemical environments: (*a*) basin-fill deposits of alluvial-lacustrine origin, particularly in semi-arid areas; (*b*) volcanic deposits; (*c*) geothermal systems; and (*d*) uranium and gold mining areas. Arsenic concentrations ranged from < 0.002 to 0.59 µg/L in rainwater and snow, from 0.20 to 264 µg/L in rivers, from 0.38 to 1000 µg/L in lakes and from 0.15 to 6.0 µg/L in seawater. Maximum observed concentrations of arsenic ranged from 130 to 48 000 µg/L in groundwater from mining areas, from 50 to 2750 in basin-fill deposits, from 170 to 3400 in volcanic areas and from 80 to 15 000 µg/L in geothermal areas. Total inorganic arsenic ranged from 1.1 to 6000 µg/L, arsenite ranged from 0.6 to 4600 µg/L and arsenate ranged from 0 to 4300 µg/L (Welch *et al*., 1988).

Within the last decade, high concentrations of arsenic exceeding 10 µg/L in groundwater have been documented in many other areas of the USA (Morton *et al*., 1976; Harrington *et al*., 1978; Page, 1981; Matisoff *et al.*, 1982; Korte & Fernando, 1991; Davis *et al*., 1994; Bates *et al*., 1995; Chen & Edwards, 1997; Frey & Edwards, 1997; Karagas *et al*., 1998; Nimick *et al.*, 1998; Lewis *et al*., 1999; Foust *et al*., 2000; Warner, 2001; Karagas *et al*., 2002) (Table 20). The US Geological Survey reported that these high concentrations most commonly result from: (*a*) upflow of geothermal water; (*b*) dissolution of, or desorption from, iron oxide; and (*c*) dissolution of sulfide minerals. Overall, analyses of approximately 17 000 groundwater samples in the USA suggest that about 40% of both large and small regulated water supplies have arsenic concentrations greater than 1 µg/L. About 5% of regulated water systems are estimated to have arsenic concentrations greater than 20 µg/L (Welch *et al*., 1999).

Using a 25-state database of compliance monitoring from community systems, the Environmental Protection Agency (2001) found that 5.3% of groundwater systems and 0.8% of surface water systems had concentrations > 10 µg/L.

In a national retrospective groundwater study of 18 850 drinking-water samples (2262 from community wells and 16 602 from private wells), the US Geological Survey found the 90th percentiles for community wells and private wells to be 8 µg/L and 13 µg/L, respectively (Focazio *et al*., 2000). A study in New Hampshire found that drinking-water from private wells contained significantly more arsenic than that from community wells. In addition, this study found that deep wells had higher arsenic concentrations than superficial wells and that samples voluntarily submitted to the state for analysis had higher concentrations than randomly selected household water samples (Peters *et al*., 1999).

1.5 Regulations and guidelines

Arsenic has been a contaminant of concern in drinking-water for several years. For example, in the USA in 1942, a maximum permissible concentration for arsenic was set at 50 µg/L by the Public Health Service. This standard was reaffirmed in 1946 and 1962; however, in 1962, the Public Health Service advised that concentrations in water should not exceed 10 µg/L when "more suitable supplies are or can be made available" (Smith *et al*., 2002). In 2002, the maximum contaminant level for arsenic in the USA was lowered from 50 µg/L to 10 µg/L (Environmental Protection Agency, 2001). Table 21

Table 21. Regulations and guidelines for arsenic in drinking-water

Region	Guideline/ regulation (μg/L)	Reference
World	10	WHO (1998)
Europe	10	European Commission (1998)
USA	10	Environmental Protection Agency (2001)
Canada	25	Health Canada (2003)
Australia	7	National Health and Medical Research Council and Agriculture and Resource Management Council of Australia and New Zealand (1996)
South-East Asia (Bangladesh, India, Viet Nam, China)	50	WHO (2000)
Laos, Mongolia, Japan and Taiwan	10	WHO (2000); Taiwan Environmental Protection Agency (2000)
Argentina, Bolivia, Brazil and Chile	50	WHO (2000); Penedo & Zigarán (2002); Chilean Institute of National Standards (1984)
Philippines and Indonesia	50	WHO (2000)
Sri Lanka and Zimbabwe	50	WHO (2000)
Namibia	10	WHO (2000)
Bahrain, Egypt, Oman and Saudi Arabia	50	WHO (2000)
Jordan and Syria	10	WHO (2000)

details the various regulations and guidelines that have been established for arsenic in drinking-water.

The WHO (1998) guideline of 10 μg/L is a provisional value. A provisional guideline is established when there is some evidence of a potential health hazard but for which available data on health effects are limited, or when an uncertainty factor greater than 1000 has been used in the derivation of the tolerable daily intake.

The Canadian guideline (Health Canada, 2003) is an interim maximum acceptable concentration, again, due to the limited data on health effects.

Several other countries have also established standards for arsenic, and several developing countries have established a standard for arsenic of 50 μg/L (WHO, 2000).

2. Studies of Cancer in Humans

Major 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 arises from case–control and cohort studies, with little, if any, evidence from ecological studies. In contrast, for arsenic in drinking-water, ecological studies provide important information on causal inference, because of large exposure contrasts and limited population migration. As a consequence of widespread exposure to local or regional water sources, ecological measures 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 review that follows, ecological studies are presented in detail.

2.1 Cancer of the urinary bladder and kidney

The findings of epidemiological studies on arsenic in drinking-water and the risk for cancers of the urinary bladder and kidney are summarized in Table 22.

Historically, several case reports have related cancers of the urinary tract with medicinal arsenic treatments or arsenic-related diseases such as Bowen disease. In 1953, a series of 27 cases with multiple skin cancers attributed to arsenical medicines was reported (Sommers & McManus, 1953). Of these cases, 10 were diagnosed as also having internal cancers at various sites, three of which were urinary tract tumours. Graham and Helwig (1959) first investigated an association between Bowen disease and primary internal cancers. Twenty-eight (80%) of 35 cases had primary internal cancers, two of which were malignant tumours of the bladder and one a tumour of the kidney. Cuzick *et al*. (1982) examined a cohort of subjects in the United Kingdom who had taken Fowler's solution (potassium arsenite) between 1945 and 1969. After further follow-up of the cohort through 1990 (Cuzick *et al*., 1992), a threefold increase in mortality from bladder cancer (standardized mortality ratio [SMR], 3.07; 95% confidence interval [CI], 1.01–7.3) was reported, strengthening the evidence on bladder cancer reported previously (Cuzick *et al*., 1982).

Bergoglio (1964) published the first report of bladder cancer associated with arsenic in drinking-water in the Province of Cordóba in Argentina. He identified 2355 deaths between 1949 and 1959 in nine towns of a highly exposed region and found that cancer was the cause of death of 24%; 11% of these cancer deaths involved cancers of the urinary tract. Biagini (1972) followed 116 patients with arsenic-related skin lesions in the same region and found that 12.5% of the cancer deaths were patients with urinary tract cancers.

Table 22. Summary of epidemiological studies of arsenic in drinking-water and risk for bladder and kidney cancers

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Ecological studies							
Taiwan							
Chen *et al.* (1985)	84 villages from four neighbouring townships on the SW coast	Mortality 1968–82	Comparison of mortality in an area endemic for Blackfoot disease with general population	*Bladder*	Obs/exp.	SMR (95% CI)	Reference: national rates
				Men	167/15.2	11.1 (9.3–12.7)	
				Women	165/8.2	20.1 (17.0–23.2)	
				Kidney			
				Men	42/5.4	7.7 (5.4–10.1)	
				Women	62/5.5	11.2 (8.4–14.0)	
Chen *et al.* (1988a)	Area endemic for Blackfoot disease	Mortality 1973–86	Village of residence; median arsenic levels of well-water samples			SMR	Age-standardized mortality rates per 100 000; 899 811 person–years
				Bladder			Reference: world population in 1976
				Men			
			General population			3.1	
			< 300 µg/L			15.7	
			300–590 µg/L			37.8	
			≥ 600 µg/L			89.1	
				Women			
			General population			1.4	
			< 300 µg/L			16.7	
			300–590 µg/L			35.1	
			≥ 600 µg/mL			91.5	
				Kidney			
				Men			
			General population			1.1	
			< 300 µg/L			5.4	
			300–590 µg/L			13.1	
			≥ 600 µg/L			21.6	
				Women			
			General population			0.9	
			< 300 µg/L			3.6	
			300–590 µg/L			12.5	
			≥ 600 µg/L			33.3	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Wu *et al*. (1989)	42 villages in an area endemic for Blackfoot disease	Mortality 1973–86	Arsenic levels: 3 groups based on median level of well-water/village		Age-adjusted mortality rates per 100 000	Reference: world population in 1976
			Group 1 (< 300 µg/L)	*Bladder*		
			Men (248 728)	Men	22.64 ($p < 0.001$)	
			Women (248 728)	Women	25.60 ($p < 0.001$)	
				Kidney		
				Men	8.42 ($p < 0.05$)	
			Group 2 (300–590 µg/L)	Women	3.42 ($p < 0.001$)	
			Men (138 562)	*Bladder*		
			Women (127 502)	Men	61.02 ($p < 0.001$)	
				Women	57.02 ($p < 0.001$)	
				Kidney		
				Men	18.90 ($p < 0.05$)	
			Group 3 (≥ 600 µg/L)	Women	19.42 ($p < 0.001$)	
			Men (79 883)	*Bladder*		
			Women (74 083)	Men	92.71 ($p < 0.001$)	
				Women	111.30 ($p < 0.001$)	
				Kidney		
				Men	25.26 ($p < 0.05$)	
				Women	57.98 ($p < 0.001$)	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Chen & Wang (1990)	314 geographical units (precincts and townships), including 4 townships in the endemic area of Blackfoot disease	Mortality 1972–83	Average arsenic levels in water samples of all geographical units. 73.9% of study precincts or townships had < 5% of wells with > 50 µg/L; 14.7% had 5–14%; 11.5% had ≥ 15%.	**All 314 precincts and townships** *Bladder* Men Women *Kidney* Men Women **170 south-western townships** *Bladder* Men Women *Kidney* Men Women		 3.9 (0.5) 4.2 (0.5) 1.1 (0.2) 1.7 (0.2) 3.7 (0.7) 4.5 (0.7) 1.2 (0.2) 1.7 (0.3)	Reference: world population in 1976. Analysis weighted by population in each group. Regression coefficients indicating an increase in age-adjusted mortality/100 000 person–years for every 0.1 µg/L increase in arsenic level (SE)
Chiang *et al.* (1993)		Incidence 1981–85	Exposure not evaluated Endemic area	*Bladder* Total Men Women	140 81 59	Incidence per 100 000 23.5 26.1 21.1	Adjusted for age
			Neighbouring endemic area	Total Men Women	13 7 6	4.5 4.7 4.3	
			All Taiwan	Total Men Women	2135 1608 527	2.3 3.3 1.2	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Guo *et al.* (1997)	National survey of 83 656 wells in 243 townships	Incidence of transitional-cell carcinoma 1980–87	Arsenic levels in town of residence (ppm) < 0.05 0.05–0.08 0.09–0.16 0.17–0.32 0.33–0.64 > 0.64	*Bladder* Men Women *Kidney* Men Women	 1185 363 158 81	 0.57 (0.07) 0.33 (0.04) 0.03 (0.02) 0.142 (0.013)	Estimates of rate difference (per 100 000 person–years) for one unit increase in the predictor and associated standard error for exposure > 0.64 ppm (SE). Results shown for transitional-cell carcinoma
Tsai *et al.* (1999)	4 townships	Mortality 1971–94	Area endemic for Blackfoot disease	**Deaths** *Bladder* Men Women *Kidney* Men Women	 312 295 94 128	 8.9 (7.96–9.96) 10.5 (9.4–11.7) 14.1 (12.5–15.8) 17.7 (5.7–19.8) 6.7 (5.5–8.3) 6.8 (5.5–8.3) 8.9 (7.4–10.6) 10.5 (8.8–12.5)	 Local reference (Chiayi-Tainan county) National reference (Taiwan) Local National Local National Local National

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
South America							
Hopenhayn-Rich *et al.* (1996a, 1998)	26 counties in the Province of Córdoba, Argentina	Mortality 1986–91	Exposure levels	**Deaths**		SMR (95% CI)	
				Bladder			
			Low (690 421)	Men	113	0.8 (0.7–0.96)	
				Women	39	1.2 (0.9–1.7)	
			Medium (406 000)	Men	116	1.3 (1.05–1.5)	
				Women	29	1.4 (0.93–1.99)	
			High (mean arsenic level, 178 µg/L) (273 014)	Men	131	2.1 (1.8–2.5)	
				Women	27	1.8 (1.2–2.6)	
				Kidney			
			Low (690 421)	Men	66	0.9 (0.7–1.1)	
				Women	38	1.0 (0.7–1.4)	
			Medium (406 000)	Men	66	1.3 (1.02–1.7)	
				Women	34	1.4 (0.94–1.9)	
			High (273 014)	Men	53	1.6 (1.2–2.1)	
				Women	27	1.8 (1.2–2.6)	
Rivara *et al.* (1997)	Chile	Mortality 1950–92	Arsenic-contaminated Region II of northern Chile versus non-contaminated region VIII	Bladder		SMR (95% CI) 10.2 (8.6–12.2)	SMR for period 1976–92
				Kidney		3.8 (3.1–4.7)	
Smith *et al.* (1998)	Chile	Mortality 1989–93	Region II of northern Chile with population-weighted average arsenic concentration up to 569 µg/L compared with rest of Chile; exposure generally < 10 µg/L			SMR (95% CI)	Population of about 400 000
				Bladder			
				Men		6.0 (4.8–7.4)	
				Women		8.2 (6.3–10.5)	
				Kidney			
				Men		1.6 (1.1–2.1)	
				Women		2.7 (1.9–3.8)	
Australia							
Hinwood *et al.* (1999)	Victoria	Incidence 1982–91	Median arsenic concentration in drinking-water ranged 1–1077 µg/L			SIR (95% CI)	State rates used as reference
				Bladder	303	0.9 (0.8–1.1)	
				Kidney	134	1.2 (0.98–1.4)	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome		Comments
Case–control studies								
Taiwan								
Chen *et al.* (1986)	4 neighbouring townships in endemic area of Blackfoot disease	Mortality 1980–82	Median arsenic content of artesian well-water, 0.78 ppm Years of artesian water consumption	Community controls	Bladder cancer cases	OR	OR from multiple logistic regression analyses	Adjusted for age and sex
			0	136	17	1.0	1.0	
			1–20	131	19	1.2	1.3	
			21–40	50	10	1.6	1.7	
			≥ 40	51	23	3.9	4.1	
USA								
Bates *et al.* (1995)	10 areas of the USA	Incident cases (aged 21–84 years) diagnosed in a 1-year period in the 1970s. Age-, sex- and area-matched controls			Bladder cancer cases	OR (90% CI)		Adjusted for sex, age, smoking, years of exposure to chlorinated surface water, history of bladder infection, educational level, urbanization of the place of longest lifetime residence, ever employed in a high-risk occupation
			Cumulative dose (mg)	Controls		All subjects		
			< 19	47	14	1.0		
			19–32	36	21	1.6 (0.8–3.2)		
			33–52	39	17	0.95 (0.4–2.0)		
			≥ 53	38	19	1.4 (0.7–2.9)		
			mg/L × years					
			< 33	42	18	1.0		
			33–52	42	16	0.7 (0.3–1.5)		
			53–73	40	16	0.5 (0.3–1.2)		
			≥ 74	36	21	1.0 (0.5–2.1)		

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Bates *et al.* (1995) (contd)			Cumulative dose (mg)		Never smoked	
			< 19		1.0	
			19–32		1.1 (0.4–3.1)	
			33–52		0.7 (0.2–2.3)	
			≥ 53		0.5 (0.1–1.9)	
			mg/L × years			
			< 33		1.0	
			33–52		0.2 (0.1–0.8)	
			53–73		0.3 (0.1–0.9)	
			≥ 74		0.9 (0.3–3.2)	
			Cumulative dose (mg)		Ever smoked	
			< 19		1.0	
			19–32		3.3 (1.0–10.8)	
			33–52		1.9 (0.6–6.2)	
			≥ 53		3.3 (1.1–10.3)	
			mg/L × years			
			< 33		1.0	
			33–52		1.95 (0.7–5.6)	
			53–73		1.2 (0.4–3.7)	
			≥ 74		1.4 (0.5–4.3)	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Europe							
Kurttio *et al.* (1999)	Areas in Finland in which < 10% of population belong to the municipal drinking-water system	Incidence 1981–95	Concentration of arsenic in water	*Short latency*	**Bladder**	Relative risk (95% CI)	Case–cohort design Adjusted for age, sex and smoking Short latency: exposure in the 3rd to 9th calendar year prior to the cancer diagnosis Long latency: exposure in the 10th calendar year and earlier prior to the cancer diagnosis
			< 0.1 µg/L	23		1.0	
			0.1–0.5 µg/L	19		1.5 (0.8–3.1)	
			≥ 0.5 µg/L	19		2.4 (1.1–5.4)	
			Total	61		1.4 (0.95–1.96)	
				Long latency			
			< 0.1 µg/L	26		1.0	
			0.1–0.5 µg/L	18		0.8 (0.4–1.6)	
			≥ 0.5 µg/L	17		1.5 (0.7–3.4)	
			Total	61		0.96 (0.6–1.6)	
			Concentration of arsenic in water	*Short latency*	**Kidney**		
			< 0.1 µg/L	23		1.0	
			0.1–0.5 µg/L	12		0.8 (0.4–1.7)	
			≥ 0.5 µg/L	14		1.5 (0.7–3.3)	
			Total	49		1.2 (0.8–1.7)	
				Long latency			
			< 0.1 µg/L	25		1.0	
			0.1–0.5 µg/L	9		0.3 (0.1–0.8)	
			≥ 0.5 µg/L	15		1.07 (0.5–2.5)	
			Total	49		0.7 (0.4–1.4)	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Cohort studies							
Taiwan							
Chen *et al.* (1988b)	4 neighbouring townships in area endemic for Blackfoot disease	Mortality 1968–93	Comparison of mortality with general and endemic population	Cancer deaths		SMR	95% CI calculated by the Working Group
				Bladder	15	38.8 [21.7–64.0]	General population as reference
				Kidney	3	19.5 [4.0–57.0]	
						SMR	
				Bladder	15	2.6 [1.4–4.2]	Area endemic for Blackfoot disease as reference
				Kidney	3	1.6 [0.3–4.7]	
Chiou *et al.* (1995)	4 neighbouring townships in area endemic for Blackfoot disease (BFD)		Cumulative index derived for each subject: Σ (Ci × Di). Cumulative exposure (mg/L × year)	BFD patients	263	*Bladder cancer* RR* (95% CI)	*Relative risk after adjustment for age, sex and smoking **Relative risk after adjustment for age, sex, smoking and BFD status Ci, median concentration of arsenic in wells of village; Di, duration of drinking water in that village
				Healthy controls	2293		
			0	Bladder cancer cases	29	1.0	
			0.1–19.9			2.1 (0.6–7.2)	
			≥ 20			5.1 (1.5–17.3)	
						RR** (95% CI)	
			0			1.0	
			0.1–19.9			1.6 (0.4–5.6)	
			≥ 20			3.6 (1.1–12.2)	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Chiou *et al.* (2001)	North-eastern Taiwan	Incidence 1991–94	Area endemic for arseniasis			RR (95% CI)	Adjusted for age, sex, smoking and duration of drinking well-water
		Person–years of observation	Arsenic concentration in well-water (µg/L)			*Urinary tract*	
		7978	< 10.0	3		1.0	
		6694	10.1–50.0	3		1.5 (0.3–8.0)	
		3013	50.1–100.0	2		2.2 (0.4–13.7)	
		5220	> 100.0	7		4.8 (1.2–19.4)	*p* for trend < 0.01
						Transitional-cell carcinoma	
		7978	< 10.0	1		1.0	
		6694	10.1–50.0	1		1.9 (0.1–32.5	
		3013	50.1–100.0	2		8.2 (0.7–99.1)	
		5220	> 100.0	6		15.3 (1.7–139.2)	*p* for trend < 0.05
Japan							
Tsuda *et al.* (1995)	Niigata prefecture	1959–92		No. of persons exposed	Urinary Obs/exp.	SMR (95% CI)	
			Arsenic concentration in well-water (ppm) from arsenic-polluted area	at concentration level (1955–59)			
			< 0.05	254	0/0.3	0 (0–12.5)	
			0.05–0.99	76	0/0.08	0 (0–47.1)	
			≥ 1	113	3/0.10	31.2 (8.6–91.8)	
			Total	443	3/0.48	6.3 (1.7–18.4)	

Table 22 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
USA						
Lewis *et al.* (1999)	Millard County, UT	Mortality	Index of exposure to arsenic calculated for each cohort member and derived from number of years of residence and median arsenic concentration in the given community		SMR (95% CI)	Confidence intervals not given for exposure categories 4058 members in cohort (2092 men and 1966 women)
					Bladder/other urinary cancers	
			Low exposure	Men	0.36	
			(< 1000 ppb–years)	Women	1.18	
			Medium exposure	Men	–	
			(1000–4999 ppb–years)	Women	–	
			High exposure	Men	0.95	
			(≥ 5000 ppb–years)	Women	1.10	
			Total	Men	0.4 (0.1–1.2)	
				Women	0.8 (0.1–2.9)	
					Kidney cancer	
			Low exposure	Men	2.51	
			(< 1000 ppb–years)	Women	2.36	
			Medium exposure	Men	1.13	
			(1000–4999 ppb–years)	Women	1.32	
			High exposure	Men	1.43	
			(≥ 5000 ppb–years)	Women	1.13	
			Total	Men	1.8 (0.8–3.3)	
				Women	1.6 (0.4–4.1)	

SMR, standardized mortality ratio; CI, confidence interval; SIR, standardized incidence ratio; OR, odds ratio; RR, relative risk; BFD, Blackfoot disease

More systematic studies were conducted in various parts of the world, the most extensive being in Taiwan, China.

2.1.1 *Studies in Taiwan, China*

There are two areas in Taiwan, China, where exposure to arsenic is endemic. One is located in the south-western coastal area where Blackfoot disease, a unique peripheral vascular disease induced by long-term ingestion of arsenic from artesian well-water, is endemic. Eighty-four villages constitute the four Blackfoot disease-endemic townships of Peimen, Hsuehchia, Putai and Ichu, and artesian wells and patients with Blackfoot disease were also found in the two neighbouring townships of Yensui and Hsiaying (Wu *et al.*, 1989). Residents in the south-western endemic areas in Taiwan drank artesian well-water with high concentrations of arsenic from the early 1910s to the late 1970s. The concentrations of arsenic in artesian well-water, tested by Natelson's method in 1964–66 in 42 villages of the six townships, ranged from 10 to 1752 μg/L, and were mostly above 100 μg/L (Kuo, 1968; Tseng *et al.*, 1968). As well-water was the only source of drinking-water in the endemic area, all residents of a given village consumed the water from a small number of shared wells in their daily home and working environments. Most residents were engaged in fishing, salt production and farming, and the migration rate was low. More than 90% of residents had lived in the study area all their lives. As the study population lived in a small area, they shared similar dietary patterns, lifestyle, socioeconomic status and health care facilities. The piped water supply system using surface water was first implemented in the south-western endemic area, but its coverage was not complete until the late 1970s (Wu *et al.*, 1989).

Another area with exposure to arsenic is located in the Lanyang Basin in north-eastern Taiwan (Chiou *et al*., 2001), and is comprised of four townships: Chiaohsi (four villages), Chuangwei (seven villages), Wuchieh (three villages) and Tungshan (four villages). Residents in this area of endemic arseniasis used river water for drinking and cooking before the Second World War, and started to use water from tubewells in their houses in the late 1940s. The concentrations of arsenic in the water of 3901 wells in the endemic area was tested from 1991 to 1994 by hydride generation combined with flame atomic absorption spectrometry, and ranged from undetectable (< 0.15 μg/L) to 3.59 ng/L, but were mostly between 10 and 100 μg/L, with a median of 27.3 μg/L. A piped water supply system using surface water was first implemented in the endemic area in late 1997, and its coverage was almost complete in 2001. Residents in the endemic area were engaged in farming, and most of them had lived in the area all their lives.

(*a*) *Ecological studies*

Chen *et al*. (1985) reported an elevation in mortality from cancers of the urinary bladder and kidney during the period 1968–82 in endemic areas of Blackfoot disease (four neighbouring townships comprising 84 villages) compared with the general population of Taiwan. The arsenic content of well-water ranged from 0.35 to 1.14 ppm

[mg/L] with a median of 0.78 ppm, while shallow well-water contained arsenic at concentrations ranging from 0 to 0.3 ppm with a median of 0.04 ppm (Chen *et al.*, 1962). The SMRs for bladder cancer and kidney cancer increased with the prevalence of Blackfoot disease. Similarly, the SMRs for cancers of the bladder and kidney were highest in villages where only artesian wells were in use and lowest in those villages using shallow wells. The high SMRs for bladder and kidney cancer were not readily explained by the higher rate of cigarette smoking in the Blackfoot disease-endemic area compared with all of Taiwan (40% versus 32%).

Chen *et al*. (1988a) briefly described a dose–response relationship between median arsenic levels in artesian well-water in the 84 villages studied by Chen *et al*. (1985) and rates of mortality from bladder cancer. The study period (1973–86) covered 899 811 person–years of observation, and exposure was stratified into three categories (< 300, 300–590 and ≥ 600 μg/L arsenic) based on concentrations from a survey of over 83 000 wells, including 313 townships in all of Taiwan, conducted from 1962 to 1964.

Wu *et al*. (1989) examined age-adjusted mortality rates for various cancers in an area of south-western Taiwan that comprised 42 villages in six townships (27 villages studied by Chen *et al*. (1988a) and another 15 villages). The arsenic content of the 155 wells sampled, measured in 1964–66, ranged from 10 to 1750 μg/L. The villages were classified according to median arsenic levels in water into three exposure groups (< 300, 300–590 and ≥ 600 μg/L). Death certificates were used to ascertain cause of death during the period 1973–86. A dose–response relationship was found with concentration of arsenic in water for cancers of the bladder and kidney for both men and women.

Chen and Wang (1990) further investigated cancer mortality rates throughout Taiwan in 1972–83. Of 361 administrative areas, 314 were included in the study following measurements of arsenic contents in well-water in 1974–76. Exposure measurements were derived from a national water survey of over 83 000 wells throughout Taiwan. About 19% of the wells contained levels of arsenic above 50 μg/L, and most of them were in north-western and south-eastern Taiwan. Indices of urbanization and industrialization were included in the analysis to adjust for the possible confounding effect of differing socioeconomic characteristics between the 314 precincts and townships. Mortality data were used to evaluate 21 malignant neoplasms, using population-weighted regression. Results were presented for increases in mortality per 100 000 that were calculated to occur for every 0.1-mg/L increase in arsenic concentration in water.

Chiang *et al*. (1993) showed that the age-adjusted incidence of bladder cancer in the period 1981–85 in the Blackfoot disease-endemic area of Taiwan was higher than that in a neighbouring area of Taiwan and in the country as a whole.

Guo *et al.* (1997) used tumour registry data and the exposure data from the 1974–76 nationwide water-quality survey used by Chen and Wang (1990), which included concentrations of arsenic in drinking-water from 243 townships with about 11.4 million residents. The annual incidence of cancers of the bladder and kidney for townships in 1980–87 and subcategories of those cancer diagnoses were regressed against a model that included six variables for the proportion of wells in each of six categories of arsenic

concentration in each township. Sex-specific models were adjusted for age and included an urbanization index and the annual number of cigarettes sold *per capita*. Regression models were weighted by the total population of each township. A total of 1962 bladder, 726 kidney, 170 ureter and 57 urethral cancers were included. The investigators found associations of high arsenic concentrations (more than 0.64 ppm) in both sexes with transitional-cell carcinomas of the bladder, kidney and ureter, and all urethral cancers combined, but they did not present relative risk estimates.

Tsai *et al*. (1999) compared mortality in people aged over 40 years in the Blackfoot disease-endemic area of Taiwan with both local and national references for the period 1971–94. Greater mortality was found for men and women with cancers of the bladder and kidney.

(*b*) *Case–control study*

In a retrospective case–control study using death certificates from 1980–82, Chen *et al*. (1986) examined the relationship between exposure to high concentrations of arsenic in artesian well-water and mortality from internal malignancies, including tumours of the bladder ($n = 69$) in four townships from the Blackfoot disease-endemic area. Controls ($n = 368$) were selected by random sampling from the same geographical areas as the cases and were frequency-matched on age and sex. The response rate was 93% for proxies of cases and 92% for matched controls. Adjustment for age, sex and other variables (smoking, tea drinking, vegetarianism and frequency of consumption of vegetables and fermented beans) was performed by logistic regression analysis. The results indicated increasing trends in odds ratios with increasing duration of intake of artesian well-water containing arsenic. The highest risks were seen for over 40 years of exposure, with an odds ratio of 4.1 for bladder cancer in a multivariate analysis. Smoking, alcohol consumption and other potential risk factors evaluated in the study did not confound the association between arsenic and cancer.

(*c*) *Cohort studies*

Chen *et al.* (1988b) studied the association between arsenic in artesian well-water in relation to Blackfoot disease and cancer from a multiple risk factor perspective. The study area included the four townships in south-western Taiwan where high rates of Blackfoot disease had been described. Levels of arsenic were reported to be high in water, soil and food, and estimates of ingestion of arsenic by local residents were up to 1 mg per day. The study examined mortality in a cohort of people who had or had since developed Blackfoot disease in 1968, totalling 789 patients and 7578 person–years of observation through 1984. Follow-up started in 1968, since this was the year that registration of deaths in Taiwan was computerized and completeness and quality of death certificate registration was improved. Mortality of persons who had died ($n = 457$) and were not lost to follow-up ($n = 84$) was compared with that of the general population of Taiwan using age- and sex-specific mortality rates from 1968 through 1983. The SMRs for cancers of the bladder and kidney (men and women combined) were 38.8 [95% CI, 21.7–64.0] and 19.5

[95% CI, 4.0–57.0], respectively. The latter result, however, is based on only three deaths. SMRs were also calculated using all residents in the Blackfoot disease-endemic area, which includes people exposed to arsenic. These much lower SMRs were 2.6 [95% CI, 1.4–4.2] for bladder cancer and 1.6 [95% CI, 0.3–4.7] for kidney cancer, indicating that patients with Blackfoot disease had somewhat higher rates for these cancers than the residents in the arsenic-exposed region combined.

Chiou *et al*. (1995) investigated the relationship between incidence of internal cancers and arsenic in relation to Blackfoot disease in 2256 subjects from 1986 to 1993. Patients with Blackfoot disease ($n = 263$) and a referent group of 2293 residents of the same region were followed for 7 years. In contrast to many other studies that evaluate mortality, incident cancer was the outcome of interest. Follow-up occurred many years after exposure to elevated concentrations of arsenic in drinking-water had ended. Information on exposure to other risk factors was gathered by individual interviews. Several measures of exposure were evaluated, including average concentration of arsenic in artesian wells and cumulative exposure to arsenic from drinking artesian well-water. Relative risks were calculated using Cox's proportional hazard regression analysis. After controlling for the effects of age, sex and smoking in the regression analysis, a dose–response relationship was observed between exposure to arsenic from drinking well-water and the incidence of bladder cancer. Patients with Blackfoot disease were found to be at increased risk even after adjustment for cumulative exposure to arsenic.

Chiou *et al.* (2001) studied the incidence of urinary tract cancers among 8102 residents in the arsenic-endemic area in north-eastern Taiwan from 1991 to 1994. Levels of arsenic in the drinking-water ranged from less than 0.15 µg/L (undetectable) to 3590 µg/L. Exposure for each member of the cohort was assessed by measuring concentrations of arsenic in the well associated with the individual's household at one point in time only, although most households had used their current wells for at least 10 years (Chen & Chiou, 2001). Using the general population as referent, the standardized incidence ratio (SIR) for bladder cancer was 1.96 (95% CI, 0.9–3.6), while that for kidney cancer was 2.8 (95% CI, 1.3–5.4). These results were based on 10 subjects with bladder cancer and nine with kidney cancer. A dose–response relationship was observed between urinary tract cancers, particularly transitional-cell carcinoma, after adjusting for age, sex and smoking.

2.1.2 *Studies in Japan*

A retrospective cohort study was conducted in a small Japanese population, which, between 1955 and 1959, used well-water contaminated with arsenic from a factory producing King's yellow (As_2O_3; Tsuda *et al*., 1995). The levels of arsenic measured in 34 of 54 wells tested in the area around the factory ranged from undetectable to 3000 µg/L, with 11 wells having levels exceeding 1000 µg/L. A total of 454 residents were enlisted in the cohort. Death certificates, autopsy records and medical records were obtained for the period 1 October 1959 to 30 September 1987. Smoking and occupational histories were ascertained from residents or close relatives. Expected numbers of deaths

were based on sex-, age- and cause-specific mortality in Niigata Prefecture from 1960 to 1989. Exposure was grouped into high (≥ 500 µg/L), medium (50–500 µg/L) and low (< 50 µg/L), based on the arsenic content of the well-water. The SMR for urinary-tract cancer in the high-exposure group was 31.2 (95% CI, 8.6–91.8) based on three observed deaths versus 0.1 expected. Two of these were deaths from bladder cancer and one from cancer of the renal pelvis. [Excluding the cancer of the renal pelvis, the SMR for bladder cancer alone would be at least 20.]

2.1.3 *Studies in South America*

(*a*) *Argentina*

As early as the beginning of the twentieth century, physicians noted an increase in the incidence of clinical skin alterations due to arsenic in well-water in certain areas of the Province of Córdoba, Argentina (Hopenhayn-Rich *et al*., 1996a). In a study of 2355 deaths in 1949–59 in a highly exposed region, Bergoglio (1964) found that 24% of deaths in the exposed region were due to cancer compared with 15% in the Province of Córdoba. In a 14-year follow-up of 116 patients diagnosed with arsenic-related skin lesions, 30.5% died of cancer, and 12.5% of these deaths were due to cancers of the urinary tract (Biagini, 1972). This was later contrasted with bladder cancer mortality in all of Argentina in 1980, with 2.9% of all cancer deaths attributable to bladder cancer (Hopenhayn-Rich *et al*., 1996a).

These early reports led to an ecological study on bladder cancer mortality for the period 1986–91 comparing counties categorized as previously having had high, medium and low concentrations of arsenic in water in Córdoba, Argentina (Hopenhayn-Rich *et al*., 1996a). The majority of reported cases of arsenic-related skin lesions were residents of two counties that were classified as having high exposure since there were extensive reports of elevated concentrations of arsenic in the water there. The average concentration of arsenic in water tested in these counties was 178 µg/L. The medium-exposure group comprised six counties based on some reports of elevated arsenic levels in the water and the occurrence of a few cases of skin lesions. The remaining 16 rural counties were classified as having low exposure. Clear trends in mortality from bladder cancer were observed. Increasing trends were also observed for mortality from kidney cancer as exposure to arsenic increased (Hopenhayn-Rich *et al.*, 1998). No differences were found between the exposure groups for chronic obstructive pulmonary disease, suggesting that the trends for bladder and kidney cancer were not attributable to confounding by smoking.

(*b*) *Chile*

Chile is a long and narrow country divided into geopolitical units called regions (like provinces in other countries), which are numbered sequentially from north to south, starting with Region I. Region II is thus located in the northern part of Chile, in an arid zone where the Atacama Desert is situated. At the time of the 1992 Census, the population in Region II was about 420 000. About 90% of the population live in the cities and towns

in this Region, and more than half of the population lives in the city of Antofagasta (Smith *et al*., 1998). In view of the extremely low level of rainfall and the inability to obtain water from wells, each city and town obtains drinking-water from rivers which originate in the Andes mountains, located on the eastern border of the Region. Many of these rivers are naturally contaminated with inorganic arsenic, some at very high concentrations. This has resulted in widespread exposure of the population of Region II to varying levels of arsenic in the drinking-water. In 1955–70, the majority of the population of Region II was exposed to very high levels of arsenic in drinking-water (see Table 17). Prior to 1955, the drinking-water supply in the main city of Antofagasta had an arsenic concentration of about 90 μg/L. A growing population, and the consequent increased need for water, led to supplementation of the drinking-water supply at Antofagasta with water from the Toconce and Holajar Rivers, which, unknown at the time, had arsenic concentrations of 800 μg/L and 1300 μg/L, respectively. The concentration of arsenic in the drinking-water at Antofagasta, together with that of neighbouring Mejillones, which shared the same supply, increased to an average of 870 μg/L. As shown in Table 17, the other towns in the region, with the exception of Taltal, also had high concentrations of arsenic in the drinking-water for variable periods. The population-weighted average concentration of arsenic in drinking-water for the entire region was about 580 μg/L over a period of approximately 15 years (1955–70). With the introduction in 1970 of a water-treatment plant, the concentration of arsenic in the water at Antofagasta initially dropped to 260 μg/L, and further reductions occurred as a result of improvements to the treatment plant. At present, levels of arsenic in water in Antofagasta are about 40 μg/L. Other cities and towns also implemented water-treatment strategies or used alternative sources that reduced arsenic levels. By the late 1980s, all of the towns with populations over 1000 had concentrations of arsenic in drinking-water of less than 100 μg/L, with the exception of San Pedro de Atacama (population about 3700, some of whom still drink the contaminated water). In contrast, most water sources in the rest of Chile have had low concentrations of arsenic (less than 10 μg/L) (Ferreccio *et al.*, 2000).

Evidence of chronic arsenic toxicity in Region II was noted in the 1960s with the emergence of classic dermatological manifestations (Borgoño & Greiber, 1971; Zaldívar, 1974; Zaldívar *et al*., 1978). In 1969, in a study of 180 residents in Antofagasta, abnormal skin pigmentation was found in 144 of the participants, 43.7% of whom also had hyperkeratoses (Borgoño *et al*., 1977). Evidence of effects on the respiratory and cardiovascular system, together with skin lesions, was also reported by Zaldívar *et al*. (1978), who conducted a series of studies concerning the effects of arsenic in Antofagasta during the high-exposure period.

Two ecological mortality studies were conducted on kidney and bladder cancer in Region II. Rivara *et al*. (1997) conducted a study comparing mortality for both sexes combined in Region II with that in Region VIII for the period 1950–92. SMRs for bladder cancer and kidney cancer were 10.2 (95% CI, 8.6–12.2) and 3.8 (95% CI, 3.1–4.7), respectively.

Smith *et al*. (1998) also investigated cancer mortality in Region II for the years 1989–93, using mortality rates in the rest of Chile (excluding Region II) in 1992, a census year, for reference. SMRs were calculated for men and women over the age of 30 years, using 10-year age groupings. The results indicated marked increases in mortality from bladder cancer and kidney cancer in Region II. Data on smoking obtained from a national survey of stratified random samples carried out in 1990, comparing the two largest cities of Antofagasta and Calama with the rest of Chile, were included. No overall increases in mortality from chronic obstructive pulmonary disease were observed in Region II: the SMR for men was 1.0 (0.8–1.1) and mortality among women was lower than expected (SMR, 0.6; 0.4–0.7). In addition, not only did the national survey not find higher rates of smoking in Region II, but in Antofagasta, 76.4% of respondents reported being non-smokers at that time compared with 75.1% of respondents in the rest of Chile. The proportion of people who smoked more than one pack per day was lower in Antofagasta (0.8%) and Calama (1.1%) than in the rest of Chile (1.5%). The SMRs for other causes of death excluding cancers of the bladder, kidney, lung, liver and skin were 1.0 (95% CI, 0.99–1.05) for men and 1.0 (95% CI, 0.97–1.03) for women.

2.1.4 *Studies in the USA*

Some ecological studies have been reported in the USA but they are not informative in view of the relatively small contrasts in exposure between counties.

(*a*) *Case–control study*

Bates *et al.* (1995) linked 71 cases of bladder cancer and 160 controls from a subsample of residents of Utah from the large national bladder cancer study conducted in 1978 (Cantor *et al.*, 1987) to levels of arsenic in water supplies. Exposures ranged from 0.5 to 160 µg/L, but most concentrations were very low, with only 1.1% of samples having concentrations greater than 50 µg/L. The findings did not provide evidence for an overall increase in the incidence of bladder cancer with the two indices of exposure used. However, among smokers only, there was an increase in risk for the highest category of cumulative dose of arsenic.

(*b*) *Cohort study*

Levels of arsenic in drinking-water and mortality were investigated in a cohort of members of the Church of Jesus Christ of Latter-day Saints in Millard County, UT (Lewis *et al*., 1999). The cohort was assembled from an earlier study that consisted of 2073 participants (Southwick *et al*., 1983). Most of these individuals had a history of at least 20 years of exposure in their respective places of residence. The cohort was expanded to include all persons who lived for any length of time in the study area, resulting in a total combined cohort of 4058 members. More than 70% of the cohort had reached the age of 60 years at the end of the follow-up period or by the time of their deaths. Approximately 7% of the cohort was lost to follow-up. Arsenic concentrations in the drinking-water supplies were

based on measurements maintained by the state of Utah dating back to 1964. The median drinking-water concentrations ranged from 14 to 166 ppb [µg/L], with wide variability in each town. An index of exposure to arsenic was calculated from the number of years of residence and the median concentration of arsenic in drinking-water in a given community, and was categorized as low (< 1000 ppb–years), medium (1000–4999 ppb–years) and high (> 5000 ppb–years). Data on confounding factors were not available; however, Church members are prohibited from using tobacco and consuming alcohol or caffeine. SMRs for kidney cancer were increased in the low- and high-exposure groups among men and in the low- and medium-exposure groups among women. The overall SMRs for cancers of the bladder and other urinary organs were below unity for both sexes, but these results were based on only three and two bladder cancers in men and women, respectively. [The Working Group noted that there were several problems in the interpretation of this study. The exposure assessment was ecological in nature because of relatively low exposures. There was widespread variability in water concentrations, which adds to the uncertainty of the study. Furthermore, the findings are influenced by lower rates of smoking for the cohort compared with all of Utah. This is manifest in the SMRs for non-malignant respiratory disease (SMR for men, 0.7; 95% CI, 0.5–0.9; SMR for women, 0.9; 95% CI, 0.7–1.2) and for mortality from chronic bronchitis, emphysema and asthma (SMR for men, 0.6; 95% CI, 0.4–0.9; SMR for women, 0.5; 95% CI, 0.1–1.2). For these reasons, the study is uninformative with regard to the relationship between exposure to arsenic and mortality from bladder and kidney cancer.]

2.1.5 *Studies in Europe*

In a type of case–control study known as case–cohort, Kurttio *et al*. (1999) investigated the association between low exposure to arsenic in well-water in Finland and the risk for cancers of the bladder and kidney. Cases of bladder and kidney cancer were identified from 1981 to 1995 within a registry-based cohort of the population who had lived at an address outside the municipal drinking-water system during 1967–80. The final study population consisted of 61 cases of bladder cancer and 49 cases of kidney cancer and an age- and sex-matched random sample of 275 subjects from the population register. The daily dose of arsenic in drinking-water was calculated from the concentration of arsenic in well-water and its reported consumption in the 1970s. Cumulative dose was defined as the integral of duration and intensity of exposure to arsenic from well-water. For the shorter latency period of 3–9 years prior to diagnosis of bladder cancer, cumulative dose was estimated from the beginning of use of well-water until 2 years before the diagnosis of cancer. For the longer latency period, the cumulative dose was calculated until 10 years before diagnosis. The concentrations of arsenic in the wells of the reference cohort ranged from less than 0.05 to 64 µg/L (median, 0.14 µg/L). After adjusting for age, sex and smoking, an increasing trend of arsenic in drinking-water and incidence of bladder cancer was observed with shorter latency but not with longer latency, whereas no evidence of an association between kidney cancer and arsenic in well-water was observed.

2.1.6 *Studies in Australia*

Two geographical areas in Victoria, Australia, were selected for study because of reports of concentrations of arsenic in the soil above 100 µg/g and/or concentrations in water above 10 µg/L (Hinwood *et al*., 1999). Median concentrations of arsenic in water were reported for various towns and showed a wide range up to a median of 1077 µg/L for Ballarat, which had a population of 43 947 in 1986. However, the extent to which contaminated water was used for drinking was not known. The authors noted that "high percentages of the population may be relying on alternative drinking-water sources such as bottled water and tank rain water". Cancer incidence was assessed for the period 1982–91 using the Victorian Cancer Registry. SIRs were estimated for the exposed population in 22 areas of Victoria, using cancer incidence rates for all of the State of Victoria as reference. SIRs for both bladder and kidney cancers were close to unity. [The Working Group noted that no information was presented on the actual use of water contaminated with arsenic for drinking by the population.]

2.2 Liver and lung cancer

2.2.1 *Liver cancer*

A previous IARC monograph on arsenic noted reports of liver angiosarcoma due to medicinal exposure to Fowler's solution (IARC, 1980). A summary of the findings of epidemiological studies on arsenic in drinking-water and risk for liver cancer, mainly hepatocarcinoma, are shown in Table 23.

(*a*) *Taiwan, China*

(i) *Ecological studies*

Chen *et al.* (1985) studied the mortality from liver cancer during the period 1968–82 among residents in 84 villages exposed to arsenic of four townships in south-western coastal Taiwan. Increased mortality was observed among both men and women. There was an exposure–response relationship between SMR and prevalence of Blackfoot disease. An exposure–response gradient for mortality from liver cancer was noted in evaluating the risk in areas with shallow wells (presumably with low exposure to arsenic), both shallow and artesian wells (intermediate exposure) and artesian wells only (highest exposure). In villages with artesian wells, the SMR was approximately 2.0 [CI not reported] for liver cancer.

Chen *et al.* (1988a) and Wu *et al.* (1989) reported the age-adjusted mortality rates for liver cancer for men and women in 42 villages in south-western Taiwan and calculated age-adjusted cancer mortality during the period 1973–86 within three groups of villages stratified by exposure concentration (< 300 µg/L, 300–590 µg/L and ≥ 600 µg/L arsenic) tested in 1964–66. Age-adjusted mortality rates (per 100 000 person–years) from liver cancer for residents of all ages in Taiwan (referent) increased with increasing concen-

Table 23. Summary of epidemiological studies on arsenic in drinking-water and risk for liver cancer

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Ecological studies							
Taiwan							
Chen *et al.* (1985)	84 villages on the SW coast	Mortality 1968–82, all ages	Endemic area for chronic arsenic toxicity (Blackfoot disease)	Men Women	305 146	Age- and sex-adjusted SMR (95% CI) 1.7 (1.5–1.9) 2.3 (1.9–2.7)	Mid-year population: 141 733 in 1968, 120 607 in 1982; national rate in 1968–82 used as the standard for estimation of SMR
Chen *et al.* (1988a)	42 villages on the SW coast	Mortality 1973–86, all ages	Average arsenic (1962–64) General population < 300 µg/L 300–590 µg/L ≥ 600 µg/mL General population < 300 µg/L 300–590 µg/L ≥ 600 µg/mL	 Men Women 		Age-adjusted SMR 28.0 32.6 42.7 68.8 8.9 14.2 18.8 31.8	899 811 person–years, rate per 100 000, age-standardized to 1976 world population
Wu *et al.* (1989)	42 villages on the SW coast	Mortality 1973–86, age ≥ 20	Average arsenic (1962–64) < 300 µg/L 300–590 µg/L ≥ 600 µg/mL < 300 µg/L 300–590 µg/L ≥ 600 µg/mL	 Men Women 	 54 42 27 25 16 10	 47.8 67.7 86.7 *p* for trend < 0.05 21.4 24.2 31.8 *p* for trend < 0.05	Men, 257 935 person–years; women, 234 519 person–years; rate per 100 000, age-standardized to 1976 world population

Table 23 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Chen & Wang (1990)	Taiwan	Mortality 1972–83, all ages	National survey of 83 656 wells (1974–76); average arsenic for each of 314 precincts or townships			*β (SE) from regression* Men 6.8 (1.3) Women 2.0 (0.5) *Percentiles of age-adjusted mortality rate/100 000 person–years* Men 25th 21.8 50th 27.0 75th 34.1 Women 25th 7.0 50th 8.7 75th 11.6	Regression coefficient (β) estimates increase in age-adjusted mortality per 100 000 per 100 µg/L arsenic increase in water
Tsai *et al.* (1999)	SW Taiwan, 4 townships	Mortality 1971–94, all ages	Arsenic-exposed area	Men Women	631 224	SMR (95% CI) 1.8 (1.7–1.98) 1.9 (1.6–2.1)	Men, 1 508 623 person–years; women, 1 404 759 person–years; national rates in 1971–94 used as the standard for estimation of SMR
South America							
Rivara *et al.* (1997)	Region II and VIII, northern Chile	Mortality 1976–92	Arsenic-contaminated Region II			Relative risk 1.2 (0.99–1.6)	Population: 411 000 in Region II, 1 700 000 in Region VIII. Antofagasta (Region II) versus Region VIII.
Hopenhayn-Rich *et al.* (1998)	Córdoba Province, Argentina, 26 counties	Mortality 1986–91, age ≥ 20	County group *Men* Low exposure (341 547) Medium exposure (201 546) High exposure (135 209) *Women* Low exposure (348 874) Medium exposure (204 454) High exposure (137 805)	 186 142 98 173 125 90		SMR 1.5 (1.3–1.8) 1.8 (1.5–2.1) 1.8 (1.5–2.2) 1.7 (1.4–1.96) 1.9 (1.6–2.2) 1.9 (1.5–2.4)	National rate in 1989 used as the standard for estimation of SMR

Table 23 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Smith *et al.* (1998)	Region II, Northern Chile	Mortality 1989–93, age ≥ 30	5-year intervals, 420 µg/L average	Men Women	48 37	SMR 1.1 (0.8–1.5) 1.1 (0.8–1.5)	National rates in 1991 used as the standard estimation of SMR; arsenic concentration is population-weighted average for major cities or towns in Region II, 1950–74
Australia							
Hinwood *et al.* (1999)	Victoria	Incidence 1982–91	Median concentration of arsenic in drinking-water ranged 1–1077 µg/L	749		SIR (95% CI) 0.5 (0.3–0.8)	State rates in 1982–91 used as the standard for estimation of SIR
Cohort studies							
Chen *et al.* (1988b)	SW Taiwan	Mortality	Area endemic for Blackfoot disease	17		SMR: 4.66 ($p < 0.001$) compared with national standard; 2.48 ($p < 0.01$) compared with regional standard	789 patients with Blackfoot disease followed from 1968 to 1984. National and regional rates in 1968–83 used as the standard for estimation of SMR
Tsuda *et al.* (1995)	Niigata Prefecture, Japan	Mortality, 1959–92, all ages	Level of arsenic < 0.05 mg/L 0.05–0.99 mg/L ≥ 1.0 mg/L Total	 0 0 2 2		SMR 0.0 (0–4.4) 0.0 (0–15.1) 7.2 (1.3–26.1) 1.5 (0.3–5.5)	113 persons who drank from industrially contaminated wells in 1955–59, then followed for 33 years; rates in Niigata Prefecture in 1960–89 used as the standard for estimation of SMR
Lewis *et al.* (1999)	Millard County, UT, USA	Mortality	Arsenic in well-water ranged 3.5–620 µg/L	Men Women	3 7	SMR 0.9 (0.2–2.5) 1.4 (0.6–2.9)	State rates in 1950–92 used as the standard for estimation of SMR.
Nakadaira *et al.* (2002)	Niigata Prefecture, Japan	Mortality	Industrially contaminated well-water in the town of Nakajo	1		O/E = 0.7	86 patients with chronic arsenic poisoning. National rates in 1959–92 used as the standard for estimation of SMR

Table 23 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Case–control study						
Chen *et al.* (1986)	SW Taiwan, 4 townships	Mortality	Duration of consumption of artesian well-water containing high levels of arsenic	65 cases 368 controls	Age- and gender-adjusted ORs by years of consuming high-arsenic artesian well-water: Never 1.00 1–20 years 0.85 21–40 years 1.24 > 40 years 2.67	ORs calculated using subjects who never consumed artesian well-water as referent Mantel-Haenszel χ^2 value: 9.01 ($p < 0.01$)

SMR, standardized mortality ratio; CI, confidence interval; SIR, standardized incidence ratio; O/E, observed/expected; OR, odds ratio

trations of arsenic in water for both men and women in the first study (Chen *et al.,* 1988a), as well as for residents aged 20 years or older in the second study (Wu *et al.*, 1989).

Chen and Wang (1990) analysed nationwide cancer mortality in Taiwan using measurements of arsenic concentrations in water from 83 656 wells located in 314 precincts and townships from 1974 to 1976. Using a multiple regression approach, the authors compared age-adjusted mortality for all ages during the period 1972–83 with arsenic concentrations in these locations. A significant association with concentration of arsenic was found for liver cancer in both men and women. Using multiple linear regression models, a regression coefficient indicating the change in age-adjusted mortality per 100 000 person–years for every 0.1 μg/L increase in arsenic in well-water was calculated, after adjusting for indices of industrialization and urbanization.

Tsai *et al.* (1999) studied mortality from liver cancer in four townships exposed to arsenic in south-western Taiwan during the period 1971–94. SMRs were calculated using two comparison groups: mortality in the whole of Taiwan and mortality in the two counties in which the four townships are located. Although differences in nutrition, socioeconomic status or other factors between populations in south-western Taiwan and the remainder of the country may influence their respective cancer rates, Tsai *et al.* (1999) provided evidence that such differences are relatively unimportant. SMRs in both men and women, using both regional and national references, were all close to 1.8.

(ii) *Case–control study*

Chen *et al.* (1986) carried out a case–control study on liver cancer and consumption of artesian well-water with high concentrations of arsenic in four townships of south-western Taiwan. A total of 65 cases of liver cancer, identified from death certificates, and 368 healthy controls were studied. Information on consumption of arsenic-contaminated artesian well-water, cigarette smoking, habitual alcohol and tea drinking, and consumption of vegetables and fermented beans was obtained through interview using a standardized questionnaire. Unconditional logistic regression was used to estimate multivariate-adjusted odds ratios for developing liver cancer and various risk factors. There was an exposure–response relationship between the duration of consumption of artesian well-water with high arsenic content and risk for liver cancer.

(*b*) *Japan*

Cohort study

Tsuda *et al.* (1995) found excess mortality from liver cancer among a cohort of 113 persons exposed to levels of arsenic above 1.0 mg/L from industrially contaminated drinking-water in villages of Niigata Prefecture, Japan. The expected number of deaths was based on sex-, age- and cause-specific mortality in Niigata Prefecture in 1960–89. Based on a subgroup of 86 study patients, Nakadaira *et al*. (2002) did not find excess mortality from liver cancer (SMR, 0.7 [95% CI, 0.02–3.9]; one case observed and 1.42 expected). [The small number of liver cancer deaths limited further analysis by severity of chronic arsenic poisoning.] [See complete comment by the Working Group in Section 2.1.2.]

(*c*) *Australia*

Hinwood *et al.* (1999) investigated the association between arsenic in drinking-water and liver cancer incidence in Victoria, Australia, in 1982–91. This study included 22 areas where the median arsenic concentration in drinking-water ranged from 14 to 166 µg/L. Using the incidence rate in Victoria, an SIR of 0.5 (95% CI, 0.3–0.8) was observed for liver cancer. [The small number of liver cancer deaths limited further analysis by severity of chronic arsenic poisoning.]

(*d*) *South America*

Ecological studies

Rivara *et al.* (1997) compared the mortality from liver cancer in Antofagasta in Region II with that in Region VIII of Chile in 1976–92. The relative risk for liver cancer was 1.2 (95% CI, 0.99–1.6) in arsenic-exposed Region II compared with the control area, Region VIII. [The data source and statistical analysis were not clearly described.]

Smith *et al.* (1998) examined liver cancer mortality during the period 1989–93 among persons 30 years of age and over in Region II of northern Chile. Concentrations of arsenic in drinking-water were well documented and had been high in all major population centres of Region II, especially before 1975. The population-weighted average in the years 1950–74 was 420 µg/L, with a maximum of 870 µg/L in Antofagasta, the largest city, between 1955 and 1969. SMRs for Region II were calculated using the national rate as the standard, and for liver cancer, were 1.1 for both men and women.

Hopenhayn-Rich *et al.* (1998) examined SMRs for liver cancer during the period 1986–91 among residents aged 20 years or older in the 26 counties of Córdoba Province, Argentina. They grouped counties into three strata according to the concentration of arsenic in drinking-water. The low- and intermediate-exposure groups were defined qualitatively. In the highest exposure group comprising two counties, the concentration of arsenic in drinking-water ranged from 40 to 433 µg/L in the towns of one county and from 50 to 353 µg/L in those of the other. Separate average concentrations in each county were 181 and 174 µg/L. SMRs were calculated using sex- and age-specific rates for Argentina as the referent. Increased mortality from liver cancer was observed for men and women, but SMRs were not related to exposure to arsenic.

(*e*) *USA*

Cohort study

Lewis *et al.* (1999) reported the association between arsenic in drinking-water and mortality from liver cancer in a cohort of residents of Millard County, UT, where the median concentration of arsenic in drinking-water ranged from 14 to 166 µg/L. [The limitations of this study are cited in Section 2.1.4.]

2.2.2 *Lung cancer*

A summary of the findings of epidemiological studies on arsenic in drinking-water and risk for lung cancer are shown in Table 24.

(*a*) *Taiwan, China*

(i) *Ecological studies*

In the study of Chen *et al.* (1985) (described in Section 2.1), increased mortality from lung cancer was observed among men and women in 1968–82 in an area endemic for Blackfoot disease. There was an exposure–response relationship between the SMR and the prevalence of Blackfoot disease. The exposure–response gradient for mortality from lung cancer was noted in evaluating the risk in areas with shallow wells (presumably with low exposure to arsenic), both shallow and artesian wells (intermediate exposure) and artesian wells only (highest exposure). In villages with artesian wells, SMRs were approximately 5.0 [CIs not reported] for lung cancer.

In the studies of Chen *et al.* (1988a) and Wu *et al.* (1989) (described in Section 2.1), age-adjusted mortality rates (per 100 000 person–years) from lung cancer increased with increasing concentrations of arsenic in water for both men and women, for residents of all ages in Taiwan (referent) (Chen *et al.*, 1988a), as well as for residents aged 20 years or older (Wu *et al.*, 1989).

In the analysis of Chen and Wang (1990) (described in Section 2.1.1), regression coefficients (SE) for lung cancer showed a significant association with concentration of arsenic for lung cancer in both men and women.

In the study of Tsai *et al.* (1999) (described in Section 2.1.1) using national and regional rates as standard, SMRs for lung cancer were also increased for both sexes.

(ii) *Cohort study*

Chiou *et al.* (1995) (described in Section 2.1.1) followed 2556 subjects in an area endemic for Blackfoot disease of south-western Taiwan for periods ranging up to approximately 7 years from 1986 to 1993, including 263 patients with Blackfoot disease and 2293 healthy individuals. Results, adjusted for cigarette smoking habits, showed an increased risk for lung cancer in relation to increasing average concentrations of arsenic and to increasing cumulative exposure to arsenic.

(iii) *Case–control study*

Chen *et al.* (1986) (described in Section 2.1.1) studied a total of 76 cases of lung cancer and 368 healthy controls and observed a dose–response relationship between the duration of consumption of artesian well-water containing high levels of arsenic and risk for lung cancer, showing the highest age- and gender-adjusted odds ratio for those who consumed artesian well-water for more than 40 years compared with those who never consumed artesian well-water.

Table 24. Summary of epidemiological studies on arsenic in drinking-water and risk for lung cancer

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Ecological studies							
Taiwan							
Chen *et al.* (1985)	84 villages on the SW coast	Mortality 1968–82, all ages	Area endemic for chronic arsenic toxicity (Blackfoot disease)	Men Women	332 233	Age- and sex-adjusted SMR (95% CI) 3.2 (2.9–3.5) 4.1 (3.6–4.7)	Mid-year population: 141 733 in 1968, 120 607 in 1982; national rate in 1968–82 used as the standard for SMR estimation
Chen *et al.* (1988a)	42 villages on the SW coast	Mortality 1973–1986, all ages	Average arsenic (1964–66) General population < 300 µg/L 300–600 µg/L ≥ 600 µg/L General population < 300 µg/L 300–600 µg/L ≥ 600 µg/L	Men Women 		Age-adjusted SMR 19.4 35.1 64.7 87.9 9.5 26.5 40.9 83.8	899 811 person–years, rate per 100 000, age-standardized to 1976 world population
Wu *et al.* (1989)	42 villages on the SW coast	Mortality 1973–86, age ≥ 20	Average arsenic (1964–66) < 300 µg/L 300–600 µg/L ≥ 600 µg/L < 300 µg/L 300–600 µg/L ≥ 600 µg/L	 Men Women 	 53 62 32 43 40 38	 49.16 100.67 104.08 (p for trend < 0.001) 36.71 60.82 122.16 (p for trend < 0.001)	Men: 257 935 person–years; females, Women: 234 519 person–years; rate per 100 000 age-standardized to 1976 world population

Table 24 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Chen & Wang (1990)	Taiwan	Mortality 1972–83, all ages	National survey of 83 656 wells (1974–76); average arsenic for each of 314 precincts or townships		*β (SE) from regression*	Regression coefficient (β) estimates increase in age-adjusted mortality per 100 000 per 100 µg/L arsenic increase in water
				Men	5.3 (0.9)	
				Women	5.3 (0.7)	
					Percentiles of age-adjusted mortality rate/100 000 person–years	
				Men	25th 11.8	
					50th 16.2	
					75th 20.7	
				Women	25th 5.2	
					50th 7.4	
					75th 10.4	
Tsai *et al.* (1999)	SW Taiwan, 4 townships	Mortality 1971–94, all ages	Arsenic-exposed area	Men 699 Women 471	SMR (95% CI)	Men: 1 508 623 person–years; Women: 1 404 759 person–years
				Men	2.6 (2.5–2.8)	National rates in 1971–94 used as the standard for estimation of SMR
				Women	3.5 (3.2–3.8)	
				Men	3.1 (2.9–3.3)	Regional rates in 1971–94
				Women	4.1 (3.8–4.5)	
South America						
Rivara *et al.* (1997)	Region II and VIII, northern Chile	Mortality 1976–92	Arsenic-contaminated Region II		Relative risk (95% CI) Region II versus region VIII 8.8 (8.1–9.5)	Population: 411 000 in Region II, 1 700 000 in Region VIII. Antofagasta (Region II) versus Region VIII.

Table 24 (contd)

Reference	Location	End-point	Exposure	No. of cases		Study outcome	Comments
Hopenhayn-Rich *et al.* (1998)	Córdoba Province, Argentina, 26 counties	Mortality 1986–91, age ≥ 20	County group: Low exposure Medium exposure High exposure Low exposure Medium exposure High exposure	 Men Women 	 826 914 708 194 138 156	 0.92 (0.85–0.98) 1.5 (1.4–1.6) 1.8 (1.6–1.9) 1.2 (1.1–1.4) 1.3 (1.1–1.6) 2.2 (1.8–2.5)	Population: low exposure, 341 547, medium exposure, 201 006; high exposure, 135 209; national rate in 1989 used as the standard for SMR estimation
Smith *et al.* (1998)	Region II, northern Chile	Mortality 1989–93, age ≥ 30	5-year intervals, 420 µg/L average	 Men Women	 544 154	SMR 3.8 (3.5–4.1) 3.1 (2.7–3.7)	National rates in 1991 used as the standard for estimation of SMR; arsenic concentration is population-weighted average for major cities or towns in Region II, 1950–74
Australia							
Hinwood *et al.* (1999)	Victoria	Incidence 1982–91	Median arsenic concentration in drinking-water ranging 1–1077 µg/L	20		SIR (95% CI) 1.0 (0.9–1.1)	State rates in 1982–91 used as the standard for estimation of SIR
Cohort studies							
Chen *et al.* (1988b)	SW Taiwan	Mortality 1968–83	Area endemic for Blackfoot disease	28		SMR: 10.49 ($p < 0.001$) compared with national standard; 2.84 ($p < 0.01$) compared with regional standard	789 patients with Blackfoot disease followed from 1968 to 1984. National and regional rates in 1968–83 used as the standard for estimation of SMR
Tsuda *et al.* (1995)	Niigata Prefecture, Japan	Mortality, 1959–92, all ages	Arsenic level: < 0.05 mg/L 0.05–0.99 mg/L ≥ 1.0 mg/L Total	 0 1 8 9		SMR 0.0 (0–2.4) 2.3 (0.1–13.4) 15.7 (7.4–31.0) 3.7 (1.8–7.0)	113 persons who drank from industrially contaminated wells in 1955–59, then followed for 33 years; rates in Niigata Prefecture in 1960–89 used as the standard for estimation of SMR

Table 24 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Chiou *et al.* (1995)	SW Taiwan; 4 neighbouring townships	Incidence 1986–93	Cumulative arsenic exposure (mg/L × year) < 0.1 0.1–19.9 ≥ 20 Average arsenic concentration (mg/L) < 0.05 0.05–0.70 ≥ 0.71	 3 7 7 5 7 7	Relative risk (95% CI) 1.0 3.1 (0.8–12.2) 4.7 (1.2–18.9) 1.0 2.1 (0.7–6.8) 2.7 (0.7–10.2)	Incidence among a cohort of 2556 subjects (263 Blackfoot disease patients and 2293 healthy individuals) followed for 7 years
Lewis *et al.* (1999)	Millard County, UT, USA	Mortality	Arsenic in well-water, 3.5–620 µg/L	Men 28 Women 6	SMR 0.6 (0.4–0.8) 0.4 (0.2–0.95)	State rates in 1950–92 used as the standard for SMR estimation.
Nakadaira *et al.* (2002)	Niigata Prefecture, Japan	Mortality	Industrially contaminated well-water in the town of Nakajo	Men 7 Women 1 Total 8	Poisson probability distribution in men: 9.6 0/E = 11.01	86 patients with chronic arsenic poisoning. National rates in 1959–92 used as the standard for SMR estimation.
Case–control studies						
Chen *et al.* (1986)	SW Taiwan, 4 townships	Mortality	Duration of consumption of artesian well-water containing high levels of arsenic	76 cases 368 controls	Age- and sex-adjusted OR by years of consuming high-arsenic artesian well-water Never 1.00 1–20 years 1.26 21–40 years 1.52 > 40 years 3.39	OR calculated using subjects who never consumed artesian well-water as referent Mantel-Haenszel $\chi 2$ value: 8.49 ($p < 0.01$)

Table 24 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Ferreccio *et al*. (2000)	Northern Chile	Incidence 1994–96	Individual ≥ 40-year average arsenic concentration from public water supply records during 1930–94	151 cases 419 matched hospital controls	Age- and sex-adjusted OR (95% CI)	OR calculated using subjects with average exposures of 0–10 µg/L as referent
			0–10 µg/L		1.0	
			10–29 µg/L		1.6 (0.5–5.3)	
			30–49 µg/L		3.9 (1.2–12.3)	
			50–199 µg/L		5.2 (2.3–11.7)	
			200–400 µg/L		8.9 (4.0–19.6)	

SMR, standardized mortality ratio; CI, confidence interval; SIR, standardized incidence ratio; OR, odds ratio

(*b*) *Japan*

Cohort study

Tsuda *et al.* (1995) (described in Section 2.1.2) found excess mortality from lung cancer among a cohort of 113 persons exposed to levels of arsenic above 1.0 mg/L from industrially contaminated drinking-water in villages of Niigata Prefecture, Japan. The expected number of deaths was based on sex-, age- and cause-specific mortality in Niigata Prefecture in 1960–89. Based on a subgroup of 86 study patients, Nakadaira *et al*. (2002) did not find excess mortality from lung cancer.

(*c*) *Australia*

Hinwood *et al.* (1999) investigated the association between levels of arsenic in drinking-water and lung cancer incidence in Victoria, Australia, during the period 1982–91. This study included 22 areas where median concentrations of arsenic in drinking-water ranged from 14 to 166 µg/L. Using the incidence rate in Victoria, an SIR of 1.0 was observed for lung cancer.

(*d*) *South America*

(i) *Ecological studies*

Rivara *et al.* (1997) compared the mortality from lung cancer in 1976–92 between Antofagasta in Region II with that in Region VIII of Chile. The relative risk for lung cancer was higher in Antofagasta compared with Region VIII. [The data source and statistical analysis were not clearly described.]

Smith *et al.* (1998) (described in Section 2.2.1) found elevated SMRs of about 3 for lung cancer for both sexes in Region II, using the national rate as standard.

In the study of Hopenhayn-Rich *et al.* (1998) (described in Section 2.2.1), significant increases in the incidence of lung cancer associated with increasing exposure to arsenic were observed for lung cancer.

(ii) *Case–control study*

Ferreccio *et al.* (2000) conducted a case–control study of incident lung cancer cases in northern Chile. Eligible cases included all lung cancer cases admitted to public hospitals in Regions I, II and III of Chile from November 1994 to July 1996. Eighty to ninety per cent of all cancer patients in the north of Chile are admitted to public hospitals, and a total of 151 cases participated. Controls were selected from all patients admitted to any public hospital in the study region and frequency-matched to cases by age and sex. Two control series were selected: cancers other than lung cancer and non-cancer controls [no response rates were indicated for cases and controls]. Potential biases in control selection were assessed by several approaches including comparisons with geographical distribution of the general population based on census data. Information regarding residential history, socioeconomic status, occupational history (to ascertain employment in copper smelting) and smoking was obtained by questionnaire interview. Historical exposure to arsenic in

drinking-water was estimated by linking information on residential history with a database of information on arsenic concentrations in public water supplies collected for the years 1950–94. Arsenic concentrations in the year prior to 1950 were based on concentrations in the 1950s. Average concentration of arsenic in the place of residence was assigned to each subject on a year-by-year basis for the period 1930–94. Population coverage of public water systems in the main cities in Regions I and II was over 90% and was between 80 and 90% in the major cities of Region III. The coverage in smaller cities varied between 64 and 91%. Odds ratios were calculated using unconditional logistic regression, adjusted for age, sex, socioeconomic status, smoking and working in a copper smelter. Results from the analysis based on average exposures during 1930–94 and using all controls showed an increase in the odds ratio with concentration of arsenic. Evidence for a synergistic effect of arsenic in water and smoking was found for those who both smoked and had high concentrations of arsenic in their drinking-water (results not shown).

(*e*) *USA*

Cohort study

Lewis *et al.* (1999) reported the association between arsenic in drinking-water and lung cancer mortality in a cohort of residents of Millard County, UT, where the median concentration of arsenic in drinking-water ranged from 14 to 166 µg/L. The SMRs for lung cancer for both men and women were below unity. [Limitations of this study have been cited in Section 2.1.4.]

2.3 Skin cancer

The recognition of arsenic as a carcinogen originally came from case series describing skin cancers following ingestion of arsenical medicine, and exposure to arsenical pesticide residues and arsenic-contaminated drinking-water. Hutchinson (1888) noted skin cancers among patients treated for psoriasis and other ailments with arsenic-containing compounds (e.g. Fowler's Solution containing 1% potassium arsenite). Neubauer (1947) summarized 143 skin cancer cases among arsenic-treated patients. Over 50% of the skin cancers developed in patients treated for 10 years or less and lesions developed after 3–40 years, and on average after 18 years. Clinical reports have described an association of chronic arsenicism with skin cancer in vineyard workers of the Moselle region, Germany (Roth, 1957; Grobe, 1977). Numerous cases of skin cancer have been documented from communities with arsenic-contaminated drinking-water. These include, but are not limited to, case reports from Silesia (Neubauer, 1947), North America (Wagner *et al*., 1979), Taiwan, China (Yeh, 1973), Argentina (Bergoglio, 1964), Mexico (Cebrían *et al*., 1983), Chile (Zaldívar, 1974; Zaldívar *et al*., 1981) and, more recently, Bangladesh (Kurokawa *et al.*, 2001), West Bengal, India (Saha, 2001) and Malaysia (Jaafar *et al.*, 1993). The characteristic arsenic-associated skin tumours include squamous-cell carcinoma arising in keratoses (including Bowen disease) and multiple basal-cell carcinomas

(e.g. Neubauer, 1947; Neuman & Schwank, 1960; Yeh *et al.*, 1968). Therefore, this section focuses on these non-melanoma skin cancers. In addition, ecological studies of skin cancer based on mortality rates in areas with low exposure to arsenic such as the USA or exposure imputed from soil levels are not considered here. Findings of epidemiological studies on arsenic in drinking-water and risk for skin cancer are summarized in Table 25.

2.3.1 *Taiwan, China*

(*a*) *Ecological studies*

(i) *Study based on prevalence of skin cancer*

In 1965, Tseng *et al*. (1968) completed a skin cancer prevalence survey on the south-west coast of Taiwan, a region known to have arsenic-contaminated artesian wells that were introduced into the region in 1910–20. A house-to-house examination was conducted of family members from 37 villages: 10 in Chai-yi County, 25 in Tainan County and two in a suburb of Tainan City. The study covered a total population of 40 421 inhabitants. A total of 428 skin cancers were identified, 238 of which were sent for histopathological review. The study was based on clinical diagnoses. In the survey region, 142 water samples from 114 wells were tested for arsenic. Arsenic concentrations ranged from 1 μg/L to 1820 μg/L, and the majority of wells in the endemic region contained between 400 and 600 μg/L arsenic. Skin cancer prevalence was computed according to the median arsenic concentrations per village, categorized as < 300 μg/L, 300–600 μg/L and > 600 μg/L. Villages with either wide-ranging arsenic concentrations or residents who no longer drank the water were deemed to be indeterminate in the analysis. Prevalence rates of skin cancer (based on clinical diagnosis) for inhabitants residing in low- (< 300 μg/L), medium- (300–600 μg/L) and high- (> 600 μg/L) arsenic areas represented over an eightfold difference from the highest to the lowest category.

(ii) *Studies based on incidence of skin cancer*

Guo *et al*. (1998, 2001) correlated incidence rates of skin cancer with levels of arsenic in well-water for 243 townships in Taiwan (with a total of about 11.4 million residents). Skin cancers were identified from the National Cancer Registry Program from 1980 to 1987 by a hospital-based registry covering both clinical and pathological diagnoses of skin cancers (reporting is not mandatory by law) (Guo *et al*., 1998). Arsenic concentrations were provided by a national survey of wells conducted by the Taiwan Provincial Institute of Environmental Sanitation and published in 1977. The investigators modelled the percentage of the population living in townships with drinking-water concentrations of < 50, 50–89, 90–169, 170–329, 330–640 and > 640 μg/L arsenic. The models assume that the same number of individuals drank from each well within a township. The results for all skin cancers combined estimated a relative risk for the highest versus lowest exposure category of about 14 in men and 19 in women, with no excess detected in the other categories (Guo *et al*., 1998).

Table 25. Summary of epidemiological studies on arsenic in drinking-water and risk for skin cancer

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Ecological studies						
Taiwan						
Tseng *et al.* (1968)	40 421 residents from 37 villages (SW)	Prevalence ≥ 20 years of age	Median arsenic concentrations of wells in village of residence (μg/L) < 300 300–600 > 600	428	Prevalence (per 1000) 2.6 10.1 21.4	Prevalence based on clinical examination of all households. Excludes villages with wells no longer in use or with variations in arsenic concentration (range, 1–1820 μg/L; most wells contained 400–600 μg/L arsenic)
Chen *et al.* (1985)	4 neighbouring townships on the SW coast	Mortality 1968–82	Areas hyperendemic (21 villages), endemic (25 villages) and not endemic (38 villages) for Blackfoot disease, corresponding to high, medium and low exposure	46 men 49 women	SMR (95% CI) 534 (379–689) 652 (469–835)	Mortality rates in all Taiwan as standard
Chen *et al.* (1988a)	Region endemic for Blackfoot disease (SW)	Mortality 1973–86	Median arsenic concentrations of well-water (μg/L) < 300 300–600 > 600	 Men Women Men Women Men Women	SMR 1.6 1.6 10.7 10.0 28.0 15.1	Age-standardized to the 1976 world standard population

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Wu *et al*. (1989)	42 villages in region endemic for Blackfoot disease (SW)	Mortality 1973–86	Median arsenic concentrations of well-water in village of residence (μg/L) in 1964–66	19 men 17 women	SMR	Age-standardized to the 1976 world standard population
			< 300	Men	2.03	
			300–600		14.01	
			> 600		32.41 ($p < 0.001$)	
			< 300	Women	1.73	
			300–600		14.75	
			> 600		18.66 ($p < 0.001$)	
Chen & Wang (1990)	314 precincts and townships	Mortality 1972–83	Average arsenic concentrations	NS	Increase (β) in mortality rate per 100 000 per 0.1 μg/L increase:	Multiple regression adjusted for age and indices of urbanization and industrialization. Mortality rates standardized to the 1976 world standard population
				Men	β (SE) = 0.9 (0.2)	
				Women	β (SE) = 1.0 (0.2)	
Guo *et al*. (1998)	243 townships, 11.4 million residents	Incidence 1980–87	Arsenic concentration in wells Exposure categories: > 50, 50–89, 90–169, 170–329, 330–640 and > 640 μg/L	952 men 595 women	Risk difference of 0.34/100 000 ($p < 0.01$) associated with a 1% increase in arsenic concentrations > 640 μg/L Relative risk of highest versus lowest exposure category: 14.21 in men; 19.25 in women No excess risk for other categories	Rates standardized using the 1976 world standard population. Model assumes that same number of individuals use each well.
Tsai *et al*. (1999)	Four townships (SW)	Mortality 1971–94	Area endemic for Blackfoot disease		Age- and sex-adjusted SMR (95% CI)	
				66 men	4.8 (3.7–6.2)	Local standard
					5.97 (4.6–7.6)	National standard
				68 women	5.7 (4.4–7.2)	Local standard
					6.8 (5.3–8.6)	National standard

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Guo *et al*. (2001)	243 townships, 11.4 million residents	Incidence 1980–89	Concentration of arsenic in well-water: Exposure categories (µg/L) arsenic	2369 (1415 men, 954 women)	Rate difference association with a 1% increase in residents with categories of arsenic (µg/L):	Cancers identified through National Cancer Registry. Models include age and urbanization index. Models assume same number of individuals use each well. BCC, basal-cell carcinoma SCC, squamous-cell carcinoma *$p < 0.05$ **$p < 0.01$
				764 BCC Men		
			Intercept	Women	0.779	
				Men	–0.002	
			50–89	Women	0.004	
				Men	–0.012	
			90–169	Women	–0.017	
				Men	0.018	
			170–329	Women	0.006	
				Men	0.004	
			330–640	Women	–0.024	
				Men	0.016	
			> 640	Women	0.128**	
				Men	0.027	
			Intercept	736 SCC Women	0.821	
				Men	1.488	
			50–89	Women	0.024	
				Men	–0.006	
			90–169	Women	–0.026	
				Men	0.006	
			170–329	Women	0.073**	
				Men	0.016	
			330–640	Women	–0.100**	
				Men	–0.064*	
			> 640	Women	0.155**	
					0.212**	
				182 melanoma	No increase associated with melanoma	

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Guo *et al.* (2001) (contd)			Three categories of township: (1) No well with arsenic > 40 µg/L; (2) Some wells > 40 µg/L but none > 640 µg/L; (3) More wells > 640 µg/L than between 320 and 640 µg/L		Dose–response relationship between basal-cell and squamous-cell skin cancer in both men and women and in all age categories (except for basal-cell < 30 years of age, which had few subjects). No consistent increase in melanoma incidence by exposure category	
Mexico						
Cebrián *et al.* (1983)	Two rural populations in Lagunera region; 2486 residents	Prevalence (time frame not specified)	Town of El Salvador de Arriba: high exposure to arsenic (410 µg/L); town of San Jose del Vinedo: low exposure (5 µg/L)	4	High exposure: 1.4% (4 cases in 57 households and 296 individuals); low exposure: 0% (0 cases in 68 households and 318 individuals)	Epidermoid or basal-cell carcinomas detected on physical exam of every 3rd household
Chile						
Zaldívar *et al.* (1974)	City of Antofagasta	Incidence of cutaneous lesions of chronic arsenic poisoning, 1968–71	Concentration of arsenic fell from 580 µg/L in 1968–69 to 8 µg/L in 1971		Incidence rates: Men: 145.5/100 000 in 1968–69, 9.1/100 000 in 1971; women: 168.0/100 000 in 1968–69 and 10.0/100 000 in 1971	
Rivara *et al.* (1997)	Regions II and VIII	Mortality 1976–92	Exposed group: Antofagasta in region II (arsenic concentration in drinking-water, 40–860 µg/L; 1950–92) Unexposed group: region VIII, no arsenic contamination (reference)	NS	SMR (95% CI) 3.2 (2.1–4.8)	
Smith *et al.* (1998)	Region II, northern Chile	Mortality 1989–93, age ≥ 30	Annual average arsenic concentrations ranging 43–569 µg/L in 1950–94	20 men 7 women	SMR (95% CI) 7.7 (4.7–11.9) 3.2 (1.3–6.6)	Age-standardized to the national rates of Chile in 1991

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
USA						
Berg & Burbank (1972)		Mortality 1950–67	Trace metals in water supplies from 10 basins throughout the USA; concentration of arsenic in water, Oct. 1962–Sept. 1967		No correlation	
Morton *et al*. (1976)	Lane County, OR	Incidence 1958–71	Mean arsenic concentration in municipal water system and single-family systems	3039	Correlation of arsenic content in drinking-water: squamous-cell carcinoma: 0.151 for men and –0.20 for women; basal-cell carcinoma: –0.064 for men and 0.10 for women	Non-melanoma cases identified by review of pathology reports
Wong *et al*. (1992)	Four counties in Montana	Incidence 1980–86	Two contaminated counties (copper smelter and copper mines); two control counties	Around 2300 in the 4 counties	Age-adjusted skin cancer incidence higher in control counties	Overall incidence rates for exposed counties within range observed for other US locations
Cohort studies						
Taiwan						
Chen *et al*. (1988b)	Four townships (SW)	Mortality 1968–83, all ages	Diagnosis of Blackfoot disease as a surrogate for high exposure to arsenic	7	SMR 28.46 ($p < 0.01$) (national standard) 4.51 ($p < 0.05$) (local standard)	871 people who developed Blackfoot disease after 1968 were followed for 15 years. National standard used for the age- and sex-standardized rates of the general Taiwanese population.
Hsueh *et al*. (1997)	Three villages in Putai township (SW)	Incidence 1989–92, age, ≥ 30 years	Duration of residence in area endemic for Blackfoot disease (years) ≥ 33 34–43 44–53 > 53	 1 4 8 20	Relative risk (95% CI) 1.0 5.01 (0.5–48.1) 4.9 (0.6–41.6) 6.8 (0.9–53.7) (p for trend = 0.07)	654 subjects (275 men and 379 women) without skin cancer followed with dermatological examinations. Total of 2239 person–years. Relative risk adjusted for age, sex and level of education

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Hsueh *et al.* (1997) (contd)			Duration of consumption of artesian well-water (years)			
			0	1	1.0	
			1–15	1	1.2 (0.4–19.7)	
			16–25	8	3.9 (0.5–32.1)	
			> 25	23	8.9 (1.1–72.9) (p for trend < 0.05)	
			Average concentration of arsenic in drinking-water (mg/L)			
			0	1	1.0	
			0.01–0.70	12	3.3 (0.4–35.8)	
			0.71–1.10	13	8.7 (1.1–65.5) (p for trend < 0.05)	
			Unknown	7	4.8 (0.6–40.4)	
			Cumulative exposure to arsenic (mg/L–years)			
			0	1	1.0	
			0.1–10.6	2	2.8 (0.3–31.9)	
			10.7–17.7	5	2.6 (0.3–22.9)	
			> 17.7	18	7.6 (0.95–60.3) (p for trend = 0.06)	
			Unknown	7	5.1 (0.6–44.4)	
			Level of serum β-carotene (μg/mL)	16 cases (61 controls)	OR (95% CI)	OR adjusted for age, sex, cumulative exposure to arsenic, serum cholesterol and triglyceride levels, cigarette smoking and alcohol drinking Incidence 14.74/1000 person–years
			≤ 0.14		1.0	
			0.15–0.18		0.4 (0.1–2.9)	
			> 0.18		0.01 (0.0–0.4) (p for trend < 0.01)	

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Case–control studies						
USA						
Karagas *et al.* (2001b, 2002)	New Hampshire	Incidence 1993–96	Concentration of arsenic in toenails (μg/g)		OR (95% CI) *Squamous-cell carcinoma*	OR adjusted for age and sex 284 cases, 524 controls
			0.009–0.089	155	1.0	
			0.090–0.133	64	0.9 (0.6–1.3)	
			0.134–0.211	33	0.98 (0.6–1.6)	
			0.212–0.280	14	1.1 (0.55–2.2)	
			0.281–0.344	5	1.0 (0.3–3.0)	
			0.345–0.81	13	2.1 (0.9–4.7)	
					Basal-cell carcinoma	587 cases, 524 controls
			0.009–0.089	281	1.0	
			0.090–0.133	156	1.01 (0.8–1.4)	
			0.134–0.211	92	1.06 (0.7–1.5)	
			0.212–0.280	22	0.7 (0.4–1.3)	
			0.281–0.344	10	0.8 (0.3–1.8)	
			0.345–0.81	26	1.4 (0.7–2.8)	
Nested case–control study						
Taiwan						
Hsueh *et al.* (1995)	Three villages in Putai Township (SW)	Prevalence	Duration of residence in area endemic for Blackfoot disease (years)		OR (95% CI)	OR adjusted for age and sex; 1081 residents (468 men, 613 women) underwent a physical examination.
			≤ 45	2	1.0	
			46–49	11	5.2 (1.1–25.8)	
			≥ 50	53	8.5 (1.96–37.2)	
					p for trend < 0.05	

Table 25 (contd)

Reference	Location	End-point	Exposure	No. of cases	Study outcome	Comments
Hsueh *et al.* (1995) (contd)			Duration of drinking artesian well-water (years)			
			≤ 13	2	1.0	
			14–25	15	5.1 (1.03–24.98)	
			≥ 60	52	6.4 (1.4–27.9)	
					p for trend < 0.05	
			Average exposure to arsenic (ppm)			
			0	2	1.0	
			0–0.70	20	3.5 (0.7–17.0)	
			> 0.71	30	5.0 (1.1–23.8)	
			Cumulative exposure to arsenic (ppm–years)			
			≤ 4	1	1.0	
			5–24	22	8.9 (1.1–73.8)	
			≥ 25	28	13.7 (1.7–111.6)	
			Chronic hepatitis B carrier and liver function status:			
			Non-carrier with normal liver function	41	1.0	
			HBsAg carrier with normal liver function	13	1.1 (0.56–2.2)	
			Non-carrier with liver dysfunction	3	2.1 (0.54–7.7)	
			HBsAg carrier with liver dysfunction	4	8.4 (2.37–29.9)	
					p for trend < 0.05	

SMR, standardized mortality ratio; CI, confidence interval; NS, not specified; OR, odds ratio

In a second study, the incidence rates of each major histological type of skin cancer (basal-cell, squamous-cell and melanoma) were modelled using the previously defined exposure categories (Guo *et al*., 2001). The model included the percentage of residents in categories of age (30–49, 50–69 and > 69 years) and urbanization index as covariates. In this analysis, they found a statistically significant increase in squamous-cell skin cancer in the highest category (> 640 μg/L arsenic) in both men and women. Similarly, for basal-cell carcinoma, there was also a positive association in the highest exposure category. Melanoma was unrelated to any category of exposure to arsenic. In a post-hoc analysis, the investigators created three exposure groups: townships with no well containing levels of arsenic above 40 μg/L, townships with some wells containing more than 40 μg/L arsenic but none above 640 μg/L and townships with more wells containing more than 640 μg/L than between 320 and 640 μg/L. A dose–response relation between basal-cell and squamous-cell skin cancer rates was observed in both men and women and in all age categories (except for basal-cell cancer below age 30 years, which had few subjects). Again, no association was seen for melanoma. [The Working Group noted that the reporting of incident skin cancer may have been incomplete.]

(iii) *Studies based on mortality from skin cancer*

A series of studies conducted in Taiwan, China (Chen *et al*., 1985, 1988a; Wu *et al*., 1989; Chen & Wang, 1990; Tsai *et al*., 1999) (described in Section 2.1.1) analysed skin cancer mortality in relation to levels of arsenic in well-water. The overall SMRs in the four counties were 534 in men and 652 in women, with 100 as the referent value. There was also a gradient of increasing SMRs for skin cancer in areas not endemic or hyperendemic for Blackfoot disease (Chen *et al*., 1985). A subsequent report (Chen *et al*., 1988a) presented SMRs for skin cancer grouped by the median levels of arsenic in well-water measured in 1962–64 into < 300, 300–600 and > 600 μg/L. The age-standardized mortality rates for skin cancer increased in the three categories for both genders (Chen *et al*., 1988a). Wu *et al*. (1989) published age-adjusted mortality rates from skin cancer for the years 1973–86 for the four townships endemic for Blackfoot disease plus 15 villages in the townships of Yensui and Hsiaying. Using the same data on well-water contents and the classification described above, skin cancer mortality rates also increased in men and women with increasing median concentrations of arsenic. Subsequently, Chen and Wang (1990) used data on wells measured from 1974 to 1976 from 314 precincts and townships throughout Taiwan. Based on a multiple regression analysis (adjusted for urbanization and age), a 0.1-μg/L increase in arsenic corresponded to an increase of 0.9 (SE, 0.2) and 1.0 (SE, 0.2) per 100 000 in skin cancer mortality in men and women, respectively. More recently, Tsai *et al*. (1999) computed SMRs for the period 1971–94 for the four townships endemic for Blackfoot disease by applying both local (Chiayi County) and national (Taiwan) rates as the standards. The SMRs for skin cancer were approximately 5.0 for men and women, using both local and national standards.

(*b*) *Cohort studies*

Chen *et al*. (1988b) conducted a retrospective cohort study of 871 patients who met clinical criteria of Blackfoot disease after January 1968. From 1968 to 1983, seven death certificates identified the underlying cause of death as skin cancer. SMRs for skin cancer were computed using both the area endemic for Blackfoot disease and the whole of Taiwan as the population standards. The age-standardized observed versus expected number of skin cancer deaths was around 28 using Taiwan as the standard and 4.5 using the Blackfoot disease townships as the standard. [Use of the national population as the standard may provide a better estimate of excess risk than use of the local population for whom arsenic concentrations in well-water were elevated.]

Hsueh *et al*. (1997) established a cohort of residents living in three villages (Homei, Fuhsin and Hsinming) in Putai Township, one of the regions of the south-west coast of Taiwan endemic for Blackfoot disease. Arsenic-contaminated well-water had existed in these villages for over 50 years, with reported values ranging from 700 to 930 μg/L. The government introduced a new water system in the 1960s, with relatively low penetration until the 1970s. Well-water remained in use for agriculture and aquaculture. The 1571 residents aged 30 years or older who lived for at least 5 days a week in the villages were recruited to take part in the study. Of these, 1081 (68.8%) participated in a physical examination (468 men and 613 women). The 1015 study subjects who did not have a prevalent skin cancer comprised the cohort. Of these, 275 men and 379 women (64%) underwent regular examinations for dermatological conditions. Thirty-three incident skin cancers developed during the follow-up period from September 1989 through December 1992, with a total of 2239 person–years and a rate of 14.74/1000 person–years. Public health nurses interviewed cohort members on length of residence, history of drinking well-water and other potentially confounding factors. An index for cumulative exposure to arsenic was derived for each subject using concentrations of arsenic in well-water for each village of residence measured in the 1960s. These data were multiplied by the duration of consumption of well-water in each village of residence and totalled for each residence. Dermatologists diagnosed clinically the skin cancers that occurred in the cohort, and 91% of clinically diagnosed carcinomas were confirmed histologically. Fasting blood samples and urine samples were collected at the time of interview. Serum was analysed for β-carotene and urine for arsenic metabolites from 16 skin cancer cases [48%] and 61 age- and sex-matched controls. Cox proportional hazard regression was used to analyse data on exposure to arsenic in relation to incidence of skin cancer adjusted for age, sex and educational level. Conditional logistic regression analysis was used to assess the effects of β-carotene and urinary metabolites on the risk for skin cancer. Risk for skin cancer was significantly related to duration of living in the area endemic for Blackfoot disease, duration of consumption of artesian well-water, average concentration of arsenic and index for cumulative exposure to arsenic. There was evidence of a reduced risk for skin cancer in the highest two tertiles of β-carotene versus the lowest tertile after adjustment for multiple potential confounders. Also, compared with controls, cases of

skin cancer had higher total urinary concentration of arsenic, percentage of MMA and ratio of MMA to inorganic arsenic and a lower percentage of DMA and ratio of DMA to MMA (not shown in Table 25).

Hsueh *et al*. (1995) conducted a nested case–control study within the cohort study of Hsueh *et al*. (1997). A total of 66 prevalent skin cancers were identified after clinical examination. Age- and sex-adjusted prevalence odds ratios were computed for the same variables of exposure to arsenic included in the cohort analysis: duration of living in the area endemic for Blackfoot disease, duration of drinking artesian well-water, and average and cumulative exposure to arsenic. Significant increases in risk for each exposure group were shown. In addition, a significantly elevated risk for skin cancer was observed among chronic hepatitis B carriers with liver dysfunction.

2.3.2 *Mexico*

Ecological study based on prevalence of skin cancer

Cebrían *et al*. (1983) reported results from a household survey of two rural Mexican towns in the Region of Lagunera. Towns were selected on the basis of levels of arsenic in drinking-water, one town with a high level of arsenic (average, 410 µg/L) and the other with a low level of arsenic (average, 5 µg/L). The towns are located 37 km apart, and their populations were of comparable size (1488 and 998 inhabitants, respectively) and socio-economic and environmental conditions. A questionnaire and physical examination were administered to all family members of every third household in each community. Seventy-five per cent of the residents had lived in these communities since birth. After clinical diagnosis, prevalence of epidermoid or basal-cell carcinoma (referred to as ulcerative lesions) was 1.4% in the exposed town, whereas no case was observed in the control town. The 20 water samples tested from the exposed town between 1975 and 1978 had arsenic concentrations ranging from 160 to 590 µg/L (standard deviation [SD], 114 µg/L), indicating significant variability. The control town showed little variability (SD, 7 µg/L) in the 18 samples collected over the same period.

2.3.3 *Chile*

Ecological study based on incidence of cutaneous lesions

Zaldívar *et al.* (1981) investigated the incidence of cutaneous lesions (leukoderma, melanoderma, hyperkeratosis, and squamous-cell carcinoma) in residents of Antofagasta in arsenic-contaminated Region II from 1968 to 1971. Among 457 patients, about 70% were children aged 0–15 years. Incidence rates decreased from 1968–69 to 1971 due to a filter plant which started operation in 1970.

Ecological studies based on mortality from skin cancer

In an ecological analysis, Rivara *et al*. (1997) compared mortality rates from skin cancer in 1976–92 between Antofagasta and the unexposed control Region VIII of Chile.

The SMR for skin cancer was 3.2 (95% CI, 2.1–4.8). In a later study, Smith *et al*. (1998) compared sex- and site-specific mortality for the years 1989–93 in Region II of Chile with national mortality rates. The SMR for skin cancer was 7.7 (95% CI, 4.7–11.9) among men and 3.2 (95% CI, 1.3–6.6) among women.

2.3.4 *USA*

(*a*) *Ecological study*

Berg and Burbank (1972) showed no correlation between trace metals from 10 water basins throughout the USA and mortality rates in 1950–67, using concentrations of arsenic measured in 1962–67.

Morton *et al*. (1976) studied the incidence of histologically confirmed basal-cell and squamous-cell skin cancers (*in situ* and invasive) for the period 1958–71 in Lane County, OR, USA. They identified skin cancers by reviewing the pathology records of facilities serving residents of the county and the biopsy files of two of five dermatologists. Water samples were tested from selected points throughout the county from public water systems and from a number of single-family systems. Single-family systems were reported to have been over-sampled in regions suspected of having a problem with arsenic. Within a given region, arsenic values ranged from undetectable to 2150 µg/L. The correlation between census tract estimates of arsenic in drinking-water and incidence of squamous-cell carcinoma was 0.151 in men and –0.20 in women; for the incidence of basal-cell carcinoma, the correlation was –0.064 in men and 0.10 in women. [A major weakness of the study is the misclassification of exposure because of widely varying concentrations of arsenic within a census tract.]

Wong *et al.* (1992) studied approximately 2300 incident cases of skin cancer in four counties in Montana (two contaminated and two controls) in 1980–86. Contamination arose from copper smelters and mines. No difference in incidence rates was observed between exposed counties and the rest of the country.

(*b*) *Case–control studies*

Karagas *et al*. (1998, 2001b, 2002) designed a case–control study of basal-cell and squamous-cell skin cancers in the population of New Hampshire to evaluate the effects of low to moderate levels of exposure to arsenic. About 40% of the population relied on private, unregulated water systems; over 10% of the private supplies contained levels of arsenic above the WHO recommended level of 10 µg/L and 1% of supplies overall contained > 50 µg/L. A biomarker of internal dose was chosen to determine exposure levels. Earlier studies had indicated the reliability of concentrations of arsenic in toenails as a measure of exposure > 1 µg/L arsenic through drinking-water (Karagas *et al*., 1996, 2000) and reproducibility of concentrations over a period of 3–6 years (Garland *et al*., 1993; Karagas *et al.*, 2001a). Cases of basal-cell and squamous-cell skin cancer diagnosed from 1 July 1993 to 30 June 1995 were identified through a statewide network of dermatologists, dermatopathologists and pathologists, with participation rates of over 90% (Karagas *et al.*,

2001b). Because of the high incidence of basal-cell carcinoma, incident cases of basal-cell carcinoma were randomly selected in a 2:1 ratio to cases of squamous-cell carcinoma. To minimize detection bias, cases of squamous-cell carcinoma were restricted to invasive disease only (cases of in-situ carcinomas were excluded). A 2:1 ratio of controls to squamous-cell carcinoma cases was randomly selected from population lists (driver's licence files for cases < 65 years and Medicare enrollment lists for cases ≥ 65 years), frequency-matched to the combined distribution of the basal-cell and squamous-cell carcinoma cases. To be eligible to participate, subjects were required to speak English and have a working telephone. Of the 1143 potential case subjects, 896 took part in the study (78%) and, of the 820 potential controls, 540 (66%) enrolled. The analysis included the 587 cases of basal-cell carcinoma, 284 cases of squamous-cell carcinoma and 524 controls (97% of subjects) who contributed a toenail sample for arsenic analysis. Study participants underwent a personal interview to obtain information on confounding factors such as exposure and sensitivity to sun, history of radiation treatment and other medical and lifestyle factors. Age- and sex-adjusted odds ratios were computed using logistic regression analysis according to percentiles of arsenic concentrations in toenails based on the control distribution. In this categorical analysis, concentrations of arsenic appeared to be unrelated to risk for squamous-cell and basal-cell carcinomas except for the highest category (the top 97th percentile; concentrations above 0.344 μg/g) versus concentrations below the median.

An analysis using continuous exposure variables was presented separately (Karagas *et al*., 2002). A quadratic and two-segment linear model fitted the data for both squamous-cell carcinoma and basal-cell carcinoma. The point at which the dose–response appeared to increase was at 0.105 μg/g (95% CI, 0.093–0.219 μg/g) for squamous-cell carcinoma using a maximum likelihood estimation of the change point for the two-segment linear model. After the change point, a 1% increase in arsenic concentration in toenails was related to a 0.61% increase in risk for squamous-cell carcinoma. The quadratic model for both squamous-cell carcinoma and basal-cell carcinoma produced a consistent nadir or change point of 0.088 and 0.091, respectively. However, it was not possible to estimate a two-segment model for basal-cell carcinoma because of sparse data at the extremes. Based on a regression analysis of concentrations of arsenic in water and toenails, a change point of 0.105 μg/g in toenails translated to 1–2 μg/L in water, with the 95% confidence interval ranging from < 1 to 10–20 μg/L.

2.4 Other organ sites

Studies on cancer at other organ sites are summarized in Table 26.

Neubauer (1947) summarized cancers that had been reported in patients treated with medicinal arsenic. His report of 143 published cases included patients who developed cutaneous tumours and other malignancies such as cancers of the stomach (one case), tongue (two cases, one who also had cancer of oral mucosa), oesophagus (two cases), uterus (one case) and urethra (two cases including one papilloma of the ureter). Among patients who had not developed cutaneous tumours, other reported malignancies included

Table 26. Summary of epidemiological studies of arsenic in drinking-water and risk for other cancers

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome	Comments
Ecological studies							
Taiwan							
Chen *et al.* (1985)	84 villages on the SW coast	Mortality 1968–82, all ages	Endemic area for chronic arsenic toxicity (Blackfoot disease)		Men	Age- and sex-adjusted SMR (95% CI)	SMRs for a total of 11 sites. Prostate not investigated; mid-year population: 141 733 in 1968, 120 607 in 1982; national rate in 1968–82 used as the standard for estimation of SMR
				Colon	54	1.6 (1.2–2.0)	
				Small intestine	17	2.98 (0.6–3.4)	
				Leukaemia	45	1.4 (1.0–1.8)	
					Women		
				Colon	61	1.7 (1.3–2.1)	
				Small intestine	5	0.97 (0.1–1.8)	
				Leukaemia	22	0.9 (0.5–1.3)	
Chen *et al.* (1988a)	42 villages on the SW coast	Mortality 1973–86, all ages	Median level of arsenic in drinking-water grouped into 3 strata, 1962–64	Prostate			899 811 person–years, rate per 100 000, age-standardized to 1976 world population
			General population			Age-adjusted SMR	
			< 300 µg/L			1.5	
			300–600 µg/L			0.5	
			> 600 µg/L			5.8	
						8.4	

Table 26 (contd)

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome	Comments
Wu *et al*. (1989)	42 villages (SW)	Mortality 1973–86, age ≥ 20 years	Median level of arsenic in drinking-water grouped into 3 strata, 1962–64			SMR	Age-adjusted mortality. All results are non-significant. Men, 257 935 person–years; women, 234 519 person–years; rate per 100 000, age-standardized to 1976 world population
			< 30 µg/L	Prostate	9 M	0.95	
				Leukaemia	11 M	4.87	
					7 F	3.03	
				Nasopharynx	11 M	3.58	
					7 F	1.59	
				Oesophagus	15 M	7.62	
					4 F	1.83	
				Stomach	46 M	25.6	
					21 F	6.71	
				Colon	17 M	7.94	
					21 F	9.05	
				Uterine cervix	6 F	0.91	
			300–600 µg/L	Prostate	9 M	9.00	
				Leukaemia	11 M	6.52	
					7 F	4.55	
				Nasopharynx	11 M	8.16	
					7 F	5.81	
				Oesophagus	15 M	9.37	
					4 F	3.64	
				Stomach	46 M	17.82	
					21 F	18.72	
				Colon	17 M	8.30	
					21 F	8.16	
				Uterine cervix	6 F	5.46	

Table 26 (contd)

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome			Comments
Wu *et al.* (1989) (contd)			> 600 µg/L	Prostate	9 M	9.18			
				Leukaemia	11 M	2.69			
					7 F	0.00			
				Nasopharynx	11 M	8.58			
					7 F	4.89			
				Oesophagus	15 M	6.55			
					4 F	0.00			
				Stomach	46 M	56.42			
					21 F	5.98			
				Colon	17 M	12.5			
					21 F	17.21			
				Uterine cervix	6 F	3.92			
Chen & Wang (1990)	42 villages on the SW coast	Mortality 1972–83, all ages	National survey of 83 656 wells (1974–76); average arsenic content for each of 314 precincts or townships			Percentiles of age-adjusted mortality rate/100 000 person–years			
					Men	*25th*	*50th*	*75th*	
				Oesophagus		3.6	6.0	9.2	
				Stomach		14.8	10.2	28.8	
				Small intestine		0.6	1.1	1.9	
				Colon		4.2	5.6	7.2	
				Rectum		1.9	2.7	3.9	
				Pancreas		1.3	2.1	3.0	
				Nasal cavity		1.1	1.3	2.6	
				Larynx		1.1	1.7	2.8	
				Bone/cartilage		1.1	1.8	2.9	
				Prostate		0.9	1.4	2.3	
				Brain		0.7	1.1	1.8	
				Leukaemia		1.3	2.1	2.7	
					Women	*25th*	*50th*	*75th*	
				Oesophagus		1.1	1.8	2.8	
				Stomach		7.2	10.0	13.7	
				Small intestine		0.6	0.9	1.6	
				Colon		3.6	5.5	6.9	

Table 26 (contd)

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome			Comments
Chen & Wang (1990) (contd)				Rectum		1.5	2.3	3.3	
				Pancreas		1.4	2.1	2.7	
				Nasal cavity		0.6	1.0	1.6	
				Larynx		0.5	0.9	1.5	
				Bone/cartilage		1.0	1.7	2.5	
				Breast		2.7	4.4	6.2	
				Cervix uteri		3.8	6.2	8.3	
				Ovary		0.8	1.4	2.0	
				Brain		1.0	1.5	2.2	
				Leukaemia		1.1	1.7	2.4	
Tsai *et al*. (1999)	Four townships (SW)	Mortality 1971–94, all ages	Endemic area for chronic arsenic toxicity		*Men*	SMR (95% CI)			SMRs with national reference, unless otherwise stated; *SMRs with local reference Men, 1 508 623 person–years; women, 1 404 759 person–years; national rates in 1971–94 used as the standard for SMR estimation
				Pharynx	24	1.1 (0.7–1.7)			
				Oesophagus	69	1.7* (1.3–2.1)			
				Stomach	195	1.4* (1.2–1.5)			
				Intestine	15	2.1 (1.2–3.5)			
				Colon	91	1.4 (1.1–1.7)			
				Rectum	46	1.2 (0.9–1.7)			
				Nasal cavity	40	3.7 (2.6–5.0)			
				Larynx	30	1.8 (1.2–2.5)			
				Bone	41	2.3 (1.7–3.2)			
				Prostate	48	1.96 (1.4–2.6)			
				Brain	19	1.1 (90.7–1.8)			
				Lymphoma	56	1.4 (1.1–1.8)			
				Leukaemia	67	1.3 (1.04–1.7)			
					Women				
				Pharynx	10	2.2 (1.1–4.1)			
				Oesophagus	12	0.8 (0.4–1.4)			
				Stomach	111	1.4* (1.2–1.7)			
				Intestine	8	1.3 (0.5–2.5)			
				Colon	83	1.4* (1.1–1.8)			
				Rectum	33	1.5* (1.03–2.11)			
				Nasal cavity	29	5.1 (3.4–7.3)			
				Larynx	13	3.8 (2.0–6.4)			
				Bone	34	2.2 (1.5–3.1)			
				Brain	21	1.8* (1.1–2.7)			
				Lymphoma	35	1.4 (1.0–2.0)			
				Leukaemia	40	1.1 (0.8–1.4)			

Table 26 (contd)

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome	Comments
Chile							
Rivara *et al.* (1997)	Regions II and VIII, northern Chile	Mortality 1950–92	Arsenic-contaminated Region II	Larynx		Relative risk (Region II versus Region VIII), 3.4 (95% CI, 1.3–8.6)	Population: 411 000 in Region II, 1 700 000 in Region VIII. Antofagasta (Region II) versus Region VIII.
USA							
Berg & Burbank (1972)	10 water basins	Mortality 1950–67	Trace metals in water supplies (As, Be, Cd, Cr, Co, Fe, Pb, Ni)			Probability of a positive association	
				Larynx		0.024	
				Eye		0.009	
				Myeloid leukaemia		0.042	
Australia							
Hinwood *et al.* (1999)	Victoria	Incidence 1982–91	Median arsenic concentration in drinking-water ranging 1–1077 µg/L		*Obs. no.*	SIR (95% CI)	State rates in 1982–91 used as the standard for estimation of SIR
				Prostate	619	1.1 (1.05–1.2)	
				Melanoma	477	1.4 (1.2–1.5)	
				Breast	762	1.1 (1.03–1.2)	
				Chronic myeloid	40	1.5 (1.1–2.1)	

Table 26 (contd)

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome		Comments
Case–control study								
Canada								
Infante-Rivard *et al.* (2001)	Québec province	Incidence 1980–93	Trihalomethanes, metals (As, Cd, Cr, Pb, Zn) and nitrates in drinking-water during prenatal and postnatal periods	Childhood acute lymphocytic leukaemia				Adjusted for maternal age and level of education
						OR (95% CI)		
			Arsenic exposure index		*Exposed cases*	*Prenatal period*	*Postnatal period*	
			Average level (> 95th versus ≤ 95th percentile [5 µg/L])		Prenatal, 18 Postnatal, 20	0.9 (0.5–1.8)	1.4 (0.7–2.8)	
			Cumulative exposure (> 95th versus ≤ 95th percentile		Prenatal, 20 Postnatal, 19	0.7 (0.4–1.3)	1.1 (0.6–2.2)	
Cohort studies								
Japan								
Tsuda *et al.* (1989)	Nakajo-machi town, Niigata Prefecture, 281 residents	Mortality 1959–87	High dose of arsenic contamination (through a factory) of well-water used for drinking (1955–59)	Uterus	17 deaths from all cancers in the entire cohort	Among the residents in high-exposure areas (low, < 0.05 ppm; medium, 0.05–0.5 ppm; high, ≥ 0.5 ppm), significant excess mortality from cancer of the uterus over expected value based on mortality for Niigata Prefecture and for all Japan		

Table 26 (contd)

Reference	Location	End-point	Exposure	Site	No. of cases	Study outcome			Comments
Tsuda *et al.* (1995)	454 residents living in Namikicho and Nakajo-machi in Niigata Prefecture	Mortality 1959–92	High-dose arsenic contamination (through a factory) of well-water used for drinking (1955–59)	Uterus	0 0 2 2	Arsenic concentration (ppm) < 0.05 0.05–0.99 ≥ 1 Total		SMR (95% CI) 0.0 (0–8.0) 0.0 (0–37.6) 13.5 (2.4–48.6) 3.0 (0.5–11.1)	113 persons who drank from industrially contaminated wells in 1955–59, then followed for 33 years; rates in Niigata Prefecture in 1960–89 used as the standard for estimation of SMR
USA									
Garland *et al.* (1996)	Nested case–control in the Nurses' Health Study Cohort, USA	Incidence 1984–86	Exposure to 5 metals (As, Cu, Cr, Fe, Zn) through any route measured	Breast, diagnosed from 1984 to 1986, 459 matched controls	Total, 433 54 67 56 62 69	Quin-tile 1 2 3 4 5	Cut-point (μg/g) < 0.059 0.059–0.078 0.079–0.103 0.104–0.138 > 0.138	Odds ratio 1.0 1.2 1.01 1.1 1.1	95% CI 0.7–1.98 0.6–1.7 0.7–1.9 0.7–1.9	Multivariate logistic regression models controlled for age, date of nail return, smoking, age at first birth, parity, history of benign breast disease, history of breast cancer in mother or sister, age at menarche, menopausal status, body mass index and alcohol consumption
Lewis *et al.* (1999)	Millard County, UT	Mortality	Exposure to arsenic in drinking-water	Prostate	50	All men (μg/L–years) Low (< 1000) Medium (1000–4999) High (≥ 5000)		SMR 1.5 (95% CI, 1.07–1.9) 1.07 1.70 ($p < 0.05$) 1.65	Exposure index relies on ecological measures of arsenic concentration, median value for the community. State rates in 1950–92 used as the standard for estimation of SMR

SMR, standardized mortality ratio; CI, confidence interval; M, male; F, female; SIR, standardized incidence ratio; OR, odds ratio

cancers of the breast (two cases, one case who had keratoses present), pancreas (one case who had keratoses present) and mouth (one patient who had treatment to the mouth for syphilis). A report of seven cases of cancer following treatment with Fowler's solution (Jackson & Grainge, 1975) included one case of bilateral breast cancer and one case of colon cancer. Multiple skin cancers were present in both cases. The literature also includes cases of meningioma and intestinal malignancies associated with ingestion of arsenic (IARC, 1987). [Case reports have helped to identify the role of ingestion of arsenic in the occurrence of skin cancer and could provide leads to occurrences of other malignancies. However, without an appropriate comparison group, it is unclear whether any of the cases represent an excess over the norm.]

2.4.1 *Taiwan, China*

Ecological studies

Chen *et al*. (1985) (described in Section 2.1.1) reported SMRs in the four-county region in South-West Taiwan that is endemic for Blackfoot disease using mortality rates for the whole country as the standard. The analyses were based on mortality data obtained from the Department of Health for the period 1968–82. To estimate dose, SMRs were computed for regions that were hyperendemic (21 villages), endemic (25 villages) and not endemic (38 villages) for Blackfoot disease. Age-standardized mortality rates for the four counties were elevated compared with national rates for cancer of the colon in both men and women. Age- and sex-standardized mortality rates for colon cancer were higher in endemic areas than in non-endemic areas, but were lower in the hyperendemic areas compared with other areas. SMRs for leukaemia were of borderline statistical significance in men and close to unity in women. The SMR for cancer of the small intestine also was increased in men but not in women, and was not statistically significant in either sex [Prostate cancer was not investigated in this report.] (Chen *et al*., 1985). A subsequent report used the median concentrations of arsenic in well-water measured in 1962–64 grouped into levels of < 300, 300–600 and ≥ 600 μg/L. A dose-related gradient in age-adjusted mortality was noted for prostate cancer. In the ≥ 600-μg/L group, the age-standardized mortality rate for prostate cancer was 5.6 times higher than that of the general population of Taiwan (Chen *et al*., 1988a) (study described in Section 2.1.1).

Wu *et al*. (1989) (study described in Section 2.1.1) published age-adjusted mortality rates for the years 1973–86 in 27 townships in the four counties endemic for Blackfoot disease together with 15 additional villages in the townships of Yensui and Hsiaying. Using the same data on well-water content and the classification scheme described above (Chen *et al*., 1988a), a dose-related trend in mortality from prostate cancer was again observed (Mantel-Haenszel test for trend, $p < 0.05$). The authors also noted dose-related increases in mortality rates from nasopharyngeal and colon cancer in men, which, however, were not statistically significant. In this analysis, leukaemia and oesophageal, stomach or uterine cancers did not appear to be related to levels of arsenic (Wu *et al*., 1989).

In a later study, Chen and Wang (1990) (described in Section 2.1.1) used data on arsenic in well-water measured from 1974 to 1976 in 314 precincts and townships throughout Taiwan. Based on a multiple regression analysis (adjusted for urbanization and age), mortality rates from prostate cancer significantly increased with higher average level of arsenic. Age-adjusted mortality rates for cancers of the nasal cavity also correlated with average arsenic concentration in men and women for all precincts and townships and for the south-western townships. Cancers of the oesophagus, stomach, small intestine, colon, rectum, pancreas, larynx, bone and cartilage, breast, cervix, ovary and brain and leukaemia were not significantly correlated. [The regression estimates were only presented for statistically significant results.]

Based on data from death certificates for the period 1971–94, Tsai *et al*. (1999) (described in Section 2.1.1) computed SMRs for the four townships that are endemic for Blackfoot disease using local (Chiayi County) and national (Taiwan) rates as the standard. Applying national rates, SMRs were elevated for cancers of the intestine, colon and prostate in men, cancers of the nasal cavity and larynx in both men and women, lymphoma in men and women, leukaemia in men and cancers of the pharynx and bone in women. These SMRs were similarly elevated using the local mortality rates as the standard. In addition, using the local rates as the standard, higher SMRs were found for cancers of the oesophagus in men, cancers of the stomach in men and women and cancers of the colon, rectum and brain in women for the four townships.

[Data from South-West Taiwan indicate a consistent pattern of increases in mortality from prostate cancer in areas with high contamination by arsenic, and there is evidence of a dose-related effect. These studies do not specifically address the issue of dose of exposure, nor do they raise issues of latency and duration. These issues cannot be addressed using mortality as an end-point for prostate cancer since the disease has a low case-fatality rate. One possible source of bias is that prostate cancer often goes undetected, and a higher mortality rate in regions with known exposure to arsenic could occur if screening for cancer deaths is enhanced in the region.]

2.4.2 *Chile*

Ecological study

Rivara *et al*. (1997) compared the mortality rates for various cancers in 1976 between Antofagasta in Region II and the non-contaminated Region VIII. An elevated risk of cancer of the larynx was observed in the arsenic-exposed Region II. Among the various other cancers (17 sites) investigated, no other elevated SMRs were reported.

2.4.3 *Japan*

Cohort studies

From about 1945 to 1959, wells in the small town of Nakajo-machi in Niigata Prefecture became contaminated with arsenic (up to 3000 µg/L) from a factory producing

King's yellow (As_2O_3). Two uterine cancer deaths occurred from 1959 to 1992 in the highest exposure category (≥ 500 µg/L), with an SMR for uterine cancer of around 3.0 using the age- and cause-specific mortality rates from Niigata Prefecture (Tsuda *et al*., 1989, 1995). [No other cancer sites were discussed.]

2.4.4 *North America*

(*a*) *Cohort studies*

Garland *et al*. (1996) conducted a nested case–control study in the USA to investigate the relationship between concentrations of arsenic and other trace elements in toenails and the incidence of breast cancer. Cases and controls were selected from the Nurses' Health Study cohort comprising 121 700 nurses aged 30–55 years living in 11 states of the USA. Toenail samples were obtained from 72% of 94 115 cohort members in 1982. A total of 62 641 women provided toenail clippings and did not have a diagnosis of breast cancer as of 1982. The nested study was based on the 433 women who had a diagnosis of breast cancer reported in mailed questionnaires in 1984 and 1986 and an age-matched control group. Compared with the quintile, the adjusted odds ratio for breast cancer was 1.1 (95% CI, 0.7–1.9) for the highest quintile of arsenic.

Lewis *et al*. (1999) reported SMRs for various cancers using a retrospective cohort of residents from Millard County, UT, USA. Of the multiple types of cancers examined, mortality was only elevated for prostate cancer [The limitations of this study have been cited in Section 2.1.4.].

(*b*) *Case–control studies*

Infante-Rivard *et al*. (2001) conducted a population-based case–control study of childhood leukaemia (occurring before the age of 9 years) in Québec Province, Canada. Cases included individuals who were newly diagnosed with childhood leukaemia from 1980 to 1993. Of 510 eligible cases, 491 participated (96.3%) and, of 588 controls, 493 took part (83.8%). The investigators sought drinking-water test data from 1970 onward through a postal questionnaire that yielded usable data from 112 of 202 municipalities (55%). Additional data was provided from the Ministry of Municipal Distribution Systems in 1986. Further analysis of tap-water was performed by the study investigators and covered 103 of the municipalities. These data were linked to information on residential history derived from interviews with the subjects' parents, and the value used for exposure to arsenic was that of the subjects' municipalities of residence for the closest year when data were available. Values from multiple test results were averaged over a given year, and individuals with private water systems were assigned the arsenic value of their municipality of residence. Separate exposure variables were computed for subjects' pre- and postnatal periods and included average arsenic levels and cumulative exposure. A logistic regression analysis included maternal age and level of schooling. The odds ratio for childhood leukaemia above versus less than or equal to the 95th percentile (5 µg/L arsenic) was 0.9 (95% CI, 0.5–1.8) for prenatal exposure and 1.4 (95% CI, 0.7–2.7) for postnatal exposure.

For cumulative exposure (above versus less than or equal to the 95th percentile), the odds ratios were 0.7 (95% CI, 0.4–1.3) for prenatal exposure and 1.1 (95% CI, 0.6–2.2) for postnatal exposure. [The study does not provide evidence of a link between childhood leukaemia and exposure to arsenic through drinking-water either prenatally or postnatally. However, the drinking-water concentrations were relatively low (95% were below 5 μg/L) and the estimates were imprecise. Furthermore, the exposure estimates are subject to misclassification, but there is no discussion on the variability of arsenic concentrations within municipalities and the fraction of residents that used private systems that were assumed to contain the same concentrations of arsenic as the public systems.]

2.4.5 *Australia*

Ecological study

In an ecological study using incidence data from the Victoria Cancer Registry for the years 1982–91, Hinwood *et al.* (1999) calculated SMRs for 14 cancer sites (in addition to liver, lung, bladder and kidney) in 22 postcode areas characterized by a level of arsenic in water > 0.01 mg/L in most areas. Incidence rates for all of Victoria were used as the standard. Cancer sites that showed elevated rates with 95% CIs that excluded 1.0 were prostate, melanoma, breast and chronic myeloid leukaemia. [The Working Group noted that no information was presented on the actual use of water contaminated with arsenic for drinking by the population.]

3. Studies of Cancer in Experimental Animals

Previous evaluation

Various inorganic arsenic compounds were tested for carcinogenicity by oral administration, skin application, inhalation and/or intratracheal administration, subcutaneous and/or intramuscular administration, intravenous administration and other experimental systems in mice, rats, hamsters, dogs or rabbits. Arsenic trioxide produced lung adenomas in mice after perinatal treatment (Rudnay & Börzsönyi, 1981) and in hamsters after its intratracheal instillation (Ishinishi *et al.*, 1983; Pershagen *et al.*, 1984). It induced a low incidence of adenocarcinomas at the site of its implantation into the stomach of rats (Katsnelson *et al.*, 1986). A higher incidence of lung carcinomas was induced in rats following a single intratracheal instillation of a Bordeaux pesticide mixture (copper sulfate and calcium oxide in a concentration of 1–2%) containing calcium arsenate (IARC, 1987). Intratracheal instillations of calcium arsenate into hamsters resulted in a borderline increase in the incidence of lung adenomas, while no such effect was observed with arsenic trisulfide (Pershagen & Björklund, 1985). These studies provide *limited evidence* for carcinogenicity of inorganic arsenics (IARC, 1980, 1987).

No adequate data on the carcinogenicity of organic arsenic compounds were available to the previous working group (IARC, 1980, 1987).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 24 male A/J mice, 6 weeks of age, were given tap-water (control) or a solution of 50, 200 or 400 ppm [μg/mL] dimethylarsinic acid (DMA^V) as drinking-water for 25 (10 mice per group) or 50 weeks (14 mice per group). The incidences of lung tumours were 2/10 (20%), 3/10 (30%), 4/10 (40%) and 3/10 (30%) in control, 50-, 200- and 400-ppm groups, after 25 weeks, with average numbers of tumours/mouse of 0.2 ± 0.42, 0.3 ± 0.48, 0.5 ± 0.71 and 0.4 ± 0.70, respectively; no significant differences were apparent, nor did average tumour size vary significantly (0.9, 0.5, 1.4 and 1.1 mm, respectively). After 50 weeks, a non-significant increase in the incidence of lung tumours (50, 71.4, 64.3 and 78.6% in 14 animals per group, respectively), a significant increase in multiplicity (0.5 ± 0.52, 1.07 ± 1.0, 1.07 ± 1.07 and 1.36 ± 1.01, respectively; $p < 0.05$ for the 400-ppm group) and an increase in average diameter (1.0, 1.2, 1.4 and 1.5 mm, respectively) were observed. The numbers of mice with papillary lung adenoma and/or adenocarcinoma at 50 weeks were two, five, seven and 10 ($p = 0.002$ for the 200- and 400-ppm groups) and increased with increasing dose of DMA^V. In animals that received 0, 50, 200 or 400 ppm DMA^V, the number of alveolar adenomas ranged from 3/14 to 5/14 per treatment group (Hayashi *et al*., 1998).

Groups of 90 female C57BL/6J mice and 140 female metallothionein heterozygous mice ($MT^{-/-}$), aged 4–5 weeks, were given drinking-water containing sodium arsenate (500 μg/L arsenic) *ad libitum* for up to 26 months. Groups of 60 control mice were given tap-water. Preliminary findings indicate that tumours were observed in the lung (C57BL/6J, 17.8%; $MT^{-/-}$, 7.1%), gastrointestinal tract (14.4%; 12.9%), liver (7.8%; 5.0%), spleen (3.3%; 0.7%), reproductive organs (3.3%; 5.0%), skin (3.3%; 1.4%), bone (2.2%; 0%) and eye (1.1%; 0%) of treated animals. No tumours were observed in the control groups (Ng *et al.*, 1999). [The Working Group decided that this study was preliminary because no histopathological findings were reported.]

Groups of 20–30 K6/ODC transgenic mice, 7 weeks of age, were administered DMA^V at either 10 or 100 ppm [μg/mL] in their drinking water or sodium arsenite at 10 ppm for 5 months. The incidence of squamous skin tumours was 0% in the controls, 8 and 22% in the 10- and 100-ppm DMA groups, respectively, and 15% in the arsenite groups (Chen *et al.*, 2000).

Groups of 29 or 30 male $p53^{+/-}$ heterozygous or $p53^{+/+}$ mice (C57BL/6J background) were exposed to 0, 50 or 200 ppm [μg/mL] DMA^V in the drinking-water for 80 weeks. In $p53^{+/+}$ mice, a significant increase in the incidence (control, 10%; 50-ppm, 30% [$p < 0.05$]; and 200-ppm, 30% [$p < 0.05$] in 30 animals per group) and multiplicity (0.2, 0.6 [$p < 0.02$] and 0.6 [$p < 0.02$] tumours per mouse, respectively) of total tumours was

observed at the terminal killing, but with no dose dependence. In the heterozygotes, a non-significant increase in incidence was observed (control, 14/29 [48.3%]; 50-ppm, 18/29 [62.1%]; and 200-ppm, 19/30 [63.3%]), but the number of tumours per mouse was significantly increased at 200 ppm ($p < 0.05$) (control, 0.8; 50-ppm, 1.1; 200-ppm, 1.2). No effects were observed in either heterozygous or $p53^{+/+}$ mice regarding the number of tumours per tumour-bearing animal (control, 1.6; 50-ppm, 1.8; 200-ppm, 1.9 in heterozygous mice; control, 2; 50 ppm, 1.9; 200 ppm, 2 in $p53^{+/+}$ mice). No significant influence on tumour development was noted in any particular organ or tissue site. The tumours induced in the $p53^{+/-}$ heterozygous mice were mainly malignant lymphomas or leukaemia (control, 8/29 [28%]; 50-ppm, 13/29 [45%]; 200-ppm, 10/30 [33%]), fibrosarcomas (5/29 [17%], 8/29 [28%], 10/30 [33%]) and osteosarcomas (3/29 [10%], 2/29 [8%], 4/30 [13%]), with lower incidences of other types of tumours such as hepatocellular carcinomas, thyroid follicular carcinomas, squamous-cell carcinomas of the skin and lung adenomas. In $p53^{+/+}$ mice, tumours were generally malignant lymphomas or leukaemia (2/30 [7%], 9/30 [30%], 9/30 [30%]) with very low incidences of the other types of tumour. No fibrosarcomas or osteosarcomas were detected in $p53^{+/+}$ mice. Tumour latency curves in DMA^V-treated $p53^{+/-}$ heterozygous and $p53^{+/+}$ mice showed a dose-dependently significant shift towards early induction ($p < 0.03$) in comparison with untreated controls (Salim *et al*., 2003).

3.1.2 *Rat*

Groups of 36 male Fischer 344/DuCrj rats, 10 weeks of age, received 0, 12.5, 50 and 200 ppm DMA^V [μg/mL] (100% pure) in the drinking-water for 104 weeks. There was no significant difference in body weight or survival (25, 28, 28 and 24 animals) among the groups at week 104. At week 97, the first tumour in the urinary bladder was observed in one animal of the 200-ppm DMA^V group. Effective numbers were considered to be the numbers of animals alive at week 97. Incidences of urinary bladder tumours were 0/28, 0/33, 8/31 (26%; two papillomas and six carcinomas; $p < 0.01$, Fisher's exact probability test) and 12/31 (39%; two papillomas and 12 carcinomas; $p < 0.001$, Fisher's exact probability test), respectively, and were multiple in two animals given the highest dose. Histopathologically, the carcinomas were transitional-cell carcinomas. Urinary pH did not differ significantly between groups during the experiment. Bladder calculi were not observed in any of the rats (Wei *et al*., 1999, 2002). In a more exhaustive examination of the urinary bladder in the same animals, preneoplastic lesions (papillary or nodular hyperplasia) were observed in 0/28, 0/33, 12/31 (39%; $p < 0.01$) and 14/31 (45%, $p < 0.01$) animals in the 0-, 12.5-, 50- and 200-ppm groups, respectively. The incidences of tumours, other than those of the urinary bladder, in all DMA^V-treated groups were not different from those of controls (Wei *et al.*, 2002).

3.2 Transplacental exposure

Mouse: Groups of 10 pregnant C3H mice were given drinking-water containing 0, 42.5 and 85 ppm [μg/mL] *ad libitum* from day 8 to 18 of gestation. Offspring were weaned at 4 weeks and then divided into separate groups of 25 males and 25 females. The offspring received no additional treatment with arsenic for the next 74 (males) or 90 (females) weeks. Transplacental exposure to arsenic did not reduce body weight in any group of offspring over the course of the experiment. In male offspring, there was a marked increase in the incidence of hepatocellular carcinomas (control, 3/24 [12%]; 42.5-ppm, 8/21 [38%]; 85-ppm, 14/23 [61%]; p for trend = 0.00006, two-sided chi-square test) and multiplicity per mouse (control, 0.13 ± 0.07; 42.5-ppm, 0.42 ± 0.13; 85-ppm, 1.30 ± 0.28; p for trend = 0.003) in a dose-related fashion. There was also a dose-related increase in the incidence of adrenal cortical adenomas (control, 9/24 [37.5%]; 42.5-ppm, 14/21 [66.7%]; 85-ppm, 21/23 [91.3%]; p for trend = 0.001) and multiplicity (control, 0.71 ± 0.20; 42.5-ppm, 1.10 ± 0.22; 85-ppm, 1.57 ± 0.32; p for trend = 0.016). In female offspring, there was a strong, dose-related increase in the incidence of ovarian tumours. Total tumour (benign and malignant) incidence was control, 2/25 (8%); 42.5-ppm, 6/23 (26%); and 85-ppm, 9/24 (38%) (p for trend = 0.015). Controls had one adenoma and one benign granulosa-cell tumour. The 42.5-ppm treatment group had three adenomas, one adenocarcinoma, one benign granulosa-cell tumour and one malignant granulosa-cell tumour. The 85-ppm treatment group developed seven adenomas, one luteoma and one haemangiosarcoma. Lung carcinomas developed (control, 0/25 [0%]; 42.5-ppm, 1/23 [4%]; 85-ppm, 5/24 [21%]; p for trend = 0.0086) in a dose-dependent manner. There were significant increases in the number of mice bearing at least one tumour (control, 11/24; 42.5-ppm, 17/21; 85-ppm, 22/23; p for trend = 0.0006 in males; control, 12/25; 42.5-ppm, 17/23; 85-ppm, 16/24; $p < 0.172$ in females) and in mice bearing at least one malignant tumour (control, 3/24; 42.5-ppm, 9/21; 85-ppm, 14/23; $p < 0.0001$ in males; control, 2/25; 42.5-ppm, 9/23; 85-ppm, 8/24; $p < 0.042$ in females) with both doses of arsenic. Exposure to arsenic also increased the incidence of hyperplasia of the uterus and oviduct. In this experiment, four of the organs that developed tumours or hyperplasia were endocrine-responsive organs: adrenal gland, liver, ovary and uterus (Waalkes *et al*., 2003).

3.3 Intratracheal administration

Hamster: Groups of 30 (20 for a control) male Syrian golden hamsters, 8 weeks of age, were given arsenic trioxide, calcium arsenate or arsenic trisulfide by intratracheal instillation once a week for 15 weeks. Each compound contained 0.25 mg arsenic suspended in 0.1 mL phosphate buffer solution. The control group received buffer solution alone. All hamsters were kept during their entire lifespan. Numbers of survivors after 15 instillations were 18/30 (60%) in the arsenic trioxide-treated group, 27/30 (90%) in the calcium arsenate-treated group, 23/30 (77%) in the arsenic trisulfide-treated group and 22/22 (100%) in the control group, showing a similar tendency in survival rates of all

groups. All hamsters had died by day 794 (arsenic trioxide group), day 806 (calcium arsenate group), day 821 (arsenic trisulfide group) and day 847 (control group) after the initial instillation. Incidences of lung tumours were 1/17 (5.8%; adenocarcinoma) arsenic trioxide-treated, 7/25 (28.0%; one adenocarcinoma, six adenomas; *p* value, significant versus controls) calcium arsenate-treated, 1/22 (4.5%; adenoma) arsenic trisulfide-treated and 1/21 (4.8%; adenosquamous carcinoma) control animals. No tumours of the upper respiratory tract including the trachea were observed in any group. Besides lung tumours, one adrenal adenoma and one liver haemangiosarcoma in the arsenic trioxide-treated group, two adrenal adenocarcinomas and one leukaemia in the calcium arsenate-treated group, one nephroblastoma and one adrenal adenoma in the arsenic trisulfide-treated group and one adrenal adenocarcinoma and one adrenal adenoma in the control group were found (Yamamoto *et al.*, 1987).

3.4 Administration with known carcinogens

3.4.1 *Mouse*

Groups of 30–32 female Swiss mice, 21–24 days of age, received concentrations of 0, 10, 50 or 100 µg/L sodium arsenate or sodium arsenite in the drinking-water for 15 weeks. At week 3, the animals were administered a single intraperitoneal injection of 1.5 mg/kg bw urethane in saline. When killed at week 15, the numbers of lung adenoma per mouse were 29.0 ± 5.4, 21.4 ± 2.6, 15.7 ± 1.8 and 16.0 ± 2.1 (p for trend = 0.0185) in the 0-, 10-, 50- and 100-µg/mL arsenate-treated groups, respectively, and 20.1 ± 1.8, 25.7 ± 4.0, 19.5 ± 2.1 and 10.8 ± 1.6 (p for trend = 0.00082) in 0-, 10-, 50- and 100-µg/mL arsenite-treated groups, respectively [suggesting that both forms of arsenic exerted an inhibitory effect]. Both arsenate and arsenite at 100 µg/mL caused a significant reduction in tumour size (0.64 ± 0.01, 0.65 ± 0.02; $p < 0.05$) compared with control animals (0.74 ± 0.01, 0.71 ± 0.02) (Blakley, 1987).

In a two-stage protocol, groups of 9–13 male ddY mice, 6 weeks of age, were given a single subcutaneous injection of 10 mg/kg bw 4-nitroquinoline 1-oxide (4NQO) and then received tap-water, a 5% glycerol solution or a 200- or 400-ppm [µg/mL] DMA^V solution in drinking-water for 25 weeks. The incidences of lung tumour-bearing mice were 2/9 (22%), 5/10 (50%), 8/13 (62%) and 10/13 (77%), respectively, while the numbers of tumours per mouse were 0.22 ± 0.15, 1.40 ± 0.62, 3.92 ± 1.79 and 4.38 ± 1.07 ($p < 0.05$ Cochran-Cox t-test). Thus, DMA^V promoted lung tumorigenesis initiated by 4NQO (Yamanaka *et al.*, 1996). [The authors described a shift from papillary-type adenomas to adenosquamous carcinomas but no quantitative data were provided.]

Groups of 60 male and female C57BL6J outbred mice [sex distribution unspecified], 2 months of age, were fed a diet containing 10% lipids and were given either 0.01% arsenic trioxide in the drinking-water for 28 weeks or 3 mg per mouse benzo[*a*]pyrene in 0.2 mL corn oil by gavage once a week for 3 weeks or both arsenic trioxide and benzo[*a*]pyrene. No significant differences between groups given benzo[*a*]pyrene with or

without arsenic trioxide were observed at the end of the 28-week experimental period with regard to focal localized hyperplasia, ulcers, focal multiple hyperplasia, papillomatosis or papillomas of the forestomach (total number per mouse, 4.20 ± 0.39 in the benzo[*a*]pyrene-treated group and 5.40 ± 0.89 in the benzo[*a*]pyrene–arsenic-treated group) (Silva *et al*., 2000).

Groups of 10–11 female Hos:HR-1 hairless mice, 6 weeks of age, were administered 0, 400 or 1000 ppm [µg/mL] DMA^V in the drinking-water and irradiated twice weekly with 2 kJ/m^2 ultraviolet B (UVB) rays for 25 weeks. DMA^V had no effect on body weight gain. The number of skin tumours per mouse was significantly increased by 1000 ppm DMA^V compared with the 0-ppm value from weeks 13 to 19, and incidence of tumour-bearing mice was significantly increased at weeks 12 and 13 [exact data not clear as there were no tabulations]. No differences were noted at later time points up to week 25 (100% incidence in all groups by week 16), showing a shift towards early tumour induction following treatment with DMA^V. Malignant tumours were observed in only two animals in the 1000-ppm group (Yamanaka *et al*., 2000).

Groups of 15 female Crl: SK1-*hr*BR hairless mice, 21 days of age, received 0 or 10 mg/L sodium arsenite in the drinking-water and were irradiated with solar lamps at a dose of 1.7 J/m^2 (lamp output: 85% in the UVB range, < 1% UVC and 4% UVA, and the remainder visible) three times weekly. The UVR dose was chosen to be approximately half of the minimal erythemic dose. Two control groups of five mice received sodium arsenite only or no treatment. Sodium arsenite did not influence body weight gain. No skin tumours were observed with arsenite alone or in the untreated controls. The first tumours were noted after 8 weeks with arsenite and UVR but after 12 weeks with UVR alone (significantly earlier appearance). All UVR-treated animals had at least one tumour at 26 weeks; however, after 19 weeks of exposure to UVR, incidences were 100% for UVR and arsenite and 33% for UVR alone. The total number of tumours in the group treated with UVR alone (15 animals) was 53 and that in the group treated with UVR and arsenite (15 animals) was 127. In UVR and arsenite-treated animals, 64/127 (50.4%) tumours were highly invasive squamous-cell carcinomas, whereas in UVR alone-treated animals, 14/53 (26.4%) were highly invasive squamous-cell carcinoma (p = 0.003) (Rossman *et al*., 2001)

Groups of 10 female Hos:HR-1 hairless mice, 6 weeks of age, were treated with a single topical application of 200 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA) dissolved in acetone and were then administered 0, 400 or 1000 ppm [µg/mL] DMA^V in the drinking-water and/or irradiated twice weekly with 0.3 kJ/m^2 UVB. All mice were killed after 50 weeks. Skin tumours occurred faster in the DMA^V-treated group. DMA^V without UVB increased the incidence of skin tumours ($p < 0.05$ at 20–22 weeks) but not dose-dependently. Greater effects were seen in combination with UVB, particularly at 1000 ppm [exact data not clear as there was no tabulation]. Incidences of papillomas in animals treated with DMA^V without UVB were 1/10, 9/10 and 7/10 in the 0-, 400- and 1000-ppm groups, respectively, and those of squamous-cell carcinomas were 2/10, 0/10 and 0/10, respectively. Incidences of papillomas in animals treated with DMA^V and UVB

were 0/10, 7/10 and 7/10, and those of squamous-cell carcinomas were 0/10, 3/10 and 1/10 in the 0-, 400- and 1000-ppm groups, respectively (Yamanaka *et al.*, 2001).

3.4.2 *Transgenic mouse*

Groups of 7–8 female *keratin (K6)/ODC* transgenic mice, 10–14 weeks of age, received two weekly applications of 3.6 mg DMA^V in neutral cream or 5 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 200 μL acetone 1 week after initiation with 50 μg DMBA in 200 μL acetone. A significantly accelerated development of skin tumours (first tumour after 8 weeks in DMA^V-treated animals and after 11 weeks in controls) was observed following treatment with DMA^V; 20 weeks after initiation, the numbers of tumours (average, 19.4 ± 10.2 per mouse compared with 9.7 ± 3.5 in controls) were increased. Promoting activity was similar to that achieved with application of 5 μg TPA (20.7 ± 8.4) twice weekly. Microscopically, most of the tumours were squamous papillomas, although squamous carcinomas occurred in some DMA^V- and some TPA-treated animals (Morikawa *et al*., 2000).

3.4.3 *Rat*

Sodium arsenite has been reported to enhance the incidence of renal tumours induced in rats by intraperitoneal injection of *N*-nitrosodiethylamine (NDEA) (Shirachi *et al*., 1983). A subsequent re-evaluation of the study indicated that not only sodium arsenite but also sodium arsenate enhanced NDEA-induced kidney tumours (Smith *et al*., 1992).

Groups of 20 male Fischer 344/DuCrj rats, 6 weeks of age, received a single intraperitoneal injection of 100 mg/kg bw NDEA, followed by intraperitoneal injections of 20 mg/kg bw *N*-methyl-*N*-nitrosourea on days 5, 8, 11 and 14 and subcutaneous injections of 50 mg/kg bw 1,2-dimethylhydrazine chloride on days 18, 22, 26 and 30. At the same time, animals received 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) in the drinking-water for the first 2 weeks then 0.1% *N*-bis(2-hydroxypropyl)nitrosamine for the next 2 weeks (so-called DMBDD model treatment). After a 2-week interval, the animals received 0, 50, 100, 200 or 400 ppm [μg/mL] DMA^V in the drinking-water from weeks 6 to 30, at which time they were killed. DMA^V significantly enhanced tumour induction in the urinary bladder (both papillomas and transitional-cell carcinomas), kidney (both adenomas and adenocarcinomas), liver (hepatocellular carcinomas) and thyroid (adenomas) (see Table 27). Values for preneoplastic lesions such as papillary or nodular hyperplasia in the urinary bladder, atypical tubules in the kidney and altered hepatocyte foci in the liver were also significantly increased. No promoting effects were noted in the lungs or the nasal cavity (Yamamoto *et al*., 1995).

To confirm the above-mentioned results and to evaluate low-dose effects of DMA in urinary bladder and liver carcinogenesis, the following two studies were conducted. Groups of 20 male Fischer 344 rats, 6 weeks of age, received 0.05% BBN in drinking-water for 4 weeks followed by 0, 2, 10, 25, 50 or 100 ppm [μg/mL] DMA^V for 32 weeks.

Table 27. Incidence of preneoplastic and neoplastic lesions in various organs of Fischer 344/Du Crj rats treated with DMA^V after initiation with DMBDD treatment

Organ and finding	DMA^V					Two-tailed Cochran-Armitage analysis[a]
	0 ppm *n* = 20 (%)	50 ppm *n* = 20 (%)	100 ppm *n* = 19 (%)	200 ppm *n* = 20 (%)	400 ppm *n* = 20 (%)	
Urinary bladder						
Papillary or nodular hyperplasia	4 (20)	13 (65)[c]	14 (73.7)[d]	11 (55)[b]	11 (55)[b]	NE
Papilloma	1 (5)	12 (60)[d]	12 (63.2)[d]	11 (55)[d]	7 (35)[b]	NE
Transitional-cell carcinoma	1 (5)	10 (50)[c]	11 (57.9)[d]	12 (60)[d]	13 (65)[d]	NE
No. of tumour-bearing animals	2 (10)	17 (85)[d]	16 (84.2)[d]	17 (85)[d]	16 (80)[d]	NE
Kidney						
Adenoma	1 (5)	3 (15)	1 (5.2)	7 (35)[b]	3 (15)	
Adenocarcinoma	0	0	2 (10.5)	1 (5)	7 (35)[c]	$p < 0.01$
Nephroblastoma	4 (20)	0	4 (12.1)	6 (30)	9 (45)	$p < 0.05$
No. of tumour-bearing animals	5 (25)	3 (15)	6 (31.6)	13 (65)[b]	13 (65)[b]	$p < 0.001$
Liver						
Altered cell foci						
Clear-cell foci	10 (50)	12 (60)	14 (73.7)	19 (95)[c]	20 (100)[d]	$p < 0.001$
Basophilic foci	1 (5)	2 (10)	3 (15.8)	10 (50)[c]	17 (85)[d]	$p < 0.001$
Eosinophilic foci	1 (5)	2 (10)	8 (42.1)[c]	15 (75)[d]	16 (80)[d]	$p < 0.001$
Hyperplastic nodule	0	0	2 (10.5)	9 (45)[d]	7 (35)[c]	$p < 0.001$
Hepatocellular carcinoma	0	2 (10)	0	8 (40)[c]	8 (40)[c]	$p < 0.001$
Cholangioma	0	0	0	1 (5)	1 (5)	
Haemangioma	0	0	0	1 (5)	0	
No. of tumour-bearing animals	0	2 (10)	2 (10.5)	17 (85)[d]	13 (65)[d]	$p < 0.001$

Table 27 (contd)

Organ and finding	DMA^V					Two-tailed Cochran-Armitage analysis[a]
	0 ppm *n* = 20 (%)	50 ppm *n* = 20 (%)	100 ppm *n* = 19 (%)	200 ppm *n* = 20 (%)	400 ppm *n* = 20 (%)	
Thyroid gland						
Hyperplasia	3 (15)	4 (20)	2 (10.5)	13 (65)[c]	13 (65)[c]	$p < 0.001$
Adenoma	2 (10)	1 (5)	3 (15.8)	1 (5)	6 (30)	
Adenocarcinoma	1 (5)	1 (5)	5 (26.3)	5 (25)	4 (20)	
No. of tumour-bearing animals	3 (15)	2 (10)	8 (42.1)	6 (30)	9 (45)[b]	$p < 0.05$

From Yamamoto *et al.* (1995)

DMA^V, dimethylarsinic acid; DMBDD, *N*-nitrosodiethylamine + *N*-methyl-*N*-nitrosourea + 1,2-dimethylhydrazine chloride + *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine + *N*-bis(2-hydroxypropyl)nitrosamine; NE, not examined

[a] The significance of differences in the incidence of lesions between groups was assessed using the Fisher's exact probability test. To evaluate the dose–response relationships of the incidences in lesions in the kidney, liver and thyroid gland, two-tailed Cochran-Armitage analysis was used.

Significantly different from 0 ppm at [b] $p < 0.05$, [c] $p < 0.01$ and [d] $p < 0.001$

Development of preneoplastic lesions and tumours of the bladder (papillary or nodular hyperplasia, papillomas and carcinomas) was enhanced in a dose-dependent manner (see Table 28). Doses of 25, 50 and 100 ppm increased the incidences (%) and multiplicities (number per rat) of bladder papillomas and carcinomas. A significant increase in multiplicity of total tumours (papillomas plus carcinomas) was observed with doses as low as 10 ppm DMA^V ($p < 0.05$): 0 ppm, 0.20; 2 ppm, 0.20; 10 ppm, 0.55; 25 ppm, 1.47; 50 ppm, 2.30; 100 ppm, 2.40. Compared with controls, doses of 50 or 100 ppm significantly increased the incidence of papillary or nodular hyperplasia (Wanibuchi *et al*., 1996).

Groups of 10 male Fischer 344 rats, 6 weeks of age, were given a single intraperitoneal injection of 0 (control) or 200 mg/kg bw NDEA in saline and 2 weeks later received 0, 25, 50 or 100 ppm [µg/mL] DMA^V in the drinking-water for 6 weeks. Partial hepatectomy was performed on all animals at the end of week 3. Final body weights were decreased dose-dependently but not significantly. No significant variation in relative liver weights was noted. Dose-dependent significant increases in both numbers and areas of glutathione *S*-transferase placental form (GST-P)-positive foci in the liver were observed after initiation with NDEA; the significance was evident at doses of 50 and 100 ppm ($p < 0.01$) for numbers and at all three doses ($p < 0.05$ or $p < 0.01$) for areas [exact values were not listed because of figure]. No GST-P-positive foci were observed in groups not initiated with NDEA (Wanibuchi *et al*., 1997).

Groups of eight male NCI-Black-Reiter rats (which lack α_{2u}-globulin), 9–14 weeks of age, received 0.05% BBN in the drinking-water for 4 weeks followed by 0 or 100 ppm [µg/mL] DMA^V for 32 weeks. When killed at the end of week 36, the incidence and multiplicity of papillary or nodular hyperplasia in the bladder was significantly increased in DMA^V-treated rats (6/8 [75%]; $p < 0.05$; number per rat, 1.1 ± 1.0, $p < 0.05$) compared with rats receiving BBN alone (0/8 [0%]; number per rat, 0). A 38% incidence of bladder papillomas or carcinomas was observed in DMA^V-treated but not in control animals (Li *et al*., 1998).

Groups of 20 male Fischer 344 rats, 10 weeks of age, received a single intraperitoneal injection of 200 mg/kg bw NDEA followed 2 weeks later by DMA^V, monomethylarsonic acid (MMA) or trimethylarsine oxide (TMAO) at a dose of 100 ppm in the drinking-water for 6 weeks. Numbers of GST-P-positive foci in the liver were significantly increased in rats treated with MMA, DMA^V and TMAO compared with the controls. Areas of GST-positive foci were also significantly increased in rats treated with MMA, DMA^V and TMAO compared with the controls (Nishikawa *et al*., 2002).

Table 28. Induction of urinary bladder lesions in Fischer 344 rats treated with BBN followed by DMA^V at various doses

DMA^V (ppm)	No. of rats examined	Papillary or nodular hyperplasia		Papilloma		Carcinoma	
		Incidence (%)	No./rat	Incidence (%)	No./rat	Incidence (%)	No./rat
0 (control)	20	14 (90)	1.05 ± 95[a]	3 (15)	0.15 ± 0.37	1 (5)	0.05 ± 0.22
2	20	13 (65)	1.30 ± 1.30	2 (10)	0.10 ± 0.31	2 (10)	0.10 ± 0.31
10	20	14 (70)	1.55 ± 1.47	7 (35)	0.40 ± 0.60	3 (15)	0.15 ± 0.37
25	19	18 (95)	2.37 ± 1.17[b]	11 (58)[c]	1.05 ± 1.18[b]	7 (37)[d]	0.42 ± 0.61[e]
50	20	20 (100)[d]	2.95 ± 1.88[b]	13 (65)[f]	1.50 ± 1.36[b]	10 (50)[f]	0.80 ± 0.95[c]
100	20	20 (100)[d]	4.10 ± 3.02[b]	17 (85)[g]	1.70 ± 1.17[b]	12 (60)[g]	0.70 ± 0.66[b]

From Wanibuchi *et al.* (1996)

BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DMA^V, dimethylarsinic acid

[a] Mean ± SD

[b] $p < 0.001$ (significantly different from control, Student's *t*-test); [c] $p < 0.01$; [d] $p < 0.05$; [e] $p < 0.05$; [f] $p < 0.01$; [g] $p < 0.001$ (significantly different from control, Fisher's exact probability test)

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

Extensive reviews of the metabolism of arsenic have been published recently (National Research Council, 1999, 2001; WHO, 2001). This section focuses on data relevant for the evaluation of carcinogenic effects. Thus, it is not a complete review on all published data. The oxidation state of arsenic and its metabolites are given if reported. If speciation of oxidation state has not been performed, the metabolites are given as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Absorption*

Arsenic in drinking-water is easily absorbed in the gastrointestinal tract. About 70–90% of a single dose of dissolved arsenite (As^{III}) or arsenate (As^{V}) was absorbed from the gastrointestinal tract of humans and experimental animals (Pomroy *et al*., 1980; Vahter & Norin, 1980; Freeman *et al*., 1995). A high rate of gastrointestinal absorption is also supported by the fact that people whose main fluid intake consists of drinking-water with elevated arsenic concentrations have very high concentrations of arsenic in their urine.

With regard to absorption of arsenic through the skin, a few experimental studies indicate a low degree of systemic absorption. Application of water solutions of radiolabelled arsenate *in vivo* to the skin of rhesus monkey and *in vitro* to human cadaver skin showed that about 2–6% and 0.98% of the applied arsenic was absorbed within 24 h, respectively (Wester *et al*., 1993). Similar in-vitro studies using dorsal skin of mice showed a much higher absorption: 33–62% of the applied dose of radiolabelled arsenate in aqueous solution was absorbed through the skin within 24 h. However, most of it, on average 60–90%, was retained in the skin (Rahman *et al*., 1994). These authors suggested that absorption of arsenic through the skin may be species-specific. For many chemicals, mouse skin is more permeable *in vitro* than human cadaver skin (Bronaugh *et al.*, 1982). In-vitro studies with human keratinocytes showed that 1–8% of the applied arsenic dose was retained per hour (Bernstam *et al*., 2002). Morphological changes, cytotoxicity and inhibition of DNA and protein syntheses were found with in-vitro doses of As^{III} as low as 10 µg/L. Thus, it seems probable that inorganic arsenic can be absorbed from the exterior, leading to a breakdown in skin barrier function.

In-vitro studies of skin absorption of DMA^{V} (10 µg in 20–100 µL water) by application to dorsal skin from adult mice mounted in flow-through diffusion cells showed that 16–25% was retained in the receptor fluid (Hanks balanced salt solution), about 15% in the skin and the remainder in the wash after 24 h (Hughes *et al*., 1995). After exposure

for 1 h only, essentially all of the applied dose was washed away. Less than 1% of the applied dose was absorbed.

A low degree of systemic skin absorption of inorganic arsenic is supported by studies showing that people in Fairbanks, AK, who used tap-water containing about 345 μg/L arsenic for washing, but only bottled water (which did not contain arsenic) for drinking, had about the same low concentrations of arsenic metabolites in urine (on average about 40 μg/L) as people with less than 50 μg/L in their tap-water (Harrington *et al*., 1978). The concentration of arsenic in hair was clearly elevated in the group drinking bottled water (5.74 μg/g compared with 0.43 μg/g in the low-arsenic group), which shows that arsenic is bound to hair and probably also to skin during washing with water rich in arsenic.

4.1.2 *Distribution*

Following its absorption, arsenate is rapidly reduced to As^{III}; the distribution of its metabolites in the body are therefore very similar to that following exposure to As^{III}. However, studies in mice given arsenite or arsenate (0.4 mg/kg bw) intravenously showed that the concentrations in stomach and intestines were higher after exposure to As^{III} than after exposure to As^{V}, while incorporation in bone was higher following exposure to As^{V} (Lindgren *et al*., 1982). The differences are less marked in the case of oral exposure, probably due to faster methylation that occurs when the absorbed arsenic passes directly to the liver. After exposure to toxic doses at which methylation capacity is exceeded or inhibited, the differences in distribution patterns for the two forms are greater (Vahter & Norin, 1980).

Absorbed arsenic is transported, mainly bound to SH groups in proteins and low-molecular-weight compounds such as glutathione (GSH) and cysteine, to different organs in the body (National Research Council, 1999, 2001). Complexation of trivalent arsenical compounds with GSH, probably mainly in the form of $As(GS)_3$, has been demonstrated, but As^{III} is easily transferred to binding sites of higher affinity, especially vicinal dithiols, such as lipoic acid and dimercaptosuccinic acid (Cullen & Reimer, 1989; Delnomdedieu *et al.*, 1993). Studies on serum arsenic in dialysis patients showed the presence of inorganic arsenic, partly bound to proteins, and DMA (Zhang *et al*., 1997, 1998a,b; De Kimpe *et al*., 1999). Transferrin was the main carrier protein, but the extent to which this occurs in healthy individuals is not known. Most of the arsenic in blood is rapidly cleared, following a three-exponential clearance curve (Mealey *et al*., 1959; Pomroy *et al*., 1980). The majority of arsenic in blood is cleared with a half-time of about 2 or 3 h. The half-times of the second and third phases are about 168 and 240 h, respectively (Mealey *et al.*, 1959; National Research Council, 1999).

In experimental studies on mammals exposed to inorganic arsenic, the tissues with the longest retention of arsenic, depending on species, were skin, hair, liver, kidney, blood, squamous epithelium of the upper gastrointestinal tract, epididymis, thyroid, skeleton and lens. Arsenic does not readily cross the blood–brain barrier, and concentrations in the brain are generally low compared with most other tissues (Lindgren *et al*., 1982; Vahter *et al*., 1982; Lindgren *et al*., 1984; Yamauchi & Yamamura, 1985).

In human subjects exposed chronically to arsenic and also at background environmental concentrations, the hair and nails generally show the highest concentrations (0.02–10 mg/kg dry wt; Hindmarsh, 2002). Thus, arsenic appears to concentrate in tissues with a high content of cysteine-containing proteins. In areas of West Bengal and Bangladesh that have high concentrations of arsenic in the drinking-water, maximal concentrations of arsenic in hair, nail and skin exceeding 40 mg/kg have been reported (Guha Mazumder *et al.*, 1988; Chowdhury *et al*., 2001; Basu *et al.*, 2002). Very few studies have been carried out on the distribution of arsenic in human tissues. Postmortem analysis of human tissues confirm that arsenic is widely distributed in the body after long-term exposure, with highest concentrations in the skin and lungs (0.01–1 mg/kg dry wt), as well as hair and nails (Liebscher & Smith, 1968; Cross *et al.*, 1979; Dang *et al*., 1983). In people exposed to high concentrations (0.2–2 mg/L) of arsenic in drinking-water, the concentration in liver was 0.6–6 mg/kg dry wt compared with 0.16 mg/kg in unexposed people (Guha Mazumder *et al*., 1988). In a case of acute intoxication by arsenic, the liver and kidneys showed the highest concentrations of total arsenic with values 350- and 63-fold higher than those in blood, respectively. In all organs, As^{III} was the predominant species, and MMA occurred at higher concentrations than DMA. MMA and DMA were more prevalent in lipid-rich organs (49% and 45% of total arsenic in cerebellum and in brain, respectively) compared with other organs (~ 20% of total arsenic). As^{V} was found in small quantities in the liver, kidneys and blood (2% of total arsenic) (Benramdane *et al*., 1999).

Dang *et al*. (1983) used neutron activation analysis (NAA) to measure total arsenic in tissues of people [age and sex not specified] dying in accidents in Mumbai, India (Table 29). Concentrations in the brain were generally low compared with most other tissues. Thus, it appears that arsenic does not readily cross the blood–brain barrier. Notably, there was a large variation in tissue concentrations of arsenic among individuals, similar to that reported in earlier studies (Liebscher & Smith, 1968; Larsen *et al.*, 1974).

Table 29. Levels of arsenic in human tissues obtained from traffic accident victims in the Mumbai area of India

Tissue	No. of samples	Mean concentration (± SD) of arsenic (mg/kg wet wt)
Brain	12	3.9 ± 1.0
Blood	8	5.9 ± 3.9
Kidney	13	12.4 ± 20.7
Liver	19	14.5 ± 6.9
Spleen	18	15.2 ± 16.6
Lung	13	19.9 ± 22.7

From Dang *et al*. (1983)

Few studies have examined the distribution of arsenic metabolites in tissues, owing to analytical difficulties. Marafante *et al*. (1982) reported predominantly inorganic arsenic in ultrafiltrates of rat and rabbit liver and kidney 1 h after intraperitoneal injection of 50 μg/kg bw [^{74}As] as sodium arsenite, using ion-exchange chromatography with radiometric detection. The fraction present as MMA was generally less than one-tenth that of inorganic arsenic. De Kimpe *et al*. (1996) studied the tissue distribution of arsenic metabolites up to 120 h after intraperitoneal injection of a trace amount of [^{74}As]-arsenate in male Flemish giant rabbits, also using ion-exchange chromatographic separation of ultrafiltrates with radiometric detection. The predominant metabolite present in tissues was DMA, followed by inorganic arsenic species and low concentrations of MMA. The percentage of DMA increased steadily over time in bone marrow, heart, liver, muscle, pancreas, small intestine and spleen, but levelled off or declined in kidney and lung.

Yamauchi and Yamamura (1985) studied the tissue distribution over time of arsenic metabolites in male Syrian golden hamsters given a single oral dose of 4.5 mg/kg bw arsenic trioxide. Speciation of arsenic metabolites was carried out by hybrid generation–atomic absorption spectrophotometry (HG–AAS) with a cold trap after alkaline digestion. The predominant form of arsenic present in all tissues up to 120 h after dosing was inorganic arsenic. In contrast to other studies, the concentrations of MMA in tissue were two- to fourfold higher than those of DMA at all time-points, while much more DMA (22% of the dose in 5 days) than MMA (2.5% of the dose) was excreted in urine. The highest concentrations of MMA were found in lungs and spleen at 12–24 h, and those of DMA in liver, lung and kidney at 24 h.

Yamauchi *et al*. (1988) reported data on the time-course tissue distribution in hamsters given a single oral dose of 50 mg/kg bw MMA. Peak MMA concentrations were achieved within 6–120 h after dosing and were highest in the kidney, followed by spleen, lung, skin, liver, muscle and brain. MMA itself accumulated in the kidney and levels declined very slowly. DMA was also detected in several tissues, with highest levels occurring in the lung, followed by kidney and liver. Trimethylated arsenic was not detected in any tissues.

The fate of DMA has been studied in mice administered [^{74}As] or [^{14}C]DMA intravenously. The highest levels of radioactivity were present in kidney at all time-points (5–60 min after injection). Tissues that retained arsenic for the longest time (24 h) were the lungs, intestinal walls, thyroid and lens (Vahter *et al*., 1984; Hughes *et al*., 2000). Yamauchi and Yamamura (1984) studied the tissue distribution of DMA in hamsters administered a single oral dose of 50 mg/kg bw DMA. Concentrations were elevated in all tissues examined, including the brain, indicating that DMA is widely distributed in the body and that it passes the blood–brain barrier, although not to a large extent. Concentrations of DMA peaked at 6 h in all tissues examined except hair, with the highest levels in lung, followed by kidney, spleen, liver, skin, muscle and brain. Part of the DMA was found to be methylated further to trimethylarsenic (TMA) *in vivo*. Concentrations of TMA peaked at 6 h in all tissues except skin and hair in which none was detected. The highest concentrations of TMA were found in lung, and were equivalent to about half of those of DMA. It is notable that the peak concentrations of DMA and TMA in the lung were over

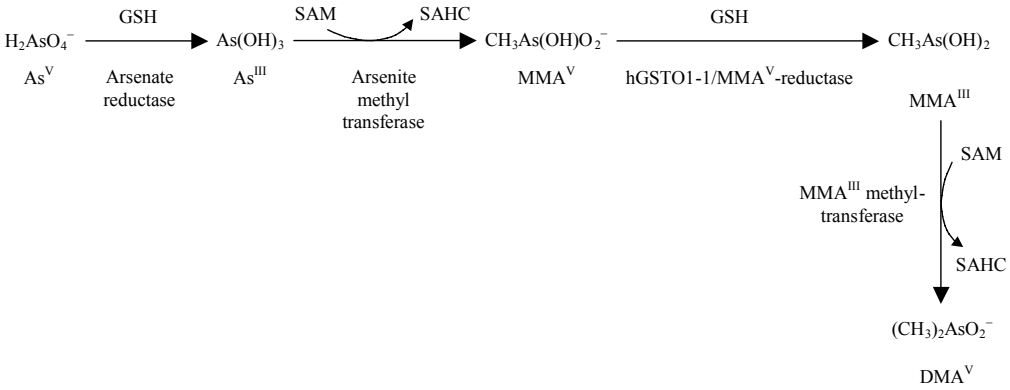
fourfold higher than those in the liver and kidney, but at 120 h after dosing, both had declined to control levels. The authors presumed that the TMA biosynthesized by hamsters is more likely to behave as an organic arsenic compound, such as arsenobetaine which is present in seafoods, than to be a very toxic substance such as trimethylarsine.

4.1.3 *Metabolism*

(*a*) *Methylation of arsenic*

For several decades, it has been known that inorganic arsenic is metabolized via methylation in microorganisms, aquatic organisms, birds and mammals. The methylation occurs through alternating reductive and oxidative methylation reactions, that is, reduction of pentavalent to trivalent arsenic followed by addition of a methyl group (Figure 3). In certain microorganisms, the methylation of inorganic arsenic may proceed to trimethylated metabolites. In humans, the relative amounts of species in urine are generally 10–30% inorganic arsenic, 10–20% MMA_{total} and 60–80% DMA_{total} (Hopenhayn-Rich *et al.*, 1993; National Research Council, 1999; Vahter, 1999a) (see below for further discussions of variations). The main metabolites excreted in the urine of humans exposed to inorganic arsenic are mono- and dimethylated arsenic acids, together with some unmetabolized inorganic arsenic. The major urinary methylated metabolites of arsenic are MMA^V and DMA^V, with arsenic in its pentavalent oxidation state. However, recent studies have demonstrated MMA^V reductase activity in different tissues that gives rise to the presence of both monomethylarsonous acid (MMA^{III}) and/or dimethylarsinous acid (DMA^{III}) in hamster liver (Sampayo-Reyes *et al.*, 2000) and of MMA^{III} in the bile of various experimental

Figure 3. Biotransformation of inorganic arsenic



Adapted from Zakharyan *et al.* (2001)

The conjugate acids and bases of the several forms of arsenic that are thought to predominate at physiological pH are shown.

SAM, *S*-adenosyl-L-methionine; SAHC, *S*-adenosyl-L-homocysteine; hGSTO1-1, human glutathione-*S*-transferase omega 1-1 (which is identical to MMA^V-reductase)

animal species (Csanaky & Gregus, 2002), as well as DMA^{III} and/or MMA^{III} in human urine (Aposhian *et al*., 2000a; Le *et al*., 2000b; Del Razo *et al*., 2001a; Mandal *et al*., 2001) after exposure to inorganic arsenic.

Following exposure of mice, hamsters, rats and humans to DMA^{V}, further methylation to trimethylarsine oxide (TMAO) has been observed (Marafante *et al*., 1987; Yoshida *et al*., 1997; Kenyon & Hughes, 2001). This probably occurs via DMA^{III} or the DMA-complex observed in urine. About 5% of urinary arsenic was in the form of TMAO. Of the species studied, demethylation of DMA to inorganic arsenic was detected only in rats, in which inorganic arsenic constituted less than 10% of the total excreted (Yoshida *et al*., 1997). Inorganic arsenic appeared in urine during the first 24–48 h after administration, whereas the highest rate of excretion of unchanged DMA had occurred by 6 h and that of TMAO between 6 and 24 h after dosing. The authors suggested that demethylation of DMA was effected by intestinal flora. TMAO was not found in an in-vitro study after incubation of inorganic arsenic with rat liver cytosol. Indeed, it was shown that the methylation of MMA to DMA was inhibited by increasing concentrations of As^{III} preventing the formation of TMAO (Buchet & Lauwerys, 1985, 1988).

It should be noted that there are pronounced species differences in the metabolism of arsenic (National Research Council, 1999; Vahter, 1999b). Most experimental animals excrete very little MMA in urine compared with humans (Vahter, 1999b), and some animals, in particular guinea-pigs and several species of non-human primates (Vahter, 1999b; Wildfang *et al*., 2001), are unable to methylate inorganic arsenic at all. In addition, rats show different kinetics of arsenic metabolism with a pronounced accumulation of DMA^{III} in red blood cells (Shiobara *et al*., 2001) and greater biliary excretion of arsenic (Klaassen, 1974; Gregus *et al*., 2000), compared with humans. The unique disposition of arsenic in rats may be due to the pronounced biliary excretion of MMA^{III} and blood cell uptake of DMA^{III} (Gregus *et al*., 2000; Shiobara *et al.*, 2001). Thus, it is difficult to evaluate human metabolism of arsenic based on much of the available experimental animal data. Studies in hamsters and rabbits seem to be the most useful because their metabolism is most similar to that in humans (National Research Council, 1999). This phenomenon has been taken into consideration here, and data from rats are not included, except for some information from in-vitro studies with rat hepatocytes, which has been used for the purpose of adding mechanistic information, where appropriate.

Compared with inorganic arsenic, the methylated metabolites containing pentavalent arsenic (MMA^{V} and DMA^{V}) are less cytotoxic, less reactive with tissue constituents and more readily excreted in urine (for review see National Research Council, 1999, 2001; Vahter & Concha, 2001). This has been taken as evidence that methylation of arsenic is an efficient detoxification process. In general, trivalent arsenic is more toxic than the pentavalent form. Recent studies, however, show that the trivalent methylated metabolites are considerably more toxic than inorganic As^{III} (National Research Council, 1999, 2001; Thomas *et al*., 2001; Sections 4.2 and 4.4). Thus, their presence in tissues and body fluids implies that the metabolism of inorganic arsenic involves important bioactivation processes, and that the toxicity of inorganic arsenic probably depends on its metabolism,

especially the capacity of cells to produce methylated intermediates that react with tissue constituents. It should be noted that there may be other mechanisms of transport out of tissues to urine. Excretion of arsenic in chimpanzees was found to be more rapid than that in humans, although methylation of arsenic does not occur in chimpanzees (Vahter *et al*., 1995a). A more complete understanding of the mechanisms of the metabolism of arsenic will provide further insight into the factors determining susceptibility to its toxicity. It should be noted that it is difficult to evaluate the tissue concentrations of MMA^{III} and DMA^{III} based on the amounts detected in urine.

A few studies have indicated a slightly larger fraction of urinary MMA and a smaller fraction of DMA in people with arsenic-related health effects, including skin lesions (Del Razo *et al*., 1997; Yu *et al*., 2000) and chromosomal aberrations (Mäki-Paakkanen *et al*., 1998). Similarly, there are indications that a relatively large amount of MMA in urine is associated with greater retention of arsenic in the body. Evaluation of data from a number of experimental studies on humans receiving specified doses of inorganic arsenic indicates that a higher percentage of DMA in urine is associated with greater overall excretion, while a higher percentage of inorganic arsenic and MMA is associated with slower excretion of total arsenic metabolites (Vahter, 2002). It should also be noted that other mammals that excrete little (rat, rabbit, hamster, beagle and mouse) or no MMA (marmoset, chimpanzee and guinea-pig) in the urine, that is, most experimental animals, show a rapid overall excretion of arsenic (Vahter, 1999b). They also seem to be less susceptible than humans to arsenic-induced toxicity, including cancer (National Research Council, 1999).

(*b*) *Mechanism of methylation of arsenic*

The mechanism of methylation of arsenic in humans has not been elucidated, but *S*-adenosylmethionine (SAM) seems to be the main methyl donor. In experimental studies, inhibition of SAM-dependent methylation pathways (by periodate-oxidized adenosine [PAD] or *S*-adenosylhomocysteine [SAH]) resulted in a marked decrease in methylation of arsenic (Marafante *et al*., 1985; De Kimpe *et al*., 1999; Csanaky & Gregus, 2001). Rabbits fed diets with a low content of methyl groups (low in methionine, protein or choline) methylated arsenic to a lesser degree (Vahter & Marafante, 1987). In-vitro studies using rat liver preparations have confirmed the requirement of SAM and thiols (reduced GSH) in the formation of MMA and DMA from As^{III} (Buchet & Lauwerys, 1988; Styblo *et al*., 1996; Healy *et al*., 1999; Thomas *et al*., 2001).

As shown in Figure 3, the methyl groups are transferred from SAM to arsenic in its trivalent form. GSH or other thiols serve as reducing agents for As^{V} and MMA^{V} (National Research Council, 1999, 2001) and are required for the methylation of arsenic. Complexation of trivalent arsenic with GSH, probably mainly in the form of $As(GS)_3$, has been demonstrated. Depletion of hepatic GSH by buthionine sulfoximine in rats and hamsters has been shown to decrease the methylation of inorganic arsenic (Buchet & Lauwerys, 1988; Hirata *et al*., 1990). Although As^{V} may be reduced non-enzymatically by GSH, enzyme-catalysed reduction seems to predominate. Studies with mice, rabbits,

and marmoset monkeys showed that a substantial fraction of absorbed arsenate (As^{V}) is rapidly reduced, probably mainly in the blood, to As^{III} (Marafante *et al.*, 1985; Vahter & Marafante, 1989; Vahter, 2002). Arsenate and pentavalent methylated metabolites may also be reduced to the corresponding trivalent form in tissues. Arsenate reductase activity has been detected in human liver (Radabaugh & Aposhian, 2000) and MMA^{V} reductase in human activity and rabbit liver (Zakharyan & Aposhian, 1999; Zakharyan *et al*., 2001) and various hamster tissues (Sampayo-Reyes *et al*., 2000). There is evidence that human MMA^{V} reductase is identical to glutathione-*S*-transferase class omega 1-1 (GSTO1-1) (Zakharyan *et al*., 2001). Based on studies on rabbit liver, it appears that reduction of MMA^{V} is the rate-limiting step in the metabolism of arsenic (Zakharyan & Aposhian, 1999). In male hamsters, MMA^{V} reductase activity was found to vary considerably among tissues: the highest activity was found in the brain followed by urinary bladder, spleen, liver, lung, heart, skin, kidney and testis. The activity in the testis was only about 10% of that in the brain (Sampayo-Reyes *et al*., 2000).

Experimental studies conducted in rabbits have indicated that the liver is the main site of arsenic methylation, especially following ingestion, when the absorbed arsenic initially passes through the liver, the only organ in which DMA is present 1 h after administration of the parent compound (Marafante *et al*., 1985). This is supported by studies showing a marked improvement in the methylation of arsenic following liver transplantation in patients with end-stage liver disease (Geubel *et al*., 1988). In-vitro studies have shown that the methylating capacity of different tissues may vary considerably. Investigation of the methylating activity of arsenic in male mice showed that the highest activity occurred in the testes, followed by kidney, liver and lung (Healy *et al*., 1998). The situation in female animals or humans remains to be elucidated. DMA was the main excretory metabolite of rat and human hepatocytes exposed to inorganic arsenic *in vitro* (Styblo *et al*., 1999). In addition to arsenic methyltransferase activity, the tissue in which arsenic is methylated may also depend on the cellular uptake of its different forms. Experimental studies show a several-fold higher uptake of As^{III} and MMA^{III} than of As^{V} and MMA^{V} in liver cells (National Research Council, 1999; Styblo *et al.*, 1999, 2000; National Research Council, 2001). In contrast, arsenate is readily taken up in the kidneys, after which it can be reduced and excreted in the urine, partly in methylated form. Whether this also occurs with MMA^{V} and DMA^{V} is not known. The uptake of MMA^{V} and DMA^{V} in most other tissues of mice seems to be low (Hughes & Kenyon, 1998; National Research Council, 2001).

The methyltransferases involved in arsenic methylation have not been fully characterized, although enzymes from liver cells of rats, rabbits, hamsters and rhesus monkeys have been partially characterized (Zakharyan *et al*., 1995, 1996; Wildfang *et al*., 1998; Lin *et al*., 2002) as cytosolic enzymes of 46–60 kDa, the activity of which requires both SAM and a thiol. As^{III} and MMA^{V} methylating activities seem to involve the same protein. Arsenate, selenate, selenite and selenide were not methylated by the purified enzyme preparations (Zakharyan *et al.*, 1995). Arsenite and $MAs^{III}O$ (methylarsine oxide) methyltransferase activities have been detected in primary cultures of human hepatocytes (Styblo *et al*., 1999). The mRNA for As^{III} methyltransferase, purified from liver cytosol of male

rats, was found to be similar to Cyt19, a putative methyltransferase expressed in human and mouse tissues (Lin *et al*., 2002), and was detected in rat tissues and in HepG2 cells, a human cell line that was reported to methylate arsenic, but not in UROtsa cells, an immortalized human urothelial cell line that does not methylate arsenite.

(*c*) *Variation in arsenic metabolism*

Although a number of studies have shown that the average relative distribution of arsenic metabolites in the urine is 10–30% inorganic arsenic, 10–20% MMA_{total} and 60–70% DMA_{total} (calculated percentages) (National Research Council, 1999), there is a wide variation among individuals (Vahter, 1999a,b). In one study, interindividual variation was found to exceed intra-individual variation considerably; the efficiency of arsenic methylation of an individual is remarkably stable over time (Concha *et al.*, 2002). Also, a few recent studies in which the trivalent and pentavalent metabolite forms have been speciated indicate a considerable variation in urinary excretion of MMA^{III} and DMA^{III} (Aposhian *et al.*, 2000a; Del Razo *et al*., 2001b; Mandal *et al*., 2001). Differences between population groups have also been reported. In one study, indigenous people living in the Andes, mainly Atacameños, excreted less MMA in urine (often only a few per cent) (Vahter *et al*., 1995b); people living in certain areas of Taiwan, China, however, seem to have an unusually high percentage of MMA in urine (20–30% on average) (Chiou *et al*., 1997b; Hsueh *et al.*, 1998). These findings indicate that the influence of genetic polymorphims is more important than environmental factors for the variation in arsenic methylation. Recently, it was reported that the methylation pattern among 11 families in Chile correlated more strongly between siblings than between father–mother pairs (Chung *et al*., 2002), supporting a genetic basis for the variation in arsenic methylation.

A few human studies indicate that high doses of arsenic may influence its methylation in humans. In humans acutely intoxicated by inorganic arsenic, there is a marked delay in the urinary excretion of DMA which exceeds all other metabolites (Mahieu *et al*., 1981; Foà *et al*., 1984). Only after 1 or 2 weeks did the fraction of DMA in urine reach 70–80%, a level commonly seen after lower exposures. In people exposed to arsenic via drinking-water, the ratio of DMA to MMA in urine decreased somewhat with increasing level of exposure (Hopenhayn-Rich *et al*., 1993). This is probably related to inhibition of methyltransferase, especially in the second methylation step, by high concentrations of arsenite, as demonstrated in experimental studies *in vitro* (Buchet & Lauwerys, 1988; Styblo *et al*., 1996). In people exposed to high concentrations of arsenic in drinking-water (several hundred micrograms per litre), there is a slight decrease in the percentage of DMA and a corresponding decrease in the percentage of MMA (Hepenhayn-Rich *et al*., 1993). In some studies, women tend to methylate arsenic more efficiently than men (Hopenhayn-Rich *et al*., 1996c), which may in part be related to the observed increase in arsenic methylation during pregnancy (Concha *et al*., 1998c).

4.1.4 *Placental transfer*

Studies in experimental animals and humans show that both inorganic arsenic and methylated metabolites cross the placenta to the fetus (Concha *et al*., 1998c). In women exposed to arsenic in drinking-water (about 200 μg/L), the concentrations of arsenic in umbilical cord blood were about as high as those in maternal blood in late gestation (about 10 μg/L) (Concha *et al*, 1998c). Placentas also had elevated concentrations of arsenic (median, 34 μg/kg wet wt; range, 17–54 μg/kg; $n = 11$). More than 90% of the arsenic in urine and plasma of both newborns and their mothers (at the time of delivery) was in the form of DMA (compared with about 70% in non-pregnant women), indicating an increase in arsenic methylation during pregnancy. The authors suggested that the DMA is much less toxic to the embryo and fetus than inorganic arsenic; the increased arsenic methylation during pregnancy could be highly protective for the developing organism.

Studies on women living in north-western Argentina indicated a low degree of arsenic excretion in human breast milk (Concha *et al*., 1998a). The average concentration of arsenic in milk was 2 μg/kg fresh wt, compared with 10 μg/L in maternal blood and 320 μg/L in maternal urine. Breastfeeding of newborns from highly exposed areas decreased the levels of arsenic in their urine (which were elevated directly after birth) because of the low concentration in maternal breast milk (compared with formula milk prepared from the local water, which would provide about 200 μg arsenic/day) (Concha *et al*., 1998c). A study of 36 German women showed that the average concentration of total arsenic in breast milk was less than 0.3 μg/L (Sternowsky *et al*., 2002). The few women who reported a high intake of seafood showed increased arsenic levels in breast milk, indicating that organic arsenic compounds of marine origin, e.g. arsenobetaine, are excreted in milk.

4.1.5 *Excretion*

In humans, the major route of excretion of most arsenic compounds is via the urine. The biological half-time of inorganic arsenic is about 4 days, but is slightly shorter following exposure to arsenate than to arsenite (Crecilius, 1977; Yamauchi & Yamamura, 1979; Tam *et al*., 1979; Pomroy *et al*., 1980; Buchet *et al*., 1981). In six human subjects who ingested radiolabelled [^{74}As]arsenate, 38% of the dose was excreted in the urine within 48 h and 58% within 5 days (Tam *et al*., 1979). The results of another study indicate that the data were best fit to a three-compartment exponential model, with 66% excreted with a half-time of 2.1 days, 30.4% with a half-time of 9.5 days and 3.7% with a half-time of 38.4 days (Pomroy *et al*., 1980). In three subjects who ingested 500 μg arsenic in the form of arsenite in water, about 33% of the dose was excreted in the urine within 48 h and 45% within 4 days (Buchet *et al*., 1981).

The administration of sodium 2,3-dimercapto-1-propane sulfonate (DMPS), a chelating agent, to humans chronically exposed to inorganic arsenic in the drinking-water resulted in increased urinary excretion of arsenic (Aposhian *et al*., 2000b; Guha Mazumder *et al*., 2001a). In particular, there was a marked increase in urinary excretion of MMA^{III} and

MMA^{V}, while the concentration and percentage of urinary DMA decreased (Aposhian *et al*., 2000b). Experimental studies supported the hypothesis that DMPS competes with endogenous ligands for MMA^{III}, forming a DMPS–MMA complex that is not a substrate for the MMA^{III} methyltransferase enzyme. This may explain the decrease in the conversion of the MMA^{III} to DMA. The DMPS–MMA complex is readily excreted in urine. Interestingly, MMA^{III} was excreted in the urine only after administration of DMPS (Aposhian *et al.*, 2000b).

4.2 Toxic effects

4.2.1 *Humans*

(*a*) *Acute and subacute toxicity*

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

(*b*) *Chronic toxicity*

Most of the reports on chronic exposure to arsenic in humans focus attention on skin manifestations because of their diagnostic specificity. However, data derived from population-based studies and clinical case series and reports relating to intake of inorganic arsenic through drinking-water, medications or occupational and environmental exposure show that chronic exposure to arsenic adversely affects multiorgan systems. The clinical appearance of non-cancerous manifestations of arsenic intoxication in humans is insidious in onset and is dependent on the magnitude of the dose and the time course of exposure.

(i) *Cutaneous manifestations*

The specific cutaneous lesions of chronic arsenic toxicity are characterized by pigmentation and keratosis. These have been reported from different regions of the world including Argentina, Bangladesh, Chile, China, India (West Bengal), Japan, Mexico and Taiwan, China, where the content of arsenic in drinking-water is elevated (Zaldívar, 1974; Borgoño *et al*., 1977; Cebrián *et al*., 1983; Saha, 1984; Chakraborty & Saha, 1987; Guha Mazumder *et al*., 1988, 1992; Ahmad *et al*., 1997; Guha Mazumder *et al*., 1998b; Biswas *et al*., 1998; Mandal *et al*., 1998; Ahmad, S.A. *et al*., 1999; Milton & Rahman, 1999; Guo *et al*., 2001). The magnitude of dose and the time frame of exposure to arsenic needed to induce the hyperpigmentation and hyperkeratosis characteristic of chronic arsenic intoxication have been investigated to a limited extent.

Among the population exposed to arsenic in drinking-water in the Antofagasta region of Chile, where levels reached 0.8 mg/L, cases of cutaneous arsenicosis, including both hyperpigmentation and hyperkeratosis, have been described in children as young as 2 years of age (Rosenberg, 1974). In a cohort of 40 421 inhabitants of south-western Taiwan, China, investigated by Tseng *et al*. (1968), the youngest subjects found to have hyperpigmentation and hyperkeratosis were reported to be aged 3 and 4 years, respectively; in a later investigation, the youngest subjects were aged 5 and 15 years (Tseng, 1977). The amount of arsenic consumed by these children was not specified. In a clinical evaluation conducted among 296 residents of Region Lagunera in northern Mexico, where ingested groundwater contained a mean arsenic concentration of approximately 0.4 mg/L, the shortest time of exposure associated with hypopigmentation was 8 years, increasing to 12 years for hyperpigmentation and palmoplantar keratosis (Cebrián *et al*., 1983).

The hyperpigmentation of chronic arsenic poisoning commonly appears in a finely freckled, raindrop pattern that is particularly pronounced on the trunk and extremities, and distributed bilaterally symmetrically, but can also involve mucous membranes such as the undersurface of the tongue or buccal mucosa (Yeh, 1973; Tay, 1974; Saha, 1984; Guha Mazumder, 1988, 1992; Saha, 1995; Guha Mazumder *et al.*, 1998b; Ahmad, S.A. *et al*., 1999; Milton & Rahman, 1999). Although less common, other patterns include diffuse hyperpigmentation (melanosis) (Tay, 1974; Saha, 1984), localized or patchy pigmentation, particularly affecting skinfolds (Tay, 1974; Szuler *et al*., 1979), and so-called leukodermia or leukomelanosis (Saha, 1984; Mandal *et al*., 1996, 1997; Chowdhury *et al*., 2000a,b) in which the hypopigmented macules take a spotty, white appearance.

Arsenical hyperkeratosis appears predominantly on the palms of the hands and on the plantar aspect of the feet, although involvement of the dorsum of the extremities and the trunk have also been described. Occasionally, lesions may be larger and have a nodular or horny appearance. In severe cases, the hands and soles present diffuse verrucous lesions. Cracks and fissures may be severe on the soles (Sommers & McManus, 1953; Black, 1967; Tseng *et al*., 1968; Yeh, 1973; Tay, 1974; Zaldívar, 1974; Borgoño *et al*., 1977; Cebrián *et al.*, 1983; Saha, 1984; Guha Mazumder *et al*., 1988; Ahmad *et al*., 1997; Guha Mazumder *et al*., 1998b; Saha & Chakraborti, 2001). Histological examination of the lesions typically reveals hyperkeratosis with or without parakeratosis, acanthosis and enlargement of the rete ridges. In some cases, there may 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 cellular atypia and those with mild cellular atypia); and a malignant type B (intraepidermal carcinoma or carcinoma *in situ*, basal-cell carcinoma or squamous-cell carcinoma). Type B arsenical keratosis is histologically similar to but not indistinguishable from Bowen disease. Skin cancer can arise in the hyperkeratotic areas or appear on non-keratotic areas of the trunk, extremities or hands (Sommers & McManus, 1953; Yeh, 1973). In epidemiological studies in West Bengal (India) and Bangladesh, a higher prevalence of arsenical skin lesions was observed in men compared with women, with a clear

dose–response relationship (Guha Mazumder *et al*., 1998c; Rahman *et al*., 1999a; Tondel *et al*., 1999).

Early studies provided estimates for the dose–response relationship of arsenic-induced skin lesions. Tseng *et al*. (1968) and Yeh (1973) evaluated 40 421 inhabitants of south-western Taiwan, China, where the drinking-water supply (artesian well-water) had been contaminated with arsenic for more than 50 years. The concentration of arsenic in the water supply varied from 0.01 to 1.82 mg/L, and most well-water in the endemic area had a range of 0.4–0.6 mg/L. The entire population at risk numbered 103 154. Of the people surveyed and examined clinically, characteristic arsenic-induced hyperpigmentation was diagnosed in 18.4%, keratosis in 7.1%, skin cancer in 1.1% and invasive skin cancer in 0.4%. Of the 428 people with clinically diagnosed skin cancer, 71.7% also had keratosis and 89.7% had hyperpigmentation. Ninety-nine per cent of the people with skin cancer had multiple skin cancers; 74.5% of the malignant lesions were on unexposed areas (Tseng *et al.*, 1968). Yeh (1973) studied 303 samples of skin cancers histologically: 57 were squamous-cell carcinomas, 45 were basal-cell carcinomas, 176 were intra-epidermal carcinomas (including 23 type B arsenical keratoses and 153 Bowen disease) and 25 were combined forms. The study in Taiwan lacked individual data on exposure to arsenic, since the levels were reported by village. In general, however, the incidence of hyperpigmentation, keratoses and skin cancer increased with increasing content of arsenic in the drinking-water and with age and length of exposure. The youngest patient with skin cancer was 2 years old. No case of melanosis, keratosis or skin cancer was identified in the nearby control population.

Guha Mazumder *et al*. (1998c) carried out the first population survey with individual data on exposure to arsenic among 7683 participants in West Bengal, India, to ascertain the prevalence of keratoses and hyperpigmentation. The arsenic content of their current water source ranged up to 3.4 mg/L, although 80% of participants consumed water containing < 0.5 mg/L arsenic. Of 4093 female and 3590 male participants, 48 and 108 had keratotic lesions and 127 and 234 had hyperpigmentation, respectively. Clear exposure–response relationships were found for levels of arsenic in water and the prevalence of these arsenic-induced skin effects. Men were affected more than women. Subjects who had body weights below 80% of the standard for their age and sex had a 1.6-fold and 1.2-fold increase in the prevalence of keratosis and hyperpigmentation, respectively. However, the survey examined only the participants' primary current drinking-water source. A similar cross-sectional study was conducted in Bangladesh by Tondel *et al*. (1999) who interviewed and examined 1481 subjects ≥ 30 years of age. A total of 430 subjects had skin lesions. Individual exposure assessment could only be estimated by present levels. Concentrations of arsenic in water 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-related skin lesions in men than in women, with a clear dose–response relationship.

Haque *et al*. (2003) recently completed a nested case–control study of a previous study (Guha Mazumder *et al*., 1998c) to examine the dose–response relationship between concentrations below 0.5 mg/L in drinking-water and arsenic-induced skin lesions using

a detailed exposure assessment that incorporated data on arsenic concentrations from current and past water sources used in households and work sites. A subset of 158 participants (69 cases and 89 controls) had complete histories of water concentrations. No case of a skin lesion was found with peak water concentrations of arsenic less than 0.1 mg/L. All of the eight cases (four men aged 31–75 years, four women aged 21–66 years), who currently had skin lesions and had ingested peak arsenic concentrations between 0.1 and 0.19 mg/L, had hyperpigmentation, and four also had keratoses.

Skin cancers are frequently associated with hyperkeratotic lesions (Yeh, 1973). Hyperkeratosis occurs more commonly and earlier in arsenic-exposed populations than skin cancer. A dose–response analysis of hyperkeratotic lesions may therefore allow the observation of a potential carcinogenic response to exposures lower than those used for skin cancer.

(ii) *Respiratory disease*

The possible role of chronic ingestion of arsenic in the genesis of non-malignant pulmonary disease has been suggested in a few case series describing medical problems among individuals chronically exposed to increased concentrations of arsenic in the drinking-water. Among a total cohort of 180 residents of Antofagasta, Chile, exposed to drinking-water containing 0.8 mg/L arsenic, 38.8% of 144 subjects with abnormal skin pigmentation complained of chronic cough, compared with 3% of 36 subjects with normal skin (Borgoño *et al*., 1977). In autopsies of four children and one adolescent from the Antofagasta region with an antecedent history of cutaneous arsenicosis and postmortem findings of extensive (non-pulmonary) vascular disease, two of the subjects were noted to have chronic bronchitis, slight bronchiectasis and slight diffuse interstitial fibrosis of the lung (Rosenberg, 1974). Symptoms of chronic lung disease were present in 89 (57%) of 156 cases of chronic arsenic toxicity caused by drinking arsenic-contaminated water in West Bengal, India (Guha Mazumder *et al*., 1998b). Lung function tests carried out on 17 patients showed features of restrictive lung disease in nine (53%) and combined obstructive and restrictive lung disease in seven (41%) cases.

To investigate the relationship between non-malignant respiratory disease and ingested arsenic, Guha Mazumder *et al*. (2000) analysed data from the cross-sectional survey of 7683 participants who were examined clinically and interviewed, and measured the arsenic content in their current primary drinking-water source. Arsenic concentrations ranged from < 0.003 mg/L to 3.4 mg/L. Because there were few smokers, analyses were confined to nonsmokers (n = 6864 participants). Study subjects had arsenic-associated skin lesions, such as hyperpigmentation and hyperkeratosis, and were also highly exposed at the time of the survey (concentration of arsenic in water ≥ 0.5 mg/L). Individuals with normal skin and low concentration of arsenic in water (< 0.05 mg/L) were used as the referent group. Participants with skin lesions had age-adjusted prevalence odds ratio estimates for cough, crepitations and shortness of breath of 7.8 (95% CI, 3.1–19.5), 9.6 (95% CI, 4.0–22.9) and 23.2 (95% CI, 5.8–92.8) in women and 5.0 (95% CI, 2.6–9.9), 6.9 (95% CI, 3.1–15.0) and 3.7 (95% CI, 1.3–10.6) in men, respectively.

The effect of chronic exposure to arsenic on the respiratory system was studied in 218 individuals (94 exposed to arsenic [0.136–1 mg/L] and 124 control cases), most of whom were non-smokers, in Bangladesh (Milton *et al*., 2001). The overall crude prevalence (or risk) of chronic cough and chronic bronchitis among exposed subjects was three times that in controls. Women were reported to be affected more than men.

The occurrence of chronic respiratory disease in the form of chronic cough or chronic bronchitis due to constant ingestion of arsenic through drinking-water has also been reported (Hotta, 1989; Chowdhury *et al*., 1997; Kilburn, 1997; Chakraborti *et al.*, 1998; Ahmad, S.A. *et al*., 1999; Ma *et al*., 1999; Chowdhury *et al.*, 2000b).

(iii) *Gastrointestinal system*

Chronic arsenic toxicity has been reported to produce various gastrointestinal symptoms. Hotta (1989) reported gastrointestinal impairment in 76% of subjects exposed to environmental arsenic at Torku, Japan. The symptoms were not serious in most patients, as they had possibly been afflicted with the initial stage of disease. Gastroenteritis was reported in a study of 1447 cases of chronic arsenicosis caused by drinking arsenic-contaminated water (0.05–1.8 mg/L) in the Inner Mongolian Autonomous region of China (Ma *et al*., 1999). Of patients suffering from chronic arsenicosis after drinking arsenic-contaminated water (0.05–14.2 mg/L) in West Bengal, India, gastrointestinal symptoms characterized by dyspepsia were present in 60/156 (38.4%) cases studied (Guha Mazumder *et al*., 1998b). Many investigators variously reported symptoms such as nausea, diarrhoea, anorexia and abdominal pain in cases of chronic arsenic toxicity (Rosenberg, 1974; Zaldívar, 1974; Borgoño *et al*., 1977; Cebrián *et al*., 1983; Guha Mazumder *et al*., 1988; Ahmad *et al*., 1997). However, in an epidemiological study carried out in the affected population in West Bengal, there was no difference in the incidence of abdominal pain among people drinking arsenic-contaminated water (0.05–3.4 mg/L) and the control population (< 0.05 mg/L) (27.84% versus 31.81%) (Guha Mazumder *et al*., 2001b).

(iv) *Liver and spleen*

Exposure to inorganic arsenic 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 trivalent inorganic arsenic (Morris *et al*., 1974; Cowlishaw *et al*., 1979; Szuler *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 given arsenic as a medication, mostly Fowler's solution, for several years. Typical cutaneous signs of long-term exposure to arsenic were also observed in some of the patients. In addition, there have been case reports on liver cirrhosis following medication with inorganic arsenic 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 levels of arsenic in their liver in Chandigarh, India, two of whom had been drinking arsenic-contaminated water (0.549 and 0.360 mg/L). Guha Mazumder *et al*. (1988) reported hepatomegaly in 62/67 (92.5%)

members of families who had drunk arsenic-contaminated water (0.2–2 mg/L) in West Bengal, India, but in only 6/96 (6.25%) people from the same area who had drunk uncontaminated water (< 0.05 mg/L). Thirteen arsenic-exposed patients who had hepatomegaly were further investigated in hospital. All showed varying degrees of portal zone expansion and liver fibrosis histologically. Four of the five patients who had splenomegaly showed evidence of increased intrasplenic pressure (30–36 cm saline), suggesting portal hypertension. Splenoportography of these cases showed evidence of intrahepatic portal vein obstruction. Although routine liver function tests were normal in all these cases, the bromosulphthalin retention test was abnormal in three. The level of arsenic in liver tissue, estimated by neutron activation analysis, was found to be elevated in 10/13 cases (0.5–6 mg/kg dry wt versus 0.16 ± 0.04 mg/kg dry wt in controls). Santra *et al*. (1999) and Guha Mazumder (2001a) subsequently reported hepatomegaly in 190/248 cases (76.6%) of chronic arsenicosis investigated in the same hospital. Evidence of noncirrhotic portal zone fibrosis of the liver was found histologically in 63/69 cases (91.30%) of hepatomegaly. Liver function tests carried out on 93 such patients showed evidence of elevated levels of alanine aminotransferase (ALT), aspartase aminotransferase and alkaline phosphatase in 25.8%, 61.3% and 29% of cases, respectively. Serum globulin was found to be high (> 3.5 g/dL) in 19 (20.7%) cases.

Liver enlargement has been reported in cases of chronic arsenic toxicity caused by drinking arsenic-contaminated water (Saha, 1984; Chakraborty & Saha, 1987; Ahmad, S.A. *et al*., 1997, 1999; Ma *et al*., 1999; Saha & Chakribori, 2001).

(v) *Chronic cardiovascular effects*

Ingested inorganic arsenic has been related to an increased incidence of cardiovascular disease, especially ischaemic heart disease. This has been reviewed extensively (WHO, 1981; Engel & Smith, 1994; Chen *et al*., 1997; National Research Council, 1999, 2001).

Arsenic has been well documented as one of the major risk factors for Blackfoot disease, a unique peripheral arterial disease characterized by severe systemic arteriosclerosis, as well as dry gangrene and spontaneous loss of affected extremities at end-stages. Histologically, Blackfoot disease can be divided into two reaction groups: arteriosclerosis obliterans and thromboangiitis obliterans. The prevalence of Blackfoot disease was reported to be 8.9/1000 among 40 421 inhabitants studied by Tseng *et al*. (1968) in Taiwan, China. The villages surveyed were arbitrarily divided according to the arsenic content of the well-water into low (< 0.3 mg/L), medium (0.3–0.6 mg/L) and high (> 0.6 mg/L) exposure. The prevalence of Blackfoot disease revealed a clear-cut ascendency gradient from low to medium to high exposure for both sexes and the three different age groups studied (Tseng, 1977). Atherogenicity and carcinogenicity of high levels of arsenic in artesian well-water was examined by Chen *et al*. (1988b). The lifetable method used to analyse cancer mortality of 789 patients with Blackfoot disease followed for 15 years showed a significantly higher mortality from cardiovascular and peripheral vascular disease among these patients compared with the general population in Taiwan and residents in the area endemic for Blackfoot

disease. Whether fluorescent humic substances isolated from artesian well-water play an etiological role in Blackfoot disease has not been ascertained by epidemiological or animal studies (Van Duuren *et al.*, 1986; Lu *et al.*, 1990). A causal role for arsenic in the induction of Blackfoot disease offers the best explanation for the observations in Taiwan (Engel *et al*., 1994).

Tsai *et al*. (1999) conducted a study in Taiwan, China, to analyse mortality from all causes in areas endemic for Blackfoot disease. They calculated standardized mortality ratios (SMRs) for cancer and non-cancer diseases, by sex, during the period 1971–94 and compared them with the local reference group (Chiayi-Tainan County) and the national reference group (population of Taiwan). The results revealed marked differences in SMR for the two reference groups. With respect to non-cancer disease, mortality was greater for men and women in the endemic area who had vascular disease, ischaemic heart disease, hypertension, diabetes mellitus and bronchitis than for the local reference groups. Mortality from other diseases including cancers of the rectum, stomach and oesophagus and cerebrovascular disease was higher among subjects in the study area than among the local reference group. These results indicated that the hazardous effect of arsenic was systemic.

Comparable peripheral vascular disorders with varying degrees of severity including Raynaud syndrome, acrocyanosis and gangrene of the feet have also been reported among people drinking arsenic-contaminated water (Rosenberg, 1974; Zaldívar, 1974; Borgoño *et al*., 1977; Tseng *et al*., 1996; Ahmad, S.A. *et al*., 1999; Ma *et al*., 1999; Guha Mazumder *et al*., 2001b). It should be emphasized that there are differences in the prevalence of peripheral vascular diseases that cause gangrene and limb loss among different populations exposed to arsenic; the incidence is high in Taiwan, China, but low in Chile, India and Bangladesh, and none has been reported from Mexico or Argentina (Engel *et al*., 1994).

An epidemiological study reported an increased prevalence of hypertension among residents in an area endemic for Blackfoot disease and a dose–response relationship with ingested inorganic arsenic (Chen *et al*., 1995). A total of 382 men and 516 women residing in areas of Taiwan, China, endemic for arsenic were studied. A 1.5-fold increase in the age- and sex-adjusted prevalence of hypertension was observed compared with residents in non-endemic areas, and was associated with higher cumulative exposure to arsenic. The dose–response relation remained significant after adjustment for age, sex, diabetes mellitus, proteinuria, body mass index and level of serum triglycerides. Increased prevalence of hypertension was also observed in 6.2% of patients affected with arsenic-induced skin lesions (144) compared with none of those with no skin lesion (36) in Antofagasta, Chile (Borgoño *et al*., 1977). Rahman *et al*. (1999b) conducted studies on arsenic-exposed people in Bangladesh and demonstrated an association between hypertension and cumulative exposure to arsenic in drinking-water (Rahman & Axelson, 2001; Rahman, 2002).

Significant dose–response relationships between the level of ingested inorganic arsenic and risk for ischaemic heart disease were observed in recent cohort and case–control studies in Taiwan, China (Chen *et al*., 1994). In an ecological correlational study in Taiwan based

on 898 806 person–years and 172 deaths from ischaemic heart disease observed from 1973 to 1986, a dose–response relationship between concentration of arsenic in drinking-water and age-adjusted mortality from ischaemic heart disease was observed. A total of 257 patients with Blackfoot disease and 753 matched healthy controls were recruited and followed-up for more than 7 years. Significantly increased mortality from ischaemic heart disease was observed for patients with Blackfoot disease and matched controls showing SMRs (95% confidence interval [CI]) of 937 (536–1519) and 248 (139–409), respectively, compared with the general population of Taiwan (SMR, 100). Cox's proportional hazard regression analysis also showed a dose–response relationship between mortality from ischaemic heart disease and cumulative exposure to arsenic after adjustment for age, sex, body mass index and disease status for hypertension and diabetes mellitus. A case–control study including 78 patients with electrocardiogram-based ischaemic heart disease and 384 healthy residents was carried out in three villages where Blackfoot disease was endemic. Based on a multiple logistic regression analysis, cumulative exposure to arsenic was found to be associated with ischaemic heart disease in a dose-related manner after adjustment for age, sex, body mass index, disease status for hypertension and diabetes mellitus, ratio between total cholesterol and high-density lipoprotein cholesterol and cumulative alcohol consumption. The occurrence of ischaemic heart disease due to chronic exposure to arsenic has also been reported by other investigators (Rosenberg, 1974; Zaldívar, 1974; Hotta, 1989; Chen *et al.*, 1994, 1995; Ma *et al*., 1999), as has the occurrence of cardiac arrhythmia (Hotta, 1989; Ma *et al*., 1999).

Mortality rates from 1973 through 1986 for ischaemic heart disease among residents in 60 villages of an area in Taiwan, China, endemic for arsenicosis were analysed by Chen *et al*. (1996) to examine their association with the concentration of arsenic in drinking-water. Based on 1 355 915 person–years and 217 deaths from ischaemic heart disease, the cumulative mortality from birth to age 79 years as 3.4%, 3.5%, 4.7% and 6.6%, respectively, for residents who lived in villages in which the median concentrations of arsenic in drinking-water were < 0.1, 0.1–0.34, 0.35–0.59 and ≥ 0.6 mg/L. A cohort of 263 patients with Blackfoot disease and 2293 residents in the endemic area of arsenicosis without Blackfoot disease were recruited and followed up for an average period of 5.0 years. There was a monotonic biological gradient relationship between cumulative exposure to arsenic through drinking artesian well-water and mortality from ischaemic heart disease. The relative risks (95% CI) were 2.5 (0.53–11.37), 4.0 (1.01–15.60) and 6.5 (1.88–22.24), respectively, for those who had cumulative exposures to arsenic of 0.1–9.9, 10.0–19.9 and ≥ 20.0 mg/L–years, compared with those with no known exposure to arsenic, after adjustment for age, sex, cigarette smoking, body mass index, serum cholesterol and triglyceride levels, and disease status for hypertension and diabetes through proportional hazard regression analysis. Patients with Blackfoot disease were found to have a significantly higher mortality from ischaemic heart disease than residents without Blackfoot disease, showing a multivariate-adjusted relative risk of 2.5 (95% CI, 1.14–5.40).

Wang *et al*. (2002) reported evidence of a dose–response relationship between long-term exposure to arsenic in drinking-water and prevalence of carotid atherosclerosis in the

arsenic-exposed area of south-western Taiwan, China. The extent of carotid atherosclerosis was assessed by duplex ultrasonography among 199 male cases and 264 residents who participated in the study. Three indices of exposure, duration of consumption of artesian well-water, average concentration of arsenic in consumed well-water and cumulative exposure to arsenic, were all significantly associated with an increased prevalence of carotid atherosclerosis with a dose–response relationship. The biological gradient remained significant after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, alcohol consumption, waist-to-hip ratio and serum levels of total and low-density lipoprotein cholesterol. The multivariate-adjusted prevalence odds ratio was 1.8 (95% CI, 0.8–3.8) and 3.1 (95% CI, 1.3–3.4) for those who had a cumulative exposure to arsenic of 0.1–19.9 and ≥ 20 mg/L–years, respectively, compared with those without exposure to arsenic from drinking artesian well-water.

(vi) *Nervous system*

Abnormal electromyographic (EMG) findings suggestive mostly of sensory neuropathy were reported in 10/32 (31.25%) subjects exposed to arsenic by drinking contaminated well-water (range, 0.06–1.4 mg/L) in Canada (Hindmarsh *et al*., 1977). Paresthaesia was present in 74/156 (47.43%) patients with chronic arsenicosis caused by drinking arsenic-contaminated water (0.05–14.2 mg/L) in West Bengal, India. Objective evaluation of neuronal involvement carried out in 29 patients showed abnormal EMGs in 10 (34.5%) and altered nerve conduction velocity and EMGs in 11 (38%) cases (Guha Mazumder *et al*., 1997). Evidence of parasthaesia or peripheral neuropathy due to chronic exposure to arsenic through drinking-water has also been reported (Saha, 1984; Hotta, 1989; Kilburn, 1997; Ahmad, S.A. *et al*., 1999; Ma *et al*., 1999; Chowdhury *et al*., 2000a; Rahman *et al*., 2001, 2003). More sensory than motor neuropathy has also been reported among arsenicosis patients in West Bengal (Basu *et al*., 1996; Mukherjee *et al*., 2003).

The relationship between the prevalence of cerebrovascular disease and ingestion of inorganic arsenic in drinking-water was reported by Chiou *et al*. (1997a) in a cross-sectional study in Taiwan, China, that recruited a total of 8102 men and women from 3901 households. The status of cerebrovascular disease of study subjects was identified through personal home interviews and ascertained by review of hospital medical records according to WHO criteria. Information on consumption of well-water, sociodemographic characteristics, cigarette smoking habits and alcohol consumption, as well as personal and family history of disease, was also obtained. The concentration of arsenic in the well-water of each household was determined by HG–AAS. A significant dose–response relationship was observed between concentration of arsenic in well-water and the 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, 3.21 (95% CI, 1.51–6.88), 4.37 (95% CI, 1.99–9.60) and 6.58 (95% CI, 2.82–15.28), respectively, for those who consumed well-water with an arsenic content of 0, 0.001–0.05, 0.051–0.299 and

> 0.3 mg/L. Increased incidences of cerebrovascular disease in cases of chronic arsenicosis have been reported elsewhere (Hotta, 1989; Chen *et al.*, 1997; Ma *et al*., 1999).

Kilburn (1997) reported the occurrence of peripheral neuritis, sleep disturbances, weakness and cognitive and memory impairment in residents of Bryan-College Station, TX, USA, exposed to arsenic in air and water from the use of arsenic trioxide to produce defoliants for cotton at an Atochem plant. Siripitayakunkit *et al*. (1999) reported retardation of intelligence among 529 children (6–9 years of age) living in Thailand who had chronic exposure to arsenic from the environment.

(vii) *Diabetes mellitus*

To examine the association between ingested inorganic arsenic and the prevalence of diabetes mellitus, Lai *et al*. (1994) studied 891 adults residing in villages in southern Taiwan, China. The status of diabetes mellitus was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylureas or insulin. They observed a dose–response relationship between cumulative exposure to arsenic and prevalence of diabetes mellitus. The relationship remained significant after adjustment for age, sex, body mass index and physical activity level at work by a multiple logistic regression analysis, giving multivariate-adjusted odds ratios of 6.61 (95% CI, 0.86–51.0) and 10.05 (95% CI, 1.30–77.9), respectively, for those who had a cumulative exposure to arsenic of 0.1–15.0 and > 15.1 mg/L–years compared with those who were unexposed.

Rahman *et al.* (1998) reported a significantly increased prevalence of diabetes mellitus in Bangladesh caused by drinking arsenic-contaminated water among subjects with keratosis compared with subjects who did not have keratosis. A significant trend in risk between an approximate, time-weighted exposure to arsenic and the prevalence of diabetes mellitus strengthened the possibility of a causal association. [The lack of comprehensive, systematic, long-term sampling of the water supplies in the study area is a limitation of the study and data on individual exposures measured directly over time would have been more informative. However, these results suggest that chronic exposure to arsenic may induce diabetes mellitus in humans.] A further study regarding glucosuria patients with and without skin lesions in relation to exposure to arsenic in drinking-water reported that the prevalence ratios among the subjects without skin lesions were 0.8 (95% CI, 0.4–1.3), 1.4 (95% CI, 0.8–2.3) and 1.4 (95% CI, 0.7–2.4), after ajustment for age and sex compared with unexposed subjects as reference. The exposure categories were < 0.5, 0.5–1 and > 1 mg/L, respectively. For those with skin lesions, the prevalence ratios were slightly higher; 1.1 (95% CI, 0.5–2.0), 2.2 (95% CI, 1.3–3.8) and 2.6 (95% CI, 1.5–4.6), respectively, in comparison with unexposed subjects (Rahman *et al.*, 1999a). [In this study also, a lack of systematic sampling of water supplies in the study area is a limitation. Furthermore, although glucosuria is a primary indicator of diabetes mellitus, identification of the hyperglycaemic patients among those with glucosuria would have been more informative.]

Tseng *et al*. (2000) reported a cohort study on 446 non-diabetic residents from an arsenic-contaminated area in south-western Taiwan, China. Diabetes mellitus was deter-

mined by an oral glucose tolerance test. The age-specific incidence density ratio of diabetes mellitus was between two- and five-fold higher in the exposed cohort than in the unexposed cohort. An exposure–response relationship was observed between incidence of diabetes mellitus and long-term exposure to ingested arsenic from artesian well-water, showing a relative risk of 2.1 (95% CI, 1.1–4.2) for those who had a cumulative exposure to arsenic ≥ 17 mg/L–years compared with those who had a lower cumulative exposure (< 17 mg/L–years).

(viii) *Study of oxidative stress in humans*

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is generated by the hydroxyl radical (Kasai & Nishimura, 1984) or singlet oxygen (Devasagayam *et al*., 1991) or by direct electron transfer, which does not involve the participation of any reactive oxygen species (Kasai *et al*., 1992). 8-OHdG is considered to be one of the main indicators of oxidative damage to DNA and may cause mutation (G:C→T:A) during DNA replication (Shibutani *et al*., 1991).

Matsui *et al*. (1999) investigated whether neoplastic and precancerous skin lesions of arsenic-exposed individuals are under oxidative stress using 8-OHdG as a marker. Biopsy samples of arsenic keratosis, arsenic-induced Bowen disease and arsenic-induced Bowen carcinoma arising in areas not exposed or less exposed to the sun were obtained from 28 individuals (aged 26–83 years) living in areas where chronic arsenicism was endemic in either Taiwan, China, Thailand or Japan. The presence of 8-OHdG was studied 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 Bowen disease unrelated to arsenic. The frequency of 8-OHdG-positive cases was significantly higher in arsenic-related skin neoplasms (22/28; 78%) than in Bowen disease unrelated to arsenic (1/11; 9%) ($p < 0.001$ by chi-square test). 8-OHdG was also detected in normal tissue adjacent to the arsenic-related Bowen disease lesion. Furthermore, arsenic was detected by neutron activation analysis in deparaffined skin tumour samples of arsenic-related disease (four of five, 80%), whereas it was not detected in control samples. The results may suggest the involvement of reactive oxygen species in arsenic-induced human skin cancer.

Wu *et al*. (2001) reported an association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity among 64 subjects aged 42–75 years from an arsenic-contaminated area in north-eastern Taiwan, China. The blood level of arsenic determined by HG–AAS ranged from undetectable to 46.5 μg/L. The capacity of subjects to methylate arsenic was determined by speciation of inorganic arsenic and its metabolites in urine using high-performance liquid chromatography (HPLC) linked with HG–AAS. The plasma level of reactive oxidants was determined by a chemiluminescence method using lucigenin as an amplifier for measuring superoxide anion (O_2^-), while the plasma level of antioxidant capacity was measured by the 2,2′-azino-di[3-ethylbenzthiazoline]-sulfonate method. There was a positive association between blood arsenic level and plasma level of reactive oxidants ($r = 0.41$; $p = 0.001$) and a negative association with plasma level of antioxidant capacity ($r = –0.30$; $p = 0.014$). Categorical analysis showed that greater

primary capacity for arsenic methylation was correlated with a higher plasma level of antioxidant capacity ($p = 0.029$).

(ix) *Other*

Generalized weakness and fatigue have been reported in people chronically exposed to arsenic-contaminated drinking-water (Zaldívar & Guillier, 1977; Saha, 1984; Guha Mazumder *et al*., 1988, 1992; Kilburn, 1997; Guha Mazumder *et al*., 1998; Guha Mazumder, 2001b; Guha Mazumder *et al.*, 2001b). Conjunctival congestion and non-pitting oedema of the legs and hands have also been reported in patients with chronic arsenic toxicity in West Bengal, India, and Bangladesh (Ahmad *et al*., 1997; Chowdhury *et al*., 1997; Guha Mazumder *et al*., 1998b; Ahmad, S.A. *et al*., 1999).

García Vargas *et al*. (1994) carried out a detailed study of the urinary excretion pattern of porphyrins in humans chronically exposed to arsenic via drinking-water in Mexico using HPLC. Thirty-six individuals (15 men and 21 women) were selected from a town which had 0.40 mg/L arsenic in the drinking-water. The control group consisted of 31 individuals (13 men and 18 women) whose concentration of arsenic in the drinking-water was 0.02 mg/L. 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 coproporphyrin III/coproporphyrin I ratio and (*b*) significant increases in uroporphyrin excretion. Both alterations were responsible for the decrease in the urinary coproporphyrin/uroporphyrin ratio. No porphyrinogenic response was found in individuals with urinary concentrations below 1 mg arsenic/g creatinine. However, as arsenic concentrations exceeded this value, the excretion of porphyrins (except coproporphyrin III) increased proportionally, and most of the individuals with high urinary arsenic concentrations had alterations in porphyrin ratios and also presented cutaneous signs of chronic arsenic poisoning. The prevalence of clinical signs of arsenicism showed a direct relationship with both concentration of arsenic in urine and time-weighted exposure to arsenic. A direct relationship between time-weighted exposure to arsenic and alterations in urinary porphyrin excretion ratios was also observed. These alterations in arsenic-exposed individuals are compatible with a lower activity of uroporphyrinogen decarboxylase, the enzyme that converts the substrate uroporphyrinogen to a coproporphyrinogen product.

Except for anaemia, no haematological abnormality (in differential lymphocyte count or in the levels of blood sugar, urea or creatinine) has been described in cases of chronic toxicity caused by drinking arsenic-contaminated water (Guha Mazumder *et al*., 1988, 1997, 1998b, 1999). Haematological consequences of subacute and chronic arsenic toxicity have been reviewed extensively (National Research Council, 1999).

4.2.2 *Experimental systems*

(*a*) *Acute toxicity*

The acute toxicity of arsenic is related to its chemical form and oxidation state. The LD_{50} (50% lethal dose) values of several arsenicals in laboratory animals have been

reviewed (Hughes, 2002). In mice, the oral lethal dose of arsenic trioxide varies from 15 to 48 mg/kg bw. In contrast, the lethal dose range of inorganic arsenic in adult humans is estimated at 1–3 mg/kg bw (Hughes, 2002). A basic tenet is that the acute toxicity of trivalent arsenic is greater than that of pentavalent arsenic. For example, in mice, the oral LD_{50} of arsenic trioxide is more than 36-fold lower than that of MMA^{V}. However, MMA^{III} has been found to be more toxic than trivalent arsenic. When MMA^{III} or sodium arsenite was administered intraperitoneally to hamsters, the LD_{50}s were found to be 29.3 and 112.0 μmol/kg bw, respectively (Petrick *et al*., 2001).

(*b*) *Chronic toxicity*

Many different systems within the body are affected by chronic exposure to inorganic arsenic. Arsenates can replace phosphate in many biochemical reactions because they have similar structure and properties. Arsenate uncouples in-vitro formation of adenosine-5′-triphosphate (ATP) by a mechanism termed arsenolysis. In the substrate, arsenolysis may occur during glycolysis. ATP is generated during glycolysis in the presence of phosphate (substrate phosphorylation), but not arsenate. In the mitochondria, arsenolysis may occur during oxidative phosphorylation. Adenosine-5′-diphosphate (ADP)-arsenate is synthesized by submitochondrial particles from ADP and arsenate, in the presence of succinate. ADP-arsenate hydrolyses easily compared with ADP-phosphate, which is formed during oxidative phosphorylation. In both the substrate and the mitochondria, arsenolysis diminishes in-vitro formation of ATP by the replacement of phosphate with arsenate in the enzymatic reactions. Depletion of ATP by arsenate has been observed in cellular systems: ATP levels are reduced in rabbit and human erythrocytes after in-vitro exposure to arsenate (rabbits, 0.8 mM; humans, 0.01–10 mM) (Delnomdedieu *et al.*, 1994a; Winski & Carter, 1998; Hughes, 2002).

Trivalent arsenic reacts readily *in vitro* with thiol-containing molecules such as GSH and cysteine (Scott *et al.*, 1993; Delnomdedieu *et al.*, 1994b). In rat red blood cells, As^{III} forms mixed complexes with protein and GSH, and the main protein-binding species is haemoglobin (Winski & Carter, 1995). Binding of MMA^{III} and DMA^{III} to protein *in vitro* occurs to a greater extent than with the pentavalent organic forms (Styblo *et al.*, 1995). Arsenite has a higher affinity for dithiols than monothiols, as shown by the highly favoured transfer of arsenite from a $(GSH)_3$–arsenic complex to the dithiol 2,3-dimercaptosuccinic acid (Delnomdedieu *et al.*, 1993). The binding of trivalent arsenic to critical thiol groups may inhibit important biochemical events that could lead to toxicity (Hughes, 2002).

(i) *Mitochondrial damage*

Hepatic phosphate resonances were evaluated by Chen *et al*. (1986) *in vivo* by ^{31}P nuclear magnetic resonance spectroscopy following a single intravenous dose of sodium arsenite (10 mg/kg bw) in rats. Acute in-vivo administration of arsenite rapidly decreased intracellular pools of ATP with concomitant increases in inorganic phosphate and phosphomonoesters (phosphocholine and adenosine monophosphate). Glycerolphosphorylcholine and glycerolphosphorylethanolamine were also increased. The data suggest

that liver cannot compensate for the rapid loss of nicotinamide-adenine dinucleotide (NAD)-linked substrate oxidation via other metabolic pathways, such as glycolysis, for the production of ATP.

Arsenic fed to laboratory animals is known to accumulate in the mitochondria and has been related to the swelling of this subcellular organelle in a number of tissues, especially the liver (Fowler *et al.*, 1977, 1979). It has been suggested that the effects of arsenic on mitochondrial utilization of pyruvate results from arsenic binding to the lipoic acid and dithiol moieties of the pyruvate dehydrogenase (PDH) complex. The initial step in the mitochondrial metabolism of pyruvate, which is catalysed by the PDH–enzyme complex, involves the formation of acetyl-coenzyme A (CoA) and the generation of CO_2 and hydrogenated NAD (NADH). This complex is composed of three enzymes: pyruvate decarboxylase (PDH), dehydrolipoate transacetylase and dihydrolipoate dehydrogenase. The latter two enzymes contain dithiol moieties. Pyruvate decarboxylase is regulated by inactivation and activation reactions, which are controlled by phosphorylation/dephosphorylation. Phosphorylation and the concomitant inactivation of PDH is catalysed by a Mg-ATP-requiring kinase, and dephosphorylation and concomitant reactivation is catalysed by a Mg^{2+}- and Ca^{2+}-requiring phosphatase. In order to examine whether this phosphorylation/dephosphorylation is a mechanism of action for arsenic, PDH activities, before and after in-vitro activation with Mg^{2+}, were measured in tissue from animals fed arsenic (Schiller *et al*., 1977). Adult male Charles River CD rats were given deionized drinking-water containing 0, 20, 40, and 85 mg/L arsenic as sodium arsenate (As^V) for 3 and 6 weeks. PDH activity was assayed in the liver tissue obtained from the animals. After 3 weeks, the effects of arsenic at the highest dose were pronounced compared with the basal activity (before activation), with up to 47.5% inhibition of the control values. The total PDH activity (after activation) was inhibited by 13.5, 15.3 and 27.6% of the control values at 20, 40 and 85 mg/L sodium arsenate, respectively. A similar pattern of inhibition of PDH activity was observed at 6 weeks, although the inhibition was lower at the highest dose. This pattern may be indicative of mitochondrial regeneration at the highest dose after 6 weeks (Schiller *et al.*, 1977). The possible metabolic effects of this inhibition are a decrease in acetyl-CoA formation, which leads to a decrease in carbon flow through the tricarboxylic acid cycle, and a decrease in the citrate available to allow mitochondria to supply acetyl-CoA for fatty acid synthesis, which in turn results in fewer storage triglycerides.

Petrick *et al*. (2001) have compared the in-vivo toxicity of MMA^{III} and arsenite in hamsters. Groups of six male golden Syrian hamsters, 11–12 weeks old and weighing 100–130 g, were injected intraperitoneally with MMA^{III} oxide or sodium arsenite. Inhibition of PDH activity of the hamster kidney or purified porcine heart by MMA^{III} or arsenite was determined. To inhibit PDH activity of hamster kidney by 50%, concentrations (mean ± SE) of 59.9 ± 6.5 μM MMA^{III} as methylarsine oxide, 62.0 ± 1.8 μM MMA^{III} as diiodomethylarsine and 115.7 ± 2.3 μM arsenite were needed. To inhibit in-vitro PDH activity of the purified porcine heart by 50%, concentrations (mean ± SE) of 17.6 ± 4.1 μM MMA^{III} as methylarsine oxide and 106.1 ± 19.8 μM arsenite were needed. These data demonstrate that MMA^{III} is more toxic than inorganic arsenite, both *in vivo* and *in vitro*.

Brown *et al*. (1976) demonstrated alteration of normal ultrastructure and respiratory ability of proximal renal tubules following administration of arsenate. Groups of male Sprague-Dawley rats weighing 70–150 g were fed laboratory chow. The control groups received deionized water while the experimental groups received 40, 85 or 125 mg/L arsenic as sodium arsenate in deionized water (n = 28, 11, 7 and 10 in each group, respectively). After 6 weeks, the rats were killed in pairs of one experimental rat and a control rat matched by weight. The kidneys were excised and the capsule removed; combined oxygen electrode and electron microscopic studies were conducted. Decreased state 3 respiration and respiratory control ratios were observed in kidneys of rats given the 85- and 125-mg/L dose levels. Ultrastructural alterations, which consisted of swollen mitochondria and an increased number of dense autophagic lysosome-like bodies, were confined to proximal tubule cells of animals at all dose levels of arsenic.

Fowler *et al*. (1977) carried out investigations to delineate the subcellular manifestations of arsenic toxicity following chronic exposure using combined ultrastructural and biochemical techniques. Four groups of 18 male Charles River CD rats were fed a casein-based purified diet and had access to deionized drinking-water containing 0, 20, 40 or 85 mg/L arsenic as sodium arsenate (As^{v}) for 6 weeks. At the end of this period, three animals from each group were killed and the livers removed. Mitochondrial respiration studies were conducted. Extensive in-situ swelling of liver mitochondria and matrix rarification with lipidic vacuolation were the most prominent ultrastructural changes observed at 40- and 80-mg/L As^{V} dose levels. Mitochondrial respiration studies indicated decreased state 3 respiration and respiratory control ratios for pyruvate/malate- but not succinate-coupled 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 an increase in specific activity of 150–200%. Activity of malate dehydrogenase, which is localized in the mitochondrial matrix, remained unchanged at all dose levels. 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 (such as haeme synthesis, carbohydrate metabolism and fatty acid synthesis), 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 has further been demonstrated (Fowler *et al*., 1979). Two groups of male Charles River CD rats were fed a casein-based semipurified diet for 6 weeks and had access to deionized drinking-water containing 0 or 40 mg/L arsenic as sodium arsenate. Ultrastructural morphometric and biochemical studies were conducted on hepatic mitochondria. Morphometric analysis disclosed an overall 1.2-fold increase in the relative mitochondrial volume density and a 1.4-fold increase in the surface density of the inner mitochondrial membrane plus cristae of arsenate-exposed rats. These observations suggest that arsenate-mediated perturbation of mitochondrial membrane integrity compromises the mechanisms of normal ion

transport. These structural changes were associated with a perturbation of mitochondrial protein synthesis as expressed by a 1.5-fold increase in [^{14}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 behaviour with depression of NAD^+-linked substrate oxidation and a subsequent approximately two-fold in-vivo increase in the mitochondrial NAD^+ to $NADH^+$ ratio. Observed changes in mitochondrial membranes from arsenate exposure also resulted in 1.5–2-fold increases in the specific activities of the membrane marker enzymes monoamine oxidase, cytochrome c oxidase and Mg^{2+}-ATPase, which are localized in both inner and outer mitochondrial membranes. Activity of malate dehydrogenase, which is localized in the mitochondrial matrix, was unchanged.

Larochette *et al*. (1999) investigated whether arsenic compounds act on mitochondria to induce apoptosis. The mechanisms by which arsenic induces apoptosis are not clear. U937 cells transfected with an SFFV.neo-vector containing the human *bcl-2* gene coding for apoptosis-inhibitory protein or the neomycin-resistance gene (*Neo*) only, or 2B4.11T cell hybridoma cells ($1–5 \times 10^5$/mL) were incubated with variable doses of sodium arsenite, sodium arsenate, phenylarsine oxide, *para*-arsanilic acid or 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195) and 100 μM of the caspase inhibitors *N*-benzyloxycarbonyl-Val-Ala-Asp.fluoromethylketone (Z-VAD.fmk) and *tert*-butyloxycarbonyl-Asp.fluoromethylketone (Boc-D.fmk) and 100 μM of the cathepsin inhibitor *N*-benzyloxycarbonyl-Phe-Ala.fluoromethylketone (Z-FA.fmk). Arsenite induced apoptosis accompanied by a loss of mitochondrial transmembrane potential ($\Delta\Psi_m$). Inhibition of caspases by Z-VAD.fmk and Boc-D.fmk prevented arsenite-induced nuclear DNA loss, but had no effect on the $\Delta\Psi_m$ dissipation and cytolysis induced by arsenite, suggesting that arsenite might cause necrosis when the caspase pathway is blocked. In contrast, *bcl-2* expression induced by gene transfer prevented all hallmarks of arsenite-induced cell death (such as generation of reactive oxygen species, hypoploidy and loss of viability), including the collapse of $\Delta\Psi_m$. PK11195, a ligand of the mitochondrial benzodiazepine receptor, neutralized the bcl-2-mediated resistance to arsenite. Mitochondria were required in a cell-free system (isolated nuclei *in vitro*) to mediate arsenite-induced nuclear apoptosis. Arsenite caused the release of an apoptosis-inducing factor from the mitochondrial intermembrane space. This effect was 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-permeabilized liposomal membranes contained the purified, reconstituted PT pore complex. Bcl-2 also inhibited the arsenite-triggered opening of the PT pore in the reconstituted system. As a control, a mutant bcl-2 $\Delta\alpha$5/6 protein, which had lost its anti-apoptotic function as a result of the deletion of a putative membrane insertion domain, failed to prevent arsenite-induced PT pore opening. Together these data suggest that arsenite could induce apoptosis via a direct effect on the mitochondrial PT pore.

(ii) *Urinary porphyrins, haeme biosynthetic enzyme activities, haeme metabolism and 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, such as δ-aminolevulinate (ALA) synthase (ALA-S), porphobilinogen deaminase, uroporphyrinogen III synthase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase, ferrochelatase and haeme oxygenase (H-O). The urinary porphyrins and several haeme enzymes can be used as early biomarkers of arsenic toxicity (García-Vargas & Hernández-Zavala, 1996).

Rodents exposed for 6 weeks to sodium arsenate in drinking-water showed a substantial increase in the urinary excretion of porphyrins, with excretion of uroporphyrin exceeding that of coproporphyrin (Woods & Fowler, 1978). Groups of 12 male Sprague-Dawley rats (CD strain) (150–200 g) or male C57 BL mice (20–30 g) were given access to laboratory chow and deionized drinking-water containing 0, 20, 40 or 85 mg/L arsenic as sodium arsenate (As^{v}) for up to 6 weeks. Livers of animals were homogenized and mitochondria and microsomal fractions were then prepared. Continuous prolonged exposure to sodium arsenate resulted in depression of hepatic δ-aminolevulinate synthase and haeme synthase, the first and last enzymes in haeme biosynthesis, respectively, in both rats and mice. ALA-S was maximally depressed to approximately 80% of control values at 40 mg/L in both species, whereas haeme synthase activity was maximally decreased to 63 and 75% of control at 85 mg/L in rats and mice, respectively. Uroporphyrinogen I synthase, the third enzyme in haeme biosynthesis, was increased at all doses in mice, whereas ALA dehydratase, the second haeme biosynthetic pathway enzyme, was unaltered in either species. Concomitantly, urinary uroporphyrin concentrations were increased up to 12-fold and coproporphyrin levels up to 9-fold the control values in rats. Similar patterns of increased porphyrin excretion were seen in mice. In contrast, no changes were observed in the activities of cytochrome oxidase or cytochrome P450, indicators of mitochondrial and microsomal haemoprotein function, respectively. These results demonstrate that prolonged exposure to low levels of arsenic results in selective alteration of hepatic haeme biosynthetic pathway enzymes, with concomitant increases in urinary porphyrin concentrations.

Cebrián *et al*. (1988) demonstrated that sodium arsenite is a potent inducer of H-O, which is the rate-limiting enzyme of haeme degradation. Male Wistar albino rats were fasted for 24 h before treatment and until they were killed. Animals received 0.1 mL sodium chloride (0.9%, w/v), or arsenic salts by subcutaneous injection. The doses of As^{III} were 12.5, 25, 50, 75 and 100 μmol/kg bw and those of As^{V} were 25, 50, 100, 150 and 200 μmol/kg bw. Animals were killed 16 h after injection. In a subchronic study, animals were exposed to As^{III} 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 h before sacrifice. The livers were excised, perfused and homogenized, and tryptophan pyrrolase (TP), ALA-S and H-O activities were measured. Cytochrome P450 and *b5* contents were also measured. Acute administration of arsenic produced a decrease in the haeme saturation of TP in rat liver,

accompanied by dose-related increased ALA-S and H-O activities, and a corresponding decrease in cytochrome P450 concentration. The decrease in the haeme saturation of TP indicates that arsenic reduced the content of cytosolic haeme in liver cells and that the increase in hepatic ALA-S activity appears to be in response to a reduction in haeme availability. The alteration in the relationship between haeme synthesis and degradation is a result of treatment with arsenic. The magnitude of these effects was related to the oxidation state of arsenic: sodium arsenite (As^{III}) was more potent than sodium arsenate (As^{V}). These results support the suggestion that haeme saturation of TP is sensitive to treatments that modify liver haeme concentration. The increase in H-O activity produced by arsenic appears to be mediated by a mechanism largely or entirely independent of haeme. Indeed, there were no indications of an increase in the free haeme pool that could trigger a positive feedback on H-O. On the contrary, it appears that one reason for the reduction in haeme saturation was the increase in H-O activity. Moreover, the concomitant increase in ALA-S activity was a further indication of cellular depletion of haeme. The main effects of continuous exposure to As^{III} were an initial decrease in the haeme saturation of TP, which remained constant during the period of treatment, and an initial increase in ALA-S activity, which after 10 days of exposure dropped somewhat but remained above control values. No significant effects on H-O or P450 activity were observed. These results were interpreted as being indicative that a new balance between haeme synthesis and degradation had been reached and that an adaptive response to the subchronic effects of As^{III} was taking place.

(iii) *Arsenic and oxidative stress*

Among the various proposed mechanisms by which arsenic induces cancer, oxidative damage may play a role in arsenic-induced carcinogenesis. Exposure to arsenite, arsenic trioxide or arsenate has been reported to result in the generation of reactive oxygen species in laboratory animals or in cultured animal and human cells by many investigators (Wang *et al*., 1996; Chen *et al*., 1998; Hei *et al*., 1998; Ahmad *et al*., 2000; Lynn *et al*., 2000; Chouchane & Snow, 2001; Liu, S.X. *et al*., 2001). The topic has been reviewed by Del Razo *et al*. (2001b), Ercal *et al*. (2001), and Thomas *et al*. (2001).

Arsenic-induced free-radical formation was indicated by Yamanaka *et al*. (1991), who studied cellular response in the lung induced by the administration of DMA^{V}, and in particular the enzymes that participate directly in protective reactions against active oxygen species, superoxide dismutase, catalase and GSH peroxidase (GPx). Male ICR mice, weighing approximately 25 g, were given an oral dose of 1500 mg/kg bw DMA^{V} after fasting for several hours. The activities of mitochondrial superoxide dismutase, GPx and glucose-6-phosphate dehydrogenase (G6PDH) significantly increased at 6 h or longer after dosing, whereas cytosolic superoxide dismutase and catalase were not. Furthermore, the NADPH levels were markedly decreased at 6–9 h after treatment with DMA^{V} while $NADP^{+}$ levels increased, resulting in a marked reduction in the NADPH/$NADP^{+}$ ratio. This change, accompanied by an increase in G6PDH activity, indicates that the pentose-phosphate pathway is activated by the oxidation of reduced GSH with hydrogen peroxide

(H_2O_2). With regard to cellular sulfhydryls, after treatment with DMA^V, levels of GSH and non-protein sulfhydryls were decreased and levels of oxidized GSH (GSSG) remained constant, whereas those of mixed disulfides were significantly increased. These cellular variations suggest that mouse pulmonary cells produced reduced oxygen species, that is, superoxide anion radical, hydrogen peroxide and subsequent radicals in the metabolism of DMA^V, and that these and the dimethylarsenic peroxyl radical were responsible for pulmonary DNA damage, the diethylarsenic peroxyl radical probably being produced from the reaction of molecular oxygene and dimethylarsine (Yamanaka *et al.*, 1990, 2001). The same investigators had demonstrated previously that oral administration of DMA^V, the main metabolite of inorganic arsenic, induces lung-specific DNA damage in mice. An in-vitro experiment indicated that the breaks were not caused directly by DMA^V but by DMA^{III}, a further metabolite of DMA. They hypothesized that this damage was partially due to the active oxygen species produced in the metabolism of DMA^V (Yamanaka *et al.*, 1989). In a further study, the authors had shown that oral administration of DMA to mice significantly enhanced the amounts of 8-oxo-2′-deoxyguanosine (8-oxodG) specifically in target organs of arsenic carcinogenesis (skin, lung, liver and urinary bladder) and in urine. The dimethyl arsenics thus may play an important role in the carcinogenesis of arsenic through the induction of oxidative damage, particularly of base-oxidation (Yamanaka *et al.*, 2001).

Ahmad, S. *et al*. (1999) investigated the biochemical effects of exposure to DMA in $B6C3F_1$ mice using six biochemical parameters: DNA damage, GSH and GSSG content, cytochrome P450 content, ornithine decarboxylase (ODC) activity in liver and/or lung and ALT activity in serum. GSH was selected as an important constituent for cellular protection against oxidative damage by free radicals and the three enzymes were employed as biological markers of cell proliferation and promotion of carcinogenesis. Groups of 10 or 12 adult female $B6C3F_1$ mice received DMA^V at a dose of 720 mg/kg bw by oral gavage at one of three times (2 h, 15 h or at both 21 and 24 h) before sacrifice. Four or five control mice were run on each of 5 experimental days and received distilled water alone. Significant ($p < 0.05$) decreases in liver GSH and GSSG contents (15–37%) were observed. Pulmonary and hepatic ODC activities were reduced (19–59%) by treatment with DMA^V. A significant decrease in hepatic cytochrome P450 content (21%) was observed only in the group treated at both 21 and 24 h before sacrifice. The mouse serum ALT activity was not reduced after in-vivo administration of DMA^V but the addition of 2.8, 28 and 280 mM DMA^V *in vitro* reduced ALT activity by 0, 8 and 6.5%, respectively.

Santra *et al*. (2000) examined the hepatic effects of chronic ingestion (for up to 15 months) of drinking-water containing arsenic (1:1 arsenite to arsenate) at 3.2 mg/L in male BALB/c mice (5–14 experimental animals, 5–10 control animals). Groups of arsenic-exposed mice and unexposed controls were killed at 3, 6, 9, 12 and 15 months for examination of hepatic histology and certain biochemical parameters of oxidative stress. Statistically significant decrements in body weight were observed in the exposed animals at 12 months and 15 months, without significant differences in the amount of food or water consumption between exposed and control groups. No abnormal hepatic morpho-

logy was observed by light microscopy during the first 9 months of exposure to arsenic, but at 12 months, 11/14 mice in the experimental group exhibited hepatocellular degeneration and focal mononuclear cell collection. After 15 months, exposed mice displayed evidence of hepatocellular necrosis, intralobular mononuclear cell infiltration, Kupffer cell proliferation and portal fibrosis. Hepatic morphology was normal in all control mice. Biochemical changes, consistent with oxidative stress, preceded the overt histological pathology. Hepatic GSH was significantly reduced after 6 months, in a time-related manner; the hepatic activities of enzymes related to GSH homeostasis, namely G6PDH, GST, GSH reductase, GPx and catalase were also reduced in a time-related manner (at 9, 12 and 15 months). There was a progressive, time-dependent increase in lipid peroxidation, as demonstrated by increased production of malondialdehyde, and concomitant time-dependent damage to hepatocellular plasma membranes, as demonstrated by decreases in membrane Na^+/K^+ ATPase activity. Depletion of GSH may result in the accumulation of free radicals that initiate lipid peroxidation and biochemical damage by covalent binding to macromolecules. Biochemical changes observed in this long-term in-vivo animal feeding experiment suggest that the adverse histological effects of arsenic on the liver may be mediated through oxidative stress.

Ishinishi *et al*. (1980) studied the chronic toxicity of arsenic trioxide in rats with special reference to liver damage. Four groups of male adult Wistar rats were given distilled water containing 0, 0.125, 12.5 or 62.5 ppm arsenic trioxide orally for 7 months and were thereafter given distilled water with no arsenic trioxide for 4 months. Despite no difference in growth among the four groups of rats, chronic exposure to arsenic trioxide induced not only liver injury but also dose-dependent proliferation of the bile duct with chronic angitis. The liver injury was 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*. (1999) demonstrated hepatic fibrosis and fibrogenesis following chronic ingestion of arsenic in Swiss albino mice fed arsenic daily (120, 240, 360 or 500 mg/L). A significant increase in hepatic collagen and its deposition in the extracellular matrix, an expression of hepatic fibrosis, were seen in arsenic-treated mice compared with controls. Hepatic 4-hydroxyproline levels, indicative of fibrogenesis, were increased four- to 14-fold with different doses of arsenic compared with controls.

Effects on levels of GSH and some related enzymes in tissues after acute exposure to arsenic were studied in rats by Maiti and Chatterjee (2001). Male Wistar rats, maintained on either an 18% or 6% protein (casein) diet, received an intraperitoneal injection of sodium arsenite at its LD_{50} dose (15.86 mg/kg bw). One hour after exposure to arsenic, the GSH concentration was significantly depleted and lipid peroxidation was increased in both the high- and low-protein diet groups. Acute exposure to arsenic significantly increased GPx activity in the liver in both groups. GST activity was significantly decreased in the liver of the animals fed 18% protein, whereas it increased in the kidneys of both groups. No significant change in GSH reductase or G6PDH activity in the liver and kidneys was observed. In this study, liver as a whole seemed to be more affected in terms of level of

GSH and GST activity. The animals fed 6% protein appeared to be less affected in terms of tissue arsenic concentration, level of GSH, level of lipid peroxidation and GST activity compared with those fed 18% protein. This might be due to the deficiency in tissues of possible target proteins for arsenic binding and a lesser availability of specific amino acid to synthesize different stress proteins in the animals fed 6% protein.

As reviewed by Del Razo *et al.* (2001b), a variety of genes related to base excision repair and oxidative stress are commensurately up-regulated by nanomolar concentrations of inorganic arsenic. Reactive oxygen species induced by low levels of As^{III} or As^{V} increase the DNA-binding activity of activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) in cultured aortic endothelial cells (Barchowsky *et al*., 1996), human MDA-MB-435 breast cancer and rat H411E hepatoma cells (Kaltreider *et al.*, 1999) and precision-cut lung slices (Wijeweera *et al.*, 2001). This results in stimulation of cell proliferation and up-regulation of gene expression including that of mdm2 protein, which is a key regulator of the critical tumour-suppressor gene *p53* (Germolec *et al.*, 1996; Hamadeh *et al.*, 1999). In contrast, high levels of inorganic arsenic inhibit the activation of NF-κB and cell proliferation and induce apoptosis in human acute myelogenous leukaemia cells, human embryonic kidney (HEK 293) cells and human bronchial epithelial (BEAS 2B) cells (Estrov *et al.*, 1999; Roussel & Barchowsky, 2000). Based on results obtained in NIH 3T3 cells exposed to arsenic, Chen *et al.* (1998) have suggested that apoptosis is triggered by generation of H_2O_2 through the activation of flavoprotein-dependent superoxide-producing enzymes (e.g. NADPH oxidase) and the increase in superoxide levels in cells. The event probably acts as a mediator to induce apoptosis through the release of cytochrome c from the mitochondria to cytosol, the activation of caspase 3 and the degradation of poly(ADP-ribose) polymerase (PARP) leading to DNA fragmentation (Chen *et al.*, 1998).

(iv) *Stress proteins*

Exposure to arsenicals either *in vitro* or *in vivo* in a variety of model systems has been shown to induce a number of the major stress protein families such as heat-shock proteins. Among them are members with a low molecular weight, such as metallothionein and ubiquitin, and others with masses of 27, 32, 60, 70, 90 and 110 kDa. In most cases, the induction of stress proteins depends on the capacity of the arsenic compound to reach the target, its valence and the type of exposure, with arsenite being the strongest inducer of most heat-shock proteins in several organs and systems. Induction of heat-shock proteins is a rapid dose-dependent response (1–8 h) to acute exposure to arsenite. Thus, the stress response appears to be useful for monitoring toxicity resulting from a single exposure to arsenite. The capacity of arsenic compounds to modulate the expression and/or accumulation of stress proteins has been studied in normal and transformed cell lines by Caltabiano *et al*. (1986), Keyse and Tyrrell (1989), van Wijk *et al*. (1993), Wu and Welsh (1996) and Wijeweera *et al*. (2001) and has been reviewed by Bernstam and Nriagu (2000) and Del Razo *et al*. (2001b).

Metallothionein is a low-molecular-weight, cysteine-rich, metal-binding protein that has been propounded 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, it is a small protein easily induced by heavy metals, hormones, acute stress and a variety of chemicals. Twenty of the 61 amino acid residues in mettalothionein molecules are cysteinyl residues, all of which are involved in metal binding (Sato & Bremner, 1993; National Research Council, 1999).

The induction of metallothionein is observed following oral administration; the doses of organic arsenic compounds (MMA and DMA) required for its induction are one order higher than those of inorganic arsenic compounds (As^{III} and As^{V}). Only a small portion of the arsenic dose was found to be associated with the metallothionein fraction, which there fore does not protect against arsenic toxicity by binding the metal (Maitani *et al*., 1987). Rather, because of its high sulfhydryl content, it has also been suggested that metallothionein reacts with organic free radicals and electrophiles (Klaassen & Cagen, 1981). Indeed, metallothionein can serve as a sacrificial scavenger for superoxide and hydroxyl radicals *in vitro* (Thornalley & Vašák, 1985). It is induced by metal chemicals that produce oxidative stress (Bauman *et al*., 1993) and has been shown to protect against oxidative damage (Sato & Bremner, 1993).

The effect of various arsenic forms on the tissue concentrations of metallothionein was determined in male CF-1 mice (25–30 g) injected subcutaneously with various doses of As^{III} (55–145 μmol/kg bw), As^{V} (165–435 μmol/kg bw), MMA (100–7250 μmol/kg bw) or DMA (2750–10 250 μmol/kg bw) (Kreppel *et al*., 1993). Controls were injected with an equal volume (0.01 mL/g bw) of saline. Metallothionein content in hepatic cytosol was quantified by the cadmium–haemoglobin assay. As^{III} was found to be a potent inducer of hepatic metallothionein, producing a 30-fold increase at a dose of 85 μmol/kg. In comparison, it took three-, 50- and 120-fold higher molar amounts of As^{V}, MMA and DMA, respectively, to produce a similar effect. MMA produced the largest increase in hepatic metallothionein (80-fold), followed by As^{III} (30-fold), As^{V} (25-fold) and DMA (10-fold). However, none of the compounds induced metallothionein in mouse primary hepatocyte cultures, suggesting that arsenicals may be considered as indirect inducers of metallothionein. Both metallothionein-I (MT-I) and metallothionein-II (MT-II) protein isoforms were commensurately induced by As^{III}, As^{V} and MMA. Induction of metallothionein by As^{III} was further characterized following subcutaneous administration of arsenite (85 μmol/kg). Induction of hepatic metallothionein peaked at 24 h. As^{III} also increased metallothionein in kidney, spleen, stomach, intestine, heart and lung and the most marked increase occurred in the liver. MT-I mRNA increased 24-, 52- and 11-fold at 3, 6 and 15 h after administration of As^{III}, respectively. This induction profile is similar to that observed after exposure to zinc or cadmium. This study showed that arsenic compounds are effective inducers of metallothionein *in vivo* and that their potency and efficacy are dependent on the chemical form of arsenic. As^{III} is a potent inducer of hepatic metallothionein for both MT-I and MT-II and this effect is associated with an increase in metallothionein mRNA, suggesting that the mechanism of this induction appears to be due, at least in part, to increased metallothionein gene transcription.

In a recent study, Liu *et al*. (2000) demonstrated that MT-I/II-null mice are more sensitive than wild-type mice to the hepatotoxic and nephrotoxic effects of chronic oral administration or injection of inorganic arsenicals. Groups of 4–6 male and female MT-I/II-null mice and corresponding wild-type mice, aged 6–8 weeks, were provided drinking-water containing As^{III} at concentrations of 7.5, 22.5 or 45 mg/L, or As^{V} at concentrations of 37.5 or 75 mg/L, or were injected subcutaneously in the dorsal thoracic midline with 10 mL/kg bw saline containing As^{III} at doses of 10 and 30 µmol/kg bw or As^{V} at a dose of 100 µmol/kg bw once daily on 5 days per week for 15 weeks. Control mice received tap-water or were injected with the same volume of saline. Chronic exposure to arsenic produced only modest increased tissue concentrations of metallothionein (two- to fivefold) in wild-type but not in MT-null mice, either following repeated injections or following oral administration. Arsenic by both routes produced damage to the liver (fatty infiltration, inflammation and focal necrosis) and kidney (tubular cell vacuolization, inflammatory cell infiltration, glomerular swelling, tubular atrophy and interstitial fibrosis) in both MT-null and wild-type mice. However, in MT-null mice, the pathological lesions were more frequent and severe compared with those in wild-type mice in either liver or kidney. This was confirmed biochemically, in that, at the higher oral doses of As^{V}, the levels of blood urea nitrogen, an indicator of kidney injury, were increased to a greater extent in MT-null mice (60%) than in wild-type mice (30%). However, As^{III} resulted in elevated levels of blood urea nitrogen in MT-null mice only. Chronic exposure to arsenic produced a two- to 10-fold increase in levels of serum interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), with greater increases seen after repeated injections than after oral exposure; again, MT-null mice had higher levels of serum cytokines than wild-type mice, following repeated injections of arsenic, but not after oral exposure. Repeated injections of arsenic also decreased hepatic GSH up to 35% but had no effect on hepatic GPx or GSH reductase activities. MT-null mice were more sensitive than wild-type mice to the effect of GSH depletion by As^{V}. Hepatic caspase 3 activity was increased (two- to threefold) in both wild-type and MT-null mice, indicating apoptotic cell death. The study demonstrated that chronic exposure to inorganic arsenic produced injuries to multiple organs, and that MT-null mice are generally more susceptible than wild-type mice to arsenic-induced toxicity regardless of route of exposure, suggesting that metallothionein could be a cellular factor in protecting against chronic arsenic toxicity.

Kato *et al*. (2000) reported from earlier studies the induction and accumulation of heat-shock protein-72 (Hsp72) in the cell nuclei of human alveolar type II (L-132) cells and DNA damage following exposure to DMA^{V} (Kato *et al.*, 1997). They also found that the accumulation of Hsp72 in cell nuclei was related to the suppression of apoptosis (Kato *et al.*, 1999). Referring to reports indicating that Hsp72 might be involved in the tumorigenic process through the function of apoptosis (Jäättelä, 1999), they assumed that Hsp72 induced by dimethylarsenics may play an important role in DNA damage and tumorigenesis. They therefore investigated whether Hsp72 was induced and accumulated in the lung, a target organ for tumorigenesis, following administration of DMA^{V} to mice. Five-week-old male A/J mice were injected intraperitoneally with DMA^{V} (100–600 mg/kg) or

arsenite (5 mg/kg bw) and then killed. Lung, kidney, liver and spleen were excised, homogenized and immunoblotting analysis was performed with anti-Hsp72 monoclonal antibody. Hsp72 in lung was also investigated immunohistochemically. Forty-eight hours after exposure to DMA, Hsp72 was observed in the lung and in the kidney, but not in the liver or spleen. Hsp72 was also detected by immunohistochemical analysis in the nuclei of alveolar flat cells containing capillary endothelium, in the lungs of DMA-treated mice. This result may be consistent with those observed in previous studies showing that oral administration of DMA to mice induces a preferential increase in heterochromatin in the vesicular endothelium of the lung, an early morphological change in the development of pulmonary carcinomas (Nakano *et al.*, 1992; Hayashi *et al.*, 1998). Kato *et al.* (2000) suggested that the increase and accumulation of Hsp72 following administration of DMA to mice occur specifically in target organs for the carcinogenesis of arsenic. It appears that arsenic compounds regulate the expression of the major families of heat-shock proteins and that inorganic As^{III} is the most potent inducer of these proteins (Del Razo *et al.*, 2001b).

Stress-related gene expression in mice treated with inorganic arsenic has been studied by Liu, J. *et al*. (2001). Adult male 129/Sv mice, aged 6–8 weeks, were injected subcutaneously in the dorsal thoracic midline with 100 μmol/kg bw As^{III}, 300 μmol/kg bw As^{V} or the same volume of saline (10 mL/kg bw). To examine stress-related gene expression, livers were removed 3 h after injection of arsenic to extract RNA and protein. The Atlas Mouse Stress/Toxicology array revealed that the expression of genes related to stress — DNA damage and repair-responsive genes — and metabolism were altered by acute exposure to arsenic. Expression of H-O-1, a hallmark for arsenic-induced stress, was increased 10-fold, together with increases in heat shock protein-60 (Hsp60), the DNA damage-inducible protein GADD45 and the DNA excision repair protein ERCC1 and growth arrest. Down-regulation of certain cytochrome P450 drug-metabolizing enzymes occurred after treatment with arsenic. Because the AP-1 complex is associated with stress-related gene activation, the effect of arsenic on AP-1 complex activation was examined. A multiprobe RNAse protection assay revealed the activation of the c-Jun–AP-1 transcription complex after treatment with arsenic. Western blot analysis further confirmed the enhanced production of arsenic-induced stress proteins such as H-O-1, Hsp70, Hsp90, metallothionein, metal-responsive transcription factor, NF-κB and c-Jun–AP-1. Increases in caspase 1 and cytokines such as TNF-α and macrophage inflammatory protein-2 were also evident. Activation of caspase has been propounded to play a role in arsenic-induced apoptosis (Chen *et al.*, 1998), and induction of inflammatory cytokines is another important aspect of arsenic toxicity. The results of this study profiled gene expression patterns in mice treated with inorganic arsenicals. The altered gene expressions following acute exposure to arsenic *in vivo* include stress-related components —DNA damage and repair-responsive genes — activation of transcription factors such as the AP-1 complex and an increase in proinflammatory cytokines.

Expression of shock proteins is regulated by a complex mechanism that requires the integration of multiple signal pathways. The inter-relationships among stress signalling,

cell death and oncogenesis after exposure to arsenic need further research (Del Razo *et al*., 2001b).

(v) *Immunotoxicity*

Although many studies have 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 aseptically, and sterile viable spleen cells were cultured with 20 µL/mL of an arsenic solution (at concentrations of 1–500 ng/mL, 0.01–50 µg/mL and 0.1–500 µg/mL, for sodium arsenite, sodium arsenate and DMA, respectively). Saline (0.9% NaCl) was added to control cultures. For plaque-forming cell (PFC) response, spleen cells ($2.5–3.0 \times 10^5$ cells/mL) were cultured in triplicate and incubated with 8×10^6 sheep erythrocytes, and the number of direct (immunoglobulin M) PFCs were enumerated. Spleen cells (2.5×10^6 cells/mL) were also cultured for 48 h with or without the mitogens, phytohaemagglutinin P (PHA) or lipopolysaccharide ω. At high doses, the three arsenic compounds (sodium arsenite, sodium arsenate and DMA) suppressed the PFC response to sheep erythrocytes and the proliferative response to mitogens whereas, at low doses, they enhanced both responses. In other studies, the authors have demonstrated that this immunoenhancing effect of arsenic on PFC response to sheep erythrocyte is not attributable to the augmentation of lymphocyte function, but to the cytotoxicity of arsenic against precursors of suppressor T cells (Yoshida *et al.*, 1987). The concentration at which each arsenic compound exerted the modulatory effects on both responses differed, and was correlated to the general toxicity of each compound.

A pilot study on arsenic-exposed humans was carried out by Ostrosky-Wegman *et al*. (1991) to determine the lymphocyte proliferation of kinetics and genotoxic effects. The exposed group comprised 11 individuals (nine women and two men) from Santa Ana, State of Coahuila, Mexico, where the drinking-water contained 0.39 mg/L arsenic (98% in pentavalent form and the rest in trivalent form). The non-exposed group (13 individuals; 11 women and two men) was chosen from Nuevo Leon, State of Coahuila, where levels of arsenic in the drinking-water ranged from 0.019 to 0.026 mg/L during 1987–89; while sampling was performed, the levels rose to 0.060 mg/L because of new piping that linked several towns in the area. Venous blood samples were taken and lymphocyte cultures were rapidly processed. The analysis of chromosomal aberrations and sister chromatid exchange 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 study the kinetics of proliferation. The highly exposed group excreted greater amounts of arsenic in urine; neverthelesss, the *Bacillus subtilis* rec-assay for genetic damage induced by urine samples showed negative results. There was a significant difference in cell-cycle kinetics between the groups: the average generation time

was longer in the highly exposed group. The lag in lymphocyte proliferation could mean an impairment of the cellular immune response due to exposure to arsenic.

Because inhibition of lymphocyte proliferation has been used to identify agents that depress the cellular immune response, Gonsebatt *et al*. (1992) investigated *in vitro* the effect of arsenic on human lymphocyte stimulation and proliferation using concentrations of arsenic similar to those found in blood. When human lymphocytes collected from healthy donors (two men, two women) 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 arsenite and arsenate for 2, 6 and 24 h at the beginning of culture in the presence or absence of PHA. Inhibition of PHA stimulation and proliferation was directly related to the length of treatment with arsenic. The results show that, at the concentrations tested, arsenite and arsenate impaired lymphocyte stimulation and proliferation and confirm that chronic exposure to arsenic 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 via drinking-water. Blood and urine samples were taken from 33 volunteers from a town where levels of arsenic in the drinking-water averaged 412 µg/L and from 30 subjects from a matched group with similar socioeconomic status, who drank water with an average level of 37.2 µg/L arsenic. Exposure was assessed by questionnaire and by determining the levels of arsenic in urine and water samples. Peripheral blood lymphocyte proliferation was evaluated at different culture times using labelling (radioactive thymidine incorporation), mitotic and replication indexes as end-points. No significant differences were seen for either labelling or mitotic indices, except for mitotic index in 72-h cultures (higher in the exposed group) and for labelling index (lower) in men and women with skin lesions versus those without lesions. Significant decreases in replication index were seen for exposed women but not for men. Correlations between labelling and mitotic indices showed that progression from 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 appears that inorganic arsenic is immunotoxic, but the mechanism of immune suppression is not clear. Harrison and McCoy (2001) showed that arsenite inhibits the enzymatic activity of lysosomal protease cathepsin L (CathL) in cultures of the murine antigen-presenting B-cell line TA3 and in lysates from unexposed TA3 cells *in vitro*. Arsenite also significantly inhibits purified CathL. This enzyme plays an important role in antigen processing, the mechanism by which antigen-presenting cells cleave foreign protein antigens to peptides to stimulate a T-cell response. Deficient proteolysis may lead to diminished immune responses. Arsenite suppressed enzymatic activity within TA3 cells after 4 h of exposure without affecting cell viability. Kinetic analyses revealed that arsenite was a reversible, partially noncompetitive inhibitor of CathL with a Ki of 90 µM for TA3-derived and 120 µM for the purified enzyme. Indeed, upon addition of excess dithiotheitol, the enzyme activity of CathL was restored; the value

of Ki was comparable to that of the arsenite concentration that maximally decreased CathL in viable TA3 cells after 4 h of exposure. However, an 18-h exposure to arsenite triggered massive cell death at concentrations that were substantially lower than those required for enzymatic inhibition. Morphological analysis (chromatin condensation, cell shrinkage) and annexin V staining showed that arsenite-exposed TA3 cells underwent apoptosis within 18 h and early stages of apoptosis began within 4 h, indicating that arsenic causes apoptosis independent of CathL. Although whether in-vivo exposure to arsenic causes apoptosis in lymphoid organs has not been assessed, these findings suggest that apoptosis could be a major mechanism of arsenic-induced immunosuppression.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

In a case–control study, Zierler *et al.* (1988) compared 270 cases of infants born with congenital heart disease and 665 controls from Massachusetts (USA). The proportional odds ratio, adjusted for all measured contaminants, source of water and maternal education, was not elevated for any congenital heart disease in relation to exposure to arsenic above the detection limit of 0.8 µg/L. However, for a specific malformation, coarctation of the aorta, there was a significant proportional odds ratio of 3.4 (95% CI, 1.3–8.9). The exposure was low, the 90th percentile level being 1 µg/L.

In a case–control study, Aschengrau *et al.* (1989) examined 286 women who experienced spontaneous abortions and 1391 controls from Boston, MA (USA), in relation to the content of their water supplies. An adjusted odds ratio of 1.5 was found for the group with the highest arsenic concentrations. [However, this exposure group had low levels of arsenic in water (1.4–1.9 µg/L), close to or lower than laboratory analytical detection limits, and the possibility of chance or unaccounted confounders could not be discounted.]

An ecological study in an area of south-east Hungary with exposure to arsenic from drinking-water examined the rates of spontaneous abortions and stillbirths for the period 1980–87. Two populations were compared: one from an area with levels of arsenic in drinking-water > 100 µg/L (n = 25 648 people) and one control area with low levels of arsenic (n = 20 836). [No information on analytical method, timing or frequency of sampling was available.] The incidences of both outcomes were significantly higher in the exposed groups, with a 1.4-fold increase in spontaneous abortions (p = 0.007) and a 2.8-fold increase in stillbirths (p = 0.028) (Borzsonyi *et al.*, 1992). [Although both populations were stated to have several similar characteristics, such as smoking, lifestyle, occupation and socioeconomic status, no data were provided, and other important factors such as maternal age were not considered. Furthermore, no mention was made of other potential environmental exposures.]

An ecological study conducted in the USA investigated mortality from vascular diseases in the 30 counties with the highest average levels of arsenic in drinking-water for

the period 1968–84. The arsenic levels ranged up to 92 μg/L in Churchill County, NV. SMRs were based on comparison with the population of the USA. When counties were grouped into three arsenic-exposure categories, defined as 5–10, 10–20 and > 20 μg/L, there appeared to be an increase in mortality from congenital anomalies of the heart only for females in the highest exposure group (SMR, 1.3; 95% CI, 1.0–1.8) and for both sexes for congenital anomalies of the circulatory system (female SMR, 2.0; 95% CI, 1.0–3.4; male SMR, 1.3; 95% CI, 0.7–2.4) (Engel & Smith, 1994).

A retrospective ecological study examined infant mortality rates in three Chilean cities over a 46-year period (1950–96). Antofagasta, in northern Chile, experienced very high levels of arsenic in drinking-water for a period of 12 years. In 1958, a new water source, which contained arsenic concentrations of around 800 μg/L, was introduced as the main supply of public water. In 1970, because of the overt signs of arsenicism observed in several studies, a plant for the removal of arsenic was installed, and levels decreased initially to around 110 μg/L, and then gradually over time to around 40 μg/L (see Table 18). The changes in late fetal, neonatal and post-neonatal mortality rates over time in Antofagasta were compared with those in Valparaiso, another Chilean city with similar demographic characteristics but with low levels of arsenic. A temporal relationship was observed between the period of high arsenic contamination and a rise in neonatal mortality rates, in particular in Antofagasta, whereas the other city had a fairly steady decline in infant mortality (Hopenhayn-Rich *et al.*, 2000). [Data on other contaminants or factors related to infant mortality were not presented, but the temporal relationship suggests a role for exposure to arsenic.]

A retrospective survey in Bangladesh compared several outcomes in women exposed to high (mean, 240 μg/L; $n = 96$) and low (< 20 μg/L; $n = 96$) concentrations of arsenic in drinking-water. Rates of spontaneous abortions, stillbirths and pre-term births were 2.9 ($p = 0.08$), 2.24 ($p = 0.046$) and 2.54 ($p = 0.018$) times higher, respectively, in the high-exposure group than in the low-exposure group. The groups were comparable in terms of age, socioeconomic status, level of education and age at marriage (Ahmad *et al.*, 2001). [This study was based on recall of previous pregnancies, however, and ascertainment of the outcomes was not clearly defined.]

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity*

(i) In vivo

Inorganic arsenic is toxic to mouse and hamster embryos and fetuses after oral or intraperitoneal administration to the dams, with arsenite being three- to 10-fold more potent than arsenate. The embryos and fetuses of hamsters are more sensitive to this effect than those of mice. The toxicity is characterized by decreases in fetal weight, crown–rump length, embryo protein content and the number of somites and by growth retardation and lethality (Baxley *et al.*, 1981; Hood & Harrison, 1982; Hood & Vedel-Macrander, 1984; Carpenter, 1987; Domingo *et al.*, 1991; Wlodarczyk *et al.*, 1996).

Sodium arsenite was given by gavage to CD-1 mice on one of days 8–15 of gestation at doses of 20, 40 or 45 mg/kg bw. The lowest dose had no effect. The two highest doses produced 19 and 36% incidences of maternal deaths, respectively, and also decreased fetal weight and increased the incidence of resorptions. Arsenite-induced lethality was dependent on dose and day of gestation (Baxley *et al*., 1981).

In hamsters, sodium arsenite administered orally (20–25 mg/kg) caused less fetal mortality than parenteral dosing (2.5–5 mg/kg) (Hood & Harrison, 1982).

Nemec *et al.* (1998) evaluated the developmental toxicity of arsenate administered by oral gavage to CD-1 mice and New Zealand white rabbits. Rabbits received doses of 0, 0.19, 0.75 or 3.0 mg/kg bw per day on gestation days 6–18 and mice received 0, 7.5, 24 or 48 mg/kg per day on gestation days 6–15. Increased fetal resorptions and decreased fetal weight were observed only at exposure levels resulting in maternal toxicity (severely decreased weight gain, mortality).

A single intravenous administration of MMA^V (disodium salt) or DMA^V (sodium salt) on day 8 of gestation at dose levels of 20–100 mg/kg elicited a low resorption rate (≤ 10%) in pregnant hamsters (Willhite, 1981). Higher doses of DMA (sodium salt, 900–1000 mg/kg) administered intraperitoneally to pregnant hamsters on one of days 8–12 of gestation induced higher resorption rates, ranging from 30–100% of the litters. MMA^V (500 mg/kg) was less toxic than DMA after intraperitoneal administration, with 6–21% of the litters resorbed. Fetal growth was retarded after administration of MMA on days 9, 10 or 12 of gestation (Hood *et al.*, 1982).

DMA^V administered orally to pregnant mice (200–600 mg/kg per day) and rats (7.5–60 mg/kg per day) on days 7–16 of gestation resulted in significant fetal mortality in mice at 600 mg/kg per day and rats at 50–60 mg/kg per day. A significant decrease in fetal weight gain was observed in mice at 400–600 mg/kg and rats at 40–60 mg/kg (Rogers *et al*., 1981).

Inorganic arsenic elicits teratogenic effects in mice (Hood & Bishop, 1972; Baxley *et al.*, 1981; Morrissey & Mottet, 1983; Wlodarczyk *et al.*, 1996), rats (Fisher, 1982) and hamsters (Hood & Harrison, 1982; Carpenter, 1987) at levels of tens of milligrams per kilogram body weight after oral or intraperitoneal administration. In these studies, the major teratogenic effect induced is cephalic axial dysraphic disorder or neural tube defect. The defect is characterized by exencephaly and encephalocele, which are characterized by non-closure and partial closure of the cephalic neural folds, respectively. Other malformations that occur to a minor extent include fused ribs, renal agenesis, micromelia, facial malformations, twisted hindlimb, microphthalmia and anophthalmia. The malformations are dose- and gestational age-dependent. Sodium arsenite is more potent than sodium arsenate in inducing a teratogenic response, and intraperitoneal administration of arsenic is more effective than oral administration.

Histological studies of the developing urogenital system in rat embryos after intraperitoneal administration of arsenate to pregnant rats revealed that the first observable change is a retardation in the growth of the mesonephric duct. This retardation led to the absence of the ureteric bud (which arises from the mesonephric duct) and resulted in the

absence of the vas deferens, seminal vesicle and part of the epididymis (Burk & Beaudoin, 1977).

Administration of inorganic arsenic to mice on days 7–9 of gestation results in neural tube defects in the developing organism. The time most sensitive to arsenate in mouse embryos is when the dams are administered the chemical on day 8. Of the fetuses that survived a single dose of sodium arsenate (45 mg/kg) administered intraperitoneally to dams on day 8, 65% or more were exencephalic. After administration of a similar dose of arsenate on day 7 or 9, 3% or less of the surviving fetuses were exencephalic (Morrissey & Mottet, 1983).

The neural tube defects seem to result from an apparent arsenic-induced arrest or delay in neural-fold apposition. Takeuchi (1979) examined the changes induced by an embryo-lethal dose of arsenate (30 mg/kg) administered to pregnant rats intraperitoneally on day 9 of gestation. At 4 h after exposure, some cellular necrosis was seen in the neuroectoderm and mesoderm of the embryos. By 12 h, abnormal mitotic and interphase cells were observed in both tissues, and necrotic cells and debris from these cells were also present. By 24 h, neurulation had stopped, as evidenced by the presence of the V-shaped neural fold that is normally closed by this time.

In studies by Morrissey and Mottet (1983), pregnant mice were killed 6–21 h after intraperitoneal administration of sodium arsenate (45 mg/kg) on day 8 of gestation. Neural folds were widely separated and not positioned for closure in the prospective hindbrain. Necrotic debris was also found primarily in the neuroepithelium of the prospective forebrain and sometimes in the mesenchyme, but it was not clear if this was the main lesion associated with exencephaly.

Fisher (1982) examined the effect on the development of embryos of sodium arsenate (45 mg/kg bw) administered intraperitonally to pregnant rats on day 10 of gestation. These rats were killed 4 h or 24 h after injection. The embryos were removed and the macromolecule levels were determined immediately, or at 24 h or 42 h after being placed in culture media. In-utero exposure to arsenate for 4 h did not affect the macromolecule levels. A 24-h in-utero exposure to arsenate resulted in a significant decrease in DNA, RNA and protein accumulation at the beginning of cultivation and after 24 h in culture. However, after 42 h in culture, protein levels had recovered. After 24 h in culture, morphological changes in the 24-h exposed embryos included a failure to rotate to a ventroflexed position, failure of closure of the anterior neuropore, no establishment of visceral yolk sac circulation, and no fusion of the allantoic sac in placental formation. The latter effect may reflect problems in the formation of the urogenital system.

Nemec *et al.* (1998) observed no teratogenic effects in mice or rabbits receiving daily oral administrations of 0–48 or 0–3 mg/kg bw arsenate on gestation days 6–15 or 6–18, respectively.

MMA^V (disodium salt, 20–100 mg/kg) and DMA^V (sodium salt, 20–100 mg/kg) induced a low percentage of fetal malformations (≤ 6%) after intravenous administration on day 8 of gestation to pregnant hamsters. The effects were characterized by fused ribs,

renal agenesis or encephalocele, with the latter anomaly was observed only with DMA. Neither MMA nor DMA caused maternal toxicity (Willhite, 1981).

The effect of continuous oral exposure of pregnant mice (200, 400, 600 mg/kg per day) and rats (7.5–60 mg/kg per day) to DMA^V during days 7–16 of gestation was examined by Rogers *et al*. (1981). In mouse fetuses, cleft palate was the major teratogenic response to DMA and was observed at the two highest doses. There was also a significant decrease in the incidence of supernumerary ribs. In the mid-dose group, four mouse fetuses had irregular palatine rugae. In rats, the average number of sternal and caudal ossifications was decreased at the two highest doses and the percentage of irregular palatine rugae increased significantly with dose. An increase in fetal lethality occurred at the highest dose in mice (39.8%) and at the two highest doses in rats (32.9 and 65.4%).

(ii) In vitro

Muller *et al.* (1986) examined the effect of sodium arsenite on mouse embryos at the two-cell pre-implantation stage, which is approximately 30–32 h after conception. Arsenite-induced lethality occured at a concentration of 100 µmol/L. After implantation, arsenite and arsenate are toxic (decreases in crown–rump length, number of somites, protein content, head length, yolk sac diameter) and lethal to embryos of mice (Chaineau *et al.*, 1990; Tabacova *et al.*, 1996) and rats (Mirkes & Cornel, 1992; Mirkes *et al.*, 1994). Tabacova *et al.* (1996) observed that as gestational age at which the mouse embryos were isolated and exposed to arsenic increased, so did resistance to toxicity or lethality. As in the in-vivo studies, arsenite was more potent than arsenate.

Inorganic arsenic is teratogenic to cultured mouse embryos (day 8), with sodium arsenite (1–4 µmol/L) being approximately 10-fold more effective than sodium arsenate (10–40 µmol/L) after a 48-h incubation. The most sensitive in-vitro effect of arsenic is hypoplasia of the prosencephalon. Other effects include failure of neural tube closure and development of limb buds and sensory placode, somite abnormalities and, in arsenate-exposed embryos, hydropericardium (Chaineau *et al.*, 1990).

Arsenite inhibits chondrogenesis in chick limb bud mesenchymal cells, with complete inhibition at 25 µmol/L. Arsenate was ineffective at concentrations up to 200 µmol/L but, when added with arsenite, gave an apparent dose-dependent additive effect (Lindgren *et al.*, 1984).

Sodium arsenite (50 µmol/L) induces dysmorphology in rat embryos (10 days old) after a 2.5-h exposure followed by a 21.5-h incubation period without arsenic. This effect is characterized by hypoplastic prosencephalon, mild swelling of the rhombencephalon and abnormal somites and flexion of the tail (Mirkes & Cornel, 1992; Mirkes *et al.*, 1994).

Tabacova *et al.* (1996) examined the teratogenicity of arsenite (1–30 µmol/L) and arsenate (5–100 µmol/L) in mouse embryos isolated from pregnant dams on day 9 of gestation. The embryos were incubated with various concentrations of arsenic, for different lengths of time and at various stages of somite development. Treatment with arsenic led to non-closure of the neural tube, collapsed neural folds, prosencephalic hypoplasia, anophthalmia, pharyngeal arch defects and abnormal somites. The malformation rates were

dependent on the dose and oxidation state of arsenic. Arsenite was generally three to four times more potent than arsenate in inducing these effects. As the age of the embryos advanced, a higher dose of arsenic was required to elicit the effect. The developmental effects most sensitive to inorganic arsenic were forebrain growth, neural tube closure, eye differentiation, axial rotation (dorso- to ventroflexion) and pharyngeal arch development, which were induced by a 1-h exposure to inorganic arsenic.

(*b*) *Gene expression*

Wlodarczyk *et al.* (1996) examined the expression of several transcription factors from embryos isolated from pregnant mice administered sodium arsenate intraperitoneally at 30–45 mg/kg, an approximately lethal dose. Expression of several genes was altered by arsenate administered on day 9 of gestation. This day corresponds to the progression of neural tube closure, which is delayed in embryos exposed to inorganic arsenic. In the neuroepithelium of arsenate-exposed embryos, there was significant down-regulation of *Hox 3.1* and up-regulation of *Pax3*, *Emx-1* and *creb*. Both *Hox 3.1* and *Pax3* play a role in the regulation of neural cellular adhesion molecules, a glycoprotein that affects neural crest cell migration and ultimately neural tube closure (Rutishauser *et al.*, 1988).

(*c*) *Induction of heat-shock proteins*

Arsenic induces the biosynthesis in embryos of several heat-shock proteins that protect cells from its detrimental effects. However, its has been proposed that induction of a heat-shock protein response could alter the normal gene programme for organogenesis (German, 1984).

(i) In vivo

Pregnant mice were administered sodium arsenite (0.5 mg/mouse, approximately 17 mg/kg bw) intraperitoneally on days 9–11 of gestation. Two proteins that were induced were isolated from the embryos and had molecular weights between 45 and 66.2 kDa. Heat-shock treatment of pregnant mice induced one embryonic protein with a molecular weight between 45 and 66.2 kDa and a second with a molecular weight between 66.2 and 92.5 kDa (German *et al.*, 1986). In mice administered sodium arsenite (19 mg/kg) intraperitoneally on day 8 and killed 1 day later, the levels of two proteins, Hsp70 and Hsp105, which are produced constitutively, were increased throughout the embryo. There was a high concentration of these proteins in the neuroepithelial tissue of the embryos after treatment with heat shock or arsenite (Honda *et al.*, 1992).

(ii) *Animal embryos* in vitro

Four proteins with molecular weights of 27, 35, 73 and 89 kDa and their mRNA were induced in chick embryo cells by sodium arsenite (50 µmol/L) or heat shock in a dose- and time-dependent manner. For example, the 35-kDa protein was induced at a concentration of 5 µmol/L sodium arsenite, but the 73- and 89-kDa proteins were minimal at this

concentration. Only the 27-kDa protein was still induced 24–48 h after treatment (Johnston *et al.*, 1980). In chick embryo fibroblasts (10–12 days old), arsenite induced the synthesis of Hsp70A and 70B (Wang & Lazarides, 1984).

Mouse embryo cells (gestation day 11) were exposed to either sodium arsenite (50 μmol/L) for 3 h or heat shock for 10 min, and proteins from cell extracts were analysed by two-dimensional gel electrophoresis. The synthesis of Hsp73 and Hsp105 was increased by both exposures (Honda *et al*., 1992).

In rat embryos (gestation day 10), exposure for 2.5–5 h to an embryotoxic level of sodium arsenite (50 μmol/L) resulted in the induction of three heat-shock proteins (Mirkes & Cornel, 1992). A monoclonal antibody specific for Hsp72 recognized one of the proteins induced by arsenite. Levels of mRNA for these heat-shock proteins were also increased in the embryos after exposure to arsenite. Hsp72 was detected 10 h after exposure, and maximal levels were observed at 24 h. However, Hsp72 was not detected at 48 h, which indicates that this protein is turned over (Mirkes *et al.*, 1994).

(iii) *Human fetal tissue* in vitro

German *et al.* (1986) treated human fetal tissue (gestational age, 77–84 days) with either sodium arsenite (50 μmol/L) for 2 h or heat shock for 6 min. The cells were then examined for induction of heat-shock proteins. Several proteins were induced by both treatments, and two with molecular weights < 45 kDa were induced only by exposure to arsenite.

Honda *et al.* (1992) treated human chorionic villus cells (gestational age, 70–119 days) with sodium arsenite (50 μmol/L) for 3 h or with heat shock for 10 min. In unstressed tissue, Hsp70, Hsp73, Hsp85 and Hsp105 were synthesized constitutively, but their levels were increased after exposure to sodium arsenite or heat.

4.4 Genetic and related effects

The genetic effects of arsenic compounds have recently been reviewed extensively (National Research Council, 1999; Basu *et al*., 2001; Gebel, 2001; National Research Council, 2001; WHO, 2001). In this section, the genotoxicity of arsenic in humans and in experimental animals is dealt with comprehensively. Relevant studies on single and combined mammalian genotoxicity have been included. Data on fungi, plants and *Drosophila* have not been reviewed.

4.4.1 *Humans*

Several studies have investigated the genotoxic effects of arsenic after long-term ingestion via drinking-water, but few studies of occupational exposure to arsenic are available. Exposures were mainly to inorganic arsenic, but since arsenic is methylated in humans, mixed internal exposures to inorganic arsenic and methylated arsenic metabolites predominate. Although MMA and DMA (as sodium salts) have been used in pesti-

cides, this use is currently decreasing and no study was available on the monitoring of human biological effects after occupational exposure to these compounds.

In a pilot study in Mexico, nine women and two men exposed to well-water containing high levels of arsenic (390 μg/L, presumably > 10 years) did not show a significantly higher frequency of chromosomal aberrations or sister chromatid exchange than controls exposed to lower levels of arsenic (11 women and two men; 19–60 μg/L arsenic in well-water). The age range for both groups was 21–62 years. Mutant frequencies at the *HPRT* locus were elevated but not significantly in the high-exposure group (Ostrosky-Wegman *et al*., 1991). In a more recent study, 35 Mexican individuals exposed to well-water containing 408 μg/L arsenic (presumably > 10 years) were compared with 34 controls (well-water concentration, 29.9 μg/L arsenic). The mean age of the two groups was 40.6 years (exposed) and 39.0 years (control), and sex distribution was said to be similar [exact data not supplied]. In the high-exposure group, chromosomal aberrations were significantly elevated, with 0.08 (exposed) versus 0.03 (control) chromosomal aberrations per cell. Moreover, the frequency of micronuclei in buccal and urothelial cells was significantly elevated (average/1000 cells, 2.21 versus 0.56 and 2.22 versus 0.48, respectively) (Gonsebatt *et al*., 1997). Among the exposed individuals, men showed more chromosomal aberrations and higher frequency of micronuclei than women. This difference could be attributed to the fact that men drank more water; in this study country, men work in the fields and, because of the dry climate, drink more water than women. The proportion of smokers was similar in the two groups: 29% of the exposed and 33% of the controls; smoking was not significantly associated with a higher incidence of chromosomal aberrations or micronuclei. People occupationally exposed to putative genotoxins or those who underwent medical treatment were excluded from the study.

No differences in sister chromatid exchange (98 exposed subjects versus 83 controls) or chromosomal aberration (104 exposed versus 86 controls) frequencies were found in the peripheral lymphocytes of subjects exposed to moderate quantities of arsenic in the drinking-water in Nevada (USA). Drinking-water with mean concentrations of 109 μg/L arsenic had been consumed for at least 5 years; control subjects had drunk water containing 12 μg/L arsenic (Vig *et al*., 1984). In the statistical evaluation, sex, age, smoking and putative occupational exposures were controlled for. The population studied was exposed to much lower levels of arsenic than the current study population and arsenic has not been shown to be associated with cancer in blood-forming tissue.

In a more recent study in Nevada (USA), 18 people (mean exposure from drinking-water, 1312 μg/L arsenic > 1 year) showed elevated frequencies of micronuclei in exfoliated bladder cells (2.79/1000 cells) in comparison with 18 control subjects exposed to low levels of arsenic (exposure from drinking-water, 16 μg/L arsenic; 1.57/1000 cells) matched for age, sex and smoking status (Warner *et al*., 1994). Occupation was included as a confounding variable. In contrast, there was no increase in micronucleated buccal cells associated with such high levels of arsenic.

The frequencies of chromosomal aberrations were determined in the peripheral lymphocytes of 32 Finnish subjects (age, 15–83 years; mean, 52 years) after long-term ingestion

of drinking-water containing a median concentration of 410 µg/L arsenic (Mäki-Paakkanen *et al*., 1998) and were compared with those of eight controls (age, 37–76 years; mean, 50 years) from the same village who consumed drinking-water containing < 1 µg/L arsenic. Estimated cumulative median doses of arsenic were 455 and 7 mg per lifetime, respectively. Smoking habits, sex, seafood consumption and residential history were included as confounders in the evaluation. The crude study results did not show elevated frequencies of chromosomal aberrations in arsenic-exposed subjects (6.9 in exposed versus 8.6 in controls) or smokers (6.0 in ex- and current smokers versus 6.9 in never-smokers). However, in the crude and adjusted linear regression analyses, numbers of chromosomal aberrations were significantly associated with levels of arsenic in urine of current users ($r^2 = 0.25$; $p = 0.08$ and $r^2 = 0.27$; $p = 0.04$, respectively).

In a pilot study in Inner Mongolia, 19 residents exposed to arsenic via drinking-water (527.5 µg/L) for 17 years (group average) were compared with 13 control subjects exposed to a low concentration of 4.4 µg/L arsenic (Tian *et al*., 2001). Data on smoking habits, occupation, diet, demographic factors, age and medical status were collected. Frequencies of micronuclei were significantly (3.4-fold) higher in cells from the buccal mucosa and sputum collected from airway epithelium. The increase observed for bladder cells was smaller: 2.7-fold over control for all subjects and 2.4-fold over control for nonsmokers. When smokers were excluded from high-exposure and control groups, the effects of arsenic were greater, although only in buccal and sputum cells, in which sixfold increases in micronuclei frequency occurred.

A nested case–control study was performed in an area endemic for Blackfoot disease in Taiwan, China (Liou *et al*., 1999). A cohort of 686 residents was assembled and, after 4 years, 31 people had developed cancer. Twenty-two blood samples obtained from these subjects at the beginning of the cohort study were successfully processed. A control comparison group was selected from among members of the cohort who had not developed cancer, matched on sex, age, history of residence (residential village) or of drinking artesian well-water and smoking. No differences were found in overall frequencies of sister chromatid exchange. The frequency of chromosomal aberrations was significantly higher among cases, which was due to the induction of chromosome-type but not chromatid-type aberrations. [The Working Group noted that there was no difference in exposure to arsenic (mean duration of drinking artesian well-water) among cases and controls.]

A study in West Bengal, India, compared 45 subjects with cutaneous signs of arsenicism (368 µg/L arsenic in drinking-water) with 21 healthy individuals considered as controls residing in two unaffected districts (5.50 µg/L arsenic in drinking-water) (Basu *et al*., 2002). The frequency of micronuclei was significantly higher in the oral mucosal cells (5.15 versus 0.77 per 1000 cells), urothelial cells (5.74 versus 0.56 per 1000 cells) and peripheral lymphocytes (6.40 versus 0.53 per 1000 cells) of exposed subjects compared with control subjects. The age distribution and socioeconomic status was reported to be similar in the two groups. Exposure of exposed subjects to arsenic via drinking-water had probably been for a mean of 11 years.

In another study, the mean frequency of sister chromatid exchange/cell in human peripheral lymphocytes was not found to be affected by voluntary ingestion of 0.15 g potassium arsenite or poisoning from 1, 10 or 20 g arsenic trioxide. At 20 g arsenic trioxide, the mean frequency of sister chromatid exchange was significantly elevated (Hantson *et al*., 1996). Doses of 10 and 20 g arsenic trioxide significantly increased the number of cells with a high sister chromatid exchange frequency and produced a shift in the distribution of the cells according to frequency of sister chromatid exchange.

Few studies have dealt with the induction of genetic damage in workers exposed to arsenic. Moreover, these subjects were exposed to other genotoxic agents. In the peripheral lymphocytes of nine smelter workers exposed to arsenic and other compounds, a significant increase in chromosomal damage was found, with 87 aberrations per 819 mitoses compared with 13 per 1012 in controls (Beckman *et al*., 1977). In this preliminary report, no data on duration of exposure or age of the workers were given. In a further study, 33 male copper smelter workers (aged 20–62 years) exposed to arsenic and other toxic compounds were studied to determine chromosomal aberrations in peripheral lymphocytes (Nordenson & Beckman, 1982). Internal exposures to arsenic were analysed in urine, but the analytical method was not given. The frequencies of chromosomal aberrations were not associated with age, smoking or degree of exposure to arsenic. Significantly increased frequencies of chromosomal aberrations were found in comparison with 15 male employees (aged 26–60 years) without known occupational exposure to arsenic or other toxic agents: 5.4 aberrations versus 2.1 per 100 cells for gaps and 1.4 aberrations versus 0.1 per 100 cells for chromosome breaks ($p < 0.001$). Chromatid breaks showed a lower significance level (1.3 versus 0.6 per 100 cells [$p < 0.05$]).

Some studies investigated whether arsenic-mediated chromosomal damage *in vivo* is caused by an aneugenic or clastogenic effect (Dulout *et al*., 1996; Moore, L.E. *et al*., 1996, 1997a). Both types of damage were induced, but clastogenicity predominated with high exposure to arsenic (Moore, L.G. *et al*., 1996, 1997a).

Apart from the pilot study of Ostrosky-Wegman *et al*. (1991), no induction of *HPRT* mutation was found in a further study of 15 male Chilean copper-roasting-plant workers (aged 24–66 years), who were categorized according to job type as being exposed to arsenic at low, medium or high levels. Their mean duration of employment in the factory was 43 months. The individual exposure was ascertained by analysing levels of arsenic in the urine. In the very highly exposed workers (internal dose, 260 μg/L arsenic in urine), no induction of *HPRT* mutations in peripheral lymphocytes was demonstrated. The authors concluded that the *HPRT* assay seems to have a low sensitivity for the detection of the genotoxicity of arsenic *in vivo* (Harrington-Brock *et al*., 1999).

Another study of 70 Chilean men with long-term exposure to 600 μg/L arsenic in drinking-water and 55 frequency-matched control subjects (15 μg/L arsenic in drinking-water) determined micronuclei in bladder cells (Biggs *et al*., 1997; Moore, L.E. *et al*., 1997a). Matching criteria were age, smoking status, time of local residence (average high exposure, 19.3 years), education and ethnicity. An exposure-related increase in the frequency of micronuclei was found in the exposure quintiles 2–4 (urinary arsenic,

54–729 µg/L), but not in the 5th quintile (urinary arsenic > 729 µg/L). The prevalence of centromer-positive micronuclei increased 3.1-fold in quintile 4 (95% CI, 1.4–6.6), and the prevalence of centromer-negative micronuclei increased 7.5-fold in quintile 3 (95% CI, 2.8–20.3), suggesting that chromosome breakage was the major cause of formation of micronuclei. An intervention study was carried out on a subset of 34 of the arsenic-exposed Chilean men of this investigation. The arsenic-contaminated drinking-water supply (600 µg/L) was changed to water containing 45 µg/L arsenic. After 8 weeks, the prevalence of micronuclei in bladder cells decreased from 2.63/1000 cells before the intervention to 1.80/1000 cells after the intervention for all individuals. The frequencies of micronuclei in exfoliated bladder cells had significantly decreased from 4.45/1000 cells before the intervention to 1.44/1000 cells after the intervention in smokers but not in nonsmokers (2.05/1000 cells versus 1.90/1000 cells), suggesting that the bladder cells of smokers could be more susceptible to genotoxic damage caused by arsenic (Moore, L.E. *et al*., 1997b).

The frequency of micronuclei in 12 Andean women and 10 children with lifetime current exposure to 200 µg/L arsenic in the drinking-water was compared with that in 10 women and 12 children exposed to 0.7 µg/L arsenic. Putative confounding variables such as smoking, consumption of alcohol and coca leaves were included in the evaluation. It was shown that the frequencies of micronuclei per 1000 binucleated cells in peripheral lymphocytes were significantly elevated in the arsenic-exposed groups as compared with controls (women, 41 versus 8.5; children, 35 versus 5.6, respectively) (Dulout *et al*., 1996). Moreover, the frequency of aneuploidy was significantly elevated (0.21% versus 0%; 12 exposed versus 17 controls). In contrast, the frequencies of sister chromatid exchange in the arsenic-exposed group were not affected (5.7 versus 5.5 per cell in exposed and control women and 4.4 versus 4.6 per cell in exposed and control children, respectively), nor were specific chromosome translocations.

Induction of sister chromatid exchange was found in peripheral lymphocytes of subjects after 20 years of exposure to arsenic in well-water (> 130 µg/L) in Argentina (Lerda, 1994). Putative exposures to other genotoxic compounds were reported to be taken into account in the study. The mean frequency of sister chromatid exchange was 10.50 per cell in exposed men and women (282 nonsmokers) versus 7.50 per cell in 155 control subjects (volunteer men and women) drinking water that contained less than 20 µg/L arsenic for more than 20 years. Exposed subjects were significantly older than the control group (mean age, 56.71 versus 38.90). In a further evaluation, to homogenize the age of the exposed group, participants older than 50 years were excluded from the analysis. In the younger subset, no correlation between sister chromatid exchange and sex, or sister chromatid exchange and age was found. Sister chromatid exchange was induced by concentrations as low as 100 µg/L arsenic for the younger subset. Moreover, the arsenic content in drinking-water was associated with the frequency of sister chromatid exchange in both sexes but was not affected by sex. [The Working Group noted that the value of the study is reduced because the statistical evaluation was not reported in detail. Moreover, arsenic in urine was quantified by an insensitive colorimetric method of analysis.]

4.4.2 *Experimental systems* (see Table 30 for details and references)

(*a*) *In-vitro studies*

The methylated forms of trivalent arsenic are the only arsenic species that cause DNA damage *in vitro* (Mass *et al.*, 2001; Nesnow *et al*., 2002).

Arsenic (sodium arsenite) did not induce tryptophan revertants in *Escherichia coli* or ouabain- or 6-thioguanine-resistant mutants in Chinese hamster lung (V79), Chinese hamster ovary or Syrian hamster embryo cells (Lee *et al.*, 1985a). Moreover, induction of SOS repair by sodium arsenite was not detected in *E. coli PQ37*. However, sodium arsenite and sodium arsenate were mutagenic in mouse lymphoma L5178Y cells, inducing trifluorothymidine-resistant mutants.

Sodium arsenite induced a significantly increased frequency of sister chromatid exchange in Chinese hamster ovary and Syrian hamster embryo cells. Sodium arsenate was one order of magnitude less potent in inducing sister chromatid exchange than sodium arsenite. It induced the formation of micronuclei in Chinese hamster ovary and V79 cells in the cytokinesis-block micronucleus test using cytochalasin B as well as in the absence of cytochalasin B in V79 cells and also induced chromosomal aberrations in mammalian cells.

Sodium arsenite significantly elevated the frequency of sister chromatid exchange and significantly enhanced micronucleus formation in isolated human peripheral lymphocytes as well as in whole blood after cytokinesis block through cytochalasin B. It induced chromosomal aberrations as chromatid gaps, fragmentation, endoreduplication and chromosomal breaks in human leukocytes, lymphocytes and primary umbilical cord fibroblasts. Moreover, induction of aneuploidy was observed in human peripheral lymphocytes treated with sodium arsenite *in vitro*, suggesting that this clastogenic agent may exhibit some weak aneuploidogenic properties.

There is some evidence that human, mouse and rat leukocytes are more sensitive to the induction of micronuclei after treatment with arsenite than guinea-pig leukocytes (Peng *et al*., 2002). This difference in the induction of micronuclei by arsenic could not be explained by a species-dependent variability in arsenite methylation. The leukocytes of all four species were able to ethylate arsenic but there was no clear correlation between the ability to methylate arsenic and the induction of micronuclei.

In assays with mouse lymphoma L5178Y cells, arsenate (As^V), MMA^V and DMA^V induced mutations at the *Tk* locus, chromosomal aberrations and micronuclei. Arsenobetaine, the major arsenic compound in seafood, did not induce neoplastic transformation in mouse fibroblast BALB/3T3 cells.

Significant increases in chromosomal aberrations were induced in human umbilical cord fibroblasts by arsenate, MMA^V, DMA^V, trimethylarsine oxide, arsenosugar, arsenocholine, arsenobetaine and tetramethylarsonium iodide. The higher potency of induction of chromosomal aberrations by DMA^V in comparison with MMA^V was probably caused by contamination of DMA^V sample by inorganic arsenic (Eguchi *et al*., 1997). Nevertheless,

Table 30. Genetic and related effects of arsenic and arsenic compounds

Test system	Results[a]	Dose[b] (LED or HID)	Reference
Arsenate (As^{V})			
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, *in vitro*	+	10	Moore, M.M. *et al.* (1997)
Gene mutation, Syrian hamster embryo cells, ouabain resistance, *in vitro*	–	31	Lee *et al.* (1985a)
Gene mutation, Syrian hamster embryo cells, 6-thioguanine resistance, *in vitro*	–	31	Lee *et al.* (1985a)
Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	+	3.1	Lee *et al.* (1985a)
Micronucleus formation, mouse lymphoma L5178Y cells *in vitro*	+	10	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	10	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	20	Lee *et al.* (1985a)
Cell transformation, Syrian hamster embryo cells	+	5	Lee *et al*. (1985a)
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	5	Oya-Ohta *et al*. (1996)
Chromosomal aberrations, human lymphocytes *in vitro*	–	1	Nordenson *et al.* (1981)
Chromosomal aberrations, human leukocytes *in vitro*	+	2.25 (0.6 ppm as As)	Nakamuro & Sayato (1981)
Arsenite (As^{III})			
Escherichia coli, gene mutation (tryptophan revertant selection) *in vitro*	–	3250	Rossman *et al*. (1980)
Escherichia coli, *LacZ* gene induction (SOS chromotest) *PQ37 in vitro*	–	105	Lantzsch & Gebel (1997)
Gene mutation, Chinese hamster ovary cells, ouabain resistance, *in vitro*	–	0.65	Rossman *et al.* (1980); Lee *et al.* (1985b)
Gene mutation, Chinese hamster ovary cells, 6-thioguanine resistance, *in vitro*	–	13	Rossman *et al.* (1980)
Gene mutation, Chinese hamster ovary cells, 6-thioguanine resistance, *in vitro*	–	1.3	Lee *et al.* (1985b)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, *in vitro*	+	1	Moore, M.M. *et al*. (1997b)
Gene mutation, Syrian hamster embryo cells, ouabain resistance, *in vitro*	–	1.3	Lee *et al.* (1985a)
Gene mutation, Syrian hamster embryo cells, 6-thioguanine resistance, *in vitro*	–	1.3	Lee *et al.* (1985a)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	0.65	Lee *et al.* (1985b)

Table 30 (contd)

Test system	Results[a]	Dose[b] (LED or HID)	Reference
Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	+	0.1	Lee *et al.* (1985a)
Micronucleus formation, Chinese hamster ovary cells *in vitro*	+	5.21[c]	Wang *et al.* (1997)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	0.325	Gebel (1998)
Micronucleus formation, mouse lymphoma L5178Y cells *in vitro*	+	1.5	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	1.5	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	0.8	Lee *et al*. (1985a)
Cell transformation, Syrian hamster embryo cells	+	0.20	Lee *et al*. (1985a)
Sister chromatid exchange, human lymphocytes *in vitro*	+	0.03	Gebel *et al.* (1997); Rasmussen & Menzel (1997); Nordenson *et al.* (1981)
Micronucleus formation, human lymphocytes *in vitro*	+	0.06	Schaumlöffel & Gebel (1998)
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	0.5	Oya-Ohta *et al*. (1996)
Chromosomal aberrations, human lymphocytes *in vitro*	+	0.09	Nordenson *et al.* (1981)
Chromosomal aberrations, human leukocytes *in vitro*	+	0.31	Nakamuro & Sayato (1981)
Aneuploidy, human lymphocytes *in vitro*	+	0.4	Eastmond & Tucker (1989)
Aneuploidy, human lymphocytes *in vitro*	+	0.31 ng/mL	Ramírez *et al.* (1997)
Single-cell gel assay (comet), Swiss albino mouse leukocytes *in vivo*	+	0.13 mg/kg po	Saleha Banu *et al.* (2001)
LacZ gene mutation, Muta^{TM} mouse lung, kidney, bladder, bone marrow *in vivo*	–	7.6 mg/kg ip × 5	Noda *et al.* (2002)
Micronucleus formation, BALB/c mouse bone marrow *in vivo*	+	10 mg/kg (24 h) or 0.5 mg/kg (30 h) ip	Deknudt *et al.* (1986)
Micronucleus formation, BALB/c/CBA/C57BL mouse bone marrow *in vivo*	+	5 mg/kg ip	Tinwell *et al.* (1991)
Micronucleus formation, B6C3F1 mouse bone marrow *in vivo*	+	5 mg/kg po × 4	Tice *et al.* (1997)

Table 30 (contd)

Test system	Results[a]	Dose[b] (LED or HID)	Reference
Micronucleus formation, Muta^{TM}mouse peripheral blood reticulocytes *in vivo*	+	7.6 mg/kg ip × 5	Noda *et al.* (2002)
Chromosomal aberrations, Swiss mouse bone marrow *in vivo*	+	0.1 mg/kg sc × 4	Roy Choudhury *et al.* (1996)
Chromosomal aberrations, Swiss mouse bone marrow *in vivo*	+	2.5 mg/kg po	Biswas *et al.* (1999)
Dominant lethal mutation, Balb/c mouse *in vivo*	–	5 mg/kg ip	Deknudt *et al.* (1986)
Monomethylarsonic acid (MMA^{V})			
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, *in vitro*	+	2500	Moore, M.M. *et al*. (1997)
Micronucleus formation, mouse lymphoma L5178Y cells *in vitro*	+	4000	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	4000	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	196	Oya-Ohta *et al*. (1996)
Dimethylarsinic acid (DMA^{V})			
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, *in vitro*	+	5000	Moore, M.M. *et al*. (1997)
Micronucleus formation, mouse lymphoma L5178Y cells *in vitro*	–	10 000	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	8000	Moore, M.M. *et al*. (1997)
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	96.6	Oya-Ohta *et al*. (1996)
DNA strand break, ICR CD-1 mouse lung *in vivo*	+	1500 mg/kg	Yamanaka *et al.* (1989); Yamanaka & Okada (1994)
DNA strand break, ICR CD-1 mouse liver, kidney and spleen *in vivo*	–	1500 mg/kg	Yamanaka *et al.* (1989); Yamanaka & Okada (1994)
LacZ gene mutation, Muta^{TM} mouse lung, kidney, bladder, bone marrow *in vivo*	–	10.6 mg/kg ip × 5	Noda *et al.* (2002)
Micronucleus formation, Muta^{TM} mouse peripheral blood reticulocytes	–	10.6 mg/kg ip × 5	Noda *et al.* (2002)
Aneuploidy, CD-1 mouse bone marrow *in vivo*	+	300 mg/kg ip	Kashiwada *et al.* (1998)

Table 30 (contd)

Test system	Results[a]	Dose[b] (LED or HID)	Reference
Trimethylarsine oxide (TMAO)			
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	503	Oya-Ohta *et al*. (1996)
Arsenocholine			
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	4950	Oya-Ohta *et al*. (1996)
Arsenobetaine			
Cell transformation, mouse BALB/3T3 cells	–	89	Sabbioni *et al*. (1991)
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	1958	Oya-Ohta *et al*. (1996)
Tetramethylarsonium iodide			
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	4978	Oya-Ohta *et al*. (1996)
Arsenosugar (2′,3′-Dihydroxypropyl-5-deoxy-5-dimethylarsinoyl-β-D-riboside)			
Chromosomal aberrations, primary human umbilical cord fibroblasts *in vitro*	+	4860	Oya-Ohta *et al*. (1996)
Methylarsonous acid (MAs^{III})			
Single-cell gel (comet) assay, human lymphocytes *in vitro*	+	2.12[c]	Mass *et al*. (2001)
Dimethylarsinous acid ($DMAs^{III}$)			
Single-cell gel (comet) assay, human lymphocytes *in vitro*	+	1.22[c]	Mass *et al*. (2001)

[a] +, positive; –, negative; without exogenous metabolic system

[b] LED, lowest effective dose; HID, highest ineffective dose unless otherwise stated; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal

[c] Estimated from graph in paper

Eguchi *et al.* (1997) had shown that pure DMA^V but not MMA^V had induced tetraploids in Chinese hamster V79 cells.

MMA^{III} and DMA^{III} were investigated using human lymphocytes in the single-cell gel assay. At low micromolar doses, these methylated trivalent arsenicals showed a comet-like tail corresponding to DNA damage. In this study, neither As^{III}, As^V, nor the methylated pentavalent arsenicals produced significant nicking, strand breaks or alkali labile lesions in DNA compared with the methylated trivalent arsenicals.

Both hypomethylation and hypermethylation of DNA were associated with exposure to arsenic in cultures of human lung A549 cells and in human kidney UOK cells (Zhong & Mass, 2001). This could be consistent with the proposal that changes in DNA methylation can activate some genes and repress others in response to exposure to arsenite.

(*b*) *In-vivo studies*

Swiss Albino mice administered arsenic trioxide (also called arsenite, As^{III}) orally showed a significantly increased DNA tail-length in leukocytes in the single-cell gel (comet) assay at the lowest dose tested.

Induction of DNA single-strand breaks was detected in the lung, but not in liver, kidney or spleen of ICR (CD-1) mice 12 h after oral administration of DMA^V. The DNA damage was completely repaired after a further 12-h interval.

No significant mutagenesis of the *lacZ* gene was observed in male transgenic Muta™ mouse lung, kidney, bladder or bone marrow after five daily intraperitoneal injections of arsenite (As^{III}) or DMA^V. However, arsenite significantly increased the frequencies of micronucleated reticulocytes in peripheral blood, whereas DMA^V had no effect. [The Working Group noted that, in comparison with other studies using DMA^V, the dose tested was more than one order of magnitude lower.]

Sodium arsenite dissolved in water and administered intraperitoneally to CBA, BALB/c and C57BL mice resulted in a significant induction of micronuclei in the polychromatic erythrocytes, as did oral administration to $B6C3F_1$ mice. Potassium arsenite tested only in C57BL mice was also positive in the micronucleus test in polychromatic erythrocytes. Arsenic sulfide (called orpiment) did not induce micronuclei to any quantifiable extent, presumably because of its low solubility and bioavailability, a reflection of elevated blood levels of arsenic in orpiment-treated animals. After oral or subcutaneous administration of sodium arsenite for either 1, 6 or 30 consecutive days, elevated frequencies of chromosomal aberrations were found in the bone-marrow cells of Swiss albino mice.

Significantly elevated numbers of aneuploid cells were detected in bone-marrow cells of ICR (CD-1) mice treated intraperitoneally with DMA^V.

In an assay to detect point mutations caused by arsenic, virgin C57BL/6J mice and female metallothionein knock-out null mice ($MT^{-/-}$) were exposed to drinking-water containing 500 µg/L arsenic for up to 26 months (Ng *et al*., 2001). Nine of 12 (75%) virgin C57BL/6J and 8/11 (72.72%) $MT^{-/-}$ mice developed one or multiple mutations in exon 5 of the *p53* gene. The most prominent mutation (mutation hot spot) appeared in codon 163

of exon 5, in 9/12 (75%) and 10/14 (71.4%) of the tissues tested in C57BL/6J and $MT^{-/-}$ mice, respectively.

C57BL/6J mice fed methyl-deficient diets were administered arsenite in the drinking-water at doses of 0, 2.6, 4.3, 9.5 or 14.6 mg/kg bw per day for 130 days. Arsenite treatment increased genomic hypomethylation in a dose-dependent manner and reduced the frequency of methylation at several cytosine sites within the promoter region of the Ha-*ras* gene (Okoji *et al*., 2002).

Co-mutagenicity/co-genotoxicity of arsenic

Trivalent arsenic was demonstrated to act as a synergistic co-mutagen in combination with many genotoxic agents including ultraviolet (UV) light.

For instance, when Chinese hamster ovary cells were treated simultaneously with UV light and sodium arsenite, chromatid and chromosomal aberrations as well as *Hprt* mutations were increased synergistically. An additive effect in the induction of sister chromatid exchange was observed with a combined treatment of low doses of UV and As^{III} but not with a combined treatment of higher doses of UV and arsenic (Lee *et al.*, 1985b). Treatment of Chinese hamster ovary cells with sodium arsenite after incubation with the DNA-alkylating agent methyl methanesulfonate also enhanced clastogenicity and *Hprt* mutagenicity synergistically (Lee *et al*., 1986a). However, pretreatment with sodium arsenite resulted in a reduction in the mutagenicity of methyl methanesulfonate. Furthermore, post-treatment of Chinese hamster ovary cells with sodium arsenite was shown to increase UV- and alkylating agent-induced chromosomal aberrations (Huang *et al*., 1986) and the clastogenicity of DNA-cross-linking agents (Lee *et al*., 1986b). In the presence of sodium arsenite, γ-ray-induced chromosomal aberration frequency was potentiated in human peripheral lymphocytes (Jha *et al*., 1992). In human UV-irradiated VH16 fibroblasts, micronuclei (but not sister chromatid exchange) were induced synergistically by post-treatment with sodium arsenite (Jha *et al*., 1992). According to the authors, the lack of synergistic effect on UV-induced sister chromatid exchange in this study may be because sodium arsenite was washed off before the cells were seeded for division.

4.5 Mechanistic considerations

Several different mechanisms of arsenic-induced carcinogenicity have been proposed, and the trivalent species are implicated in most of these mechanisms (National Research Council, 1999, 2001; Simeonova & Luster, 2000; Kitchin, 2001; Hughes, 2002). It should be noted, however, that the trivalent species are formed *in vivo* after exposure to pentavalent arsenic. Methylated trivalent arsenic is more toxic, and genotoxic, than trivalent inorganic arsenic; in contrast, methylated pentavalent arsenic is less toxic, and genotoxic, than pentavalent inorganic arsenic.

4.5.1 *Genotoxicity*

Arsenic induces chromosomal aberrations, micronuclei, aneuploidy, endoreduplication and gene amplification. These may play a role in the genomic instability that can result from treatment with arsenic. Arsenic appears to have little if any ability to induce point mutations (National Research Council, 1999, 2001). The methylated trivalent molecules of arsenic are potent forms for the induction of DNA damage in cells *in vitro*, and they are the only forms of arsenic that cause DNA breakage *in vitro*, a reaction that is mediated by reactive oxygen species (Yamanaka & Okada, 1994; Nesnow *et al*., 2002; Kitchin & Ahmad, 2003).

4.5.2 *Altered DNA repair*

Trivalent arsenic (As^{III}) inhibits nucleotide-excision repair of UVC-induced DNA damage in human fibroblasts by interacting with distinct steps of the repair process. It impaired the incision step at low concentrations and the ligation step at higher concentrations (Hartwig *et al.*, 1997).

As^{III} inhibits several DNA-repair enzymes including DNA ligases I and II (Li & Rossman, 1989; Lee-Chen *et al.*, 1992), and zinc-finger proteins bearing covalent disulfide linkages seem to be potential targets of this metal. The activity of PARP, one of the zinc-finger DNA-repair enzymes, is inhibited in a human T-cell lymphoma-derived Molt-3 cell line and HeLa cells by low concentrations of arsenic (5 μM and 10 nM, respectively) (Yager & Wiencke, 1997; Hartwig *et al.*, 2003). However, other zinc-finger DNA-repair enzymes such as mammalian xeroderma pigmentosum group A protein and bacterial formamido-pyrimidine-DNA glycosylase are not inhibited by As^{III} (Asmuss *et al.*, 2000).

4.5.3 *Induction of oxidative stress*

Exposure to arsenic results in the generation of reactive oxygen species both *in vitro* and *in vivo*. There is evidence that these may be involved in the DNA-damaging activities of As^{III}, MMA^{III} and DMA^{III}. Arsenic species, particularly DMA^{III}, release iron from ferritin (Ahmad *et al.*, 2000); this free iron can produce reactive oxygen species via Fenton and/or Haber-Weiss type reactions. Reactive oxygen species are detected in human–hamster hybrid cells exposed to arsenite (As^{III}) (Liu, S.X. *et al.*, 2001) and in ϕX174 DNA incubated *in vitro* with MMA^{III} or DMA^{III} (Nesnow *et al.*, 2002). They are also involved in stress responses that may alter DNA and gene expression. For example, 8-OHdG formation and cyclooxygenase Cox-2 expression, most commonly used as a marker for the evaluation of oxidative DNA damage, are increased in the urinary bladder cancers of rats treated with dimethyl arsenite (Wei *et al.*, 2002). The DMA^{III} produced *in vivo* in the urine of rats treated with DMA^{V} (Cohen *et al.*, 2002) and subsequent generation of reactive oxygen species may be important factors in the arsenic-induced bladder cancer observed in these animals (Wei *et al.*, 2002).

4.5.4 *Altered DNA methylation*

The alteration of DNA methylation by arsenic may also play a role in the development of cancer. In-vitro and in-vivo studies indicate that the carcinogenicity of arsenic may be mediated by alterations in the methylation status of DNA either by hypermethylation or hypomethylation (Mass & Wang, 1997; Zhao *et al.*, 1997; Okoji *et al.*, 2002).

4.5.5 *Cell transformation*

Arsenic induces cell transformation in Syrian hamster embryo cells, BALB/3T3 cells and in the rat liver cell line TRL1215. Inoculation of the latter cells into nude mice gave rise to malignant tumours (fibrosarcoma and metastases to the lung) (Lee *et al.*, 1985a; Bertolero *et al.*, 1987; Zhao *et al.*, 1997).

4.5.6 *Altered cell proliferation*

Increased cell proliferation has been demonstrated directly or indirectly in various experimental systems after exposure to arsenic (Germolec *et al.*, 1997; Kitchin, 2001; Hughes, 2002).

Increases in ODC activity, a biomarker of cell proliferation, have been observed in the kidney or liver of rats treated with arsenic (Yamamoto *et al.*, 1995; Brown & Kitchin, 1996). Stimulation of cell proliferation had been shown in normal human epidermal keratinocytes treated *in vitro* by arsenic (Germolec *et al.*, 1997).

Hyperplasia has been observed in the bladder of rats treated with DMA^V (Cohen *et al.*, 2002).

4.5.7 *Altered cell signalling*

Arsenic stimulates the activity of Jun kinases, which belong to the mitogen-activated protein kinase family, and increases the DNA binding of transcriptional factor AP-1. Arsenic also induces the expression of proto-oncogenes such as C-*JUN*, C-*FOS*, C-*MYC* and tumour growth factor-α (Cavigelli *et al.*, 1996; Germolec *et al.*, 1998; Simeonova *et al.*, 2000; Chen *et al.*, 2001). A reduction in p53 protein levels concomitant with an increase in mdm_2 protein levels were also observed in a keratinocyte (HaCaT) cell line treated with arsenic. The disruption of *P53-*MDM_2 loop-regulating cell-cycle arrest as a model for arsenic-related skin carcinogenesis has been proposed (Hamadeh *et al.*, 1999).

4.5.8 *Altered steroid receptor binding and gene expression*

Arsenic inhibited steroid binding to glycocorticoid receptors but had no effect on the binding of ligands to androgen, estrogen, mineral corticoid or progesterone receptors. This specific inhibition may provide a method of using arsenic to block glucocorticoid receptors selectively in assays of the progesterone receptor content of breast cancer tissues

(Lopez *et al.*, 1990). In MCF-7 cells, arsenite blocked the binding of estradiol to oestrogen receptor-α (ER-α) (Stoica *et al.*, 2000).

Moreover, arsenic inhibited expression of ER-α but had no effect on expression of ER-β in breast cancer cell lines (Chen *et al.*, 2002). Thus, the authors concluded that the role of arsenic in the expression of ER-α provides a novel therapeutic approach for ER-α-positive breast cancer (Chen *et al.*, 2002).

4.5.9 *Gene amplification*

Arsenic enhanced the amplification of the dihydrofolate reductase (*DHFR*) gene in mouse 3T6 cells and gene amplification has been suggested as a possible mechanism of the carcinogenicity of arsenic (Lee *et al.*, 1988).

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^{V}), although in reducing environments significant concentrations of arsenite (As^{III}) 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 β-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 $p53^{+/-}$ and $p53^{+/+}$ 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 genotoxi 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)*.

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