Review

Arsenic in the aetiology of cancer

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Abstract

Arsenic, one of the most significant hazards in the environment affecting millions of people around the world, is associated with several diseases including cancers of skin, lung, urinary bladder, kidney and liver. Groundwater contamination by arsenic is the main route of exposure. Inhalation of airborne arsenic or arsenic-contaminated dust is a common health problem in many ore mines. This review deals with the questions raised in the epidemiological studies such as the dose–response relationship, putative confounders and synergistic effects, and methods evaluating arsenic exposure. Furthermore, it describes the metabolic pathways of arsenic, and its biological modes of action. The role of arsenic in the development of cancer is elucidated in the context of combined epidemiological and biological studies. However, further analyses by means of molecular epidemiology are needed to improve the understanding of cancer aetiology induced by arsenic.

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Keywords: Arsenic; Cancer; Drinking water; Mines; Molecular epidemiology

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1. Introduction

Arsenic (As), a widely distributed semi-metallic element occurring in various compounds in the crust of the earth, is considered one of the most significant hazards in the environment. The exposure to the trivalent inorganic form iAs(III) and its mono- and dimethylated derivatives MMA(III) and DMA(III), respectively, are associated with cancers of skin, lung, urinary bladder, kidney and liver [1–6], as well as several non-cancer diseases such as diabetes mellitus, hypertension, and cardiovascular and cerebrovascular diseases [7–10]. Drinking water contaminated with inorganic arsenic is the primary route of exposure. A second major exposure source is the diet. However, in food, especially seafood, arsenic is mostly present in its organic forms such as arsenocholine, arsenobetain or arsenosugars, not presently known to be toxic. Inhalation of airborne iAs and iAs-contaminated dust is a common healthy problem in tin, gold and uranium mines [11–16] and copper smelters [17,18]. Arsenicals can also be emitted to the air by coal combustion [19]. Other occupational sources of arsenic exposure include glass smelters [20] and its use in semiconductors, pesticides and wood preservatives [21,22]. Paradoxically, arsenic administered in its trioxide form appears to be a valuable therapeutic tool in cancer treatment [23].

The latency period in humans of arsenic-related carcinogenesis is considered to be 30–50 years. Therefore, the current epidemiological data on arsenic-induced cancers available now is coming from countries like Taiwan, the USA, Chile and Argentina, where people have consumed contaminated water for more than 50 years [2,24–26]. The first signs of chronic exposure are skin pigmentation, depigmentation, hyperkeratosis of palms and soles and skin lesions. A unique peripheral vascular disease in Taiwan is the black foot disease, starting with numbness and ulceration of extremities and ending in gangrene and spontaneous amputations [27]. The affected populations in Taiwan or South America represent a minor fraction of the total population. Contrarily, in countries like China, Bangladesh or India millions of people are exposed to arsenic-contaminated drinking water since 1980s [28–30]. Formerly mainly shallow well water or surface water was used in households. Efforts to improve the quality of drinking water by drilling deeper wells led to the unanticipated opposite effect by increasing the amount of arsenic leaking from the soil. Being mainly a problem in the countryside, arsenic concentrations often exceed several folds the limit of 10 μg/l recommended in the WHO drinking water quality guideline [31]. In these large areas of endemic arsenic poisoning, the rate of malignancies is expected to explode within the next several decades.

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Countries with arsenic-affected populations are summarised in Table 1.

This review describes epidemiological effects of arsenic contamination, either being the sole source of exposure or in combination with other toxicants, as well as cellular mechanisms known to be involved in arsenic carcinogenesis.

2. Methods evaluating arsenic exposure

Once ingested, soluble forms of arsenic are quickly absorbed from the gastrointestinal tract to blood and delivered through the liver to different organs and tissues. The concentration of arsenic in blood decreases to normal levels within a few hours. After being metabolised most of arsenic is excreted usually in dimethyl arsenic forms from the body via urine within days. In other tissues like finger and toe nails and hair, arsenic can be monitored for months or years after the exposure, respectively [32].

Samples of urine and blood can be used to estimate the individual dose of ongoing exposure, whereas hair and nail samples give the average exposure over a longer duration of time. Since the concentration of arsenic in urine is stable throughout the day [33], most researchers prefer to use spot urine samples adjusted with creatinine instead of the more troublesome 24 h urine collection samples. Peripheral blood samples are reliable but difficult to use in mass screening. Whereas urine and blood samples are free of outer contamination, external contamination by sweating or fixation of dust in nail and hair samples has to be considered. Concentrations of arsenic in drinking water or air and dust are usually expressed as mg/l or µg/m³, alternatively mg/m³, respectively.

Water consumption can vary significantly depending on the weather, labour intensity and body size; this variation has to be taken into account when estimating the daily arsenic dose. In retrospective studies the estimates of the daily water consumption are prone to be affected by recall bias. The average arsenic concentration is calculated in a time-weighted manner by using measured arsenic concentration in water multiplied by duration of consumption and divided by total duration. If the arsenic concentration of the exposure source stays relatively constant for a longer period of time, indexes depending on the arsenic concentration in drinking water or those depending on tissue samples can both be used with equal confidence. For the development of diseases such as cancer having a long latency time the use of cumulative arsenic exposure is recommended. Cumulative arsenic exposure is the sum of multiplying different arsenic concentrations by the duration of consumption and usually expressed as mg × year/l [34].

In occupational arsenic exposure situations, the dose is usually estimated by using individual job exposure matrices (JEM), being the product of average arsenic concentration in air or dust and the number of years of exposure. Cumulative arsenic exposure levels are estimated by summing JEMs representing the different exposure levels of varying occupational environments. Some researchers use the term ‘index of arsenic exposure month’ (IAEM) instead of JEMs [14].

The carcinogenicity of arsenic depends on its chemical form and oxidation status [35,36]. Recently, techniques have been developed to specify the different states of arsenic in biological samples [37,38]. The speciation of arsenic compounds is especially important in urine which often contains arsenobetaine, arsenocholine and other arsenosugars from sources other than drinking water. Arsenosugars are considered much less toxic than iAs [39].

3. Epidemiological studies

Although many epidemiological studies dealing with the health effects of arsenic have been reported worldwide, data concerning dose–response relationships are scarce. This may be due to difficulties in accurately evaluating individual doses of arsenic exposure, an issue of extreme importance in estimating cancer risks. Also the type of arsenic exposure, whether

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of people affected</th>
<th>Concentration (µg/l)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>50–75 million</td>
<td>&lt;10–&gt;1000</td>
<td>[343,344]</td>
</tr>
<tr>
<td>West Bengal (India)</td>
<td>&gt;6 million</td>
<td>3–3700</td>
<td>[344,345]</td>
</tr>
<tr>
<td>China</td>
<td>&gt;2 million</td>
<td>50–2000</td>
<td>[346]</td>
</tr>
<tr>
<td>Taiwan</td>
<td>120,000 (1982)</td>
<td>200–2500</td>
<td>[347,348]</td>
</tr>
<tr>
<td>Thailand</td>
<td>n.d.</td>
<td>1–5114</td>
<td>[344]</td>
</tr>
<tr>
<td>Vietnam</td>
<td>11 million</td>
<td>1–3050</td>
<td>[349]</td>
</tr>
<tr>
<td>Mexico</td>
<td>400,000</td>
<td>8–624</td>
<td>[350]</td>
</tr>
<tr>
<td>Chile</td>
<td>250,000</td>
<td>470–770</td>
<td>[351]</td>
</tr>
<tr>
<td>Argentina</td>
<td>2 million</td>
<td>&gt;100</td>
<td>[25,344]</td>
</tr>
<tr>
<td>United States</td>
<td>350,000</td>
<td>1–1160</td>
<td>[344]</td>
</tr>
<tr>
<td>Finland</td>
<td>10,000</td>
<td>17–980</td>
<td>[352]</td>
</tr>
<tr>
<td>Hungary</td>
<td>n.d.</td>
<td>1–174</td>
<td>[344]</td>
</tr>
</tbody>
</table>

n.d., not determined.
present in drinking water or in air, seems to influence the shape of the dose–response curve.

3.1. Environmental studies

Due to the long latency period of human cancer, the calculations of the dose–response relationship between arsenic and internal and external cancers have been based on studies in areas with a long exposure history such as Taiwan, the USA or Chile. Only recently small numbers of skin cancer cases, mostly squamous cell carcinoma, have started to emerge in the areas of contaminated drinking water in Bangladesh and India [40]. Earlier data, based mainly on Taiwanese drinking water studies, suggests a linear dose–response relationship, at least with higher doses [34].

3.1.1. Cohort studies

Wu et al. [41] calculated age-adjusted mortality rates from various cancers and vascular diseases by gender in a Taiwanese cohort study using the 1976 world population as the standard (Table 2a). A significant dose–response relation was observed between arsenic levels in well water and cancers of the bladder, kidney, skin, and lung in both males and females, and cancers of the prostate and liver in males.

In a Taiwanese study by Chiou et al. a total of 263 patients with blackfoot disease and 2293 healthy residents in the endemic area were recruited and followed up for 7 years (Table 2a) [2]. A dose–response relationship was observed between cumulative arsenic exposure or the duration of drinking contaminated water in years and the incidence of lung cancer, bladder

<table>
<thead>
<tr>
<th>Expose pathway</th>
<th>Type of cancer</th>
<th>Arsenic range</th>
<th>Number of deaths (observed)</th>
<th>SMR/RR (95% CI)</th>
<th>Area</th>
<th>Reference/remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>Bladder</td>
<td>Males</td>
<td>23</td>
<td>22.64*</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.30 mg/l</td>
<td>23</td>
<td>22.64*</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.30–0.59 mg/l</td>
<td>36</td>
<td>61.02</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥0.60 mg/l</td>
<td>26</td>
<td>92.71</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
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<td></td>
<td></td>
<td>Females</td>
<td>30</td>
<td>25.60</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
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<td></td>
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<td>&lt;0.30 mg/l</td>
<td>30</td>
<td>25.60</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<tr>
<td></td>
<td></td>
<td>0.30–0.59 mg/l</td>
<td>36</td>
<td>57.02</td>
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<td>≥0.60 mg/l</td>
<td>30</td>
<td>11.30</td>
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<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<tr>
<td></td>
<td>Kidney</td>
<td>Males</td>
<td>9</td>
<td>8.42</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.30 mg/l</td>
<td>9</td>
<td>8.42</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<tr>
<td></td>
<td></td>
<td>0.30–0.59 mg/l</td>
<td>11</td>
<td>18.90</td>
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<td></td>
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<td>≥0.60 mg/l</td>
<td>6</td>
<td>25.26</td>
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<td></td>
<td></td>
<td>Females</td>
<td>4</td>
<td>3.42</td>
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<tr>
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<td></td>
<td>&lt;0.30 mg/l</td>
<td>4</td>
<td>3.42</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<td>19.42</td>
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<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<td>≥0.60 mg/l</td>
<td>16</td>
<td>57.98</td>
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<td>Skin</td>
<td>Males</td>
<td>2</td>
<td>2.03</td>
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<tr>
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<td>&lt;0.30 mg/l</td>
<td>2</td>
<td>2.03</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
<tr>
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<td></td>
<td>0.30–0.59 mg/l</td>
<td>8</td>
<td>14.01</td>
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<tr>
<td></td>
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<td>Females</td>
<td>2</td>
<td>1.73</td>
<td>Taiwan</td>
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<tr>
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<td>&lt;0.30 mg/l</td>
<td>2</td>
<td>1.73</td>
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<td>10</td>
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<td>5</td>
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<td></td>
<td>Lung</td>
<td>Males</td>
<td>53</td>
<td>49.16</td>
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<td></td>
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<td>&lt;0.30 mg/l</td>
<td>53</td>
<td>49.16</td>
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<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<td>0.30–0.59 mg/l</td>
<td>62</td>
<td>100.67</td>
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<td>≥0.60 mg/l</td>
<td>32</td>
<td>104.08</td>
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<td>Females</td>
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<td>36.71</td>
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<td>&lt;0.30 mg/l</td>
<td>43</td>
<td>36.71</td>
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<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<td>0.30–0.59 mg/l</td>
<td>40</td>
<td>60.82</td>
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<td>≥0.60 mg/l</td>
<td>38</td>
<td>122.16</td>
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<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<tr>
<td></td>
<td>Liver</td>
<td>Males</td>
<td>54</td>
<td>47.78</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<td>&lt;0.30 mg/l</td>
<td>54</td>
<td>47.78</td>
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<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
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<td>0.30–0.59 mg/l</td>
<td>42</td>
<td>67.62</td>
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<td></td>
<td>≥0.60 mg/l</td>
<td>27</td>
<td>86.73</td>
<td>Taiwan</td>
<td>[41]. *Age adjusted mortality rate per 100,000. Cohort size 203,344. In the original paper arsenic concentration in ppm</td>
</tr>
</tbody>
</table>
Table 2a (Continued)

<table>
<thead>
<tr>
<th>Expose pathway</th>
<th>Type of cancer</th>
<th>Arsenic range</th>
<th>Number of deaths (observed)</th>
<th>SMR/RR (95% CI)</th>
<th>Area</th>
<th>Reference/remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>All</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25</td>
<td>1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Taiwan</td>
<td>[2]. &lt;sup&gt;bmg/l × years.&lt;/sup&gt; RR. Cohort size 2556. Age-, sex-, and smoking-adjusted</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>1.0</td>
<td>Taiwan</td>
<td>[42]. &lt;sup&gt;dRR&lt;/sup&gt;. Multivariate (age, sex, smoking, alcohol, SES) adjusted</td>
</tr>
<tr>
<td></td>
<td>Bladder</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>1.0</td>
<td>Taiwan</td>
<td>[43]. &lt;sup&gt;eSMR&lt;/sup&gt;. Cohort size 454</td>
</tr>
<tr>
<td>Drinking water</td>
<td>Lung</td>
<td>&lt;0.05 mg/l</td>
<td>11</td>
<td>0.78 (0.41–1.41)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Niigata, Japan</td>
<td>[44]. &lt;sup&gt;fSMR&lt;/sup&gt;. Cohort size?</td>
</tr>
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<td></td>
<td>Bladder</td>
<td>&lt;0.05 mg/l</td>
<td>0</td>
<td>0 (0–2.43)</td>
<td>Cordoba, Argentina</td>
<td>[25]. &lt;sup&gt;gSMR&lt;/sup&gt;. Cohort size?</td>
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<td>0.05–0.99 mg/l</td>
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<td>1.30 (0.51–3.06)</td>
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<td>≥1 mg/l</td>
<td>18</td>
<td>3.63 (2.25–5.71)</td>
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<tr>
<td>Drinking water</td>
<td>Bladder</td>
<td>Males</td>
<td>Low (0.04 µg/l)</td>
<td>113</td>
<td>0.80 (0.66–0.96)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Finland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium (120 µg/l)</td>
<td>116</td>
<td>1.28 (1.05–1.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High (178 µg/l)</td>
<td>131</td>
<td>2.14 (1.78–2.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>Low (0.04 µg/l)</td>
<td>39</td>
<td>1.22 (0.86–1.67)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Medium (120 µg/l)</td>
<td>29</td>
<td>1.39 (0.93–1.99)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>High (178 µg/l)</td>
<td>27</td>
<td>1.81 (1.19–2.64)</td>
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<tr>
<td></td>
<td>Kidney</td>
<td>Males</td>
<td>Low (0.04 µg/l)</td>
<td>66</td>
<td>0.87 (0–66–1.10)</td>
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<tr>
<td></td>
<td></td>
<td>Medium (120 µg/l)</td>
<td>66</td>
<td>1.33 (1.02–1.68)</td>
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<tr>
<td></td>
<td></td>
<td>High (178 µg/l)</td>
<td>53</td>
<td>1.57 (1.17–2.05)</td>
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<td>Females</td>
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<td>1.00 (0.71–1.37)</td>
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<tr>
<td></td>
<td></td>
<td>Medium (120 µg/l)</td>
<td>34</td>
<td>1.36 (0.94–1.89)</td>
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<td></td>
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<td>High (178 µg/l)</td>
<td>27</td>
<td>1.81 (1.19–2.64)</td>
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<td></td>
<td>Lung</td>
<td>Males</td>
<td>Low (0.04 µg/l)</td>
<td>826</td>
<td>0.92 (0.85–0.98)</td>
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<tr>
<td></td>
<td></td>
<td>Medium (120 µg/l)</td>
<td>914</td>
<td>1.54 (1.44–1.64)</td>
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<td></td>
<td>High (178 µg/l)</td>
<td>708</td>
<td>1.77 (1.63–1.90)</td>
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<td>Females</td>
<td>Low (0.04 µg/l)</td>
<td>194</td>
<td>1.24 (1.06–1.42)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Medium (120 µg/l)</td>
<td>138</td>
<td>1.34 (1.12–1.58)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>High (178 µg/l)</td>
<td>156</td>
<td>2.16 (1.83–2.52)</td>
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</tr>
<tr>
<td>Drinking water</td>
<td>Bladder</td>
<td>&lt;0.1 µg/l</td>
<td>23</td>
<td>1.0&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Finland</td>
<td>[44]. &lt;sup&gt;iRR&lt;/sup&gt;. Cohort size 4590. Age-, sex-, and smoking-adjusted</td>
</tr>
</tbody>
</table>
cancer, and cancers of all sites combined, after adjustment for age, sex, and cigarette smoking. Blackfoot disease patients had a significantly increased cancer incidence after adjustment for cumulative arsenic exposure.

A second 8-year follow-up study by Chen et al. included a total of 10,591 residents in the arsenic-polluted areas in Taiwan (Table 2a) [42]. After adjustment for cigarette smoking and other risk factors, there was an increasing trend of lung cancer risk by increasing arsenic level in drinking water varying from 10 to 700 µg/l or more (p < 0.001). The relative risk was 3.29 [95% confidence interval (CI) 1.60–6.78] for the highest arsenic level compared with the lowest. There was an indication of a synergistic effect of ingested arsenic and cigarette smoking on lung cancer.

In a retrospective cohort study in Japan, Tsuda et al. reported standardised mortality ratios (SMR) in a group consuming water with high concentrations of arsenic (>1 ppm) of 3.63 (95% CI 2.25–5.71) for all cancers,

### Table 2b
Case-control studies on environmental arsenic exposure

<table>
<thead>
<tr>
<th>Expose pathway</th>
<th>Type of cancer</th>
<th>Arsenic range</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>Area</th>
<th>Reference/remarks</th>
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<tbody>
<tr>
<td>Drinking water</td>
<td>Lung</td>
<td>0–10 µg/l</td>
<td>9</td>
<td>104</td>
<td>1</td>
<td>Chile [24]. Adjusted for age, sex, smoking, SES, and working in copper smelting</td>
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<tr>
<td></td>
<td></td>
<td>10–29 µg/l</td>
<td>5</td>
<td>39</td>
<td>1.6 (0.5–5.3)</td>
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<td></td>
<td></td>
<td>30–49 µg/l</td>
<td>8</td>
<td>23</td>
<td>3.9 (1.2–12.3)</td>
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<td></td>
<td></td>
<td>50–199 µg/l</td>
<td>50</td>
<td>124</td>
<td>5.2 (2.3–11.7)</td>
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<td></td>
<td>200–400 µg/l</td>
<td>79</td>
<td>129</td>
<td>8.9 (4.0–19.6)</td>
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<tr>
<td>Drinking water</td>
<td>Bladder</td>
<td>Non-smokers</td>
<td></td>
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<td></td>
<td>Utah, USA [26]. Cumulative dose</td>
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<tr>
<td></td>
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<td>&lt;19 mg&quot;a&quot;</td>
<td>10</td>
<td>25</td>
<td>1.00</td>
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<td></td>
<td></td>
<td>19–33 mg&quot;a&quot;</td>
<td>10</td>
<td>19</td>
<td>1.09 (0.4–3.1)</td>
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<td></td>
<td></td>
<td>33–53 mg&quot;a&quot;</td>
<td>7</td>
<td>20</td>
<td>0.68 (0.2–2.3)</td>
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<td></td>
<td></td>
<td>≥53 mg&quot;a&quot;</td>
<td>4</td>
<td>17</td>
<td>0.53 (0.1–1.9)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Smokers</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>&lt;19 mg&quot;a&quot;</td>
<td>4</td>
<td>22</td>
<td>1.00</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>19–33 mg&quot;a&quot;</td>
<td>11</td>
<td>17</td>
<td>3.33 (1.0–10.8)</td>
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<td></td>
<td></td>
<td>33–53 mg&quot;a&quot;</td>
<td>10</td>
<td>19</td>
<td>1.93 (0.6–6.2)</td>
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<td></td>
<td></td>
<td>≥53 mg&quot;a&quot;</td>
<td>15</td>
<td>21</td>
<td>3.32 (1.1–10.3)</td>
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<tr>
<td>Drinking water</td>
<td>Bladder</td>
<td>Never smokers, 5-year lag</td>
<td></td>
<td></td>
<td></td>
<td>Western USA [45]. Cumulative dose</td>
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<tr>
<td></td>
<td></td>
<td>&lt;6.4 mg&quot;b&quot;</td>
<td>8</td>
<td>38</td>
<td>1.00</td>
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<td></td>
<td></td>
<td>6.4–82.8 mg&quot;b&quot;</td>
<td>11</td>
<td>32</td>
<td>1.55 (0.51–4.72)</td>
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<td></td>
<td></td>
<td>&gt;82.8 mg&quot;b&quot;</td>
<td>10</td>
<td>49</td>
<td>0.83 (0.28–2.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ever smokers, 5-year lag</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>&lt;6.4 mg&quot;b&quot;</td>
<td>58</td>
<td>63</td>
<td>1.00</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>6.4–82.8 mg&quot;b&quot;</td>
<td>46</td>
<td>79</td>
<td>0.69 (0.40–1.18)</td>
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<td>&gt;82.8 mg&quot;b&quot;</td>
<td>48</td>
<td>66</td>
<td>0.76 (0.44–1.30)</td>
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<tr>
<td></td>
<td></td>
<td>Never smokers, 40-year lag</td>
<td></td>
<td></td>
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<td>&lt;6.4 mg&quot;b&quot;</td>
<td>23</td>
<td>92</td>
<td>1.00</td>
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<tr>
<td></td>
<td></td>
<td>6.4–82.8 mg&quot;b&quot;</td>
<td>3</td>
<td>5</td>
<td>2.65 (0.49–14.24)</td>
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<tr>
<td></td>
<td></td>
<td>&gt;82.8 mg&quot;b&quot;</td>
<td>3</td>
<td>22</td>
<td>0.50 (0.12–2.05)</td>
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<tr>
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<td>Ever smokers, 40-year lag</td>
<td></td>
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<td>&lt;6.4 mg&quot;b&quot;</td>
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<td>189</td>
<td>1.00</td>
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<td></td>
<td></td>
<td>6.4–82.8 mg&quot;b&quot;</td>
<td>6</td>
<td>8</td>
<td>1.06 (0.34–3.33)</td>
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<td></td>
<td>&gt;82.8 mg&quot;b&quot;</td>
<td>16</td>
<td>11</td>
<td>2.25 (0.97–5.20)</td>
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<tr>
<td>Drinking water</td>
<td>Bladder</td>
<td>Never smoked</td>
<td></td>
<td></td>
<td></td>
<td>Cordoba, Argentina [46]. Excluding proxy-well measurements</td>
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<tr>
<td></td>
<td></td>
<td>0–0.5 µg/l</td>
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<td>14</td>
<td>1.00</td>
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<td></td>
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<td>0.6–1.2 µg/l</td>
<td>5</td>
<td>13</td>
<td>2.15 (0.4–11)</td>
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<td>1.3–35 µg/l</td>
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<td>11</td>
<td>4.03 (0.9–18)</td>
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<tr>
<td></td>
<td></td>
<td>&gt;35 µg/l</td>
<td>6</td>
<td>12</td>
<td>2.27 (0.4–12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ever smoked</td>
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<td>0–0.5 µg/l</td>
<td>20</td>
<td>17</td>
<td>1.00</td>
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<td>0.6–1.2 µg/l</td>
<td>23</td>
<td>15</td>
<td>1.19 (0.5–3.1)</td>
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<td>1.3–35 µg/l</td>
<td>20</td>
<td>14</td>
<td>1.06 (0.4–2.8)</td>
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<tr>
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<td>&gt;35 µg/l</td>
<td>21</td>
<td>17</td>
<td>1.05 (0.4–2.8)</td>
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</tr>
</tbody>
</table>
and 15.69 (95% CI 7.38–31.02) for lung cancer [43]. There was evidence for synergism between smoking and arsenic (Table 2a).

A study in regions with low (0.04 µg/l), medium (120 µg/l) and high (178 µg/l in average) arsenic exposure in Argentina showed increasing trends for kidney and lung cancer mortality with increasing arsenic exposure for both men and women (Table 2a). [25]. The association between arsenic exposure and mortality from liver and skin cancers was not clear.

In spite of very modest exposure levels some evidence was found in a Finnish cohort study for an association between arsenic and bladder cancer [44]. The total arsenic concentrations in the wells of the reference cohort were low (median = 0.1 µg/l; maximum = 64 µg/l). Arsenic exposure was estimated as arsenic concentration in the well, daily dose, and cumulative dose of arsenic. Bladder cancer tended to be associated with arsenic concentration and daily dose during the third to ninth years prior to the cancer diagnosis; the risk ratios for arsenic concentration categories 0.1–0.5 and ≥0.5 µg/l relative to the category with <0.1 µg/l were 1.53 (95% CI 0.75–3.09) and 2.44 (CI 1.11–5.37), respectively.

The results of the cohort studies on environmental arsenic exposure are summarised in Table 2a.

3.1.2. Case-control studies

A Chilean study by Ferreccio et al. identified 152 lung cancer cases and 419 controls [24]. Logistic regression analysis revealed a clear trend in lung cancer odds ratios with increasing concentration of arsenic in drinking water (Table 2b). There was evidence of synergism between cigarette smoking and ingestion of arsenic in drinking water; the odds ratio for lung cancer was 32.0 (95% CI = 7.2–198.0) among smokers exposed to more than 200 µg/l of arsenic in drinking water (lifetime average) compared to non-smokers exposed to less than 50 µg/l.

Case-control studies on arsenic-induced bladder cancer risk in the USA [26,45] and in Argentina [46] showed no association with the exposure. However, among smokers, but not among non-smokers, positive trends in risk were found for exposures estimated for decade-long time periods, especially in the periods of 30–50 years prior to diagnosis (Table 2b).

In general, studies with relatively low doses (0.005–0.200 µg/l) of arsenic in drinking water as in the USA and Argentina showed no association with increased risk of cancer, with the exception of bladder cancer among smokers. This data suggest lower bladder cancer risk for arsenic than predicted from Taiwanese and Japanese studies but adds to evidence that the latency for arsenic-induced bladder cancers may be longer than previously thought [26,45,46].

3.2. Occupational studies

In contrast to the environmental studies mainly suggesting a linear dose–response relationship between arsenic exposure and SMR, at least with high doses, almost all studies among miners with quantitative data of lung cancer are consistent with a supralinear dose–response relationship [47]. Confounding by age, smoking or other workplace carcinogens does not appear to explain this curvilinearity.

3.2.1. Cohort studies

A study on the copper smelter cohort of Tacoma by Enterline et al. showed a strong correlation between the respiratory cancer rate and cumulative arsenic exposure (Table 3a) [48]. Here, when a dose–response relation was based on airborne concentrations of arsenic, it was clearly concave downward, but when based on urine concentrations, it appeared to be linear. The effect of duration of exposure appeared to depend on air arsenic concentration, being smallest in the lowest level of exposure and largest in the highest category.

An additional follow-up in the Tacoma cohort showed significant increases in the rates of large intestine and bone cancers, but a positive relation between arsenic exposure and increased cancer rates could only be shown in the case of kidney cancer. However, the number of cases was small (Table 3a) [49].

A Chinese cohort study by Qiao et al., including 8346 tin miners with a total of 243 lung cancer cases, emphasised the complex influence of several risk factors on the cancer rate. Radon and arsenic exposures were the predominant risk factors, but lung cancer risk was also associated with chronic bronchitis and silicosis, as well as a number measures of exposure to tobacco smoke, including early age of first use, duration, and cumulative exposure [13].

Cohort studies on occupational arsenic exposure are summarised in Table 3a.

3.2.2. Case-control studies

Taylor et al. started one of the first investigations of lung cancer rates among Yunnan tin miners in China exposed to the effects of mining and smelting and thus to arsenic [14]. The case-control study included 107 living tin miners having lung cancer and an equal number of age matched healthy controls from among tin miners. The cases and controls were interviewed
to obtain information on risk factors for lung cancer including detailed history of employment and tobacco use. Occupational history was combined with industrial hygiene data to estimate cumulative arsenic exposure. Similar methods were also used to estimate radon exposure for simultaneous evaluation. A clear dose–response relationship between arsenic exposure and lung cancer rate could be shown both among smokers and non-smokers (Table 3b). In contrast to many other studies, no synergistic effect between smoking and arsenic exposure could be observed, perhaps due to the fact that assessment of tobacco exposure was limited to water pipe use. Among arsenic-exposed individuals, cases had longer duration but lower average intensity of arsenic exposure than controls, indicating that duration of exposure may be more important than intensity in the aetiology of lung cancer.

Similarly, a dose–response relationship between arsenic exposure and lung cancer rate was found in a nested case-control study by Chen et al. in four additional Chinese tin mines (Table 3b) [12]. Interestingly, in these mines negligible amounts of radon were present. No excess of lung cancer was found among silicotic subjects although there was a high prevalence of silicosis.

Data representing a nested case-control study at the Swedish copper smelter in Rönnskär area with 107 lung
cancer cases and 214 controls showed that lung cancer risks were positively related to cumulative arsenic exposure (Table 3b) [50]. A negative confounding by smoking was suggested in the higher exposure categories. The interaction between arsenic and smoking for the risk of developing lung cancer was intermediate between additive and multiplicative and appeared less pronounced among heavy smokers.

### 3.3. Reasons for non-linearity of dose–response relationship

Arsenic is cleared by the body much faster after drinking water exposure as compared to inhalation exposures. In the latter case the arsenic-contaminated dust particles often remain insoluble for a long period of time. The local concentrations of inhaled arsenic in the lung can be as much as 17 times higher in the case of exposed miners compared to the unexposed [3]. However, once the arsenic is changed from insoluble forms to soluble forms, it will be rapidly methylated and cleared from the body. Deviations due to the different methylation rates with low and high arsenic concentrations appear to be of minor importance. A preferable explanation for the differences in the dose–response relationship kinetics between ingested and inhaled arsenic may have to do with the capacity of the lung as a target tissue to deal with the exposure. A mechanism by which a more efficient tracheobronchial clearance would be induced at high concentrations of arsenic could be a possible explanation for the supralinearity in the case of inhaled arsenic [47].

Another explanation may be the healthy worker survivor effect, suggesting that workers who are less healthy are most likely to leave work. This difference may attenuate the dose–response relation and has the
potential to alter the shape of the curve. However, Arrighi and Hertz-Picciotto concluded that this effect does not explain the non-linear shape found in the analysis unadjusted for the healthy worker survivor effect, since the adjusted curves were strikingly similar to the unadjusted curve across all the methods examined [51].

Furthermore, a dose-dependent synergism with smoking, described in more detail in the next section, being larger at low arsenic exposures, is a plausible explanation for the reduction in slope at high arsenic concentrations. There are some epidemiological data supporting this view which indicate that the interaction between arsenic and smoking for the risk of developing lung cancer is intermediate between additive and multiplicative and appears less pronounced among heavy smokers [50,52].

4. Confounders and synergisms

When estimating cancer risks in arsenic-exposed populations the influence of confounders, such as gender, coexisting carcinogenic elements in water, air or dust, smoking, nutrition, sun exposure, and genetic polymorphisms, has to be taken into account.

4.1. Gender

Many reports from regions with arsenic-contaminated drinking water indicate that non-malignant skin lesions occur more often in men than in women, although the urinary arsenic concentrations are on a similar level [53–56]. The incidence of arsenic-induced peripheral vascular diseases, cough, or skin cancer appears to be higher in the male population, whereas malignancies of kidney and lung are dominant in the female population [34]. A Danish study analysing the influence of gender and age on urinary arsenic showed that men had markedly higher urinary arsenic levels than women at 40 and 50 years, but this difference disappeared with increasing age [57]. The reasons for the gender-related differences are not known but both biochemical factors as well as confounding factors such as smoking, alcohol consumption, sun exposure, and occupations with heavy duty hand work may be possible explanations.

4.2. Radon

Radon and its progenies, naturally decaying products, are carcinogens commonly existing in nature in extremely low concentrations. In metal mines arsenic usually coexists with radon. The high correlation between arsenic and radon exposure makes assessing the individual effect of each exposure difficult.

Confounding by radon has been studied extensively in Chinese tin mines. A 40-year investigation of the Lao, Ma and Song mines showed that incidence of lung cancer decreased in parallel with a reduction in the arsenic concentration against a background in which the concentration of radon remained essentially unchanged. This suggested causality with the arsenic exposure [3].

Taylor et al. [14] estimated the relative increase in lung cancer risk in the Yunnan tin mine for increasing arsenic exposure in three different categories (low, medium and high) of radon exposure. At the lowest radon category the risk increased linearly with the increasing arsenic concentration, whereas risk estimates in the medium and high radon categories were highly variable.

4.3. Smoking

Most studies concerning the relationship between active smoking and environmental or occupational exposure to arsenic indicate that these two exposures act synergistically with a more than additive interaction on the rate of lung cancer (Tables 2b and 3b) [24,26,42,43,45,46,50].

The magnitude of synergism seems to vary according to the level of arsenic exposure, often being multiplicative with lower arsenic concentrations but less than multiplicative, though still synergistic, with higher arsenic concentrations [47]. This phenomenon has been described as negative confounding by smoking with high arsenic concentrations and has been observed in several studies [50,58].

The mechanism leading to synergism of lung cancer from arsenic and tobacco smoking is not clear. The two exposures may act on the same causal pathway, however, potentially at different points [52]. The putative mechanisms of arsenic carcinogenicity are described later in this review. Generally, cigarette smoke impedes tracheobronchial clearance, possibly prolonging the exposure of bronchial epithelium to arsenic-containing dust particles, thus increasing the dose to the target pulmonary tissue [52].

Simultaneous exposure to multiple agents such as arsenic, radon and smoking and their putative interaction complicate the analysis of the influence of the single factors. The two-stage clonal expansion model was used to analyse the lung cancer risk in the retrospective Yunnan cohort consisting of 12,011 males. Complete exposure records with initial surveying in
1976 and followed through 1988 were utilised [59]. According to this model, 21.4% of 842 lung cancer deaths were attributable to smoking, 19.7% to a combination of smoking and arsenic, 15.8% to arsenic alone, 11% to a combination of arsenic and radon, 8.7% to combination of arsenic, radon and smoking, 5.5% to radon alone, and 8.7% to background. The attributable risk for lung cancer was dominated by the influence of arsenic and smoking. Notably, only 14.2% of the risk was attributable to radon alone together with the background.

4.4. Nutritional factors

There are several reports available showing that undernourishment may exacerbate arsenic toxicity. Hsueh et al. reported that undernourishment, indexed by a high consumption of dried sweet potato as a staple food, was significantly associated with an increased prevalence of arsenic-induced skin cancer in the Taiwanese population [60]. This risk factor remained statistically significant in the multiple logistic regression analysis.

Studies among subjects in West Bengal who were below 80% of the standard body weight had a 1.6-fold increase in the prevalence of keratoses [55]. A nutritional study in this region showed that low intake of calcium, animal protein, folate, and fiber may increase susceptibility to arsenic-induced skin lesions [61]. However, arsenic-induced skin lesions in Chilean population were not dependent on the nutritional status [62]. The discrepancy may be due to the small number of individuals (44) in the Chilean study compared to the Taiwanese and West Bengalese data.

Diets low in selenium seem to influence the toxicity of arsenic. Selenium has been shown to increase the rate of arsenic metabolism by forming methylated arsenic compounds. Lower selenium intake in residents of the Lanyang Basin in Taiwan led to reduced arsenic methylation and higher retention of iAs in the body [8,63]. On the other hand, since selenium is able to form a complex with arsenic, it has been argued that ingested high levels of arsenic in drinking water will accelerate the excretion of selenium, lowering the concentration of this essential trace element in the body and increasing the toxicity of arsenic over time [64]. Data from animals studies indicate that antioxidants like zinc, folic acid and vitamins A, C and E together with selenium may protect against arsenic-induced oxidative injury and lipid peroxidation [65–67].

The effect of dietary intake of fruits and vegetables on the odds ratio of lung cancer among Yunnan tin miners was analysed [68]. Tin miners with reduced intake of yellow and light green vegetables as well as tomatoes had statistically significant increased adjusted odds ratios of lung cancer. High serum carotenoid levels were significantly associated with decreased lung cancer risk among non-alcohol drinkers but among alcohol drinkers high carotenoid levels increased lung cancer risk [69]. Low serum beta-carotene levels have been associated with increased incidence of arsenic-induced skin lesions [8,70].

4.5. Genetic susceptibility

Human sensitivity to the toxic effects of arsenic is likely to vary not only based on diet, health status or gender but also genetic differences, especially concerning genes involved in the biotransformation of arsenic. The influence of genetic susceptibility on the risk of arsenic-induced skin lesions was estimated in a case-control study in Bangladesh by determining the mutational statuses of the codon 751 of the DNA repair gene XPD [71] as well as codons 463 and 262 in the oxidative stress genes myeloperoxidase (MPO) and catalase (CAT) [72]. Carriers of the AA genotype of the XPD gene and the high-risk genotypes of MPO and CAT had an elevated risk of developing hyperkeratosis, as compared to the low-risk genotypes.

Genetic polymorphisms of glutathione-S-transferase (GST) M1 and T1 were studied among subjects from Lanyang Basin in Taiwan [73]. The polymorphisms of M1 and G1 were significantly associated with arsenic methylation status and the body retention of arsenic. There is evidence showing that methylation patterns aggregate in families; a family pedigree with high prevalence of arsenic-induced effects has been reported [74].

5. Biotransformation of arsenic

Arsenic taken up by the body is mainly in the inorganic form, either as arsenite [iAs(III)] or arsenate [iAs(V)] [35]. In anaerobic conditions iAs(III) is the predominant form. It is more toxic than iAs(V), probably due to its faster rate of cellular uptake [36,75–77]. Both iAs(III) and iAs(V) are actively transported to the cell, the former by aquaglycoporins, normally transporting water and glycerol [78], the latter by the phosphate transporter [79]. After entering the cell iAs(V) is rapidly reduced to iAs(III).

In general, trivalent arsenicals are more toxic than the corresponding pentavalent forms because of greater
cellular uptake, much greater ability to bind to sulfhydryl sites of proteins [80] and the greater tendency to form free radicals and thus oxidative stress [81].

The most common arsenic compounds are shown in Fig. 1.

### 5.1. Methylation of arsenic

In humans, inorganic arsenic is metabolised by methylation followed by excretion in the urine of iAs as well as mono- and dimethylated forms. Methylated species are excreted more rapidly than unmethylated species [82]. The reduction of iAs(V) to iAs(III) is a prerequisite for the methylation to occur. Reduction and oxidation between iAs(III) and iAs(V) forms takes place in the plasma, whereas methylation reactions occur primarily in the liver and to much lesser extent in the kidney and lung [35]. Isolated rat liver mitochondria have been shown to rapidly reduce As(V) to As(III) [83].

iAs(III), as well as trivalent methyl compounds of arsenic, can be oxidised to their pentavalent counter-parts, probably via hydrogen peroxide [84]. Hydrogen peroxide is formed in the cell via several pathways such as xanthine oxidase reaction [85], NADH–NADPH oxidase system [86], or aldehyde oxidase system [87]. It is inactivated by catalase and peroxidases such as selenium-containing glutathione peroxidase [88].

Zakharyan and Aposhian showed that methylvitamin B12 produced substantial amounts of methylated arsenic compounds in vitro in the absence of enzymes. This non-enzymatic methylation of iAs was increased by the presence of sodium selenite [89]. Contrarily, selenium inhibited methylation of iAs in primary rat and human hepatocytes [90,91]. Also antimony has been shown to inhibit arsenic methylation in rat liver [92,93] and to suppress arsenic-induced sister chromatid exchanges and micronuclei formation in V79 cells [94,95].

Similarly, glutathione (GSH) and other thiols are able to reduce iAs(V) non-enzymatically [75,96,97]. This reduction step results in a formation of As(III)(GS)3 complex that readily donates As(III) to dithiol groups of proteins [98]. The hydrolysis of an As(III)(GS)3 complex to As(III) and monomethylarsonous acid [MMA(III)] can be prevented by high intracellular concentrations of GSH [99].

Furthermore, two mammalian enzymes are able to reduce iAs(V). Purine nucleoside phosphorylase (PNP) is able to reduce iAs(V), if its substrate, a purine nucleoside, and an appropriate dithiol are present [100–102]. A novel study, however, suggests that PNP does not appear to play a significant role in iAs(V) reduction in human erythrocytes and in rats in vivo [103]. iAs(V) can also be reduced by a second enzyme, monomethylarsonic acid [MMA(V)] reductase, identical to glutathione-S-transferase omega class 1-1 (GSTM1-1) [104]. It is the rate-limiting enzyme in the iAs metabolism with an absolute requirement for GSH as a reductant [105] and seems to be induced by arsenite [106].

iAs(III) is methylated by enzymatic transfer of the methyl group from S-adenosylmethionine (SAM) to iAs(III) to form MMA(V). A 60 kDa protein, purified from rabbit liver cytosol [105,107] and a 41 kDa enzyme, purified from rat liver cytosol [108], both having methyltransferase activity, have been isolated. The 41 kDa enzyme is identical with arsenic(III)-methyltransferase [As(III)MT], a putative methyltransferase expressed in human tissues. As(III)MT seems to be able to catalyse all steps in a pathway leading from iAs(III) to dimethylarsinous acid [DMA(III)], thus combining the functions of a methyltransferase and a reductase in a single protein [109]. As(III)MT utilises thioredoxin and NADPH as a reductant.
MMA(V) can also be reduced by (GSTO1-1) to form MMA(III) [104]. In a second methylation reaction MMA(III) forms dimethylarsinic acid [DMA(V)]. Subsequently, some DMA(V) is reduced to DMA(III) either by As(III)MT, GSTO1-1 or non-enzymatic means. A schematic presentation of the methylation pathway of arsenic is shown in Fig. 2.

Usually mammals excrete arsenic with two or fewer methyl groups on it. However, the arsenic methylation or reduction pathways are not exclusively terminated at the level of dimethylation. Arsenic compounds containing three or four methyl groups have been isolated in rats [110]. The regulation of this pathway seems to be affected by the intracellular glutathione concentration [111].

5.2. Toxicity of the methyl compounds

Historically, the methylation of arsenic was considered to be a detoxification process [112,113]. Indeed, the pentavalent arsenicals MMA(V) and DMA(V) are less toxic and excreted with a higher rate than iAs(V) or iAs(III) [82]. However, recent studies show that the trivalent methylated forms MMA(III) and DMA(III) often exceed iAs(III) in cytotoxicity and genotoxicity. As noted by Kligerman et al., methylation of the trivalent forms increases the genotoxicity and cytotoxicity, whereas methylation of the pentavalent forms decreases them [114]. The biological effects of the methyl forms of arsenic have recently been reviewed [115].

Nesnow et al. showed that the trivalent forms MMA(III) and DMA(III) are more clastogenic than their pentavalent counterparts and are able to produce reactive oxygen species (ROS) [116]. Interestingly, the trivalent methylated forms of arsenic are able to cause chromosomal aberrations that are consistently found in people exposed to arsenic and in arsenic-associated tumours.

In Chang human hepatocytes, MMA(III) was shown to be more cytotoxic [117] and a more potent inhibitor of pyruvate dehydrogenase [118] than iAs(III) or the pentavalent arsenicals [80]. MMA(III) has also shown to be a potent inhibitor of thioredoxin reductase [119], yeast glutathione reductase [120] and *Escherichia coli* zinc finger protein Fpg, involved in nucleotide excision repair (NER) and base excision repair (BER) [121]. The ability of MMA(III) and DMA(III), but not of MMA(V) or DMA(V), to bind to cytosolic proteins may be responsible for some of the toxic effects after arsenic exposure [122].

MMA(III) and DMA(III) have shown to be more potent in generating DNA strand breaks in human lymphocytes than iAs(III) [123]. DMA(III) has been shown to cause plasmid DNA damage in an iron-dependent manner [124]. Furthermore, MMA(III) and DMA(III) induce cytotoxic and genotoxic effects such as micronucleus formation, chromosome aberrations and sister chromatide exchange to a greater extent than MMA(V) or DMA(V) [125] and effect cell signalling pathways [126]. Taken together, MMA(III) and DMA(III) are highly potent in generating both cytotoxic and genotoxic damage and a likely basis for the carcinogenicity of arsenic.

MMA(V) and DMA(V), although being less cytotoxic, mutagenic, and clastogenic than the corresponding methylated trivalent arsenicals, have been shown to affect DNA repair of lymphocytes in the Comet assay [127] and induce DNA damage via ROS [128–130].

5.3. Genotypic variation of methylation status

Genotypic variation may affect the metabolism of arsenic or modify susceptibility to arsenic-induced disease. The recent development of separational and analytical techniques has enabled the speciation of arsenic compounds according to oxidation states in biological samples [37,131,132]. There are marked differences in the metabolism of arsenic between mammalian species, population groups and individuals. Normally, humans exposed to arsenic excrete 10–30% total iAs, 10–20% total MMA and 60–80% total DMA [133]. Humans differ from other species in that they excrete much more total MMA than other species [112]. There are indications that subjects with low total MMA in urine have faster elimination of ingested arsenic, compared to those with more total MMA in urine [112].

Two cases of single nucleotide polymorphisms in the gene encoding GSTO1-1, being the rate-limiting enzyme for As(V) to As(III) reduction, were recently characterised [134]. The result of this polymorphism appeared to be that 76 and 64%, respectively, of the total urinary arsenic were in the unmethylated form, a totally different pattern as seen in the normal population.

![Fig. 2. The enzymatic methylation pathway of arsenic.](image-url)
The relationship between genotypes for glutathione-S-transferases (GST) M1 and T1 and arsenic methylation capacity and body retention was studied in a Taiwanese population exposed to arsenic [73]. Subjects having null genotype of GST M1 had an increased percentage of iAs in urine, while those with null genotype of GST T1 had an elevated percentage of DMA in urine. Among subjects having null phenotypes, arsenic contents in hair and toenail were negatively associated with MMA percentage and positively associated with DMA percentage. This seems to suggest that GST M1 may facilitate the methylation and that the methylation capability indexed by relative percentages of various arsenic species in urine is associated with the body retention of arsenic in humans.

Primary human hepatocytes obtained from different donors showed a great interindividual variation in the arsenic metabolism [135,136]. DMAs were the major methylated metabolites in cultures exposed to low concentrations of iAs, whereas at higher concentrations, MMAs were always predominant. The methylation rates for iAs were not strictly correlated with variations in the mutational status of As(III)MT, suggesting that other cellular factors such as GSTs are contributing to the differences.

The fact that methylation patterns aggregate in families suggests a strong genotypic component in the interindividual variation of metabolism of arsenic [74]. Other factors associated with differences in individual arsenic methylation patterns include age and gender [137,138], nutrition [60], ethnicity [139,140], pregnancy [141], and smoking and alcohol use [140].

6. Mode of action of arsenic

6.1. Oxidative stress

Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radical can directly or indirectly damage DNA or proteins [142]. Many transition metals such as chromium, nickel, cobalt, and iron, existing in multiple oxidation states, are able to catalyse formation of ROS by donating or accepting single electrons in Fenton-type or Haber–Weiss-type reactions [143,144]. The most aggressive form of ROS and mainly responsible for DNA damage is currently considered to be the hydroxyl radical.

The fact that the level of ferritin is increased in arsenic-exposed human cells suggests the involvement of iron in the arsenic-induced formation of ROS [145,146]. Both DMA(V) and DMA(III) have been shown to release iron from ferritin. The presence of ascorbic acid increased the iron release and DNA damage by DMA(III) in a synergistic way [124].

The induction of heme oxygenase (HO), an indicator of oxidative stress [147,148], has been shown to occur in several animal and human cell lines exposed to arsenic, such as human fibroblasts, human keratinocytes, human embryonic kidney cells and Chinese hamster ovary (CHO) cells as well as in rat liver and kidney [145,146,149–152]. HO cleaves the heme tetrapyrrole ring in hemoproteins, resulting in the production of carbon monoxide, biliverdin, and free iron, and shifting cellular redox potential towards reduction [35]. HO induction may thus directly cause formation of ROS. It may also be involved in the mechanism of arsenic resistance in cells [153]. In arsenic-exposed mice or rats, the induction of HO showed a dose-dependent response and therefore may be used as a biological biomarker for iAs exposure [154,155].

Dimethylarsine, a trivalent gaseous arsenic form, is a minor in vivo metabolite of DMA(V) produced by a process of reduction [156]. It can react with molecular oxygen forming (CH$_3$)$_2$As$^+$ and (CH$_3$)$_2$AsOO$^+$ radicals and a superoxide anion. Subsequently, hydroxyl radicals may be produced via cellular iron and other transition metals [157]. Dimethylarsine, as well as trimethylarsine, are considered to be highly genotoxic compounds.

Direct evidence for induction of oxyradicals by arsenic was supplied by confocal scanning microscopy, showing a dose-dependent increase of up to three-fold in intracellular ROS production within 5 min after arsenic treatment in human–hamster hybrid cells [158]. Concurrent treatment with the radical scavenger dimethylsulfoxide (DMSO) reduced the induction to control level.

A possible mechanism for accumulation of intracellular ROS after arsenic exposure is the activation of flavoprotein-dependent enzymes such as NAD(P)H oxidase [159,160]. Superoxide production and DNA strand breaks in arsenic-treated human vascular smooth muscle cells were suppressed by transfecting antisense oligonucleotides of p22phox, an essential component of NAD(P)H oxidase. Treatment with arsenite also increased the mRNA level of p22phox [160]. These results suggest that arsenite activates NAD(P)H oxidase to produce superoxide which then causes oxidative DNA damage.

Data emerging from animal studies emphasise the role of DMA(III) in generating oxidative stress. Elevated levels of 8-hydroxy-2$'$-deoxyguanosine (8-OHdG) derivatives, characteristic DNA adducts of
oxidative injury, were found in the lung, skin, liver, bladder and kidney of mice and rats after oral ingestion of DMA [128,161,162]. These organs are associated with the arsenic-induced carcinogenesis. The induction of 8-OHdG could be reduced in vitro and in vivo by using antioxidants [163,164].

The endogenous glutathione system plays a key regulatory role in redox signalling, potentially protecting cells from the damaging effects of arsenic [165]. In mammalian cells, catalase and glutathione peroxidase are main enzymes regulating the levels of ROS. The addition of catalase or glutathione peroxidase to cultures reduced the arsenic-induced micronuclei in xrs-5 cells, an X-ray hypersensitive CHO cell line, [166] and in diploid human fibroblasts [167]. Catalase modulated the arsenic-induced DNA damage in CHO and human leukemia cells [168].

Cellular levels of GSH play an important role, in addition to the reduction of As(V) to As(III) as mentioned before, in resistance to arsenic toxicity and proto-oncogene activation [169]. Human cell lines treated with iAs(III) showed increased levels of GSH related enzymes like glutathione reductase (GR) and GSTO1-1 [170,171].

A large amount of indirect data support the suggestion that arsenicals increase levels of ROS in the cell. Protection against arsenic genotoxicity is accomplished by GSH elevation [145,163], antioxidants such as vitamin E, melatonin [116], sodium azide [145], and dimethyl sulfoxide (DMSO) [145], sugars such as pyruvate and D-mannitol [160], or antioxidative enzymes such as catalase (CAT) [145,160,166,168], glutathione peroxidase [166], superoxide dismutase [163] and myeloperoxidase (MPO) [72].

The presence of arsenic-induced oxidative damage is also evident in epidemiological studies. The frequency of 8-OHdG positive cases was significantly higher in arsenic-related skin neoplasms than in arsenic-unrelated Bowen’s disease in a Japanese population [172]. In a study carried out in Inner Mongolia, the mean serum level of lipid peroxides (LPO) was significantly higher among the high-arsenic-exposed compared with the low-exposed group [173]. Elevated serum LPO concentrations were correlated with blood levels of iAs and its methylated metabolites. In addition, they showed an inverse correlation with non-protein sulphydryl (NPSH) levels in whole blood. Blood NPSH levels were inversely correlated with the concentrations of iAs and its methylated metabolites in blood and with the ratio of MMA to iAs.

Genetic susceptibility, determined as single nucleotide exchanges in the genes coding for MPO and CAT, was shown to be associated with the risk of arsenic-induced hyperkeratotic skin lesions, presumably precursors of skin cancer, in a case-control study in Bangladesh. Carriers of the susceptible MPO and CAT genotypes were at elevated risk of hyperkeratosis after adjustment for arsenic exposure [72].

A recent review by Hughes and Kitchin describes in detail the role of arsenic-induced oxidative stress in carcinogenesis [81].

6.2. Cellular signalling

Important cellular events such as proliferation, differentiation, and apoptosis are co-ordinated and mediated by signal transduction pathways. Recent data suggest that arsenic perturbs these pathways. The impact of arsenic on cellular signalling has been reviewed recently in detail [144,174,175]. Therefore, it is sufficient to briefly describe the influence of arsenic on the main signal transduction pathways such as the tyrosine phosphorylation system, mitogen-activated protein kinases (MAPKs) and transcription factor families NFκB and AP-1.

The tyrosine phosphorylation system is mediated by a complicated interplay of protein tyrosine kinases and protein tyrosine phosphatases [176]. Protein tyrosine kinases can be divided in two groups, namely receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). Most RTKs are protein growth factor receptors. NRTKs, including Src family members [177] are under cellular control of RTKs and can be activated either by binding to the activated RTKs or by dephosphorylation by protein tyrosine phosphatases.

A constitutive increase in the level of tyrosine phosphorylation is associated with aberrant cell signalling, uncontrolled cell growth, and the development of cancer such as leukemia, lymphoma, cancer of the prostate, lung, kidney, bladder and colon [178–182]. Arsenic has been shown to elevate the level of total cellular tyrosine phosphorylation in a dose-dependent manner [183], the target of the phosphorylation being the epidermal growth factor receptor (EGFR) [184–186].

MAPKs are a family of protein serine-threonine kinases. At present there are four known families of MAPK proteins: the extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), p38, and BMAPK-1 [187–190]. Recent studies indicate that arsenic can induce ERKs, JNKs and p38 kinases in concentrations varying from 0.1 to 500 μM in a variety of human cell lines [175]. However, the arsenic-dependent induction of different MAPK families is highly dependent on the concentration, the length of
exposure, and cell-type and may mediate opposite effects in terms of the biological pathway chosen. The induction of ERKs may lead to uncontrolled cell proliferation and transformation, whereas the induction of JNKs is associated with growth arrest and apoptosis [174,175].

The arsenic-induced stimulation of EGFR and MAPK pathways may be coupled processes. Wu et al. demonstrated in human airway epithelial cells that arsenic-induced tyrosine phosphorylation of EGFR leads to the activation of MAPKs via the Ras protein mediated signal transduction [185,191].

It has been shown in several cell lines and in arsenic-exposed mice that arsenic induces Src, in addition to EGFR and MAPKs. Src is a member of the NTK family [192–194]. The data demonstrate that the activation of EGFR and MAPKs is dependent on Src, the mechanism still being unknown. Furthermore, the arsenic-stimulated activation of MAPKs by Src probably includes two parallel pathways, one being dependent on EGFR and the other one not [193].

The induction of MAPKs subsequently influence the activation of transcriptional pathways determined by transcription factors AP-1 and NFκB, playing a key role in cellular transformation and apoptosis. AP-1 consists of homo- or heterodimers of the gene products of early response gene families c-fos and c-jun [195]. Several studies confirm the induction of AP-1 transactivation and DNA binding, as well as the enhanced expression c-jun and c-fos genes and gene products by arsenic [175,196–198].

NFκB is a general term for heterodimeric transcription factors of the rel family, typically composed of p50 and p65 (RelA). It may be activated by a variety of stimuli such as ROS, cytokines, and MAPK signalling, especially p38 induction [199–201]. The mechanism of NFκB activation includes the phosphorylation of its inhibitor IκB, a subsequent degradation of IκB by the 26S proteasome, and the translocation of the activated NFκB to the nucleus. NFκB activation is associated with the turning-on of numerous genes involved in the inhibition of apoptosis, induction of proliferation and initiation or acceleration of tumourigenesis [202–204].

Arsenic has a dual role on the activity of NFκB, from having no effect to inhibition or activation depending on treatment duration, concentration and cell-type. Generally, low and non-cytotoxic concentrations of arsenic (1–10 μM) usually activate NFκB, while higher concentrations (>10 μM) inhibit the transcription factor [175,205]. Generation of ROS is a prerequisite for the arsenic-induced activation of NFκB in endothelial cells [206]. Several mechanisms for the inhibition of NFκB by arsenic have been suggested, including the repression of an upstream kinase of IκB, and inhibition of the phosphorylation and degradation of IκB, as well as translocation of NFκB to nucleus and DNA binding of NFκB [205,207–212].

6.3. Apoptosis

ROS formation and arsenic-induced apoptosis seem to be coupled. The activation of NAD(P)H oxidase and the subsequent increase in the intracellular peroxide levels may play an essential role in this pathway [213–215]. Arsenic-induced apoptosis has been intensively studied with arsenic trioxide, As2O3, a cyotoxic drug used for patients with acute promyelocytic leukemia (APL) [23]. Arsenite, in contrast to arsenate, is able to induce apoptosis [213]. As2O3 is able to generate arsenite in aqueous solutions.

On the one hand, mitochondria are an important organelle involved in arsenic-induced apoptosis [216,217]. Mitochondria are both a source and a target of excessive ROS generation [218]. Excess ROS increases the mitochondrial membrane permeability and disrupts the respiratory chain, resulting in enhanced ROS production. The instability of the mitochondrial membrane leads to the release of cytochrome c, which binds to apoptotic activating factor-1 (APAF-1) and activates caspase-9 and subsequently caspase-3. Caspase-3 is responsible for the cleavage of poly(ADP-ribose) polymerase (PARP) and induction of apoptotic cell death [219]. Mitochondrial membrane permeability is regulated by the bcl-2 family of proto-oncogenes, being either anti-apoptotic (bcl-2) or pro-apoptotic (Bad, Bax). Arsenic has been shown to increase the activities of caspases-3, -8 and -9, to suppress bcl-2 expression, to activate MAPKs and release cytochrome c, leading subsequently to apoptosis [208,216,217,220–223].

On the other hand, in human keratinocytes, arsenic was shown to induce receptor-mediated apoptosis by the Fas/Fas ligand pathway not depending on the mitochondrial status [224]. Thus, arsenic may able to trigger both mitochondria-mediated and receptor-mediated apoptosis. Both mechanisms of apoptosis require caspase-3 activation [225]. Recently, the activation of JNK-dependent pathway was described as a link between mitochondria-mediated and receptor-mediated apoptosis [226].

The data concerning the role of p53 in the arsenic-induced apoptosis are contradictory. Previous studies showed that the p53 activation was not necessary
The eukaryotic cell cycle, composed of four phases M, G₁, S, and G₂, is controlled by regulatory proteins called cyclins and cyclin-dependent kinases (CDKs). The control of the cell cycle is achieved through checkpoints in the G₁, G₂, and M phases that prevent late events from being initiated until earlier events have been completed. DNA damage results in an accumulation of p53 protein [235] and arrests the cell cycle at the check points, allowing time for DNA repair [236], or, in heavily damaged cells, the onset of apoptosis [237]. The G₁ check point control occurs via p53-regulated signal pathways, involving downstream gene products of p21, cyclin E/CDK2 and cyclin D/CDK4/6. Serine-threonine kinase ATM-regulated signal pathways, involving GADD45, CDC25 and CDC2/cyclin B, are responsible for the G₂ check point control, whereas microtubules are responsible for the M phase check point control [205,238].

Arsenic has been observed to damage DNA and induce the cell arrest at G₁ or at G₂–M phase [239]. The exact mechanism is not known, but some evidence indicates that arsenic is able to reduce steady-state levels of CDK/cyclin complexes as well as cyclins in some human cancer cell lines [231,240]. Furthermore, data are accruing showing that p53 is involved in arsenic-induced G₁ and G₂–M phase arrest [229,241]. However, p53 expression in response to arsenic is complex and probably dose-, cell-, and tissue-type specific. Arsenic can both induce, inhibit or have no influence on the p53 expression and its regulation of down-stream genes like p21 [228,242–244]. Interestingly, arsenite has been shown to greatly suppress the radiation-induced, p53-dependent increase of P21 protein in human fibroblasts [244]. Blocking P21 expression after DNA damage allowed cell cycle progression and DNA replication of the damaged template.

Arsenite is known to inhibit more than 200 enzymes [21]. In addition to a moderate affinity for single sulfhydryl groups, trivalent arsenicals can effectively bind to two proximate sulfhydryl groups of proteins, leading to conformational alteration of protein structure and inhibition of enzyme activity. However, no enzymes of the DNA repair system have been shown to be inhibited by arsenic, with the exception of human poly(ADP-ribose)polymerase (PARP) [245]. Thus, it is unlikely that the effect of arsenic on DNA repair occurs via direct interaction with DNA repair enzymes, but rather indirectly via arsenic-induced gene modulation [246,247]. Recent human data suggest that exposure to low levels of arsenic in drinking water indirectly inhibits the nucleotide excision repair (NER) system, including enzymes like ERCC1, XPF, and XPB [248,249].

6.4. Cell cycle control and DNA repair

Maintenance of the proper methylation pattern at the CpG sites of the promoter region is critical to the regulation of gene transcription. The fact that arsenic metabolism and DNA methylation share a common cofactor, 5'-adenosylmethionine (SAM), has led to the hypothesis postulating a perturbation of normal DNA methylation by arsenic [250]. This type of perturbation, leading either to hyper- or hypomethylation, has been shown in vitro and in vivo. In human adenocarcinoma cells the presence of arsenite increased the cytosine methylation of the p53 promoter [251,252]. Both hyper- and hypomethylation of different genes were found in arsenite-treated human kidney UOK cells [252]. Widespread hypomethylation resulted in up-regulation of several genes involved in cell cycle regulation including c-myc, c-H-ras, c-ras, c-met, and cyclin D1, cyclin D2 and ERK [253,254]. Chen et al. observed DNA hypomethylation in male mice liver samples exposed to arsenic concentration of 45 ppm in the drinking water for 48 weeks [255].

Gene amplification can be considered as a sign of genomic instability. Arsenite has been shown to induce gene amplification at the dhfr locus of both human and rodent cells [256,257]. Arsenite-induced gene amplification may be caused by p53-dependent abnormalities of the cellular checkpoint pathways [35,258]. Extremely low concentrations of arsenite have been shown to induce delayed mutagenesis after more than 20 generations of culture, as well as gene amplification, at the hprt locus in human osteosarcoma cells [256].

Genomic instability has been associated with inhibition of the telomerase function. In human leukemia cells, arsenic trioxide induces chromosomal end fusions that correlate with the inhibition of telomerase activity [259]. In HaCaT and HL-60 cells in vitro low concentrations (0.1–1 μM in HaCaT and 0.1–0.5 μM in...
HL-60) of arsenite increased telomerase activity, maintained or elongated telomere length, and promoted cellular proliferation. High concentrations (>1–40 μM) of arsenite decreased telomerase activity, telomere length and induced apoptosis [260]. ROS may play an important role in the shortening of telomeres and apoptosis induced by arsenic [260]. In normal human somatic cells, cell senescence is believed to occur via progressive telomere shortening [261]. Loss of telomeres can lead to genomic instability and tumourigenesis [262,263].

6.6. Chromosomal abnormalities

There are many positive reports of arsenic-induced chromosomal aberrations including micronuclei formation, deletions, sister chromatide exchanges and aneuploidy, found in both in vitro and animal studies [264–266]. Furthermore, epidemiological studies demonstrate that arsenic exposure results in increased micronuclei incidence and sister chromatide exchanges in bladder cells [267–269]. It may be that arsenic-induced chromosomal abnormalities are not immediate effects of its toxicity but rather a consequence of secondary effects such as oxidative stress or perturbed cellular signalling [270].

7. Arsenic carcinogenesis

In carcinogenesis, the terms initiation and promotion are operationally defined. In multi-stage carcinogenesis, mutations are important in initiation and progression during which malignancy and metastatic potential develops in a cell. In contrast, cellular proliferation is an important driving force in promotion of carcinogenesis.

It has been clearly shown that exposure to low concentrations of arsenic increases proliferation in human [77,271–273] and other mammalian cells [192,274]. This enhanced proliferation is probably mediated by multiple biological mechanisms, some of which were mentioned in the previous section. However, the inorganic forms of arsenic, iAs(III) and iAs(V), show little or no mutagenic potential in bacterial or animal models [275]. Only in combination with other DNA-damaging agents such as UV light [276], ionising radiation [277], alkylating agents [278] or DNA-crosslinking agents [279] the genotoxic effects are enhanced already at low non-toxic arsenic concentrations. Therefore, previously arsenic was not considered to be complete carcinogen but rather have a promoting role in the development of cancer.

There is increasing evidence showing that low concentrations of the trivalent forms MMA(III) and DMA(III) are highly cytotoxic and genotoxic in several cell lines such as human hepatocytes and human and rat bladder, skin, and lung cells [36,77,116,117,280]. There are strong indications that oxidative stress is the causative factor in the formation of MMA(III) and DMA(III). Animal models of DMA-induced promotion of carcinogenesis have been reported for all five target organs of arsenic-induced carcinogenesis, namely skin, lung, bladder, kidney and liver [281–285]. In rat bladder DMA has been shown to be not only a promoter but a complete carcinogen [286]. The role of DMA in tumourigenesis using animal models is reviewed by Wanibuchi et al. [319].

However important the role of methylated arsenic compounds in tumourigenesis may be, we must bear in mind that the inorganic arsenic compounds, especially arsenite, are extremely cytotoxic and clastogenic as well. Furthermore, there seems to be no apparent positive relationship between the cell’s methylation capacity and the arsenite-induced cytotoxicity [287–289]. In studies with human bladder epithelial cell line (UROtsa), sodium arsenite was a more effective inducer of cell growth and AP-1 activation than the methylated forms [198]. Both UROtsa cells and keratinocytes are extremely poor methylators of arsenic when compared to other cell types such as hepatocytes or HeLa cells. These findings have lead to the hypothesis that tissues failing to metabolise arsenic are the most susceptible targets [198].

7.1. Lung cancer

There is a clear correlation between the rate of human lung cancer and arsenic exposure, either via ingestion or inhalation [1,17,18,24,25,49,50,290]. In a recently proposed mechanism, trivalent methylated forms of arsenic, especially DMA(III), are able to produce dimethyl arsine gas, a powerful producer of free radicals [157,249]. The lung may function as a target tissue for arsenic carcinogenesis due to the fact that dimethylarsine is excreted via lungs [156]. Secondly, high partial pressures of molecular oxygen found in the lung may be relevant for the development of cancer, if the oxidative stress theory is correct [157,291,292].

7.1.1. Ingestion of arsenic

The association between lung cancer and the concentration of arsenic in drinking water is well established (Tables 2a, 2b, 3a and 3b). However, there
seems to be some confounding by gender, women being more at risk [25].

The distribution of pathological cell types of lung cancer in the regions with arsenic-contaminated drinking water was compared to other areas in Taiwan [293]. To control for gender and age, data on men and women was analysed separately and patients were divided into four age groups. A total of 37,290 lung cancer patients, including 26,850 men and 10,440 women, were diagnosed. Patients from the endemic area had higher proportions of squamous cell and small cell carcinomas, but a lower proportion of adenocarcinomas. These findings were similar across all age groups showing that squamous cell and small cell carcinomas appeared to be related to arsenic ingestion [293].

7.1.2. Inhalation of arsenic

In mines arsenic is inhaled as dust particles. The iAs in ore mines is mainly composed of arsenopyrite (FeAsS) having a low solubility. Large amounts of arsenic particles have been found in the lungs of miners having lung cancer [3]. These particles are slowly oxidised to soluble compounds like arsenite and arsenate.

There are some indications showing that in mines the duration of exposure may be more important than the intensity with respect to the aetiology of cancer [14]. This is in agreement with the hypothesis that arsenic-containing dust particles act partly by mechanical irritation, followed by inflammation of the lung tissue. The toxic effects of arsenic are time-dependent due to the low solubility of the arsenic-containing minerals. Both mechanical irritation, as in the case of quartz dust, and arsenic’s toxicity are important factors in the development of arsenic-induced human lung cancer in mining.

In copper smelters, the airborne arsenic is not bound to dust particles. The major arsenic compound in smelters is arsenic trioxide, although there is also evidence of arsenic sulfides being present [294]. Several epidemiological studies among copper smelter workers in Sweden, Montana, and Tacoma, Washington show a clear association between the rate of lung cancer and the concentration of airborne arsenic [17,18,50,295,296]. Contrary to the studies on lung cancer-associated arsenic ingestion, some data emerging from studies in mines or smelters suggest a higher proportion of adenocarcinoma and a lower proportion of squamous cell carcinoma [297–300]. However, diagnosis of 174 lung cancer cases in Chinese Yunnan tin mines based on either cytology or histology showed 75% squamous cell and only 10% adenocarcinoma cases [13].

7.2. Skin cancer

Hyperkeratosis in the palms of the hands and soles of the feet and pigmentation or hypopigmentation of the trunk have been used as diagnostic criteria for defining arsenic exposure from drinking water. Arsenic-induced skin lesions are persistent and some of them progress to skin ulceration and skin cancer [19].

A study on cell-type specificity of arsenic-induced skin cancers in Taiwan showed that squamous and basal cell carcinoma appeared to be associated with ingestion of arsenic but such an association was not observed for malignant melanoma [301]. Arsenic-related squamous cell carcinomas of the skin can develop either de novo or progress from Bowen’s disease lesions, whereas arsenic-related basal cell carcinomas develop usually in non-sun-exposed areas and are multiple [302].

It has been estimated that the p53 gene is mutated in more than 50% of all human cancers [144]. The role of p53 in arsenic-induced skin cancer is still unclear. Overexpression of p53 was found in 44% of squamous cell carcinomas but only in 14% of basal cell carcinomas in Taiwan [303]. Accumulation of p53 protein was found in 78% of the pre-malignant skin lesions of patients treated with arsenic-containing medications but mutations of the p53 gene were found only in 30% of the cases; however, the number of cases was small (8 patients with 29 lesions) [302]. In an Australian study, arsenic-induced basal cell carcinomas were found to overexpress p53 less frequently than sporadic basal cell carcinomas [304]. This result is consistent with the hypothesis that the p53 gene is down-regulated by methylation in arsenic-related basal cell carcinoma, particularly in cases from less sun-exposed sites [251].

The role of ultraviolet radiation (UVR) from sunlight in the arsenic-induced skin cancer is controversial. Experiments with hairless mice showed that tumours, mostly squamous cell carcinomas, appearing after the combined exposure to UVR and drinking-water arsenite appeared earlier and were much larger and more invasive than in mice exposed to UVR alone [305]. Arsenite alone induced no tumours in mice. However, in humans, most arsenic-induced skin tumours appear on unexposed surfaces like palms and soles, suggesting that UVR may not be needed for arsenic carcinogenesis.

Whether arsenic needs an additional carcinogenic factor to induce skin cancer remains to be elucidated. The individually varying arsenic methylation capacity, measured as an elevated level of MMA in urine, was
shown to be correlated with an increased risk of skin cancer [70]. Skin cancer cases also had a significantly lower serum level of beta-carotene than the controls. In another study individuals with a higher percentage of MMA in urine (>15.5%) had an odds ratio of developing skin disorders of 5.5 (95% CI, 1.22–24.81) compared to those having a lower percentage of MMA [306].

8-Hydroxy-2'-deoxyguanosine is one of the major ROS-induced DNA base modifications and widely accepted as a sensitive marker of oxidative DNA damage. The frequency of 8-hydroxy-2'-deoxyguanosine positive cases was significantly higher in arsenic-related skin neoplasms (22 of 28 cases) than in arsenic-unrelated Bowen’s disease (one of 11 cases), suggesting that oxidative damage is involved in the development of arsenic-induced skin cancer [172]. In vitro, the involvement of ROS and the subsequent activation of the transcription factor Nfr2 has also been shown in cultured keratinocytes [307].

7.3. Bladder cancer

There is accruing evidence showing that the estimations of arsenic-related risk of bladder cancer based on high dose studies from Taiwan require adjusting for the low-level exposure. On the one hand, there seems to be no elevated risk with arsenic concentrations below 200 μg/day, except for smokers [45]. In spite of some methodological flaws in their data analysis [308], a big American study seems to support this view [309]. However, the latency for arsenic-induced bladder cancer may be longer than 20–30 years as previously thought [46]. On the other hand, in spite of very low exposure levels (0.5 μg/l), a Finnish study found some association between arsenic and bladder cancer risk and provided evidence for synergistic effects of smoking and nutritional factors with arsenic [44].

A study on cell-type specificity of arsenic-induced bladder cancer in Taiwan showed that the arsenic concentration correlated with the development of transitional cell carcinomas of the bladder, kidney, and ureter cancers as well as adenocarcinomas of the bladder in males, but not with squamous cell carcinomas of the bladder or renal cell carcinomas or nephroblastomas in the kidney [310].

The role of p53 in the development of arsenic-induced bladder cancer, as in the case of other arsenic-induced cancer types remains unclear. In a Taiwanese study bladder tumours from people chronically exposed to arsenic showed mutations in the p53 gene at codon 175 and transitions at points 9 and 10 [311]. However, in a larger South American study arsenic exposure was not associated with an increased prevalence of p53 mutations or immunopositivity of p53 protein in bladder tumours investigated [312]. This study showed a strong correlation between arsenic exposure and other genetic alterations, especially deletions of part or all of chromosome 17p [313].

Cellular studies support epidemiological data suggesting an involvement of p53 status in carcinogenesis. A study with bladder epithelial cells showed an arsenic-induced increase in the proliferation accompanied by increased AP-1 DNA binding activity and upregulation of gene expression of proto-oncogenes c-fos and c-jun [198]. Furthermore, arsenite was shown to influence the cell cycle progression in a human bladder cell line, increasing the proportion of cells in the S-phase [314]. P53 level increased in a time-dependent manner whereas P21 content showed a peak after about 8 h, then decreasing drastically to undetectable levels at 24 h, suggesting that P21 was unable to block the cells in the G1 phase. The arsenic-induced degradation of P21 probably occurred via the ubiquitin-proteasome degradation pathway [314]. The accumulation of ubiquinated proteins has been observed in human urothelial cells, suggesting that arsenic-induced alterations in the ubiquitine-proteasome pathway may contribute to carcinogenesis in the bladder [106,315].

Rat bladder is the most studied and probably best understood experimental model of arsenic carcinogenesis [157]. There are four studies showing DMA as either a promoter or a complete carcinogen in rat bladder [283,284,286,316] whereas administration of arsenite and arsenate have failed to show any carcinogenic potential in animal models. However, the accumulation of arsenite, and to a lesser extent DMA, arsenate, and MMA, has been observed in bladder tissue of mice. Urinary excretion has been shown to be mostly in the form of MMA and DMA, suggesting that intracellular concentrations of arsenite in bladder epithelial cells may be considerably high [317,318]. Bladder cells receive their dose of arsenicals both from arterial circulation and from the lumen of the bladder, a situation not existing for other target organs of arsenic-induced carcinogenesis.

7.4. Liver cancer

Liver cancers including hepatic angiosarcoma and hepatocellular carcinoma have been associated with a long-term exposure to ingested or inhaled arsenic. The main exposure sources include contaminated drinking
water or contaminated wine, Fowler’s solution and copper smelting [1,4,41,320,321]. A Taiwanese study found an association between arsenic exposure and liver cancer only in the female population, probably due to confounding by social habits such as cigarette smoking and alcohol drinking in the aetiology of male liver cancers [322].

A study in an Indian cohort of 29 arsenic-exposed patients showed that hepatomegaly was present in 76.6% of the cases, and non-cirrhotic portal fibrosis (91.3%) was the predominant lesion in the liver histology [323]. Hepatic fibrosis due to long-term arsenic exposure has also been demonstrated in BALB/c mice [324]. Two-month exposure caused significant elevation of hepatic glutathione (GSH) and glutathione-S-transferases (GSTs), followed by a sharp decrease in the enzyme levels and a depletion of GSH after 6 months. Continued arsenic feeding resulted in fatty liver with serum aminotransferases elevated at 12 months and hepatic fibrosis at 15 months, suggesting that oxidative stress plays a role in the initiation of hepatic damage by arsenic [325].

8. Setting the limits for arsenic exposure

In spite of its toxicity, some human and animal data indicate that low levels of arsenic induce beneficial effects such as thermo-tolerance [326] or cross-tolerance to toxicants like nickel [327] and endotoxin [328]. Self-tolerance to arsenic toxicity in humans occurring after chronic exposure has been reported as beginning 23 January 2006. The new American limit (OELs). OELs can be supplemented by further advice on the setting of Occupational Exposure Limits (SCOEL) to evaluate risks related to chemical substances at the workplace. Its major task is to give occupational limits

8.1. Environmental limits

The first regulatory limit was set in the United Kingdom as a result of a public inquiry because of arsenic found in beer, via six members chaired by the physicist William Thompson, first Lord Kelvin, in 1903 [336]. They recommended a limit of 100 grains of arsenious oxide per gallon, corresponding to about 90 μg/l arsenic. This was reduced two-fold over the next century and until recently the limit set by Bangladesh, the European Union and the United States was 50 μg/l, corresponding to 50 parts per billion (ppb). The discovery that there are adverse effects of continuous chronic exposure led WHO to lower their recommendation to 10 μg/l. The European Union (EU) accepted a new drinking water standard of 10 μg/l for arsenic in the year 2003 [337]. Developing countries such as Bangladesh with large populations exposed to arsenic in water have kept their arsenic standards in drinking water at 50 μg/l [338].

The risk at low doses of arsenic has been a subject of a controversial debate in the USA [270,339]. After the requirement of The Safe Drinking Water Act to revise the existing 50 μg/l standard for arsenic in drinking water, the Environmental Protection Agency (EPA) adopted a new standard of 10 μg/l in 2001 and public water systems complied with the new standard beginning 23 January 2006. The new American limit is mainly based on the Taiwanese data with very high concentrations of arsenic, the mean exposure being 780 μg/l and the assumption that the dose–response curve is linear [1,27,41].

More information on the effect of lower exposure doses coming from both biological and epidemiological studies are needed for setting the future limits for arsenic in drinking water.

8.2. Occupational limits

In 1995 the European Union set up a Scientific Committee on Occupational Exposure Limits (SCOEL) to evaluate risks related to chemical substances at the workplace. Its major task is to give advice on the setting of Occupational Exposure Limits (OELs). OELs can be supplemented by further notations such as 8-h time-weighted average (TWA-8h), short-term exposure limit (STEL) or biological
limit value (BLV) [337]. In most European countries the TWA-8h for arsenic is 0.1 mg/m³ or less [340]. In the USA the Permissible Exposure Limit given by the Mine Safety and Health Administration (MSHA) [341] for arsenic is 0.5 mg/m³ and the STEL-15 min value set up by the National Institute for Occupational Safety and Health (NIOSH) is 0.002 mg/m³ [342].

9. Conclusions

Inorganic arsenic in drinking water and in mining environments is a recognised cause of various cancers. Arsenic exposures affect millions of people worldwide. Epidemiological studies provide the useful quantitative data for guiding risk assessment at levels commonly occurring in drinking water or arsenic-contaminated air or dust. To date, most estimates of risk at low levels of exposure have been based on extrapolations from data on highly exposed populations. However, there is strong evidence showing that the dose–response curve between arsenic concentration and the risk of various cancers is linear only among highly exposed populations. Furthermore, the type of arsenic exposure, ingestion or inhalation, seems to influence the shape of the curve. There are several factors such as gender, ionising radiation, smoking, diet or genetic susceptibility which may act synergistically or as confounders influencing the dose–response curve. The risk at low doses of the arsenic-induced carcinogenesis is a subject of controversial debate and may not be solved solely by epidemiological means. Therefore, it is essential to learn more about arsenic’s mode of action in biological systems. In addition to the important role of methylated trivalent arsenic compounds in the development of cancer, the oxidative stress theory seems to be the mechanism of action best explaining the capacity of arsenic compounds to induce human cancer in the lung, skin, bladder, and liver.

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References


