Oral exposure to inorganic arsenic: evaluation of its carcinogenic and non-carcinogenic effects

Ulrike Schuhmacher–Wolz1, Hermann H. Dieter2, Dominik Klein3, and Klaus Schneider1

1Forschungs- und Beratungsinstitut Gefahrstoffe GmbH (FoBiG), Freiburg i. Br., Germany, 2Umweltbundesamt, Berlin, Germany, and 3Helmholtz Zentrum München, GmbH, Institute for Toxicology, Neuherberg, Germany

Abstract
Inorganic arsenic, which is extensively metabolised in humans into even more toxic methylated arsenicals, is a potent carcinogen, causing tumours of the skin, lung, urinary bladder, and other organs. It also induces a number of non-cancer effects. Consumption of drinking water highly contaminated by arsenic causes serious health problems in some countries in southeastern Asia, and arsenic poses problems for drinking-water safety worldwide. Existing risk assessments are based on epidemiological studies from regions with high exposure concentrations (in the mg/L range). It is a matter of debate whether these findings are useful at predicting arsenic-induced effects at low concentrations. In recent years numerous epidemiological studies on cancer and non-cancer effects of inorganic arsenic have been published. This work aims at reviewing recent toxicological and epidemiological data on inorganic arsenic with emphasis on effects at low exposure concentrations. Information obtained from epidemiological studies is supplemented with mechanistic data from in vitro and in vivo studies. Various modes of action for arsenic carcinogenicity are discussed. The information gathered was used to evaluate the reliability of existing cancer-risk assessments and to improve current assessments of non-cancer health effects. A tolerable daily dose, based on epidemiological studies on arsenic-induced skin disorders, is presented.

Keywords: Cancer; drinking water; inorganic arsenic; risk assessment; skin lesions

Introduction
Arsenic is an important contaminant, whose occurrence in environmental media (air, soil, water) results from natural (geogenic) sources and from anthropogenic activities. Humans are mainly exposed to inorganic arsenic species (arsenite, AsO3\(^{3-}\), and arsenate, AsO4\(^{3-}\)). Millions of people in Bangladesh, India, and China, as well as large populations in North and South America, are exposed to inorganic arsenic via drinking water, partly at high concentrations (up to several thousands µg/L) from geogenic sources (Tapio and Grosche, 2006). In regions with low levels in the drinking water, it is technically demanding and costly to comply with health-based standards for arsenic levels in drinking water.

Oral intake of inorganic arsenic compounds can cause a plethora of adverse effects in humans: skin lesions (dyspigmentation, keratosis); peripheral vascular diseases; reproductive toxicity; and neurological effects. Inorganic arsenic has been shown to cause cancer in humans after inhalation or ingestion, resulting in tumours of the skin, lung, urinary bladder, and other locations, and has been classified as a proven human carcinogen by the International Agency for Research on Cancer (IARC, 2004), in the EU (European Chemicals Bureau, 2007), and by the US Environmental Protection Agency (EPA, 2007).

Interestingly, the well-known effects of arsenic on humans are difficult to reproduce in experimental animals, and the toxicological evaluations of recent years of inorganic arsenic are mainly based on human data. But despite the well-known effects of inorganic arsenic and the wealth of human data, the toxicological significance of low-level oral exposure to arsenic and the dose–response relationship for carcinogenic effects in humans has been the subject of controversial discussions.

Current estimates of the excess lifetime cancer risk are mainly based on several epidemiological studies of an ecological study design that observed populations in Taiwan highly exposed to arsenic via drinking water. These studies were criticised for shortcomings in the exposure assessment...
(Brown and Ross, 2002; Lamm et al., 2006; Brown, 2007). In 2001, re-evaluations of the cancer risks posed by arsenic in drinking water took place in the US. Cancer-risk estimates were developed by the US National Research Council (NRC), US EPA, and others, using models extrapolating linearly from the range of observations to low exposure levels (NRC, 2001; Morales et al., 2000). This approach was considered overly conservative by others, who assume that thresholds for carcinogenic effects of arsenic exist (Lamm et al., 2006).

A legal-limit value of 10 µg As/L was set in Germany in 1990 and came into force on 1 January 1996 (Federal Republic of Germany, 1991). It was based on a 200 µg/day effect threshold for the carcinogenic potential of arsenic (Dieter, 1991). A provisional drinking-water guideline value of 10 µg/L was proposed by the World Health Organisation (WHO) in 1993 (WHO, 1993). The Drinking Water Directive, Republic of Germany, 1991). It was based on a 200 µg/day effect threshold for the carcinogenic potential of arsenic (Dieter, 1991). A provisional drinking-water guideline value of 10 µg/L was proposed by the World Health Organisation (WHO) in 1993 (WHO, 1993). The Drinking Water Directive, containing the same drinking-water limit value, was put into force in the EU in 1998 (CEU, 1998). In the US, the maximum contaminant level for arsenic was lowered from 50 µg/L to 10 µg/L in 2001.

A national project was initiated by the German Federal Environment Agency (Umweltbundesamt) to reassess the available toxicological data on the oral intake of inorganic arsenic with a view to the possible implications for existing limits to arsenic levels in environmental media. To this end, we reviewed toxicological information published in the last decade relevant to the assessment of low-dose arsenic exposure. The review is focused on epidemiological data on both cancer and non-cancer endpoints, but also takes into account in vitro and other data for the discussion of the modes of action involved in arsenic-related adverse effects.

Methods

This evaluation of the effects of arsenic on human health was performed within the framework of a research project in Germany that started in 2006, commissioned by the Umweltbundesamt. The evaluation aimed to assess carcinogenic and non-carcinogenic effects of oral intake of inorganic arsenic and to re-evaluate existing assessments in the light of new evidence that has emerged in the last decade. An enormous number of studies on arsenic toxicology have been published, covering epidemiological (referring to tumour incidences, non-cancer effects, individual susceptibility, and the like), in vitro (especially investigations into the mechanisms of action, role of methylated metabolites), and in vivo studies (e.g. animal experiments on tumour induction by methylated arsenicals). A central point of discussion was whether there exist epidemiological data suitable to describe carcinogenic risks in the low-dose range.

A comprehensive literature search was performed to identify relevant publications published since 2000 dealing with modes of action, the role of methylated metabolites, and epidemiological findings on cancer and non-cancer effects in the low-exposure range (<200 µg/L drinking water).

Results and conclusions of the assessment were presented to a national panel of experts at a one day workshop at the Umweltbundesamt on 5 July 2007 in Berlin. The results of the assessment, after consideration of workshop discussions, are presented in this publication.

Toxicokinetics of inorganic arsenic

The toxicokinetics of inorganic arsenic have been studied extensively. Comprehensive reviews of toxicokinetic data on inorganic arsenic are presented, for example, by the Agency for Toxic Substances and Disease Registry (ATSDR, 2005), the WHO (2001), and IARC (2004). The following description focuses on the metabolic pathways in humans. Since relevant qualitative and quantitative species differences exist in the metabolism of inorganic arsenic (e.g. methylated metabolites have not been found in guinea pigs and primates), data from other species are presented only where this enhances understanding and risk assessment. Detailed descriptions of existing physiologically based pharmacokinetic models can be found in the literature (Mann et al., 1996a, 1996b, Gentry et al., 2004).

Absorption

Several studies in humans indicate that soluble salts of inorganic arsenic are rapidly and nearly completely (about 95%) absorbed after ingestion. Absorption of insoluble salts is much lower (ATSDR, 2005). On the basis of urinary excretion studies with volunteers, it seems that the methylated metabolites monomethylarsonic acid (MMA\textsuperscript{\text{III}}) and dimethylarsinic acid (DMA\textsuperscript{\text{III}}) are absorbed from the gastrointestinal tract to a somewhat lesser degree (at least 75–85%; ATSDR, 2005; WHO, 2001).

Distribution

After absorption, inorganic arsenic is readily distributed and taken up by cells in tissues. Both trivalent arsenite (As\textsuperscript{\text{III}}) and pentavalent arsenate (As\textsuperscript{\text{V}}) are actively transported to the cell; the former by aquaglyceroporins, which normally transport water and glycerol, and the latter by phosphate transporters (Tapio and Groeschle, 2006). Arsenic accumulates in skin, hair, and nails. It can pass into the brain, placenta, and breast milk (ATSDR, 2005; Cohen et al., 2006; WHO, 2001).

Metabolism

Pentavalent arsenate is rapidly reduced to trivalent arsenite, mainly in blood and the liver, before it is further metabolized (see Figure 1). Approximately 50–70% of a single dose of arsenate is rapidly reduced to arsenite. This reaction can occur non-enzymatically, via glutathione as an electron donor, or can it be enzymatically catalyzed by glutathione-S-transferase (GST, omega class 1-1 (GSTO1-1), which is identical to MMA\textsuperscript{\text{III}}-reductase. Transformation by GSTO1-1 is the rate-limiting step in inorganic-arsenic metabolism and requires glutathione as a reductant (Cohen et al., 2006; Pott et al., 2001; Tapio and Groeschle, 2006; Tseng, 2007).

Arsenite is methylated to dimethylarsinous acid (DMA\textsuperscript{\text{III}}) in a multistage process. The oxidative methylation of As\textsuperscript{\text{III}} to MMA\textsuperscript{\text{V}} and of monomethylarsonous acid (MMA\textsuperscript{\text{III}}) to DMA\textsuperscript{\text{V}}
Oral exposure to inorganic arsenic

is catalysed by arsenic(III)methyltransferase (As(III)MT). This human enzyme seems to combine the functions of a methyltransferase and a reductase in a single protein. As(III)MT uses thioredoxin and nicotinamide adenine dinucleotide phosphate (NADPH) as reductants. S-Adenosylmethionine supplies the methyl group for methylation. As(III)MT also seems to be able to catalyse the reduction of MMAV to MMAIII and the reduction of DMAV to DMAIII. Reduction of the pentavalent to the trivalent forms can also be catalysed by GSTO1-1 or non-enzymatically (Cohen et al., 2006; Tapio and Grosche, 2006). It is probable that further enzymes like other methyltransferases and glutathione-S-transferases are also involved (Lindberg et al., 2007; Steinmaus et al., 2007).

The arsenic methylation or reduction pathways do not all end at the level of DMA. Arsenic compounds containing three or four methyl groups, such as trimethyl arsine and trimethyl arsine oxide, have been isolated from rats. However, in humans, MMAIII and DMAIII are the most relevant metabolites (Cohen et al., 2006; Tapio and Grosche, 2006). The primary site of arsenic metabolism in mammals is the liver, although there is also high methylating activity in testes, kidney, and lung tissues (Cohen et al., 2006).

An alternative scheme to that presented in Figure 1 has been proposed by Hayakawa et al. (2005). They postulated that inorganic arsenic is metabolised via arsenic-glutathione complexes with sequential transfer of methyl groups without oxidation of trivalent arsenic. Currently, the exact mechanism of arsenic metabolism is still unclear (Thomas, 2007).

Unlike inorganic arsenic, organic arsenicals (MMAV and DMAV) are metabolised only to a small extent in humans (about 10%). The greater part is excreted unchanged. By contrast, rats that received MMAV via drinking water excreted about 65% of it unchanged, and only about 27% DMAV was found in the urine unchanged, besides a number of further metabolites. Demethylation of DMAV may possibly be due to microbial demethylation in the intestine (Gebel, 2001).

Elimination

The major route of the excretion of arsenic compounds is via urine. DMA is the primary metabolite excreted with urine. Humans also excrete significant amounts of MMA. Urine in humans typically contains 10–30% inorganic arsenic, 10–20% MMA, and 60–80% DMA, indicating relatively efficient methylation (Cohen et al., 2006; NRC, 1999; Vahter and Concha, 2001). Improvement of the analytical methods has allowed differentiation between trivalent and pentavalent methylated metabolites. As shown in Table 1, trivalent metabolites contribute to a relevant extent to the overall excretion of

**Table 1. Distribution of urinary arsenicals according to arsenic concentrations in drinking water.**

<table>
<thead>
<tr>
<th>Study population</th>
<th>Arsenic in drinking water (µg/L)</th>
<th>Urinary content in µg/L (SD)</th>
<th>AsIII</th>
<th>MMAIII</th>
<th>MMAV</th>
<th>DMAIII</th>
<th>DMAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>India, West Bengal†</td>
<td>29.0</td>
<td>9.27 (4.0)</td>
<td>6.49 (0.9)</td>
<td>8.63 (4.3)</td>
<td>6.31 (3.1)</td>
<td>25.8 (14)</td>
<td></td>
</tr>
<tr>
<td>India, West Bengal†</td>
<td>55.0</td>
<td>11.2 (8.9)</td>
<td>7.44 (2.5)</td>
<td>10.3 (3.8)</td>
<td>16.8 (7.5)</td>
<td>31.5 (11)</td>
<td></td>
</tr>
<tr>
<td>India, West Bengal†</td>
<td>130.0</td>
<td>19.9 (15)</td>
<td>9.37 (2.9)</td>
<td>14.9 (5.2)</td>
<td>23.1 (9.6)</td>
<td>80.8 (45)</td>
<td></td>
</tr>
<tr>
<td>India, West Bengal†</td>
<td>163</td>
<td>22.9 (16)</td>
<td>11.9 (5.2)</td>
<td>28.5 (13)</td>
<td>32.2 (11)</td>
<td>171 (71)</td>
<td></td>
</tr>
</tbody>
</table>

Note: †Mandal et al., 2004 (mean values).
‡Le et al., 2000 (data presented as range of four samples).

**Figure 1.** Enzymatic methylation pathway of arsenic.
methylated metabolites (up to 50%; Mandal et al., 2004). Valenzuela et al. (2005) investigated urinary arsenic excretion in a collective from central Mexico and found that the amount of the trivalent methylated metabolites was about twice that of the pentavalent methylated metabolites (7.4% MMAIII, 2.8% MMAV, 49.0% DMAIII, 23.7% DMAV; figures are the percentage of total arsenic in urine). The rat is the only species that excretes significant amounts of trimethylarsine oxide (WHO, 2001).

After 4 days, about 50% of a single oral dose of sodium arsenite is excreted in urine (Buchet et al., 1981a; WHO, 2001).

Factors influencing the metabolism of inorganic arsenic and individual susceptibility

While millions of people are exposed to drinking water with elevated arsenic concentrations, only 15-20% exhibit arsenic-specified skin lesions (Banerjee et al., 2007). Individual differences in arsenic metabolism are discussed as a main factor contributing to individual susceptibility. Large inter-individual variation has been observed in the excretion patterns of arsenic species in urine. For example, urinary excretion of MMA in some residents of the Andes (Atacamenos in northern Chile) is less than 5%, whereas in northeastern Taiwan, MMA can comprise up to 27% of the urinary arsenic excretion (Chiou et al., 1997a; WHO, 1999).

The so-called primary methylation index (PMI) and secondary methylation index (SMI), characterising the relationship between MMAV and inorganic arsenic and the relationship between DMAV and MMAV, respectively (Pu et al., 2007), are used to describe individual methylation capacity. An increased PMI and a decreased SMI point to an incomplete methylation of inorganic arsenic to DMAV and an accumulation of MMAV.

Several studies of populations with skin or bladder tumours due to exposure to high arsenic concentrations revealed that persons with cancer have higher urinary levels of MMA than persons without cancer (Hsuheu et al., 1997; Chen et al., 2003a, 2003b; Yu et al., 2000). Interestingly, methylation capacity also seems to influence the susceptibility of persons with low arsenic exposure. Investigating individuals from Taipeh (Taiwan) who were exposed to low levels of arsenic via drinking water (0.7–4.0µg/L), Pu et al. (2007) found that the SMI of individuals with bladder tumours was lower (≤11.2) than the SMI of persons without tumours (≥12.8).

The risk of developing non-cancer health effects due to arsenic exposure also seems to be influenced by individual methylation capacity. Persons with skin lesions had higher urinary concentrations of MMA than persons without skin lesions (Ahsan et al., 2007; McCarthy et al., 2007; Valenzuela et al., 2005). Hypertension risk was also associated with a reduced SMI and increased MMA concentrations in urine (Huang et al., 2007). Several factors have been discussed as possibly contributing to metabolic differences (NRC, 1999): exposure concentration, sex; age, nutritional status; and genetic polymorphisms.

Influence of exposure concentration (dose)

The data on how dose influences arsenic metabolism are contradictory. In vitro data point to a decreased methylation at higher doses of arsenic. Also, in a study with human volunteers, the excretion of DMA was diminished at the highest dose (1000µg arsenic/day for 5 consecutive days; Buchet et al., 1981b). As only one person was tested per dose-group, the reliability of these data is uncertain. Data from persons exposed to drinking water with different arsenic concentrations indicate that the percentage of urinary DMA (relative to total arsenic metabolites in urine) decreased and that of MMA increased with increasing arsenic concentrations in drinking water. However, these changes were small (less than 5%; Hopenhayn-Rich et al., 1996; NRC, 1999). Findings from a population in Bangladesh also suggest that arsenic metabolism, particularly the conversion of MMA to DMA, may be saturable (Ahsan et al., 2007). On the other hand, data from adults of northeastern Taiwan show an increase in the proportion of urinary DMA with increasing arsenic concentrations in drinking water. Overall, the effect of arsenic dose on the methylation efficiency seems to be small and mainly affects the MMA:DMA ratio (NRC, 1999).

Sex

There is evidence that women methylate arsenic more efficiently than men. An increase in the methylation capacity has been observed, especially during pregnancy (NRC, 1999). This finding indicates that hormonal effects might be responsible for the differences in methylation capacity. Whereas older investigations, such as those from northeastern Taiwan and Finland, partly failed to find sex-related differences in methylation capacity (NRC, 1999; IARC, 2004; Loffredo et al., 2003), recent publications added to the evidence that women have a higher methylation capacity compared with men (Ahsan et al., 2007; Lindberg et al., 2007, Steinmaus et al., 2007; Vähter et al., 2007).

Age

The proportion of DMA in urine of children from northern Argentina and from Finland was lower than the proportion of DMA in urine in adults (NRC, 1999). On the other hand, children (7–11 years of age) from Mexico excreted proportionally more DMA in urine than adults. Moreover, the DMAV:MMAV ratio was higher in these children than in adults. A genetic analysis of As(III)MT polymorphisms indicated that different As(III)MT genotypes are associated with different DMAV:MMAV ratios (Meza et al., 2005).

Nutritional status

There is evidence that nutritional status influences individual susceptibility to arsenic toxicity. Arsenic-exposed people from Bangladesh and West Bengal (India) with a low body weight showed higher incidences of skin lesions than persons with a higher body weight (Ahsan et al., 2006a; Guha Mazumder et al., 1998; Milton et al., 2004; Mitra et al., 2004). The body mass index (BMI) of participants with skin cancer from southwestern Taiwan was lower than that of persons...
without arsenic metabolism; fasting for a period of 12 h results in a significant increase in the percentage of urinary MMA. The MMA detection frequency in volunteers at the end of the fasting period is almost ninefold higher than at the beginning (Brima et al., 2007). In experimental investigations, animals receiving low-protein diets (especially low in amino acids with sulfhydryl groups) showed a marked decrease in urinary excretion of DMA and increased tissue retention of arsenic (NRC, 1999). Similar findings have been reported in a US population (Steinmaus et al., 2005). Folic-acid supplementation increased the amount of urinary arsenic excreted as DMA in a population from Bangladesh. Folic acid is essential for the allocation of methyl group donors like S-Adenosylmethionine (Gamble et al., 2005, 2006). Sufficient supply of other nutritional factors, like selenium, also reduces the incidence of arsenic-related lesions (Chen et al., 2007a, 2007b).

Genetic polymorphisms

The potential correlation between interindividual variation in arsenic metabolism and genetic polymorphisms of several enzymes involved in arsenic metabolism has been intensively investigated. Most studies concentrate on the polymorphisms of As(III)MT, GST (especially GSTO1-1), and other enzymes, such as methylenetetrahydrofolate reductase (MTHFR, a key enzyme in the metabolism of folate) and 5-methyltetrahydrofolate-homocysteine methyltransferase, relevant in arsenic metabolism. Schläwicke-Engström et al. (2007) showed that a significant part of the variation in urinary arsenic metabolite distribution in an Andean population (northern Argentina) was due to hereditary differences in genes related to arsenic metabolism. Specific single nucleotide polymorphisms of As(III)MT were related to an approximately 50% reduction of MMA in urine. Investigations in an arsenic-exposed population from Argentina revealed that subjects with the TT/AA polymorphism at MTHFR 677 and 1298, which correlates with a lower MTHFR activity, excreted a significantly higher proportion of ingested arsenic as inorganic arsenic and a lower proportion as DMA. Women with the null genotype of GSTM1 (i.e. no enzyme activity) excreted a significantly higher proportion of arsenic as MMA than women with the active genotype (Steinmaus et al., 2007).

Lindberg et al. (2007) investigated the influence of genetic polymorphisms on the arsenic metabolism in a central European population. The polymorphisms of As(III)MT, MTHFR, and GSTO1-1 investigated in this study only partially explained the variation seen in arsenic metabolism (about 20% of the variation seen in men and around 4% among women). In addition, Marcos et al. (2006) found that the polymorphic expression of several genes of GST isoforms only partially explained the variations in the urinary profile of arsenic metabolites. Various ethnic groups show differences in the frequency and form of genetic polymorphisms (Mukherjee et al., 2006) indicating that differences in the susceptibility of different populations towards arsenic may exist. Table 2 summarises relevant aspects of arsenic metabolism.

### Table 2. Characteristics of arsenic metabolism in humans.

At least 75% of any chemical form of As are resorbed in the digestive tract.

Inorganic arsenic is metabolised to methylated arsenicals with dimethylarsinic acid (DMA\textsuperscript{VI}) as the main metabolite in humans.

Methylation is not complete and inorganic arsenic and monomethylated metabolites are also excreted via urine.

Human urine typically contains about 10–30% inorganic arsenic, 10–20% MMA, and 60–80% DMA.

Inter-individual differences are probably due to variations in exposure concentration, sex (women methylate arsenic more efficiently than men), age, nutritional status, and genetic polymorphisms.

Increased urinary MMA-levels (i.e. diminished metabolism of arsenic to DMA) are associated with an increased risk of developing cancer or non-cancer lesions.

#### Mode of carcinogenic action

The carcinogenicity of inorganic arsenic after oral (drinking water) and inhalative exposure has been substantiated by epidemiological data (IARC, 2004). Arsenic-induced tumours in humans were observed in particular in populations exposed to high arsenic concentrations (data on populations with exposure in the low-dose range are reported below): Arsenic does not directly interact with DNA, and it is uncertain whether linear extrapolation to low doses is appropriate (Lamm et al., 2006, 2007).

To improve the quality of risk assessments, efforts have been undertaken in recent years to investigate the mode of carcinogenic action of arsenic. Numerous animal studies have been performed. However, no suitable animal model for the human situation has been found to date, possibly due to interspecies differences in toxicokinetics and toxicodynamics. Inorganic arsenic was not carcinogenic in most animal experiments, except when As\textsuperscript{V} was given to pregnant mice (Waalkes et al., 2003, 2006a, 2003b). Besides sodium arsenite, MMA\textsuperscript{V} and DMA\textsuperscript{V} showed carcinogenic activity in animal experiments. In a 2-year carcinogenicity study, MMA\textsuperscript{V} (500 μg/L drinking water) induced tumours (mainly lymphoma) in C57BL/6J-mice (Krishnamohan et al., 2006). DMA\textsuperscript{V} is carcinogenic in rats. Exposure to high cytotoxic concentrations via diet or drinking water induced bladder tumours (Arnold et al., 2006; Wei et al., 2002). MMA\textsuperscript{V} was not carcinogenic in mice and rats (Arnold et al., 2003). No data on the carcinogenic potential of DMA\textsuperscript{V} exist. Skin tumours, one of the typical forms of arsenic induced tumours in humans, were not observed in chronic animal studies.

The following modes of action have been suggested to be relevant for tumour induction: genotoxicity; oxidative damage; inhibition of DNA repair; influence on DNA methylation; modification of cell signalling; and changes in cell proliferation.

#### Genotoxicity

Arsenite and arsenate were not mutagenic to bacteria (Escherichia coli, Salmonella typhimurium). They did not induce ouabain-resistant and thioguanine-resistant mutants in mammalian V79 or Chinese-hampster-ovary cells (Gebel, 2001). However, sodium arsenite induced micronuclei...
in V79 and Chinese-hamster-ovary cells and in human peripheral lymphocytes (Gebel, 1998; Wang et al., 1997; Schaumlöffel and Gebel, 1998). Furthermore, the induction of sister chromatid exchanges (Gebel et al., 1997; Lee et al., 1985) and DNA strand breaks was observed in mammalian cells (Gómez et al., 2005; Sordo et al., 2001). Increased incidences of chromosomal aberrations and aneuploidy were observed in human peripheral lymphocytes (Eastmond and Tucker, 1989; Kligerman et al., 2003; Mahata et al., 2004; Nordenson et al., 1981; Oya-Ohta et al., 1996; Ramirez et al., 1997). Arsenite-induced gene amplifications in human osteosarcoma cells (Rossman, 2003) and cell transformations in Syrian-hamster-embryo cells (Lee et al., 1985). Effective doses were usually below 1 µg/mL. Similar effects (induction of sister chromatid exchanges, micronuclei, chromosomal aberrations and cell transformations) were induced by arsenate; however, at concentrations around 10 times higher than with arsenite (Gebel, 2001; Rossman, 2003; IARC, 2004). Arsenite enhanced the mutagenicity and or clastogenicity of ultraviolet light, N-methyl-N-nitrosurea, diepoxybutane, X-rays, and methylmethane sulfonate in E. coli and mammalian cells (Rossman, 2003).

Arsenic was also genotoxic in vivo. Animal experiments produced evidence of the induction of micronuclei, chromosomal aberrations, and strand breaks (Biswas et al., 1999; Deknudt et al., 1986; RoyChoudhury et al., 1996; Saleha Banu et al., 2001; Tice et al., 1997), supporting the numerous findings in humans. Humans exposed to inorganic arsenic (mostly to arsenite or arsenate via drinking water, but there are also data on occupational exposure) had increased incidences of chromosomal aberrations and aneuploidy and DNA strand breaks at very high concentrations (Kashiwada et al., 1998; Yamanaka et al., 1989). Arsenic trioxide has been demonstrated to vary significantly (Dopp et al., 2004). So the concentrations given in Table 3 represent only the lowest effective dose in the relevant test system, and should not be regarded as absolute values. These data further demonstrate that methylation of inorganic arsenic does not automatically mean a detoxification. Regarding genotoxicity, formation of trivalent methylated arslenicals is a toxification process.

Effective concentrations observed in several in vitro assays were in the range of arsenic concentrations measured in urine of arsenic-exposed humans.

### Table 3. Comparison of the genotoxic activity of different arsenicals in vitro.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>DMA&lt;sub&gt;III&lt;/sub&gt;</th>
<th>MMA&lt;sub&gt;III&lt;/sub&gt;</th>
<th>As&lt;sub&gt;III&lt;/sub&gt;</th>
<th>As&lt;sub&gt;V&lt;/sub&gt;</th>
<th>MMA&lt;sub&gt;V&lt;/sub&gt;</th>
<th>DMA&lt;sub&gt;V&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal aberration, human umbilical cord fibroblasts (Oya-Ohta et al., 1996)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>3.8µM</td>
<td>16µM</td>
<td>1400µM</td>
<td>700µM&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chromosomal aberration, HPL (Kligerman et al., 2003)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35µM</td>
<td>0.6µM</td>
<td>2.5µM</td>
<td>10µM</td>
<td>3000µM</td>
<td>3000µM negative up to 7mM</td>
</tr>
<tr>
<td>Micronuclei, CHO (Dopp et al., 2005)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1µM</td>
<td>5µM</td>
<td>0.5µM</td>
<td>1µM</td>
<td>negative up to 7mM</td>
<td>negative up to 1mM</td>
</tr>
<tr>
<td>Micronuclei, CHO (Dopp et al., 2004)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1µM</td>
<td>10µM</td>
<td>Negative up to 500µM</td>
<td>Negative up to 500µM</td>
<td>Negative up to 500µM</td>
<td>Negative up to 500µM</td>
</tr>
<tr>
<td>Chromosomal aberration, CHO (Dopp et al., 2004)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50µM&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10µM</td>
<td>1mM</td>
<td>1mM</td>
<td>negative up to 10mM</td>
<td>negative up to 10mM</td>
</tr>
<tr>
<td>Strand breaks, lymphoblastic cell line (Gómez et al., 2005)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10µM</td>
<td>0.2µM</td>
<td>10µM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: CHO, Chinese-hamster-ovary cells; HPL: human peripheral lymphocytes.

<sup>a</sup>Incubation time was 24 h.
<sup>b</sup>Incubation time was 1 h.
<sup>c</sup>Incubation time was 0.5 h.
<sup>d</sup>Incubation time was 2 h (DMA<sub>III</sub>) or 4 h (MMA<sub>III</sub>, As<sub>III</sub>).
<sup>e</sup>Contaminated with As<sub>V</sub>.
<sup>f</sup>Lowest tested dose.

### Dose–response analysis of genotoxic data

Rudel et al. (1996) analysed the dose–response relationships observed in genotoxicity assays performed with arsenic.
Oral exposure to inorganic arsenic

They came to the conclusion “that arsenic indirectly induces genetic damage with a sublinear dose–response in humans”. Gebel (2001) criticized this interpretation and showed that insufficient consideration of cytotoxic effects may erroneously have led them to conclude a sublinear dose–response relationship. According to Gebel (2001), the variation in shapes of dose–response relationships observed in genotoxicity assays is caused by the use of different cell types or different biological endpoints and by experimental scatter, and that no final conclusions can be drawn from these data.

Information on the shape of dose–response relationships may also be obtained by analysing genotoxicity data from humans. Unfortunately, most of the published studies on arsenic genotoxicity in humans do not allow a detailed dose–response analysis to be performed, because they provide no data on individual exposure or effects or no data for the dose range of interest (< 50 µg/L drinking water). Ghosh et al. (2006) presented a differentiated analysis of genotoxic effects in a population from India. They distinguished between individuals with and without skin lesions. Both subgroups were exposed to similar arsenic concentrations in drinking water and individuals were assigned to different exposure categories (see Table 4).

The incidence of micronuclei in different cell types (lymphocytes, mucosa cells, and urothelial cells) and the incidence of aberrant lymphocytes were significantly increased in exposed individuals (with and without skin lesions) in comparison to controls. A clear dose–response relationship was observed for the effects in individuals with skin lesions, whereas no such dose–response relationship was found for individuals without skin lesions, except for induction of aberrant lymphocytes. Therefore, no general conclusion can be drawn from these data with respect to the dose–response relationship for arsenic-induced genotoxic effects.

The correlation between the incidence of chromosomal aberrations (CA) in peripheral lymphocytes and the urinary arsenic (As) content in individuals from West Bengal, India (data from Mahata et al., 2003).

Table 4. Induction of micronuclei and aberrant cells in arsenic-exposed individuals from West Bengal, India, with and without skin lesions (data from Ghosh et al., 2006).

<table>
<thead>
<tr>
<th>Arsenic content of drinking water (µg/L)</th>
<th>Micronuclei /1000 lymphocytes</th>
<th>Micronuclei /1000 mucosa cells</th>
<th>Micronuclei /1000 urothelial cells</th>
<th>% aberrant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.16</td>
<td>2.03</td>
<td>1.67</td>
<td>1.70</td>
</tr>
<tr>
<td>Participants without skin lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51–150</td>
<td>6.35</td>
<td>3.44</td>
<td>4.17</td>
<td>5.89</td>
</tr>
<tr>
<td>151–250</td>
<td>6.16</td>
<td>3.91</td>
<td>4.16</td>
<td>6.31</td>
</tr>
<tr>
<td>&gt;250</td>
<td>6.35</td>
<td>3.41</td>
<td>4.25</td>
<td>7.19</td>
</tr>
<tr>
<td>Participants with skin lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51–150</td>
<td>8.54</td>
<td>4.95</td>
<td>5.25</td>
<td>9.23</td>
</tr>
<tr>
<td>151–250</td>
<td>8.84</td>
<td>5.45</td>
<td>5.84</td>
<td>8.77</td>
</tr>
<tr>
<td>&gt;250</td>
<td>9.48</td>
<td>5.83</td>
<td>6.21</td>
<td>9.49</td>
</tr>
</tbody>
</table>

Note: *Determined in lymphocytes.

*Effects were dose dependent (Cochran–Armitage trend test).

Figure 2. Correlation between the incidence of chromosomal aberrations (CA) in peripheral lymphocytes and the urinary arsenic (As) content in individuals from West Bengal, India (data from Mahata et al., 2003).
are superimposed by as yet unknown disturbances. Similar findings were described by others (Martinez et al., 2004; Lewinska et al., 2007), who reported significant differences in the occurrence of micronuclei in exposed and control individuals, but no correlation between the genotoxic effect and the arsenic content in fingernails or urine.

**Oxidative damage**

There is increasing evidence that the induction of reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide and hydroxy radical, and oxidative stress play a crucial role in arsenic toxicity: cytotoxic and genotoxic effects of arsenicals *in vitro* are suppressed by the addition of inhibitors of oxidative stress like catalase, superoxide dismutase, and glutathione peroxidase or antioxidative substances like glutathione and vitamin E (Nesnow et al., 2002; Rossmann, 2003; Schoen et al., 2004; Shi et al., 2004). Furthermore, arsenic causes the induction of metallothionein, which has been shown to be involved in oxidative stress, and heme oxygenase, an indicator of oxidative stress, in several animal and human cell lines and in mice (Rossman, 2003; Tapio and Grosche, 2006). Radical generation was detected by confocal fluorescence microscopy after treatment of cells with As$^{III}$ (1 µM and 10 µM) or MMA$^{III}$ (50 nM and 500 nM; Eblin et al., 2006) and by electron-spin-resonance spectroscopy after DMA$^{III}$-treatment (1 mM; Nesnow et al., 2002).

Oxidative damage has also been observed after arsenic treatment *in vivo*. Oral administration of DMA induced the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the kidneys of mice and rats. Such oxidative lesions have also been observed in rodents in other target organs of arsenic toxicity, like the lungs, liver, skin, and urinary bladder (IARC, 2004; Shi et al., 2004). Free radicals have been detected in the liver of mice that received arsenite or arsenate (Rossman et al., 2003). Furthermore, increased lipid peroxidation has been observed in animal experiments (Shi et al., 2004). Shi et al. (2004) described the influence of arsenic on glutathione levels. Depending on the arsenic concentration glutathione levels may increase (low arsenic concentration) or decrease (high arsenic concentration).

Epidemiological data support the finding that arsenic causes oxidative stress: 8-OHdG was detected by immuno-histochemical methods in tumour tissues and healthy skin of all individuals (n = 28) with arsenic-induced skin tumours. But only in 15% (3/20) of the individuals with non-arsenic induced skin tumours was 8-OHdG detectable in tumour tissues or other parts of the skin (An et al., 2004). Increased 8-OHdG concentrations were detected in the urine of persons with an acute arsenic intoxication (Yamauchi et al., 2004). Increased lipid peroxide serum levels and decreased nonprotein sulfhydryl serum levels, two indicators of oxidative stress, were reported for persons from a contaminated area in Inner Mongolia. Individuals from northeastern Taiwan who were exposed to arsenic via drinking water showed increased levels of ROS and decreased levels of antioxidants in plasma (Schoen et al., 2004).

The exact mechanism of ROS formation by arsenic is still open to debate. The following mechanisms are under discussion:

- Formation of ROS in mitochondria via arsenic interference with the respiratory chain (Shi et al., 2004).
- Activation of NAD(P)H oxidase: NAD(P)H oxidases catalyse the one electron reduction of O$_2$ to superoxide; its activation leads to increased levels of superoxide and other ROS (Rossman, 2003; Shi et al., 2004; Kumagai and Sumi, 2007).
- Formation of dimethylated arsenic peroxide, dimethylarsonic radical, and dimethylarsenic peroxy radical via reaction of DMA$^{III}$ with molecular oxygen was discussed by Yamanaka et al. (2004).
- Trivalent arsenicals, especially DMA$^{III}$, may lead to a release of iron from ferritin. The free iron may lead to the formation of ROS via Fenton or Haber–Weiss reaction (IARC, 2004; Yamanaka et al., 2004).
- Hydrogen peroxide is assumed to be formed during oxidation of arsenite (As$^{III}$) to arsenate (As$^{V}$) under physiological conditions (Shi et al., 2004).

Induction of ROS (Shi et al., 2004) and genotoxic effects of inorganic arsenicals and trivalent methylated arsenicals (see above) occurred in nearly the same concentration range (1–20 µM).

Eblin et al. (2006) detected the formation of ROS in UROtsa cells in the presence of 1 µM As$^{III}$ (maximum effect at 10 µM) or 50 nM MMA$^{III}$ (maximum effect at 500 nM; 5 µM was cytotoxic). Additionally, the formation of 8-OHdG was detected. ROS formation in the presence of As$^{III}$ occurred a few minutes after start of incubation, whereas 30 minutes of preincubation were necessary for the reaction with MMA$^{III}$.

Both catalase and superoxide dismutase prevented ROS formation; however, less effectively in the presence of MMA$^{III}$ than in the presence of As$^{III}$. Eblin et al. (2006) suggested that this may indicate the formation of different ROS by As$^{III}$ and MMA$^{III}$. Additionally, 1 µM As$^{III}$ and 50 nM MMA$^{III}$ led to an increase in levels of heat shock protein Hsp70 and metallothionein, two indicators of oxidative stress. Concentrations of As$^{III}$ and MMA$^{III}$ tested *in vitro* are in the range of urinary concentrations of arsenicals measured *in vivo*. The concordance between the concentration ranges that led to the induction of genotoxic effects and that induced ROS after treatment with arsenicals supports the assumption of a causal relationship between both events. These data are supported by the findings of Schwerdtle et al. (2003a) and Wang et al. (2002), who reported the formation of oxidative DNA damage (oxidative DNA base modifications) by arsenite and its trivalent and pentavalent methylated metabolites at concentrations in the nanomolar and micromolar range.

Induction of oxidative stress does not seem to be the only mechanism by which arsenicals induce genotoxicity: in some cases, addition of catalase or superoxide dismutase had no effect on the sodium-arsenite-induced formation of micronuclei in human peripheral lymphocytes *in vitro* (Gebel,
Arsenic may disturb the cellular redox balance and induce oxidative stress by glutathione depletion, as arsenic binds to free sulfhydryl-groups, glutathione is needed for the reduction of AsIII to AsII, and glutathione is involved in the inactivation of free radicals (Shi et al., 2004). Besides the formation of ROS, arsenic treatment may also induce reactive nitrogen species, especially nitrogen oxide, which was described to be mutagenic (Florea et al., 2005; Shi et al., 2004).

**Influence on DNA repair**

Inhibition of DNA repair seems to be one mechanism by which arsenic may lead to genotoxic effects (Gebel, 2001). This is supported by the observation that arsenic itself does not induce point mutations, but amplifies the mutagenic effects of other point mutagens (Rossman, 2003). Inhibition of nucleotide excision repair in the presence of arsenite or its methylated metabolites was detected in vitro, e.g., after ultraviolet-irradiation or pretreatment of cells with benzo[a]pyrene (Danaee et al., 2004; Hartwig et al., 1997; Schwerdtle et al., 2003b; Shen et al., 2008). At low doses, arsenite impaired the incision step, and at higher doses, the ligation step (Hartwig et al., 1997). Inhibition of nucleotide excision repair has also been observed in vivo in rats treated with benzo[a]pyrene (Tran et al., 2002). Interaction of arsenite with base excision repair has been reported, for example by Li and Rossman (1989) and Sykora and Snow (2008). Inhibition of DNA repair has been shown to take place via two different mechanisms: (a) by direct interaction of arsenic with DNA repair enzymes, leading to their inhibition; and (b) by arsenic influencing the expression of the DNA repair enzymes via modulation of signal transduction.

A decrease in PARP-1 [poly(ADP-ribose) polymerase 1] activity was demonstrated by Walter et al. (2007) in HeLa cells in the presence of very low concentrations of MMAIII and DMAIII (1nM). PARP activity was also decreased by arsenite (AsIII, 10 nM; Hartwig et al., 2003). MMAV and DMAV did not influence PARP activity, even at cytotoxic concentrations (MMAV 500 µM and DMAV 250µM). PARP expression was not influenced by these arsenic species in HeLa cells (Walter et al., 2007). Reduced poly(ADP-ribose)ylation might possibly be due to an interaction of the arsenicals with the zinc finger domain of PARP-1 (Kitchin and Wallace, 2008; Walter et al., 2007; Witkiewicz-Kucharczyk and Bal, 2006).

Andrew et al. (2003) demonstrated an inverse correlation between the expression of DNA repair enzymes (endonucleases ERCC1, XPF, and helicase XPB) in a small US population (n = 16) and the toenail arsenic content. Arsenic concentrations in drinking water were in the range of 0–75 µg/L. These data were confirmed by an investigation with a larger population (n = 53): Expression of the DNA repair enzyme ERCC1 was significantly reduced in individuals with arsenic concentrations in drinking water >5µg/L in comparison with those with drinking-water concentrations ≤5µg/L (Andrew et al., 2006).

Banerjee et al. (2007) recently described that polymorphism of excision-repair gene ERCC2 (codon 751) is associated with arsenic-induced hyperkeratosis, possibly because of impaired DNA-repair capacity. Arsenic also may impair DNA repair by influencing p53 expression (Schoen et al., 2004).

**Influence on DNA methylation**

Maintenance of the proper methylation pattern of the promotor region is critical for the regulation of gene transcription. Disturbances of gene expression may result in tumour induction (NRC, 1999). Arsenic metabolism and DNA methylation share a common cofactor, S-adenosylmethionine. It was hypothesized that, in the presence of arsenic, normal DNA methylation may be disturbed (Tapio and Grosche, 2006). This theory was supported by several in vitro and in vivo investigations.

In vitro investigations demonstrated that arsenic induces hypomethylation in cells, which is accompanied by the up-regulation of several genes that influence the cell-cycle, such as c-myc, c-ras, and cyclin D1 (Schoen et al., 2004). Reichard et al. (2007) demonstrated in human keratinocytes in vitro that sodium arsenite may inhibit DNA methylation by S-Adenosylmethionine depletion and by inhibition of DNA methyltransferases (DNMT1 and DNMT3A).

C57Bl/6j mice administered sodium arsenite via drinking water for 130 days (up to 14.6 mg/kg a day) exhibited dose-dependent DNA hypomethylation in the liver. Feeding a methyl-deficient diet and sodium arsenite reduced the frequency of methylation at several cytosine sites within the promotor region of the oncogenic gene, Ha-ras (Okoji et al., 2002).

On the other hand, the presence of arsenic was shown to coincide with hypermethylation. In human lung adenocarcinoma A549 cells, exposure to arsenic was associated with the hypermethylation of the promotor region of p53. Hypermethylation of this gene inhibits p53 expression, which could lead to compromised DNA-repair capacity. Effects of arsenic on p53 expression seem to be cell-type specific and dose dependent (Schoen et al., 2004). Whereas sodium arsenite and arsenate induced hypermethylation at concentrations of 0.08–2.00 µM and 30–300 µM, respectively, DMA did not induce hypermethylation, even at much higher concentrations (tests were performed in the dose range of 2–2000 µM; NRC, 1999).

Hypermethylation of the tumour suppressor genes p53 and p16 has also been observed in individuals from West Bengal and reported in patients with arsenic-induced skin tumours (although effects were not statistically significant in comparison with patients with non-arsenic induced skin tumours). A small subgroup of cases with exposure to high arsenic concentrations showed p53 hypomethylation (Chanda et al., 2006). Silencing of the tumour-suppressor genes RASSF1A and PRSS3 by hypermethylation of the promotor regions was also reported for bladder-tumour...
cases with relatively high arsenic exposure. Silencing of the tumour-suppressor genes was associated with advanced tumour stages (Marsit et al., 2006a, 2006b).

The mechanisms leading to hypermethylation have not yet been revealed (Chanda et al., 2006; Marsit et al., 2006a, 2006b).

Modification of cell signalling

Important cellular events like proliferation, differentiation, and apoptosis are controlled and coordinated by complex signal-transduction pathways. Arsenic may disrupt these pathways at different levels. Arsenic influences the tyrosine phosphorylation system, mitogen-activated protein kinases (MAPK), and transcription-factor families NFkappaB and AP-1, which interact with each other. For a comprehensive description of signal transduction and possible arsenic interaction with it, we refer to the literature (e.g. Florea et al., 2005; Kumagai and Sumi, 2007; Luster and Simeonova, 2004; Rossman, 2003; Shi et al., 2004; Yang and Frenkel, 2002).

Arsenic has been shown to elevate the level of total cellular tyrosine phosphorylation, which is associated with aberrant cell signalling, uncontrolled cell growth, and the development of cancer (Tapio and Grosche, 2006).

The induction of different MAPK families is highly dependent on concentration, exposure period, and cell type. Opposite effects may be mediated, depending on the biological pathway targeted. This may result in proliferation and transformation or in growth arrest and apoptosis (He et al., 2007; Schoen et al., 2004; Shi et al., 2004). Effects on NFkappaB also depend on concentration and cell type (Tapio and Grosche, 2006).

The arsenicals show different potential: methylated arsenicals (MMAIII and DMAIII) were more potent transactivators of AP-1 than AsIII (MMAIII about 0.5 µM; AsIII about 5 µM; Stýblo et al., 2002).

Arsenic probably influences signal transduction via induction of ROS or direct interaction with enzymatic sulfhydryl groups (Rossman, 2003; Shi et al., 2004; Yang and Frenkel, 2002).

<table>
<thead>
<tr>
<th>Table 5. Modes of carcinogenic action of arsenic.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotoxicity</td>
</tr>
<tr>
<td>Oxidative damage</td>
</tr>
<tr>
<td>Influence on DNA repair</td>
</tr>
<tr>
<td>Influence on DNA methylation</td>
</tr>
<tr>
<td>Modification of cell signalling</td>
</tr>
<tr>
<td>Changes in cell proliferation</td>
</tr>
</tbody>
</table>

Changes in cell proliferation

Increased cell proliferation due to mitogenic or cytotoxic effects may enhance tumour development. Increased cell proliferation after arsenic exposure has been observed in several systems.

Arsenite-induced increased cell proliferation in vitro in human keratinocytes and HL-60 cells (0.1–1.0 µM and 0.1–0.5 µM, respectively). Apoptosis was induced at higher concentrations (1–40 µM; Zhang et al., 2003).

The trivalent arsenicals AsIII, MMAIII and DMAIII induced cell proliferation at concentrations of 0.001–0.010 µM, while at high concentrations (>0.5 µM) cell proliferation was inhibited. Pentavalent arsenicals did not influence cell proliferation. Proliferation was accompanied by an increased secretion of growth factors like tumour necrosis factor alpha and granulocyte macrophage colony stimulating factor (Vega et al., 2001).

To summarize, arsenic is clearly genotoxic in vitro and in vivo, but not a point mutagen in bacteria and mammalian cells. Despite earlier interpretations that methylation of inorganic arsenic to monomethylated and dimethylated metabolites is a detoxificant, current findings point to a central role for these arsenic metabolites in arsenic toxicity. The trivalent arsenicals especially (whether methylated or not) are highly reactive compounds and seem to be responsible for various genotoxic activities, which might partly be explained by the binding of these trivalent arsenicals to different proteins (Kitchin and Wallace, 2008). Even very low concentrations of AsIII and MMAIII in the nanomolar range (10–50 nM) were sufficient to induce ROS, cell transformation, and cell proliferation and to influence DNA repair. Oxidative stress with formation of 8-OHdG has been detected in vitro and in vivo in the presence of arsenic. Whilst arsenicals do not directly interact with the DNA, tumour induction seems to be a result of complex interactions of arsenicals with cellular mechanisms such as DNA repair, DNA methylation, signal transduction, and proliferation (see Table 5).

Epidemiological data

Carcinogenic effects

The IARC of the WHO has concluded that there is sufficient evidence for a carcinogenic activity of inorganic arsenic in humans for lung, bladder, and skin tumours. Some epidemiological studies also point to carcinogenic activity in the liver (IARC, 2004; Chiu et al., 2004) and prostate (Benbrahim-Tallaa and Waalkes, 2008).

Early evidence of carcinogenic risks from oral intake came from populations in southwestern Taiwan highly exposed to
inorganic arsenic. These studies used an ecological design, producing only a geographic relationship between tumour frequency and exposure. Early-cancer-risk estimates of the US EPA (EPA, 2007; Table 6) were based on studies by Tseng and coworkers (Tseng et al., 1968; Tseng, 1977), who investigated non-melanoma skin-cancer prevalence in 37 villages in southwestern Taiwan.

In rural Taiwan, mobility is low, which makes ecological studies more meaningful. In addition, the inclusion of large study populations enhances their predictive power. On the other hand, individual measures of exposure are lacking and they do not control for confounding factors, such as smoking, because of the absence of individual data. The studies used more recently by NRC (2001) and the US EPA (2001) (Table 6) for deriving cancer-risk estimates were, therefore, criticised for their weak exposure data (Lamm et al., 2006; Brown and Ross, 2002; Brown, 2007). Brown and Ross (2002) and Lamm et al. (2003) discuss factors influencing the outcome of the ecological studies on arsenic carcinogenicity from Taiwan, such as humic acids contained in artesian-well water in southwestern Taiwan and nutritional and disease status.

Supportive evidence used for the NRC risk assessment came from Chiu et al. (2001), who investigated the incidence of urinary-tract tumours (transitional-cell carcinomas) in a cohort of 8,102 residents of northeastern Taiwan. Only 10 cases occurred in the total cohort in the observation period, from 1991 to 1994. A high relative risk (RR = 8.1, 95%CI 0.7–98.2) was observed in the group with 50–100 µg/L exposure, but, because of the low number of cases, only results from the highest-exposure group reached statistical significance (>100 µg/L; RR = 15.1, 95%CI 1.7–138.5). Statistically significant higher risks were shown for cigarette smokers by multivariate analysis, but no significant synergistic interaction between arsenic exposure and smoking was evident.

Ferrecio et al. (2000) also found increased cancer risks at drinking-water arsenic concentrations <100 µg/L. In a case-control study, they compared 151 cases of lung cancer from northern Chile with 419 control cases. Community tap water is supplied to >90% of the population in the main cities and >64% of the population in small towns. Arsenic levels were measured regularly by water companies. Increased odds ratios (OR), adjusted for sex, age, smoking, socioeconomic status, and employment in copper smelting, were obtained for even the lowest-exposure category (10–29 µg/L, compared with <10 µg/L, OR = 1.6, 95%CI 0.4–4.6), and were statistically significant at the next-highest-exposure group (30–49 µg/L, OR = 3.9, 95%CI 1.2–12.3; 50–199 µg/L, OR = 5.2, 95%CI 2.3–11.7; 200–400 µg/L, OR = 8.9, 95%CI 4.0–19.6). The authors found a synergistic interaction between smoking and arsenic exposure.

Several new epidemiological studies have been published since the last NRC review (Table 7), covering various ranges of exposure to arsenic via drinking water below 100 µg/L. The following analysis focuses on new studies published since 2001 for the bladder, lungs, and skin. For reviews of the data until 2001, the reader is referred to IARC (2004) and NRC (2001).

### Table 7. Epidemiological studies since 2001 on cancer from arsenic in drinking water that include concentration–response data.

<table>
<thead>
<tr>
<th>Target organ</th>
<th>Population</th>
<th>Study design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>USA</td>
<td>Ecological</td>
<td>Lamm et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Case-control</td>
<td>Steinmaus et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Case-control</td>
<td>Karagas et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>Case-control</td>
<td>Bates et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Finland</td>
<td>Case-control</td>
<td>Michaud et al. (2004)</td>
</tr>
<tr>
<td>Lungs</td>
<td>Southwestern and Northeastern Taiwan</td>
<td>Cohort</td>
<td>Chen et al. (2004)</td>
</tr>
<tr>
<td>Skin</td>
<td>USA</td>
<td>Case-control</td>
<td>Karagas et al. (2001a)</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Case-control</td>
<td>Beane Freeman (2004)</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Cross-sectional</td>
<td>Knobelot et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>Cross-sectional</td>
<td>Lamm et al. (2007)</td>
</tr>
</tbody>
</table>

#### Bladder tumours

In a case-control study, Steinmaus et al. (2003) analysed 181 bladder-tumour cases from Nevada and California. Arsenic concentrations in the tap water of the participants’ communities were provided by State health services, and cumulative exposure was calculated by consideration of tap-water consumption. Slightly, although not statistically significant, increased cancer risks were obtained for exposure periods of 40 years (prior to diagnosis), but not for shorter exposure periods. For 40-year exposure at 10–80 µg As/day (highest 20-year average), the OR was 1.28 (95%CI 0.53–3.11), and for 40-year exposure at >80 µg As/day, the OR was 1.70 (95%CI 0.73–3.96). For the same exposure period, smokers in the highest arsenic-exposure group (>80 µg As/day) showed a significantly increased OR of 3.87 (95%CI 1.41–10.6).

In New Hampshire, USA, arsenic concentration in groundwater is high because of geological factors, and more than 30% of the population consume water from private wells (Karagas et al., 2004). In a case-control study, these authors used arsenic concentrations in toenails from New Hampshire residents as an exposure indicator and analysed data from 383 cases of transitional-cell carcinoma of the bladder and 641 control cases. No increased cancer risk was observed after correcting for sex, age, and smoking status. Smokers with the highest arsenic exposure (>0.33 µg As/g toenail) had an elevated OR for bladder cancer of 2.17 (95%CI 0.92–5.11). From an estimation presented in Exponent (2005), based on data from Karagas et al. (2001b), it can be deduced that toenail concentrations of >0.33 µg/g may be associated with a chronic drinking-water concentration of about 60 µg/L and higher.

In a further study of bladder-cancer cases in New Hampshire, Marsit et al. (2006a) investigated whether epigenetic silencing of tumour-suppressor genes by hypermethylation is associated with arsenic exposure. Cases were identified by the New Hampshire State Cancer Register and arsenic exposure was determined by the analysis of toenail clippings. Promotor methylation of tumour-suppressor gene p16INK4A was associated with smoking but not with arsenic exposure. By contrast, tumour-suppressor genes PRSS3 and
RASSF1A were methylated significantly more often in individuals with toenail concentrations ≥0.26 µg/g, compared with those with lower exposure.

An ecological study in the US, similar in design to the studies from southeastern Taiwan (Wu et al., 1989) and showing similar shortcomings with respect to exposure estimates and control of confounders, reviewed more than 4,500 cases of bladder cancer from 133 counties. No increases in standard mortality ratios were found for any of the exposure groups (Lamm et al., 2004). Drinking-water concentrations, even in the highest-exposure group, were below 60 µg/L. Another ecological study from the US investigated the relationship between bladder-cancer mortality and use of private-well water in New Hampshire, without estimation of arsenic exposure (Aytte et al., 2006). The authors found a significant relationship for both sexes.

A recent ecological study from northern Chile investigated the variation in RRs over the years (from 1950 to 2000) in the region of Antofagasta. Starting in 1958, the region experienced strong increases in drinking-water arsenic contamination (with a maximum of up to 870 µg/L). Concentrations declined in the 1970s after the installation of treatment plants. The RRs for bladder cancer peaked in the years 1980–2000, with RRs of 4–6 for bladder cancer in men and 7–14 in women (Marshall et al., 2007). The relationship between exposure concentration and cancer risk was not investigated in this study.

A recent case–control study with bladder-cancer cases in Argentina compared arsenic exposure via drinking water for 114 case–control pairs (Bates et al., 2004). Exposure concentrations ranged up to 200 µg/L, but in most cases (61%) were below 50 µg/L. No association between bladder cancer and arsenic drinking-water concentrations was found. When well-water consumption was used as an exposure marker, a significant OR was determined for smokers who had used well-water for >50 or more >60 years. This result may indicate a longer latency period for arsenic-induced bladder cancer, but it cannot be ruled out that the observed association was by chance.

Using arsenic concentrations in toenails as an exposure marker, Michaud et al. (2004) investigated 280 bladder-cancer cases and 293 matched control cases. Cases were from a cohort of Finnish male smokers and had a median exposure of 0.110 µg/g (range 0.014–2.62 µg/g), similar to the control cases. Only 34 cases (12%) had toenail concentrations above 0.26 µg/g. No significant association was found between arsenic toenail concentrations and bladder cancer risk. For those cases with a smoking history of >45 years the OR was 2.3 (not statistically significant) in the highest-exposure tertile, and the OR tended to increase with increasing cigarette consumption per day in the second-highest-exposure tertile.

**Lung cancer**

In a cohort study, Chen et al. (2004) followed residents of southwestern and northeastern Taiwan for 8 years in order to study the relationship between arsenic exposure and lung-cancer risk. Individual information on smoking, location of wells, and other risk factors was collected by interview using a standardised questionnaire. Assessment of arsenic concentrations in well water differed for the two subcohorts: measurements of arsenic in well water from the 1960s were used in the case of the southwestern Taiwan residents, whereas current measurement data were used for well water used by northeastern residents. Analytical RR in the lowest-exposure category (10–99 µg/L) was not significantly higher than for residents consuming water with arsenic levels <10 µg/L (RR 1.09, 95% CI 0.63–1.91). Higher exposure levels were significantly associated with elevated lung-cancer risk as a function of concentration (100–299 µg/L, RR 2.28, 95% CI 1.22–4.27; 300–699 µg/L, RR 3.03, 95% CI 1.62–5.69; >700 µg/L, RR 3.29, 95% CI 1.60–6.78). The authors noted a marked relationship between arsenic exposure and cigarette smoking, with high RRs for highly arsenic-exposed smokers.

An ecological study of residents in 10 townships in southwestern Taiwan compared lung-cancer mortality cases village by village and investigated possible associations with village well-water arsenic concentrations (Guo, 2004). After adjusting for age, significant increases in mortality from lung cancer were found for arsenic concentrations >0.64 mg/L, but not for lower exposures. Due to its ecological design, the study did not account for the risk factor cigarette smoking.

Nakadeira et al. (2002) investigated lung-cancer mortality in a small cohort of residents in Japan exposed to arsenic because of industrial contamination of well water. Residents were exposed between 1954 and 1959 to high levels of arsenic in their drinking water (up to 400 mg/L). As individual exposure data were not available, the authors used the severity of signs of chronic arsenic intake (hyperpigmentation, hepatomegaly, pancytopenia) as a surrogate exposure marker. Significantly more male members of the cohort died from lung cancer than expected when compared with control participants (7 observed versus 0.64 expected). Lung-cancer cases were linked to severity of symptoms. Results were not controlled for smoking habits.

Smith et al. (2006) investigated mortality ratios in Antofagasta (Chile), which experienced a period of high-level arsenic exposure in the 1960s, in comparison with the levels for the rest of Chile. They noticed that mortality from lung cancer and bronchiectasis in the period 1989 to 2000 was especially high for persons exposed to arsenic from drinking water in utero or in early childhood.

**Skin cancer**

An association between arsenic exposure and increased rates of non-melanoma skin cancer was found in southwestern Taiwan, where residents were exposed to high levels of arsenic in drinking water (Tseng et al., 1968). A well-conducted, recent study on arsenic-induced skin cancer comes from the US. In their case–control study, Karagas et al. (2001a) investigated diagnosed cases of basal-cell (n = 587) and squamous-cell skin cancer (n = 284) and their relationship to arsenic exposure via drinking water. As with the bladder-cancer study by the same group, arsenic toenail concentration was used as a
marker of exposure. A borderline elevated OR was observed for squamous-cell carcinoma in the highest exposure group (arsenic toenail concentrations >0.345 µg/g, OR 2.07, 95%CI 0.92–4.66). For basal-cell carcinoma in this group, the OR was 1.44 (95%CI 0.74–2.81). No increased OR was observed for lower exposure. Most cases occurred in the two lowest-exposure categories, limiting the power to detect significant responses in the higher-exposure groups.

Another study from the US investigated the correlation of skin cancer incidence and arsenic exposure from private well use of residents in Wisconsin (Knobeloch et al., 2006). In this cross-sectional study, exposure was determined by analysis of well-water samples submitted by study participants. Cancer status was determined by asking participants whether they had ever been diagnosed with cancer. For positive answers, information provided by participants was evaluated. Other individual information was obtained by questionnaire. Analysis was restricted to residents aged 35 years or older who had consumed their water for ≥10 years. Significantly increased ORs were obtained even for low exposure levels. After adjustment for age, sex, and smoking status, residents exposed to drinking-water arsenic levels of 1.0–9.9 µg/L had an OR of 1.81 (95%CI 1.10–3.14), compared with those with exposures <1 µg/L. The OR was 1.92 (95%CI 1.01–3.68) for individuals with exposures ≥10 µg/L. For arsenic-exposed smokers aged 65 or older, higher ORs were obtained than for the same age and exposure group of non-smokers, which points towards a relationship between smoking status and arsenic exposure. Only a few people were able to provide information on the type of skin cancer, and reliance on information provided by study participants can be a source of misclassifications.

Additional evidence for arsenic-induced skin cancer comes from a cross-sectional study of a population in Inner Mongolia, China (Lamm et al., 2007). By conducting dermatological examinations, the authors investigated the prevalence of skin cancer and skin disorders in the study group. Eight cases of skin cancer were found in this cohort of 3,179 persons with a median age of 29 years. Analytical data on arsenic levels in well water and the use of wells were compiled. All skin-cancer cases were associated with use of wells containing arsenic in concentrations >150 µg/L.

Beane Freeman and coworkers (2004) were the first researchers to establish an association between arsenic exposure and cutaneous melanoma in an analytical epidemiological study. They examined 368 melanoma cases and 373 matched controls (cases of colorectal cancer) from Iowa, USA. Participants answered a questionnaire and provided toenail clippings for the assessment of arsenic exposure. Diagnoses were ascertained by checking the Iowa Cancer Registry. Exposure to arsenic was quite low, the highest-exposure group (toenail concentrations ≥0.084 µg/L) comprising 121 of 326 cases. After adjusting for age, sex, and education, exposure concentrations were significantly associated with melanoma risk. The ORs were 1.7 (95%CI 1.1–2.7) and 2.1 (95%CI 1.4–3.3) for toenail-concentration groups 0.04–0.083 µg/gand ≥0.084 µg/L, respectively, compared with the exposure group with toenail concentrations ≤0.02 µg/L. According to the authors, genetic factors may be responsible for the lower susceptibility of Asian populations towards arsenic-induced melanoma and cutaneous melanoma in general, which might explain why no increased melanoma risks have been reported in studies with Asian populations, despite high levels of arsenic exposure.

Non-Carcinogenic effects
Soluble inorganic arsenicals are acutely toxic. Oral intake may induce gastrointestinal symptoms (vomiting, diarrhea, gastrointestinal hemorrhage), disturbances of the cardiovascular and neurological system, and eventual death. The acute lethal dose for humans has been estimated at about 1 mg/kg (ATSDR, 2005). Further consequences of acute poisoning can include bone-marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy, and encephalopathy (ATSDR, 2005; WHO, 2001).

Chronic arsenic exposure has been associated with peripheral vascular diseases (black foot disease—a severe form of peripheral vascular disease, in which the blood vessels in the lower limbs are severely damaged, resulting eventually in progressive gangrene; Raynaud’s syndrome—constriction of the small arteries in fingers and toes), skin lesions (dyspigmentation, keratoses), cardiovascular and cerebrovascular diseases, diabetes mellitus, neurological diseases, as well as diseases of the respiratory tract, the liver and the spleen. Comprehensive reviews on toxic effects of arsenic have been published (e.g. ATSDR, 2005; Kapaj et al., 2006; WHO, 2001). The following presentation focuses on epidemiological data regarding effects for which causal relationships with arsenic exposure have been demonstrated and which are supposed to occur at lower doses. Special emphasis is placed on studies considered relevant for the derivation of a tolerable dose for non-cancer health effects.

Reproductive effects
Animal experiments have indicated the developmental toxicity of inorganic arsenic (e.g. growth retardation, lethality and malformations like neural-tube defects and facial malformations after intraperitoneal application) and its effects on fertility, such as reduced sperm count, reduced weight of sexual organs, decreased hormonal concentrations (Wang et al., 2006; WHO, 2001). Epidemiological data support these findings from animal experiments. In a cross-sectional study on women in Bangladesh, an excessive risk of spontaneous abortion and stillbirth were observed among the participants chronically exposed to arsenic in drinking water. Data were adjusted for participants’ height, history of hypertension and diabetes, and age at first pregnancy. Comparing risks for groups with an exposure concentration >50 µg/L with those with a concentration of 50 µg/L or less, the ORs for spontaneous abortion were 2.5 (95%CI 1.5–4.3), 2.5 (95%CI 1.3–4.9) for stillbirth and 1.8 (95%CI 0.9–3.6) for neonatal death (Milton et al., 2005). No clear dose–response relationship for spontaneous abortion and neonatal death was observed when a more differentiated exposure analysis

Non-Carcinogenic effects
Soluble inorganic arsenicals are acutely toxic. Oral intake may induce gastrointestinal symptoms (vomiting, diarrhea, gastrointestinal hemorrhage), disturbances of the cardiovascular and neurological system, and eventual death. The acute lethal dose for humans has been estimated at about 1 mg/kg (ATSDR, 2005). Further consequences of acute poisoning can include bone-marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy, and encephalopathy (ATSDR, 2005; WHO, 2001).

Chronic arsenic exposure has been associated with peripheral vascular diseases (black foot disease—a severe form of peripheral vascular disease, in which the blood vessels in the lower limbs are severely damaged, resulting eventually in progressive gangrene; Raynaud’s syndrome—constriction of the small arteries in fingers and toes), skin lesions (dyspigmentation, keratoses), cardiovascular and cerebrovascular diseases, diabetes mellitus, neurological diseases, as well as diseases of the respiratory tract, the liver and the spleen. Comprehensive reviews on toxic effects of arsenic have been published (e.g. ATSDR, 2005; Kapaj et al., 2006; WHO, 2001). The following presentation focuses on epidemiological data regarding effects for which causal relationships with arsenic exposure have been demonstrated and which are supposed to occur at lower doses. Special emphasis is placed on studies considered relevant for the derivation of a tolerable dose for non-cancer health effects.

Reproductive effects
Animal experiments have indicated the developmental toxicity of inorganic arsenic (e.g. growth retardation, lethality and malformations like neural-tube defects and facial malformations after intraperitoneal application) and its effects on fertility, such as reduced sperm count, reduced weight of sexual organs, decreased hormonal concentrations (Wang et al., 2006; WHO, 2001). Epidemiological data support these findings from animal experiments. In a cross-sectional study on women in Bangladesh, an excessive risk of spontaneous abortion and stillbirth were observed among the participants chronically exposed to arsenic in drinking water. Data were adjusted for participants’ height, history of hypertension and diabetes, and age at first pregnancy. Comparing risks for groups with an exposure concentration >50 µg/L with those with a concentration of 50 µg/L or less, the ORs for spontaneous abortion were 2.5 (95%CI 1.5–4.3), 2.5 (95%CI 1.3–4.9) for stillbirth and 1.8 (95%CI 0.9–3.6) for neonatal death (Milton et al., 2005). No clear dose–response relationship for spontaneous abortion and neonatal death was observed when a more differentiated exposure analysis
was performed. Furthermore, data on drinking-water consumption and possible confounding by exposure to other chemicals were not considered, which impairs the validity of the study.

An increased risk of stillbirth, spontaneous abortion, and preterm birth in Bangladesh women chronically exposed to arsenic through drinking water (>100 µg/L) was also described by Ahmad et al. (2001). Concentrations >200 µg/L were associated with an increased incidence of stillbirth in a study by von Ehrenstein et al. (2006). However, no effect for arsenic exposure on spontaneous abortion and survival of newborns was observed.

One study from Chile indicated that increased exposure to inorganic arsenic (about 40 µg/L) is associated with a decreased body weight at birth (Hopenhayn et al., 2003). However, no differentiated dose–response analysis has been performed.

Cognitive abilities and neurological effects
Exposure to arsenic in drinking water has been associated with a decline in intellectual function in children. Such associations have been established in several studies. Some of them, involving exposure in the low-dose range, are reported in the following pages.

In an ecological study of 720 children between 8 and 12 years of age in rural villages in China, mean IQ scores (assessed with the Combined Raven’s Test—The Rural in China method) were inversely correlated with increasing arsenic content of the drinking water. The children were exposed to arsenic at mean concentrations of 142 µg/L (medium arsenic group) and 190 µg/L (high arsenic group) in drinking water, compared with the control group, which was exposed to low concentrations of arsenic (about 2 µg/L; Wang et al., 2007). The validity of the study is impaired because of missing data on individual exposure. Furthermore, the children were exposed towards small amounts of fluoride in drinking water, which may also have affected intelligence performance (Li et al., 1995; Wang et al., 2007).

In a cross-sectional study of 201 10-year-old children in Araihazar, Bangladesh, exposure to arsenic in drinking water (mean exposure 117.8±145.2 µg/L) has been shown to lower scores on tests that measure children’s intellectual function (Wechsler Intelligence Scale for Children, version III; Wasserman et al., 2004). Arsenic concentration in water was associated with reduced intellectual function, in a dose-related manner. Children with water arsenic levels >50 µg/L achieved significantly lower performance and full-scale scores than did children with water arsenic levels <5.5 µg/L. The association was generally stronger for arsenic concentrations in well water than in urine. In this study, well water at each child’s home was sampled and analysed. But no data on individual water consumption or arsenic content of food were presented. These data were confirmed by a study on 6-year-old children from Araihazar, Bangladesh, who were exposed to average arsenic concentrations in drinking water of 120.1 µg/L (range 0.1–864.0 µg/L; Wasserman et al., 2007). The associations were weaker than in the previous study on 10-year-old children (Wasserman et al., 2004).

von Ehrenstein et al. (2007) performed a cross-sectional study among 351 children aged 5–15 years in West Bengal, India. Current arsenic concentrations in urine, which reflect all sources of recent exposure, including water and food, were associated with small decrements in intellectual-test scores in school-aged children. There was no evidence of an association between test results and arsenic concentrations during pregnancy or childhood.

Tsai et al. (2003) reported impaired development of cognitive function among adolescents because of long-term arsenic exposure. Average exposure was about 185 µg/L in the high-exposure group and about 131 µg/L in the low-exposure group. The study comprised only 49 exposed cases and 60 control cases.

The motor and sensory nerve conduction velocity of peripheral nerves in the right upper and lower limbs was measured in a cross-sectional study of 130 students 12–14 years of age from northeastern Taiwan. Development of slow nerve conduction velocity of sural sensory action potential was observed among subjects who drank well water containing arsenic concentrations of >50 µg/L or among subjects with a cumulative arsenic dosage of >100 mg (Tseng et al., 2006). Exposure assessment, however, was based on measurement data from the year 1991, and might not be representative of actual exposure.

Cerebrovascular effects
The prevalence of cerebrovascular disease among residents of northeastern Taiwan was surveyed to examine its association with exposure to arsenic in well water (Chiou et al., 1997b). The cross-sectional study comprised 8,102 persons from 3,901 households. A significant dose–response relationship was observed between arsenic concentrations in well water and the prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The correlation between arsenic exposure and cerebral infarction was even more prominent than for arsenic exposure and cerebral diseases in general. ORs for cerebral infarction were already significantly increased at arsenic concentrations 0.1–50.0 µg/L (compared with concentrations <0.1 µg/L). However, other studies could not demonstrate an interrelation between arsenic exposure and cerebrovascular diseases (WHO, 2001).

Cardiovascular effects
A dose–response analysis of the relationship between chronic arsenic exposure and mortality due to ischemic heart disease (ISHD) has been performed for a population in southwestern Taiwan by Chen et al. (1996). The cumulative ISHD mortalities from birth to age 79 years were 3.4%, 3.5%, 4.7%, and 6.6%, respectively, for persons exposed to arsenic drinking water concentrations <0.1 mg/L, 0.1–0.34 mg/L, 0.35–0.59 mg/L, and ≥0.6 mg/L. The 217 ISHD deaths that occurred between 1973 and 1986 were analysed in this ecological study. Chen et al. (1996) reported the RRs for ISHD mortality to be 2.5, 4.0, and 6.5, respectively, for a cohort...
with a cumulative arsenic exposure of 0.1–9.9, 10.0–19.0 and ≥20.0 mg/L and years compared with persons without arsenic exposure. Data from this prospective cohort study were adjusted for age, sex, cigarette smoking, BMI, serum cholesterol and triglyceride levels, and disease status for hypertension and diabetes. Exposure analysis for these studies was based on information obtained from the participants by a questionnaire and analytical data from the early 1960s.

### Skin lesions

The potential of arsenic to induce skin lesions such as dyspigmentation and hyperkeratoses has been well known since the 1960s (Tseng et al., 1968). The dose–response relationship between arsenic exposure via drinking water and the induction of these skin lesions has been investigated in a number of recent studies from southeastern Asia. The risk for pigment disturbances—but not for keratoses—was increased in a Chinese population from Inner Mongolia (227 cases, 221 controls) with drinking water concentrations >50 µg/L (Guo et al., 2006).

In an ecological study that considered all women aged 18 years or more from four districts from Bangladesh (about 14,000 women), an increased incidence of skin lesions was observed at drinking-water concentrations above 50 µg/L (McDonald et al., 2006).

Between April 1995 and March 1996, Guha Mazumder et al. (1998) conducted a cross-sectional survey in West Bengal (India). They collected individual exposure data from 7,683 individuals. Study participants were questioned about their sources of drinking water, diet, and water intake. Water samples from tube wells used for drinking and cooking purposes by each recruited household were analysed. Arsenic exposure from drinking water at the workplace and from food was not measured. The arsenic content of drinking water was <3400 µg/L, and over 80% of participants consumed water containing <500 µg/L of arsenic. Of the 4,093 female and 3,590 male participants, 48 women and 108 men had keratotic skin lesions and 127 women and 234 men exhibited hyperpigmentation. A clear relationship was apparent between water levels of arsenic and the prevalence of keratosis (Table 8). Calculation of exposure as dose per body weight showed that men had about 2–3 times the prevalence of both keratosis and hyperpigmentation compared with women. About 30% of women and 38% of men with known body weights were ≥20% below the standard weight and must be considered malnourished. Compared with those with adequate nutrition, women ≥20% below the standard weight had higher age-adjusted prevalences of keratosis. No such distinct influence of nutrition was observable for men or for hyperpigmentation. Some cases with skin lesions were identified who apparently had low levels of arsenic in their drinking water. The authors suggested that these individuals might have been exposed to high levels of arsenic from drinking-water sources other than the one measured in the survey. Haque et al. (2003) further posited that false-positive classifications might have occurred due to misdiagnosis. The significance of the study is, therefore, impaired.

Haque et al. (2003) performed a nested case–control study with the participants from the survey of Guha Mazumder et al. (1998) whose arsenic exposure was below 500 µg/L. Participants were re-examined between 1998 and 2000. The study by Haque et al. (2003) largely confirmed the data collected by Guha Mazumder et al. (1998). However, when the field diagnosis was reevaluated by photographic review, 29 of the controls were found to manifest skin lesions. Therefore, the validity of this study is also limited.

A population-based case–referent study was performed in Madab, Bangladesh (Rahman et al., 2006). All inhabitants above 4 years of age who had lived in the region for at least 6 months before the start of the study were screened for skin lesions. Initial skin examination in the field was followed by verification by physicians in a clinic and a final confirmation by experts reviewing photographs. Exposure information was constructed using drinking-water histories and data on water arsenic concentrations since 1970. Exposure levels went up to 3644 µg/L. In total, 504 cases and 1830 controls were selected. The risk for skin lesions increased dose dependently, with men having a significantly higher prevalence of skin lesions (see Table 9). In the highest-exposure category (≥300 µg/L), age-adjusted ORs for men and women were 9.56 (95%CI 4.20–21.8) and 6.88 (95%CI 3.06–15.5), respectively (Table 9). Increased ORs, albeit not statistically significant, already occurred in the exposure category 10–49 µg/L in comparison with exposures below 10 µg/L (reference group). No information on drinking-water consumption, arsenic intake via diet, and tube wells other than private wells of the household was available.

Hall et al. (2006) reported data from a case–cohort analysis of 303 newly diagnosed cases of skin lesions (melanosis, leukomelanosis, and keratosis) and 849 subcohort members randomly selected from the participants in the Health Effects of As Longitudinal Study (HEALS) in Araihaazar, Bangladesh. Rate ratios for skin lesions were slightly increased (RR 1.2,

### Table 8. Age-adjusted keratosis and hyperpigmentation prevalence per 100 participants in West Bengal, India (data from Guha Mazumder et al., 1998).

<table>
<thead>
<tr>
<th>Arsenic concentration in drinking water (µg/L)</th>
<th>Keratosis</th>
<th>Hyperpigmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>0.0</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>0.1–9.9</td>
<td>2.3</td>
<td>4.7</td>
</tr>
<tr>
<td>10.0–19.0</td>
<td>2.0</td>
<td>4.9</td>
</tr>
<tr>
<td>20.0–349</td>
<td>2.7</td>
<td>9.0</td>
</tr>
<tr>
<td>350–499</td>
<td>3.1</td>
<td>8.9</td>
</tr>
<tr>
<td>500–799</td>
<td>8.3</td>
<td>10.7</td>
</tr>
<tr>
<td>&gt;800</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arsenic concentration in drinking water (µg/L)</th>
<th>Keratosis</th>
<th>Hyperpigmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>0.0</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>0.1–9.9</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>10.0–19.0</td>
<td>5.7</td>
<td>4.7</td>
</tr>
<tr>
<td>20.0–349</td>
<td>5.1</td>
<td>4.9</td>
</tr>
<tr>
<td>350–499</td>
<td>6.5</td>
<td>9.0</td>
</tr>
<tr>
<td>500–799</td>
<td>9.5</td>
<td>8.9</td>
</tr>
<tr>
<td>&gt;800</td>
<td>5.3</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Note: The validity of the data is impaired because of possible false-positive classification due to misdiagnosis (Haque et al., 2003).
95% CI 0.69–2.08) at a mean arsenic drinking water concentration of 65µg/L (range 39–94µg/L) and above. The effect became statistically significant (RR 1.9, 95% CI 1.14–3.17) at a mean arsenic concentration of 138µg/L (range 95–189µg/L).

A dose-dependent increased incidence of skin lesions for men and women from southeastern Bangladesh was described in a cross-sectional study (Ahsan et al., 2006a). The participants were recruited in 2000–2002 for the HEALS. The study comprised approximately 11,000 participants, of whom 714 had skin lesions confirmed by field diagnosis. The field diagnosis was confirmed by further clinical review: 337 men and 84 women had melanosis only, and 247 men and 46 women had melanosis and hyperkeratosis. The prevalence ORs for skin lesions by levels of arsenic exposure are presented in Table 10. The estimates of prevalence ORs increased monotonically with levels of arsenic exposure. Males were more likely than females to have skin lesions (Figure 3).

Individual exposure data were calculated on the basis of analyses of tube-well water and of individual information on drinking-water consumption. The analysis was adjusted for the following confounding variables: age; gender socioeconomic status; sun exposure; and BMI. The prevalence of skin lesions increased with increasing age, decreasing BMI and educational status, findings which are in agreement with those of other studies (Guha Mazumder et al., 1998).

### Evaluation and discussion

#### Carcinogenic effects

In 2001, the NRC based its revision of cancer-risk estimates on ecological studies of southwestern Taiwanese populations (Table 6). Using a model previously developed for estimating lung-cancer risk from radon exposure (BEIR IV) with linear extrapolation, background cancer rates from the US and corrections for differences in body weight and water consumption between US residents and Taiwanese, the NRC derived maximum-likelihood estimates for the combined excess risk for bladder and lung tumours of 3.0 (female) to 3.7 (males) cases per 1,000 individuals exposed to 10µg/L of arsenic. Risks lower by a factor of 5 were obtained by Morales et al.

### Table 9. Odds ratios for skin lesions (hyperkeratosis, dyspigmentation) in arsenic-exposed individuals from Bangladesh (data from Rahman et al., 2006).

<table>
<thead>
<tr>
<th>Mean arsenic exposure (µg/L)</th>
<th>Crude odds ratio (95% CI)</th>
<th>Adjusted odds ratioa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>10–49</td>
<td>2.51 (1.27–4.97)</td>
<td>3.25 (1.43–7.38)</td>
</tr>
<tr>
<td>50–149</td>
<td>1.77 (0.93–3.37)</td>
<td>2.28 (1.04–4.98)</td>
</tr>
<tr>
<td>150–299</td>
<td>3.47 (1.87–6.45)</td>
<td>5.41 (2.52–1.62)</td>
</tr>
<tr>
<td>≥300</td>
<td>4.34 (2.22–8.46)</td>
<td>9.56 (4.20–21.8)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>10–49</td>
<td>1.13 (0.51–2.50)</td>
<td>1.66 (0.65–4.24)</td>
</tr>
<tr>
<td>50–149</td>
<td>2.40 (1.25–4.59)</td>
<td>3.06 (1.39–6.74)</td>
</tr>
<tr>
<td>150–299</td>
<td>2.96 (1.56–5.62)</td>
<td>4.08 (1.86–8.93)</td>
</tr>
<tr>
<td>≥300</td>
<td>4.17 (2.14–8.14)</td>
<td>6.88 (3.06–15.5)</td>
</tr>
</tbody>
</table>

Note: aAdjusted for age and asset score.

### Table 10. Prevalence odds ratios for skin lesions by levels of arsenic exposure (data from Ahsan et al., 2006a).

<table>
<thead>
<tr>
<th>Arsenic-exposure measure (quintiles; µg/L)</th>
<th>Median</th>
<th>Total n</th>
<th>Cases</th>
<th>Prevalence odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-weighted well arsenic concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1–8.0</td>
<td>1.8</td>
<td>2259</td>
<td>57</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>8.1–40.0</td>
<td>23.0</td>
<td>2122</td>
<td>90</td>
<td>1.91</td>
<td>1.26–2.89</td>
</tr>
<tr>
<td>40.1–91.0</td>
<td>62.0</td>
<td>2202</td>
<td>144</td>
<td>3.03</td>
<td>2.05–4.50</td>
</tr>
<tr>
<td>91.1–175.0</td>
<td>125.0</td>
<td>2185</td>
<td>162</td>
<td>3.71</td>
<td>2.53–5.44</td>
</tr>
<tr>
<td>175.1–864.0</td>
<td>255.0</td>
<td>2183</td>
<td>242</td>
<td>5.39</td>
<td>3.69–7.86</td>
</tr>
</tbody>
</table>

Figure 3. Prevalence of skin lesions by levels of arsenic exposure from drinking water (median arsenic level of exposure quintiles), sex, and body mass index (no distinction by sex). (Data from Ahsan et al., 2006a.)
Critical Reviews in Toxicology Downloaded from informahealthcare.com by University Of Arizona on 11/04/10

For personal use only.

years (Table 7). Their main advantages, compared with earlier misclassifications probable.

arsenic concentrations from all wells, which makes exposure of a village were assumed to be exposed to the average of used by an individual. In the ecological study, all residents were assumed to have much higher arsenic concentrations than the shallow wells (Lamm et al., 2003). Villages with more than one well may have had mixed water supplies, with drinking water coming from both artesian and shallow wells (with water from a depth of 100–200 m) or shallow (near-surface water, depths 6–8 m); the former were assumed to have much higher arsenic concentrations than the shallow wells (Lamm et al., 2003). Villages with more than one well may have had mixed water supplies, with drinking water coming from both artesian and shallow water. Individual exposure levels, therefore, can be expected to vary substantially within a village, depending on the well used by an individual. In the ecological study, all residents of a village were assumed to be exposed to the average of arsenic concentrations from all wells, which makes exposure misclassifications probable.

Several epidemiological studies investigating the carcinogenicity of oral arsenic intake have been published in recent years (Table 7). Their main advantages, compared with earlier ecological studies, are their study design (mostly case–control and cross-sectional studies) and the fact that they use individual exposure data for exposure assessment. Furthermore, several of the studies investigate the lower drinking-water arsenic concentration range of about or below 100 µg/L. Their main disadvantage lies in their much smaller study groups, compared with the ecological studies with study populations of tens of thousands of people, which limits their power to detect subtle increases in cancer risk. According to Frost et al. (2002), sample sizes in a cohort study of more than 7,000 people would be necessary to detect with statistical significance (with a power of 0.8 and $p = 0.95$) an added lifetime risk for bladder cancer of 1 in 100 (from lifetime exposure to 50 µg As/L, as predicted by Morales et al. 2000). Because of the higher background incidence, sample sizes even larger than this are necessary for detecting similar lung-cancer risks.

In light of these considerations, epidemiological studies as described above cannot always be expected to produce statistically significant results at concentrations below 100 µg/L. Taking this into account, consideration of dose–response relationships for (not statistically) elevated risks at low concentrations and consistency of results from several studies are as important as statistical significance. Cantor and Lubin (2007) argued that risks were difficult to detect in existing low-exposure studies, because exposure misclassification errs towards underestimating risks. A large-scale case–control study with improved past-exposure assessment is in preparation by these authors for a study population in northern New England, USA.

The studies described above used either arsenic concentration in drinking water together with participant history of use of drinking-water sources or the analysis of arsenic in the toenail clippings of study participants as a means for exposure assessment. Both methods have advantages and disadvantages. Arsenic concentrations in drinking water may have changed for individual wells over the years, and to truly reflect internal exposure must be combined with individual data on water consumption. By contrast, toenail analysis covers internal arsenic exposure over the last 3–12 months and, thus, does not detect possible changes in previous periods. Also, toenail concentrations may integrate exposures from other sources of arsenic, such as food or occupational exposures. Karagas et al. (2000) found arsenic toenail concentration to be closely correlated with arsenic in drinking water at arsenic concentrations in water above, but not below, 1 µg/L.

Recent studies on bladder cancer failed to demonstrate elevated risks in study populations in the US, Argentina, or Finland at drinking-water concentrations below 100 µg/L. This is in agreement with earlier studies from the US (Bates et al., 1995; Lewis et al., 1999). Several studies, discussed above, showed slight increases in bladder cancer risk at these low exposures for the subgroup of smokers and suggested a synergistic effect between smoking and arsenic exposure, leading, partly, to high RRs. This observation, again, is in agreement with earlier findings by Bates et al. (1995), who found a positive trend for higher bladder-cancer risk with increasing arsenic exposure among smokers. Drinking-water arsenic concentrations in this study were in the range 0.5–160.0 µg/L. Elevated risks for smokers and a possible synergistic interaction with arsenic exposure were also observed in new studies on lung cancer and in one study on non-melanoma skin tumours. On the basis of the risks observed, it can be concluded that the current cancer-risk estimate by the NRC (2001) probably overestimates the bladder-tumour risk for the general (non-smoking) population, but might be a reasonable worst-case estimate for smokers. In a meta-analysis of eight analytical studies on bladder cancer with modest exposures, Exponent (2005) observed no increased risk for non-smokers and smokers combined, but obtained a borderline significant RR estimate for smokers (mRR 1.24, 95%CI 0.99–1.56, with high heterogeneity). This RR was at the lower end of the risk estimates derived by NRC (2001).

The data for arsenic-induced lung cancer shows less improvement. New studies reinforced the evidence of arsenic lung carcinogenicity (Nakadeira et al. 2002; Chen et al., 2004), but failed to elucidate dose–response relationships at low concentrations. In an ecological study, Guo (2004) did not find elevated risks in a population in southwestern Taiwan with exposure to drinking-water concentrations below 0.64 mg/L, which is in contrast to the case–control study of Ferreccio et al. (2000) from Chile.

Contradictory results were also obtained from studies on skin cancer. Karagas et al., (2001a) found slight increases in
risks for basal-cell and squamous-cell tumours at toenail concentrations >0.345 µg/g, but not at lower concentrations. In a cross-sectional study, Knobeloch et al. (2006) showed increased risk for skin tumours even at concentrations below 10 µg/L. No characterisation of tumours was available for this study, as case identification was based on patient records only. A well-conducted study by researchers from the US National Cancer Institute and the University of Iowa was the first to find a significant relationship between oral exposure to arsenic and melanoma (Beane Freeman et al., 2004).

Several influencing factors have to be carefully discussed in epidemiological studies on arsenic cancer risk. Smoking is the most important confounding parameter, especially for studies on bladder and lung tumours. This might partially be explained by arsenic-mediated inhibition of repair of polycyclic aromatic hydrocarbon mediated DNA damage (Schwerdtle et al., 2003b; Tran et al., 2002). In all but the ecological studies cited above, smoking has been taken into account as a confounding factor.

As discussed above, the capacity to methylate arsenic is influenced by nutritional status, and malnourishment has been associated with an increased risk of non-cancer and cancer effects (Ahsan et al., 2006a; Chen et al., 2003a). Insufficient intake of several nutritional factors, like folate and proteins, may result in impaired methylation of arsenic (NRC, 1999; Steinmaus et al., 2005). Consequently, the PMI and SMI may increase or decrease, respectively, either of which is associated with an increased risk of adverse health effects (Chen et al., 2003a; 2003b; Pu et al., 2007).

Several attempts have been made to identify thresholds for the carcinogenic action of arsenic based on epidemiological data. In a reanalysis of the data from studies from southwestern Taiwan (Chen et al., 1985; Wu et al., 1989) Lamm et al. (2006) regrouped the data with respect to exposure. When dividing villages into high-dose (average well concentrations >250 µg/L) and low-dose villages, the authors found a clear association only for the high-dose villages. The authors further stratified the data according to whether or not townships (comprising several villages) showed associations between bladder and lung cancer cases and arsenic concentration. For townships that had an association, a linear dose-response model showed an intercept with the x-axis (structure metabolism relationship = 100) at 119 µg/L (95%CI 70–229) for males and 191 µg/L (95%CI 66–280 µg/L) for females (Lamm et al., 2006). Taking into account the uncertainty of exposure estimates, the strong dependence of risk estimates on the type of background cancer rates used, and the highly scattered data, any attempt to infer thresholds from the Taiwanese data should be made with caution.

Lamm and co-workers (2007) also applied threshold dose-response models to data for skin cancer prevalence in residents of Inner Mongolia, China. They found that the models applied are consistent with the existence of thresholds for arsenic-induced skin cancer in the range 120–150 µg/L. The authors did not try to fit non-threshold models and, considering the fact that only 8 cases were observed in this study, the conclusions regarding thresholds must be considered very uncertain. In general, dose-response modelling should not be pre-informed by assuming or not assuming specific dose-response curves. Rather, a broad range of models should be applied and the observed dose-response data alone should direct to those fittings that are in agreement with the observed data (Slob, 1999).

Using data from their case-control study on non-melanoma skin cancer in New Hampshire (Karagas et al., 2001a), Karagas et al. (2002) applied various dose-response models and obtained change-points (points after which increasing risks with increasing exposure are observed) for squamous-cell carcinoma in the range of 1–2 µg/L (95%CI <1–20 µg/L).

From a general point of view, and considering the uncertainty and variability of epidemiological data, it remains doubtful whether thresholds can be derived in principle from these data. This is especially true when inter-individual variability of susceptibility is taken into consideration.

Studies investigating toxicokinetics, genotoxicity, and other mechanistic aspects have been reviewed to investigate whether mode of actions can be identified for the carcinogenic effects of inorganic arsenic and whether conclusions on dose-response relationships can be derived. Several modes of action, which are partly interrelated, seem to be active in the case of inorganic arsenic: (a) the production of ROS; (b) induction of cell proliferation and transformation; (c) genotoxicity (induction of chromosomal aberrations); (d) inhibition of DNA repair; and (e) changes in cell signalling pathways and in gene expression (e.g. by hypermethylation of gene promoter regions).

Methylation of arsenic has formerly been considered a detoxification pathway. In view of recent results from in vitro investigations into the genotoxicity of MMAIII and DMAIII, this position has to be changed. AsIII, MMAIII, and DMAIII seem to be responsible for various effects at low concentrations. For example, MMAIII and DMAIII exerted genotoxic effects in vitro at or below 1 µM, and MMAIII at 50 nM induced malignant transformation of UROtsa cells and ROS production in vitro (Eblin et al., 2006; Bredfeldt et al., 2006). Whereas receptor-mediated processes and, hence, thresholds might be anticipated in principle for ROS production, inhibition of DNA repair, and related activities, no inactive concentrations could be identified in several of the investigations described above, MMAIII and DMAIII being active at the lowest concentrations tested.

Some of these assumed modes of action have been corroborated by human data. For example, Andrew et al. (2006) found reduced expression of DNA repair genes in subjects with arsenic concentrations in drinking water >5 µg/L. Marsit et al. (2006a) reported suppression of tumour-suppressor genes in bladder-cancer cases with moderate arsenic exposure.

For concluding about a threshold for carcinogenicity of a chemical agent, two conditions must be met: identification
of the most relevant modes of action and proof of non-effective doses in suitable test systems. Taken together, the data described above do not allow us to conclude that arsenic carcinogenicity involves a threshold dose–response relationship. Currently available estimates of cancer risks may overestimate the risks for humans in the general population, at least for bladder cancer, but may be reasonable worst-case estimates for smokers. More precise cancer-risk estimates would need large analytical epidemiological studies with careful exposure analysis (Cantor and Lubin, 2007) and a thorough consideration of inter-individual differences in susceptibility due to polymorphic expression of enzymes involved in arsenic metabolism and other, possibly yet unknown, factors.

**Non-Carcinogenic effects**

**Selection of the most relevant study for the derivation of a tolerable DOSE**

Arsenic shows a broad spectrum of non-cancer effects that can occur in the low-dose range (at or below 100 µg/L in drinking water): reproductive toxicity; neurotoxicity (impairment of intellectual function); cardiovascular and possibly cerebrovascular effects; and skin lesions. Also, the induction of diabetes mellitus by chronic arsenic exposure has been discussed (Rahman et al., 1998; Lai et al., 1994), but evidence for an association is inconclusive (Navas-Acien et al., 2006).

A number of epidemiologic studies on non-cancer effects of arsenic were published in recent years and contributed to a better understanding of chronic arsenic toxicity, but most of them have some shortcomings (mainly insufficient data on individual exposure), which exclude them from further risk assessment.

Data on reproductive toxicity indicate that developmental toxicity may occur after long-term exposure to arsenic concentrations >50 µg/L drinking water. However, due to shortcomings in exposure assessments, a lack of reliable dose–response data, and insufficient consideration of confounders, the currently available data are not suitable for a quantitative risk assessment. Other studies have shown evidence of impairment of cognitive and neurological abilities and adverse effects on the cardiovascular system. Whereas some of these studies are limited because of insufficient data on individual exposure, possible false classifications, and difficulties in the interpretation of some of the performed intelligence tests, they generally support the possibility of long-term health effects for arsenic concentrations at and above >100 µg/L.

Consistent dose–response data for arsenic-induced skin lesions due to lower exposure levels are available from several studies. Substantial effort has been put into the proper diagnosis of these skin effects in the studies of Ahsan et al. (2006a) and Rahman et al. (2006). The trunk, palms, and soles are the typical locations of the lesions, which at higher exposure levels show a characteristic appearance (Saha, 2003; Schwartz, 1996). Hypertipism usually consists of diffuse dark-brown or blackish areas on the skin or mucous membranes, and diffuse or spotted dense pigmentation. Hypopigmentation is characterized by whitish or pallid patches commonly referred to as raindrop pigmentation. Keratosis is characterized as bilateral thickening of the palms and soles, and small protrusions (Rahman et al., 2006). Non-malignant skin lesions are supposed to have a shorter latency period than skin tumours and may appear within a few years of exposure, whereas the latency period for tumours is about 20 years (Ahsan et al., 2006a; Chen et al., 2006; Parish and Burnett, 1987).

Hyperkeratosis is assumed to be a precursor of skin cancer, as the majority of the basal-cell and squamous-cell skin cancers in arsenic-exposed persons are thought to develop from these lesions (Ahsan et al., 2003; 2006a; Alain et al., 1993). But skin tumours may also develop on unaffected areas (Shannon and Strayer, 1989).

The occurrence of typical arsenic-induced skin lesions has been described for several populations from India and Bangladesh with low exposure concentrations in drinking water (<100 µg As/L; Table 11). Arsenic-induced skin lesions have also been observed in populations from Germany, Hungary, and the US, which were exposed to arsenic occupationally (e.g. vine growers), therapeutically (persons with psoriasis), or via drinking water (Grobe, 1977, 1982; Lüchtrath, 1983; Piontek et al., 1989; Nagy et al., 1975; Nagy and Korom, 1983; Tollesstrup et al., 2005; EPA, 1981). These older studies generally failed to produce enough reliable dose–response data to allow for quantitative assessment.

In conclusion, arsenic-induced skin lesions are seen as a relevant and reliable effect marker that occur at exposure levels at which other non-carcinogenic health effects are not yet observable.

Existing risk assessments on non-carcinogenic effects of arsenic (ATSDR, 2005; EPA, 2007; Hassauer and Kalberlah, 1999)

**Table 11. Summary of some relevant epidemiologic studies concerning arsenic-induced skin lesions.**

<table>
<thead>
<tr>
<th>Location of study</th>
<th>Total n</th>
<th>Participants with skin lesions</th>
<th>Level at which risk is increased&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lowest exposure category</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Bengal, India (cross-sectional study)</td>
<td>7683</td>
<td>517</td>
<td>50–99 µg/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;50 µg/L</td>
<td>Guha Mazumder et al., 1998</td>
</tr>
<tr>
<td>West Bengal, India (case–control study)</td>
<td>405</td>
<td>192</td>
<td>50–99 µg/L</td>
<td>&lt;50 µg/L</td>
<td>Haque et al., 2003</td>
</tr>
<tr>
<td>Bangladesh (case–control study)</td>
<td>2334</td>
<td>504</td>
<td>10–49 µg/L</td>
<td>&lt;10 µg/L</td>
<td>Rahman et al., 2006</td>
</tr>
<tr>
<td>Bangladesh (case–cohort study)</td>
<td>1152</td>
<td>303</td>
<td>95–189 µg/L</td>
<td>3–38 µg/L</td>
<td>Hall et al., 2006</td>
</tr>
<tr>
<td>Bangladesh (cross-sectional study)</td>
<td>10951</td>
<td>714</td>
<td>8.1–40 µg/L</td>
<td>0.1–8.0 µg/L</td>
<td>Ahsan et al., 2006a</td>
</tr>
</tbody>
</table>

Note: *Given as Odds Ratio, Rate Ratio, or prevalence.

<sup>a</sup>No data for statistical significance.
are based on arsenic-induced skin effects, as reported in the ecological studies of Tseng and colleagues (Tseng et al. 1968; Tseng, 1977). No individual exposure data and only restricted dose–response information are available from these studies. With the studies from Guha Mazumder et al. (1998), Haque et al. (2003), Rahman et al. (2006), Hall et al. (2006), and Ahsan et al. (2006a), improvement of the exposure assessment (chemical analysis of the arsenic content of the tube wells, an individual questionnaire on water consumption) has been achieved, which allowed the analysis of dose–response relationships between the occurrence of skin lesions and different exposure categories. Taken together, the new studies consistently point to the occurrence of skin lesions at arsenic concentrations below 100 µg/L (Table 11). The lowest observed adverse-effect level in the studies of Tseng and colleagues (Tseng et al. 1968; Tseng, 1977) was 170 µg/L.

The studies by Guha Mazumder et al. (1998) and Haque et al. (2003) were excluded from further analysis because of possible failures in diagnosis and uncertainties in exposure assessment (skin lesions have been observed in the lowest-exposure category, exposure was probably due to unknown sources). Furthermore, Haque et al. (2003) did not evaluate the data for men and women separately. The case–cohort-analysis of Hall et al. (2006) included only a relatively small number of participants, and the concentrations that showed statistical significance for the effects were higher (about 140 µg/L) in their analysis than in the other studies reported.

As in the studies by Guha Mazumder et al. (1998) and Ahsan et al. (2006a), men proved to be more sensitive than women in the study by Rahman et al. (2006). However, the influence of nutritional status was not considered by Rahman et al. (2006).

The cross-sectional study of Ahsan et al. (2006a) involved more than 10,000 participants, individual data on arsenic exposure via drinking water, and careful diagnosis of skin lesions. It is the only study that investigated the influence of both sex and nutritional status on the occurrence of skin lesions and is, therefore, selected as the basis for the assessment of non-cancer health effects and the derivation of a tolerable dose.

Data on the prevalence of skin lesions (melanosis and hyperkeratosis), as described by Ahsan et al. (2006a), are presented in Table 12 and Figure 3. Analysis has separately been performed for men and women and according to the nutritional status (BMI >20.4, 18.1–20.4, and <18.1).

### Selection of the most susceptible subgroup

Men show the highest prevalence of skin lesions and are considered to be the most susceptible subgroup (Figure 3). Furthermore, nutritional status influences individual susceptibility: malnourished persons are more sensitive than persons with a better nutritional status. A BMI <18.5 has been classified as underweight by the WHO (see also Maharjan et al., 2007). Ahsan et al. (2006a) grouped participants according to a BMI cutoff level of 18.1. Persons with a BMI of 18.1–20.4 can be regarded as being in the lower end of the normal range. Persons with a BMI >20.4 are in the normal range or are overweight (a BMI ≥25 has been defined as overweight by WHO). A concomitant analysis of the data from Ahsan et al. (2006a) according to BMI and sex was not possible, as these details were not presented in the publication.

Several factors have been suggested to contribute to the increased susceptibility of men compared with women. Because of their higher methylation capacity, lower MMA-concentrations are found in the urine of women than in the urine of men, and these lower concentrations seem to be associated with a lower risk for cancer and non-cancer lesions in women (Ahsan et al., 2007; Lindberg et al., 2007).

### Table 12. Prevalence data for skin lesions by level of arsenic exposure in a population from Bangladesh (data from Ahsan et al., 2006a).

<table>
<thead>
<tr>
<th>Median arsenic level of exposure quintile (µg/L)</th>
<th>Total n</th>
<th>n cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.8</td>
<td>980</td>
<td>47</td>
</tr>
<tr>
<td>23.0</td>
<td>897</td>
<td>72</td>
</tr>
<tr>
<td>62.0</td>
<td>923</td>
<td>118</td>
</tr>
<tr>
<td>126.0</td>
<td>946</td>
<td>141</td>
</tr>
<tr>
<td>254.0</td>
<td>938</td>
<td>191</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.8</td>
<td>1287</td>
<td>12</td>
</tr>
<tr>
<td>23.0</td>
<td>1218</td>
<td>15</td>
</tr>
<tr>
<td>63.0</td>
<td>1269</td>
<td>27</td>
</tr>
<tr>
<td>125.0</td>
<td>1245</td>
<td>24</td>
</tr>
<tr>
<td>256.7</td>
<td>1248</td>
<td>48</td>
</tr>
<tr>
<td>BMI &lt;18.1*</td>
<td>1.9</td>
<td>729</td>
</tr>
<tr>
<td>22.0</td>
<td>681</td>
<td>36</td>
</tr>
<tr>
<td>62.0</td>
<td>677</td>
<td>57</td>
</tr>
<tr>
<td>124.2</td>
<td>750</td>
<td>78</td>
</tr>
<tr>
<td>252.0</td>
<td>776</td>
<td>112</td>
</tr>
<tr>
<td>BMI 18.1–20.4*</td>
<td>1.7</td>
<td>701</td>
</tr>
<tr>
<td>24.0</td>
<td>715</td>
<td>30</td>
</tr>
<tr>
<td>64.2</td>
<td>753</td>
<td>48</td>
</tr>
<tr>
<td>126.0</td>
<td>714</td>
<td>55</td>
</tr>
<tr>
<td>257.0</td>
<td>731</td>
<td>82</td>
</tr>
<tr>
<td>BMI &gt;20.4*</td>
<td>1.8</td>
<td>837</td>
</tr>
<tr>
<td>23.0</td>
<td>719</td>
<td>21</td>
</tr>
<tr>
<td>61.9</td>
<td>762</td>
<td>40</td>
</tr>
<tr>
<td>126.0</td>
<td>727</td>
<td>32</td>
</tr>
<tr>
<td>259.0</td>
<td>679</td>
<td>45</td>
</tr>
</tbody>
</table>

Note: *No differentiation according to sex.

### Table 13. Estimated arsenic intake of males in rural Bangladesh (data from Watanabe et al., 2004).

<table>
<thead>
<tr>
<th>Well-water arsenic concentration (µg/L)</th>
<th>10</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total intake (µg per day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water for food preparation</td>
<td>16</td>
<td>160</td>
<td>800</td>
</tr>
<tr>
<td>Food without water and without fish</td>
<td>154</td>
<td>154</td>
<td>154</td>
</tr>
<tr>
<td>Sub-total (without drinking water)</td>
<td>170</td>
<td>314</td>
<td>954</td>
</tr>
<tr>
<td>Drinking water*</td>
<td>30</td>
<td>300</td>
<td>1500</td>
</tr>
<tr>
<td>Total (with drinking water)</td>
<td>200</td>
<td>614</td>
<td>2454</td>
</tr>
</tbody>
</table>

Note: *Uptake of inorganic arsenic from fish can be regarded as negligible, according to Borak et al. (2007). *Drinking-water consumption of 3L per day has been assumed for the derivation of the tolerable resorbed dose.
Steinmaus et al. 2007; Vahter et al., 2007). These differences are probably due to genetic polymorphisms of several key metabolic enzymes (Schläwicke Engström et al., 2007; Steinmaus et al., 2007).

Another factor leading to differences in susceptibility to arsenic between men and women is the higher arsenic intake of men from food and drinking water in comparison with women. This will be elaborated on below.

Sunlight exposure, smoking status, and occupational exposure to fertilizer were discussed by Chen et al. (2006) as possible reasons for the increased susceptibility of men. Although an interrelation between arsenic exposure, sunlight, and smoking status cannot be excluded, no data are available to quantify the influence of sunlight or other factors on the prevalence of skin lesions.

**Arsenic background exposure**

The prevalence of skin lesions was about 5% for men in the lowest-exposure category (1.8 µg/L on average) in the study by Ahsan et al. (2006a). As no causal factors other than arsenic exposure exist for such skin lesions, these data indicate that arsenic exposure from sources other than drinking water must occur and contribute to the development of skin lesions.

No information on background exposure was initially given by Ahsan et al. (2006a). Additional information on the collective was presented by Ahsan et al. (2006b) in another paper: “...because arsenic exposure from food to this population is likely to be negligible in comparison to As exposure from drinking water, we did not ... assess dietary exposure to As.” This statement is incompatible with findings from several other studies, which reported relevant arsenic intake via food (Kile et al., 2007; Ohno et al., 2007; Smith, N.M. et al., 2006; Watanabe, 2001, Watanabe et al., 2004). These authors demonstrated that at low concentrations of arsenic in drinking water (<10 µg/L), the arsenic content of food substantially contributes to total arsenic intake. With increasing arsenic concentrations in drinking water, arsenic intake via drinking water or water used for the preparation of food becomes increasingly important (see Table 13; Watanabe et al., 2004). Mean food-consumption rates of female study participants in Bangladesh are about 70% of those of male participants (Ohno et al., 2007), probably partially due to the higher body weight of men. This results in different background exposures for men and women who are exposed to drinking water with identical arsenic content (Kile et al., 2007). In Table 13, the background exposure of men in Bangladesh from food and the resulting total exposure from food and drinking water at several arsenic water concentrations is calculated.

To this end, arsenic intake from food without water added for preparation, water added for food preparation, and drinking water were summarized. Watanabe et al. (2004) calculated a total arsenic intake from major food items of 214 µg per day for men. Arsenic intake from fish has been estimated to be about 60 µg per day for men. As the proportion of inorganic arsenic in fish is negligible (Borak et al., 2007) it was considered irrelevant for the calculation of the total intake of inorganic arsenic. Staple foods like rice, but also cereals, vegetables, and fruits, contribute to total arsenic intake via food. The arsenic content of rice in Bangladesh varies greatly according to the producing area, the rice variety, and the season (dry or rainy). Arsenic levels between 0.10 µg/g and 0.95 µg/g rice have been reported for Bangladesh (Williams et al., 2005). Arsenic in rice, cereals, and vegetables in Bangladesh is mainly (90% or more) inorganic arsenic (Ohno et al., 2007; Williams et al., 2005, 2006). For the following calculations, it has been assumed to be 100%. For further calculations, the total intake of inorganic arsenic from food without water has been assumed to be 154 µg per day. Arsenic intake from water used in food preparation can be calculated to be 1.6 times the arsenic concentration in drinking water (Watanabe et al., 2004).

According to Ahsan et al. (2006a), the drinking-water consumption of the study population was about 2.5–3.0L per day. Milton et al. (2006) reported that drinking-water consumption in a cross-sectional study of the rural population of northwestern Bangladesh (n = 640) was about 3.53 L per day. The average drinking-water consumption of males and females has been estimated to be about 3L per day (cup method; Watanabe et al., 2004; Ohno et al., 2007). So a drinking-water consumption of 3L per day has been assumed for the derivation of the TRD (tolerable resorbed dose) value.

These data clearly demonstrate that, especially at low arsenic concentrations in drinking water (<10 µg/L), arsenic intake via food relevantly contributes to the total intake.

Benchmark dose modelling was performed based on data from Ahsan et al. (2006a) regarding the prevalence of skin lesions depending on inorganic arsenic content in drinking water. To this end, for individual exposure groups, total

---

**Table 14.** Calculation of the total intake of inorganic arsenic of men in the study by Ahsan et al. (2006a) with regard to the arsenic intake via food (data from Watanabe et al., 2004).

<table>
<thead>
<tr>
<th>Drinking-water arsenic concentration in Ahsan et al. (2006a) (µg As/L)</th>
<th>Arsenic intake via food according to Watanabe et al. (2004) (µg As/person and day)</th>
<th>Arsenic-intake via 3L per day drinking water (µg As/person and day)</th>
<th>Total arsenic-intake (µg As/person and day)</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>156.9</td>
<td>5.4</td>
<td>162.3</td>
<td>47/980</td>
</tr>
<tr>
<td>23.0</td>
<td>190.8</td>
<td>69.0</td>
<td>259.8</td>
<td>72/897</td>
</tr>
<tr>
<td>62.0</td>
<td>253.2</td>
<td>186.0</td>
<td>439.2</td>
<td>118/923</td>
</tr>
<tr>
<td>126.0</td>
<td>355.6</td>
<td>378.0</td>
<td>733.6</td>
<td>141/946</td>
</tr>
<tr>
<td>254.0</td>
<td>560.4</td>
<td>762.0</td>
<td>1322.4</td>
<td>191/938</td>
</tr>
</tbody>
</table>

Note: *154 µg arsenic per day (food without water added for preparation) + drinking-water concentration × 1.6 (to account for the water added for preparation).*
Table 15. Result of the benchmark dose–response modelling of the data for men in the study of Ahsan et al. (2006a) with regard to the total arsenic exposure as described in Table 14.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Log-probit</th>
<th>Log-logistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>p value</td>
<td>0.263</td>
<td>0.160</td>
</tr>
<tr>
<td>AIC</td>
<td>3336.85</td>
<td>3338.04</td>
</tr>
<tr>
<td>1% prevalence</td>
<td>BMD01 (µg/day)</td>
<td>22.8</td>
</tr>
<tr>
<td>BMDL01 (µg/day)</td>
<td>13.2</td>
<td>6.4</td>
</tr>
<tr>
<td>5% prevalence</td>
<td>BMD05 (µg/day)</td>
<td>140.8</td>
</tr>
<tr>
<td>BMDL05 (µg/day)</td>
<td>109.2</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Table 16. Derivation of a tolerable daily intake for inorganic arsenic.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMDL05</td>
<td>109.2 µg per day</td>
</tr>
<tr>
<td>Body weight</td>
<td>50 kg</td>
</tr>
<tr>
<td>Daily dose per kg body weight at BMDL05</td>
<td>2.18 µg/kg per day</td>
</tr>
<tr>
<td>Extrapolation factor to consider effect level (5%) at BMDL</td>
<td>5</td>
</tr>
<tr>
<td>Tolerable daily intake</td>
<td>0.45 µg/kg per day</td>
</tr>
</tbody>
</table>

tolerable daily intake for inorganic arsenic from all sources of 0.45 µg/kg body weight per day (Table 16).

This evaluation does not include any consideration of carcinogenic effects. The tolerable daily intake derived is marginally higher than the value of 0.3 µg/(kg ∙ day), derived previously by the US EPA (2007), ATSDR (2005), and others (Hassauer and Kalberlah, 1999). These evaluations are based on the data of Tseng and colleagues (Tseng et al. 1968; Tseng, 1977) on skin lesions in Taiwanese populations, which included very limited dose–response information and uncertainties regarding the size of effects and associated doses.

Assuming background exposure levels from food in western countries at about 14 µg per day (Hughes, 2006) this tolerable dose would be exceeded when drinking-water levels of arsenic exceed 9 µg/L (assuming consumption of 2 L per day by an adult of 70 kg). The drinking water limit value of 10 µg/L recommended by WHO and established in Europe and the US seems justified from this perspective.

The proposed tolerable daily intake considers differences related to sex and nutrition status. Other inter-individual differences in susceptibility exist, such as those resulting from polymorphisms of enzymes relevant for arsenic metabolism or DNA repair. However, because of the large site of the study population, susceptible subgroups are supposed to be sufficiently represented in the data from Ahsan et al. (2006a).

The skin lesions evaluated here as the critical effect are areas of Bangladesh is 50.5 kg) and Abdullah and Wheeler (1985; average body weight 48.5).

The study of Ahsan et al. (2006a) included a large population of more than 10,000 persons, and the evaluation is based on the group considered the most susceptible—men. For this database, no extrapolation factor for considering inter-individual differences in susceptibility is considered necessary. BMD and BMDL were calculated at a response level of 5%, which is not an acceptable effect level. Therefore, an extrapolation factor of 5 is used to derive a tolerable daily intake for inorganic arsenic from all sources of 0.45 µg/kg body weight per day (Table 16).

Figure 4. Modulation of the dose–response relationship between arsenic exposure and the prevalence of skin lesions in men from Bangladesh, using the log-probit model. Exposure concentration is given as daily intake of inorganic arsenic (data from Ahsan et al., 2006a and Watanabe et al., 2004).

Conclusions

Information on the potential carcinogenic effects of inorganic arsenic at low exposure concentrations in drinking water has improved substantially thanks to epidemiological...
data gathered in recent years. These data suggest a low or negligible risk of bladder cancer in populations exposed to concentrations of up to 100 µg/L. From these studies and others, evidence of a synergistic relationship between arsenic and smoking has accumulated, and smokers may bear an increased risk of arsenic-induced bladder cancer, even at moderate exposure concentrations. Information on the low-dose range is less convincing and partly contradictory for lung and skin tumours. Data from a study pointing for the first time to an increased risk for melanoma await confirmation.

With respect to non-carcinogenic effects, recent epidemiological studies indicate that effects on the development of cognitive functions, cardiovascular diseases, and reproductive effects are caused by oral arsenic exposure at drinking-water concentrations below 200 µg/L. At even lower exposure concentrations, skin disorders such as dyspigmentation and keratosis occur. Studies from Bangladesh and other countries deliver convincing dose–response data, showing increases in the prevalence of skin effects at concentrations below 100 µg/L. Despite differences between the Asian populations studied and populations in Western countries (e.g. with respect to background exposure and nutritional status), these data can be used to derive a generally applicable tolerable daily intake, supposed to protect nutritional status), these data can be used to derive a generally applicable tolerable daily intake, supposed to protect the Asian populations studied and populations in Western countries (e.g. with respect to background exposure and nutritional status), these data can be used to derive a generally applicable tolerable daily intake, supposed to protect nutritional status.

Endnotes

1 The different valence states (trivalent or pentavalent) of arsenicals are not specifically noted in all publications. Where available from the original literature, we indicated the valence state with a superscript. In those cases, where the data were not obvious the valence state is not given.

Acknowledgments

This publication is part of a research project on the toxicological activity of arsenic in soil, groundwater and drinking-water sponsored by the Federal Environmental Agency (Umweltbundesamt), Berlin, Germany (grant no. FKZ 206 61 201).

References


Critical Reviews in Toxicology Downloaded from informahealthcare.com by University Of Arizona on 11/04/10


arsenic compounds and the different roles of glutathione in such induction. Res. 357:123–129.


