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Inorganic arsenic: a nongenotoxic threshold carcinogen

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Summary

Inorganic arsenic (iA) in the drinking water is a human carcinogen (bladder, lung, and skin). The mode of action involves metabolism to trivalent arsenicals that react with sulfhydryl groups in critical proteins, leading to cytotoxicity with regenerative proliferation, involving a threshold at in vitro concentrations >0.1 μM. Adverse biologic effects at such tissue concentrations in rodents occur with ≥10 ppm of iAs in diet or drinking water. On the basis of mode of action, in vitro, and in vivo studies, anticipated drinking water exposures of 50–150 μg/L exceed a tissue concentration of >0.1 μM in humans. Epidemiologic investigations evaluating populations exposed at levels <150 μg/L iAs in drinking water are consistent with such a threshold for cancer.

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Inorganic arsenic, threshold, Risk assessment, bladder cancer, lung cancer, skin cancer.

Inorganic arsenic (iA) is a known human carcinogen, causing skin, urinary bladder, and lung cancer, with more limited evidence for an increased risk of kidney, liver, and prostate carcinomas [1]. The increased kidney cancer risk is due to kidney pelvis urothelial carcinomas, not renal cell carcinomas [2].

iAs levels are regulated by government authorities [3]. In the United States, Europe, and the World Health Organization, 10 μg/L of iAs is an acceptable drinking water level. For some beverages, such as for various fruit juices, the level is also 10 μg/L. Regulatory levels for foods are difficult to develop since many foods have high levels of various organic arsenicals. Nevertheless, rice, a common food component in the diets of many people around the world, has attracted considerable attention. In the United States, 100 ppb for iAs has been set by the Food and Drug Administration for infant rice cereal and by the European Commission for rice and rice products for infants and children; whereas, Codex has set levels for iAs in polished rice at 200 ppb and brown rice at 350 ppb. Although an acceptable level in rice remains to be established in the United States, it is reassuring that large epidemiology studies concerning the relationship of rice consumption with cancer and with cardiovascular disease have shown no increased risk for either [4–7].

Increased risk of lung cancer was first identified in an occupational setting because of iAs inhalation exposure [8]. However, considerable evidence indicates that not only lung cancer but also urinary bladder and skin cancer are the primary cancers associated with elevated iAs exposures through the drinking water [1,3]. Contributions from iAs in food are small in comparison, unless exposures in drinking water are low (<10 μg/L). Increased risk of cancers due to iAs have been identified in populations with high exposures in drinking water, generally >200 μg/L. Risk assessments concerning iAs in the drinking water have traditionally involved evaluations of populations with high and low exposures, with extrapolations from high to low exposure levels without a threshold [3]. However, the possibility of iAs acting as a threshold carcinogen has been discussed in the literature for more than 20 years [9]. This is a critical determinant for our understanding of risk to human populations.

Although epidemiology studies are valuable in our understanding of the dose response of human exposures to iAs, such studies have limitations at low doses for discerning the dose-response relationship. Ultimately, the rationale for dose-response at low doses must rely on a mechanistic understanding of iAs carcinogenicity.

Various modes of action (MOAs) have been proposed for iAs carcinogenesis but can be classified overall as genotoxicity or nongenotoxicity [1,3]. Genotoxicity can be direct, involving covalent binding of arsenic or metabolite with DNA, forming DNA adducts, leading to

mutations and increased risk of cancer. However, a direct DNA reactive effect does not occur [10] with iAs. Others have suggested the formation of free radicals producing an interaction with DNA, but free radical generation occurs only at exceptionally high iAs concentrations, not attainable in vivo, or under circumstances that would not occur in vivo [1]. Thus, a direct DNA reactive MOA for iAs can be excluded.

Evidence for indirect genotoxic effects of iAs, such as micronucleus formation or chromosomal aberrations, have been demonstrated predominantly in vitro, and only at concentrations that exceed those that could be attained in vivo [1,3]. This is a major shortcoming for genotoxicity assays, in general, as the concentrations used for evaluation are cytotoxic, which by definition would lead to an increase in DNA damage. The in vitro concentrations utilized in these genotoxicity assays greatly exceed the lethal in vitro concentrations, for several cell types (10−20 μM). In vivo demonstration of genotoxicity has been limited and again has involved exposures to extremely high concentrations in rodents, in the ppm range rather than in the ppb range to which humans are exposed.

There have also been evaluations of indirect genotoxicity in humans, predominantly involving assessing micronuclei in bladder urothelial cells exfoliated into urine, but also blood lymphocytes and buccal mucosa squamous cells in individuals exposed to high levels of iAs in drinking water [11]. These studies have purported to demonstrate increased micronuclei with high iAs exposures compared with individuals exposed at lower levels. However, these studies have numerous methodological issues, most notably the lack of a dose response with respect to iAs exposure. In addition, there were inadequate controls for exposure to tobacco (some studies have controlled for cigarette smoking but not for betel quid consumption), nutrition (particularly folate and selenium), and definition of exposure. Urinary levels of arsenic have been used as the exposure metric, although drinking water levels have also been used. However, a single time point is evaluated, despite variability in water consumption and urinary concentrations. In addition, urinary levels are measured as either total arsenic or dimethylarsinic acid (DMA), which poses several difficulties. Total arsenic includes inorganic and organic forms, with organic forms highly variable based on diet. Seafood and rice are particularly noteworthy in their content of various organic arsenicals, many of which are considered to have limited or no human toxicity, such as arsenobetaine and arsenocholine. Most of the iAs is excreted in urine as DMA [12]. However, measurement of DMA in urine can be markedly influenced by organic arsenicals in food, including DMA as well as organic precursors that are metabolized to DMA. In addition, arsenic-containing lipid inclusions in urothelial cells can be mistaken for micronuclei because of their similar appearance by Giemsa stain. These lipid inclusions do not contain DNA and thus are not actually micronuclei [13]. Such inclusions have been demonstrated in urothelial cells in humans exposed to high levels of iAs, such as in patients treated with arsenic trioxide for promyelocytic leukemia [14]. They are present in urothelial cells of mice exposed to high levels of iAs but also at lower exposures [13]. Moreover, in the rat model of DMA urinary bladder carcinogenesis, there is no evidence of micronuclei in the target cell population, the urothelial cells [15].

iAs also shows inhibition of DNA repair and interaction with tubulin [1,3]. As discussed below, trivalent arsenicals react with sulfhydryl groups of proteins leading to effects on their functions. These proteins include certain DNA repair enzymes and tubulin. However, the effects on either of these involves a threshold because there is a high reserve of these proteins available before an effect would occur biologically. Furthermore, other tubulin inhibitors, such as colchicine, have been widely used clinically to treat gout with no evidence of carcinogenicity. Colchicine is used at much higher concentrations than iAs exposures.

In summary, there is strong evidence against direct interaction of arsenicals with DNA, and the weight of the evidence supports a lack of genotoxicity in vivo at human exposure levels. Excluding a direct DNA reactive effect eliminates the possibility of linear extrapolation to low exposure levels.

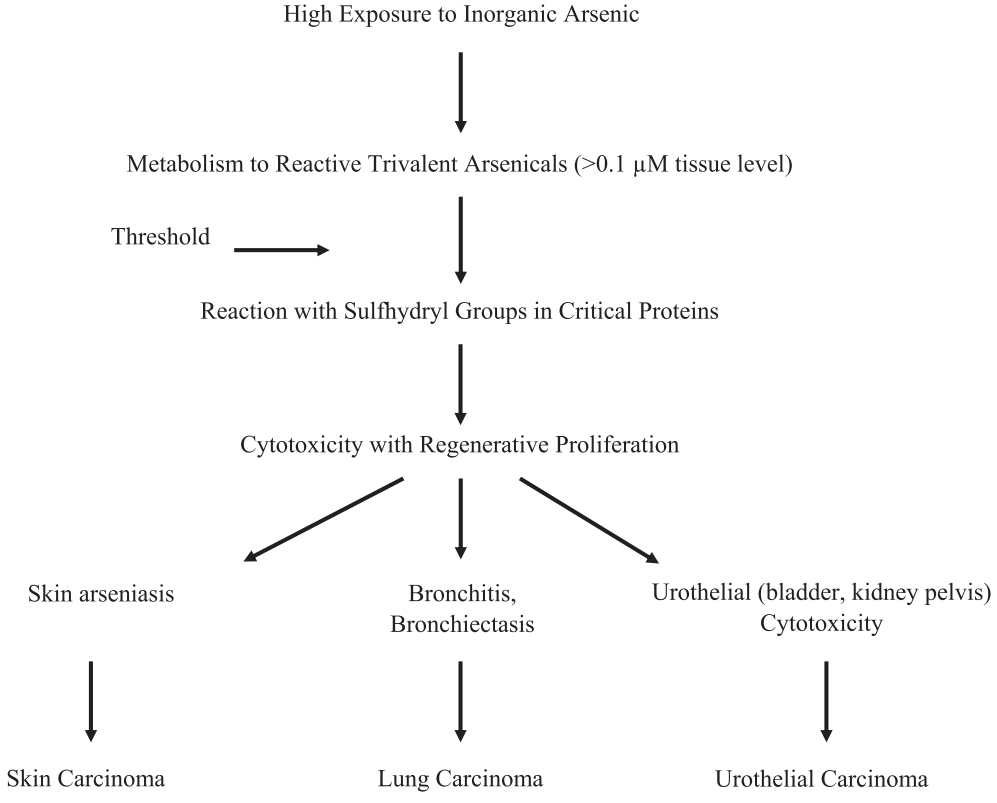
iAs is primarily metabolized to methylated forms, with only small amounts being excreted in the urine as iAs [12]. Methylation has traditionally been considered to occur through a series of reductions and oxidative methylations, from arsenate to arsenite, monomethylarsonic acid (MMA^V), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^V), dimethylarsinous acid (DMA^{III}), and trimethylarsine oxide ($TMAO^v$). Pentavalent methylated arsenicals are biologically inactive, with IC50s for cytotoxicity in vitro in the mM range. In contrast, trivalent arsenicals are reactive, with IC50 cytotoxicity in vitro in the μM range or less (>0.2 μM) [1,16]. Trivalent arsenicals do not react with DNA but, rather, are known to avidly react with thiol groups, whether in small molecules (e.g., glutathione) or in cysteine residues in proteins [17]. There is considerable evidence that the biological effects of arsenicals, other than effects on oxidative phosphorylation related to arsenate, are because of reaction of trivalent arsenicals with thiol groups of critical proteins. Available free thiol groups in proteins differ between species. For example, rat hemoglobin has a free alpha chain cysteine thiol group not present in other species, resulting in binding of trivalent arsenicals to the hemoglobin and thereby altering the toxicokinetics [18]. Similarly, trivalent arsenicals react with the

estrogen receptor in certain species but not in others [19]. These differences result in considerable variation between species regarding kinetics, tissue specificity, and overall biological responsiveness. Because of the half-life of many of these proteins, particularly those active in important cellular regulatory functions such as proliferation and differentiation, the biologic response involves a threshold. Until the binding reaches a level sufficient to supersede the regeneration of these proteins, there will be no biological response.

More recently, thiolated metabolites of arsenicals have been identified as generated by a chemical, nonenzymatic reaction between hydrogen sulfide (H_2S) and oxygenated arsenicals [12,20]. Pentavalent oxygenated arsenicals slowly traverse the cell membrane whereas trivalent oxygenated forms rapidly cross the cell membrane. In contrast, pentavalent and trivalent thiolated arsenicals readily cross the cell membrane, giving an appearance of increased cytotoxicity for the pentavalent thiolated forms compared with oxygenated forms [21]. However, once inside the cell, the pentavalent thiolated arsenicals are rapidly converted to the trivalent, reactive, cytotoxic oxygenated forms. Thus, the MOA of arsenicals relies on an evaluation of the total amount of trivalent oxygenated arsenicals present in a given tissue.

Although several MOAs have been postulated for various arsenicals, most evidence supports cytotoxicity with regenerative proliferation (Figure 1) as the basis for their carcinogenicity [1,3]. This has been best demonstrated in the rat model of DMA^V-induced urinary bladder tumors when administered in diet or drinking water [22]. Urothelial hyperplasia occurs at 10 ppm and tumors at 25 ppm or higher. However, at 2 ppm and below, there is no evidence of an effect on the bladder. By scanning electron microscopy (SEM), superficial cytotoxicity is identified, with consequent regenerative proliferation identified on the basis of hyperplasia by light microscopy, piling up of cells by SEM, and increased DNA replication labeling index (bromodeoxyuridine or Ki-67). The cytotoxicity is because of metabolism of DMA^V to DMA^{III}, which is concentrated and excreted in the urine. In vitro, cytotoxicity does not occur below 0.2 μM DMA^{III}, so this urinary concentration is required for DMA^{III} to produce urothelial cytotoxicity in vivo. This requires oral administration of >2 ppm DMA^V to rats.

Figure 1



Mode of action for inorganic arsenic carcinogenesis involving a threshold.

Preneoplastic changes are also observed in the urinary bladder of rats and mice administered arsenate or arsenite in the diet or drinking water, but not urinary bladder tumors, likely related to changes in arsenical kinetics with age [22−24]. By age 26 weeks, there is no longer evidence of hyperplasia [24].

A major drawback for evaluating the MOA of iAs has been the lack of animal models of arsenical carcinogenesis. Waalkes et al. showed increased lung tumors in mice using transplacental administration [25,26]. However, reproducibility of this model has been difficult. Although they stated that there is a tumorigenic effect at low doses (50 ppb), closer examination of the data [27,28] shows no effect below 12,000 ppb, when one accounts for the high variability of lung tumors in this mouse strain, applying the Haseman rule [29] for statistical significance of common tumors (using $p < 0.01$ rather than $p < 0.05$) and also incorporating information from historical controls. Furthermore, a Japanese laboratory has been unable to reproduce the induction of lung tumors in this mouse strain, identifying a slight increase in liver tumors instead [30]. A critical review of this model was reported [31].

On the basis of extensive examination of in vitro studies, Gentry et al. [16,32,33] identified 0.1 μM as a threshold for the adverse in vitro effects of arsenicals. Concentrations >10 μM were lethal to cells regardless of cell type, whether using established cell lines or primary cells. Utilizing various markers, including genomics, Yager et al. utilizing primary human urothelial cells demonstrated that the range of effects between humans was approximately threefold [34]. On the basis of their various in vitro studies combined with the in vivo studies in rats and mice, an estimated threshold for humans could be calculated at 50−150 μg/L in the drinking water [3].

Although deficits in exposure assessment, incomplete control of confounding factors, limited understanding of interactions, the potential for bias, and the likelihood of chance findings from multiple hypothesis testing seriously limit epidemiologic studies of iAs for determining a threshold [3], critical review of observational human studies can be used to corroborate mechanistic information derived from experimental in vivo and in vitro investigations. Numerous epidemiology studies concerning iAs and cancer have been reported especially concerning bladder, lung, and skin cancer. Most dose-response assessments utilize populations having high and low iAs drinking water exposures, then calculate an extrapolation from the positive findings at high exposures to lower exposures where risks are often not statistically significantly elevated or may even show an inverse association. On the basis of the above discussion, iAs should have a threshold biologically. This was described in detail by Lynch et al. [35], but they performed a meta-regression analysis extending to zero for risk assessment. In contrast, Tsuji et al. [3] recently reported a threshold-based risk assessment utilizing the above mechanistic information, in vivo and in vitro investigations, and examination of epidemiologic evidence, but restricting the evaluation to populations exposed to low drinking water levels of iAs (<150 μg/L) meeting inclusion criteria for study quality (e.g. individual-level control for smoking). Utilizing the low exposure populations avoids the marked influence by including high exposure populations in the evaluation. When restricted to these low exposure populations, there is no clear evidence of increased cancer risks for the urinary bladder, lung, or skin cancer or benign changes in the skin. On the basis of this examination, an estimated threshold for humans is approximately 100 μg/L in the drinking water, with a lower limit of approximately 50 μg/L, corroborating the estimated threshold calculated on the basis of in vitro and in vivo investigations This is also in keeping with previous meta-analyses of low exposure populations [36−38] and various other studies by Lamm et al. [39,40]. For example, Tsuji et al. [37] showed that predicted risks based on extrapolation from high doses in Southwestern Taiwan [41] were statistically incompatible with the meta-analysis results of low dose studies, particularly in never smokers.

Evidence of cytotoxicity and regeneration in human populations has also been identified [1]. Noncancer arsenic-related skin lesions involve hyperkeratosis, inflammation, and regenerative proliferation, eventually leading to invasive carcinoma. In a detailed examination of the West Bengal population exposed to various iAs levels, Haque et al. [42] did not identify any individuals with arsenic-related benign skin changes exposed to a drinking water level below 115 μg/L at any time in their past exposure history. In addition, there is accumulating evidence that high iAs exposures in drinking water are associated with an increased incidence of chronic bronchitis and bronchiectasis, cytotoxic inflammatory respiratory tract disorders that can be lung cancer precursors [1]. For the urinary bladder, animal models clearly show cytotoxicity, but there are no adequate markers of superficial cytotoxicity and hyperplasia in humans. However, in an occupational accident involving high iAs exposures, approximately 1/3 developed hematuria, indicating urothelial cytotoxicity [43]. Similar to nongenotoxic carcinogens generally, a risk assessment based on thresholds for the precursor, noncancer toxicities will also be protective of cancer.

In summary, there is strong support both mechanistically, in vitro, in vivo, and epidemiologically for a threshold for iAs carcinogenesis. Direct arsenical interaction with DNA does not occur, and the weight of the evidence strongly supports a nongenotoxic MOA for iAs. Reaction of trivalent arsenicals with free thiol groups in

critical proteins is the basis for the biologic effects of arsenicals and is supportive of a threshold response. On the basis of these findings, a threshold for iAs in drinking water for humans is approximately 100 μg/L, with a range extending down to approximately 50 μg/L. This approach should be incorporated into a risk assessment for human populations rather than the default extrapolations from high to low exposures without a threshold.

Conflict of interest

The research on arsenic of S.M. Cohen and L.L. Arnold has been supported by the US National Cancer Institute, ILSI North America Technical Committee on Food and Chemical Safety, EPRI, ASTF, the Organic Arsenical Products Task Force (OAPTF) (trade organization of companies producing methylated arsenicals for pesticide use), USEPA, Alberta Health (from the Canadian Province of Alberta), Alberta Innovates (from the Canadian Province of Alberta), Canada Research Chair's Program, Canadian Institute of Health Research, and Natural Sciences and Engineering Research Council of Canada. S.M. Cohen has also provided public comments on USEPA IRIS assessment for arsenic. Drs. Cohen and Tsuji received funding from the Texas Commission on Environmental Quality for a risk assessment of inorganic arsenic in drinking water [3].

J.S. Tsuji has provided scientific consultation to the Arsenic Science Task Force (ASTF), the Wood Preservatives Science Council (WPSC), and the Electric Power Research Institute (EPRI) regarding arsenic dose response issues, including providing public comments on USEPA Integrated Risk and Information System (IRIS) assessments for arsenic. The ASTF represents trade associations of industries, manufacturers, and agricultural producers with interests in the scientific and regulatory developments on arsenic. WPSC is a trade organization funded by manufacturers of wood preservative chemicals including those containing arsenic. EPRI is a nonprofit organization that conducts research, development, and demonstration projects on scientific topics of interest to electric utilities. Its members are mostly electric utilities and also include businesses, government agencies, regulators, and public and private entities. Some of J.S. Tsuji published papers on arsenic were partially funded by the ASTF and EPRI. Some of J.S. Tsuji's publications were also partially funded by Rio Tinto (a mining company), the American Chemistry Council (specifically, companies within the antimicrobial sector of this chemical trade organization that funds research on health, safety, and the environment), or ILSI North America Technical Committee on Food and Chemical Safety. ILSI is a nonprofit foundation (funded by member food companies) focused on scientific issues related to nutritional quality and safety of the food supply. The arsenic biomonitoring study in New York published by J.S. Tsuji was funded by FMC Corporation (a former manufacturer of arsenic-based pesticides); FMC had no role in providing comments on the reporting of the biomonitoring study results or in funding its publication. J.S. Tsuji has also provided public comments on arsenic exposure and health risks on behalf of the above parties, and scientific consultation to a number of mining companies, the Tennessee Valley Authority, and the USEPA for conducting health risk assessments of arsenic at contaminated sites, and in guiding cleanup of sites. J.S. Tsuji has also provided expert testimony on behalf of both defendants and plaintiffs on arsenic exposure and health risk in legal cases.

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