

Toxicity and Risk Assessment of Trace Elements

Health Effects and Risk Assessment of Arsenic^{1,2}

Charles O. Abernathy,^{*3} David J. Thomas[†] and Rebecca L. Calderon^{**}

^{*}Office of Science and Technology, U.S. Environmental Protection Agency, Washington, D.C. 20422 and

[†]Experimental Toxicology Division and ^{**}Human Studies Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

ABSTRACT Humans can be exposed to arsenic (As) through the intake of air, food and water. Although food is usually the major source of As exposure for people, most adverse effects are seen after As exposure from drinking water. The two main reasons for this situation are that most food arsenicals are organic and have little or no toxicity, and in many cases, As exposures from drinking water sources are to the more toxic inorganic form and occur at relatively high doses, e.g., hundreds of micrograms per day. In various parts of the world, As in drinking water is associated with such effects as gastroenteritis, neurological manifestations, vascular changes, diabetes and cancers (bladder, lung, liver, kidney and prostate). After reviewing the As database, the U.S. Environmental Protection Agency promulgated a maximum contaminant level for As in drinking water of 10 $\mu\text{g/L}$. *J. Nutr.* 133: 1536S–1538S, 2003.

KEY WORDS: • arsenic • methylation • cancer • diabetes • vascular disease

In this review, we focus on the effects of arsenic (As)⁴ exposure from drinking water sources. The primary inorganic As species in water are arsenate [As(V)] and/or arsenite [As(III)]; their proportions depend on the water's redox potential and pH (1). Many As contamination sources are natural. Concentrations of As in the earth's crust vary but usually range from 1.5 to 5 mg of As/kg in igneous and sedimentary rocks. As is also present in metal ores and minerals (2), and geothermal wells can be a source of As in surface and ground waters (3). Anthropogenic sources include pesticides, wood preservatives and industrial, mining and smelting wastes. As levels in water

depend on factors such as the level of human activity, distance from pollution sources and dispersion and fate of the released As (2). Excluding As exposure from polluted sources, people are exposed to As in their water, food and air. In the U.S., air exposure is usually minimal. For most people, the major exposure source is the diet; total food intake is $\sim 50 \mu\text{g}$ of As/d of which $\sim 10 \mu\text{g}$ of As/d is inorganic (4). However, the organic As in food and seafood appears to be much less toxic than the inorganic As forms (4,5). Generally, $< 4 \mu\text{g}$ of As/d comes from drinking water (4).

Effects on humans

Acute poisoning incidents. Ingestion of toxic quantities of As usually has effects within 30–60 min, and severe toxicity has been reported with as little as 1 mg of As₂O₃ (6). The clinical presentation of acute As poisoning occurs in two distinct forms: acute paralytic syndrome and acute gastrointestinal syndrome. Acute paralytic syndrome is characterized by cardiovascular collapse [secondary to a direct toxic effect; (7)], central nervous depression [caused by vasodilation resulting in hemorrhagic necrosis of both white and gray matter; (8)] and death within hours. Acute gastrointestinal syndrome starts with a metallic or garliclike taste associated with dry mouth, burning lips and dysphagia. Violent vomiting may ensue and may eventually lead to hematemesis (7).

Cardiovascular effects. A Taiwanese study (9) concluded that a dose-response relationship exists between the As concentration in well water and cerebrovascular disease. Another study (10) found that As was associated with a 1.5-fold increase in age- and sex-adjusted cases of hypertension; these researchers concluded that long-term As exposure may induce hypertension in humans. A U.S. study in Utah (11) found

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³ To whom correspondence should be addressed. E-mail: abernathy.charles@epa.gov.

⁴ Abbreviations used: As, arsenic; As(III), arsenite; As(V), arsenate; DMAs, dimethyl arsenic; MAs, methyl arsenic; NRC, National Research Council.

a significant excess of deaths for cardiovascular diseases including hypertensive heart disease in males and females and all other heart disease in females that was associated with As exposure.

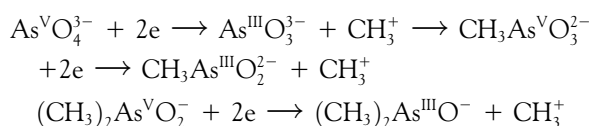
Diabetes mellitus. Lai et al. (12) found a dose-response relationship between cumulative As exposure and diabetes mellitus. A similar association was also found in a study in Bangladesh (13) that used the presence of keratosis as an indicator of As exposure and showed elevated risks for diabetes for those exposed to As in their drinking water.

Cancer. Prevalence studies of skin cancer in Taiwan (14) indicate some degree of dose-response activity between amount of As exposure and skin cancer and other manifestations including keratosis and hyperpigmentation. They found that ascending rates for skin cancer, keratosis and hyperpigmentation corresponded with As content of well water and identified a dose-response relationship between As concentration and blackfoot disease.

Studies in Taiwan (15), Japan (16) and Chile (17) report significant associations between lung cancer and exposure to As in drinking water. Two bladder cancer-incidence studies from Taiwan (18,19) found a higher incidence of bladder cancer associated with consumption of well water with high As concentration. Increased bladder cancer mortality associated with increased drinking water As concentration was found in a mortality study in the eastern region of the Cordoba province in Argentina (20) and in northern Chile (17).

Studies in the U.S. and Belgium have not identified a positive association between bladder cancer and As ingestion. Exposures in the U.S. and Belgian studies are lower than in Taiwan, and therefore the evaluation of an association with bladder cancer is more difficult. Exposures reported in a case-control study in Utah (21) ranged from 0.5 to 160 μg of As/L (average 5.0 μg of As/L). A retrospective cohort study in Belgium that combined inhaled and ingested exposure to As (22) reported drinking water exposures that ranged from 20 to 50 μg of As/L.

Role of metabolism of arsenic in its toxicity. Understanding the biomethylation of As is central to elucidating its action as a toxin and a carcinogen. In humans as in many other species, inorganic As is enzymatically converted to the methylated products methyl arsenic (MAs) and dimethyl arsenic [DMAs (23)]. Hence, exposure to As results in concurrent exposure to the parent compound and two unique metabolites. A reaction scheme (24) illustrates that the metabolism of As can be divided into two distinct but interrelated processes as follows:



Here, As is reduced from As(V) to As(III) and then it is oxidatively methylated (23). Although the reduction of As(V) to As(III) can be accomplished by the concurrent chemical oxidation of a thiol-containing molecule (e.g., glutathione), this process is likely enzymatic in mammals. Several enzymes including purine nucleoside phosphorylase function as As(V) reductases. The S-adenosylmethionine-dependent methyltransferases that catalyze the formation of MAs and DMAs from As have been characterized. Because arsenicals that contain As(III) are the preferred substrates for enzymatically catalyzed methylation, trivalent MAs is an intermediate in the metabolic pathway and the obligatory substrate for the second methylation reaction that yields DMAs (23). Both trivalent MAs and trivalent DMAs are persistent metabolites that can be

identified in the urine of individuals chronically exposed to As in drinking water (25). These metabolites exceed inorganic As in potency as cytotoxins (26), genotoxins (27) and enzyme inhibitors (28). Because the metabolites are more toxic than the parent compound, the methylation of As should be considered an activation and not a detoxification process.

Interindividual variability in the metabolism of As may also affect the consequences of As exposure. Studies in populations that ingest drinking water that contains As have found that individuals who develop As skin lesions also excrete more MAs in their urine than those who do not have lesions (29). The increased urinary excretion of MAs in these individuals may be due to differences in the metabolism, disposition or retention of As or its metabolites. These differences probably reflect polymorphisms in the genes that encode As(V) reductases and/or As methyltransferases. Differences in the capacity to form and retain the metabolites of As could also be affected by host factors such as age, sex or nutritional status. For example, selenium nutrition can affect the metabolism and retention of As (30).

Modes of action. For As, cancer bioassays in lab animals have been generally negative, and the key events in the cancer process are unknown. However, several possibilities exist (31,32). The kinds of genetic alterations that are seen include inhibition of DNA repair enzymes, changes in DNA methylation patterns that could affect gene expression and/or repair, oxidative stress, potentiation of effects of mutations by other agents and cell proliferative effects. The National Research Council (NRC) recommends that the study of biomarkers of As exposure might better characterize the dose-response effects of As at lower exposure levels. For noncancer effects, inhibition of cellular respiration in mitochondria by As may be a focal point of its toxicity. In addition, As causes oxidative stress, which could also play a role. A greater understanding of the effects of As on cellular respiration and the subsequent effects on methylation and oxidative stress is needed (31,32).

Risk characterization. Exposure to As in drinking water is reported to cause many different human cancer and noncancer diseases. The NRC (31,32) reviewed the As health-effects database and concluded that the studies on Taiwan bladder cancer provide the current best-available data for the risk assessment of As-induced cancer. The consensus opinion of the NRC (31) was that the then "current EPA MCL [(Environmental Protection Agency Maximum Contaminant Level)] for arsenic in drinking water of 50 $\mu\text{g}/\text{L}$ does not achieve EPA's goal for public-health protection and, therefore, requires downward revision as promptly as possible." On January 22, 2001, the EPA promulgated a final As regulation of 10 $\mu\text{g}/\text{L}$.

LITERATURE CITED

- Irgolic, K. J. (1994) Determination of total arsenic and arsenic compounds in drinking water. In: *Arsenic: Exposure and Health* (Chappell, W. R., Abernathy, C. O. & Cothorn, C. R., eds.), pp. 51-60. Science and Technology Letters, Northwood, England.
- National Academy of Sciences (1977) *Arsenic: Medical and Biological Effects of Environmental Pollutants*. National Academy Press, Washington, D.C.
- Welch, A. H., Lico, M. & Hughes, J. (1988) Arsenic in groundwater of the western United States. *Ground Water* 26: 333-347.
- Borum, D. R. & Abernathy, C. O. (1994) Human oral exposure to inorganic arsenic. In: *Arsenic: Exposure and Health* (Chappell, W. R., Abernathy, C. O. & Cothorn, C. R., eds.), pp. 21-29. Science and Technology Letters, Northwood, England.
- Donohue, J. M. & Abernathy, C. O. (1999) Exposure to inorganic arsenic from fish and shellfish. In: *Arsenic Exposure and Health Effects* (Chappell, W. R., Abernathy, C. O. & Calderon, R. L., eds.), pp. 89-98. Elsevier, Amsterdam.
- Cullen, N. M., Wolf, L. R. & St. Clair, D. (1995) Pediatric arsenic ingestion. *Am. J. Emerg. Med.* 13: 432-435.

7. Civantos, D. P., Lopez Rodriguez, A., Aguado-Borruey, J. M. & Narvaez, J. A. (1995) Fulminant malignant arrhythmia and multiorgan failure in acute arsenic poisoning. *Chest* 108: 1774–1775.
8. Brouwer, O. F., Okenhout, W., Edelbroek, P. M., de Kom, J. F. M., de Wolff, F. A. & Peters, A. C. B. (1992) Increased neurotoxicity of arsenic in methylenetetrahydrofolate reductase deficiency. *Clin. Neurol. Neurosurg.* 94: 307–310.
9. Chiou, H.-Y., Huang, W.-I., Su, C.-L., Chang, S.-F., Hsu, Y.-H. & Chen, C.-J. (1997) Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. *Stroke* 28: 1717–1723.
10. Chen, C.-J., Hsueh, Y.-M., Lai, M.-S., Shyu, P., Chen, S.-Y., Wu, M., Kuo, T. L. & Tai, T. Y. (1995) Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* 25: 53–60.
11. Lewis, D. R., Southwick, J. W., Ouellet-Helstrom, R., Rench, J. & Calderon, R. L. (1999) Drinking water arsenic in the United States: a cohort mortality study. *Environ. Health Perspect.* 107: 359–365.
12. Lai, M.-S., Hsueh, Y.-M., Chen, C.-J., Shyu, M.-P., Chen, S.-Y., Kuo, T.-L., Wu, M.-M. & Tai, T.-Y. (1994) Ingested inorganic arsenic and prevalence of diabetes mellitus. *Am. J. Epidemiol.* 139: 484–492.
13. Rahman, M., Tondel, M., Ahmad, S. A. & Axelson, O. (1998) Diabetes mellitus associated with arsenic exposure in Bangladesh. *Am. J. Epidemiol.* 148: 198–203.
14. Tseng, W. P., Chu, H. M., How, S. W., Fong, J. M., Lim, C. S. & Yeh, S. (1968) Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst.* 40: 453–463.
15. Chiou, H.-Y., Hsueh, Y.-M., Liaw, K.-F., Horng, S.-F., Chiang, M.-H., Pu, Y.-S., Lin, J. S.-N., Huang, C.-H. & Chen, C.-J. (1995) Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study in Taiwan. *Cancer Res.* 55: 1296–1300.
16. Tsuda, T., Babazono, A., Yamamoto, E., Kurumatani, N., Mino, Y., Ogawa, T., Kishi, Y. & Aoyama, H. (1995) Ingested arsenic and internal cancer: a historical cohort study followed for 33 years. *Am. J. Epidemiol.* 141: 198–209.
17. Smith, A. H., Goycolea, M., Haque, R. & Biggs, M. L. (1998) Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. *Am. J. Epidemiol.* 147: 660–669.
18. Chiang, H.-S., Guo, H.-R., Hong, C.-L., Lin, S.-M. & Lee, E.-F. (1993) The incidence of bladder cancer in the blackfoot disease endemic area in Taiwan. *Br. J. Urology* 71: 274–278.
19. Guo, H.-R., Chiang, H.-S., Hu, H., Lipsitz, S. R. & Monson, R. R. (1994) Arsenic in drinking water and urinary cancers: a preliminary report. In: *Arsenic: Exposure and Health* (Chappell, W. R., Abernathy, C. O. & Cothorn, C. R., eds.), pp. 119–128. Science and Technology Letters, Northwood, England.
20. Hopenhayn-Rich, C., Biggs, M. L., Fuchs, A., Bergoglio, R., Tello, E. E., Nicoli, H. & Smith, A. H. (1996) Bladder cancer mortality associated with arsenic in drinking water in Argentina. *Epidemiology* 7: 117–124.
21. Bates, M. N., Smith, A. H. & Cantor, K. P. (1995) Case-control study of bladder cancer and arsenic in drinking water. *Am. J. Epidemiol.* 141: 523–530.
22. Buchet, J. P. & Lison, D. (1998) Mortality by cancer in groups of the Belgian population with a moderately increased intake of arsenic. *Int. Arch. Occup. Environ. Health* 71: 125–130.
23. Thomas, D. J., Styblo, M. & Lin, S. (2001) The cellular metabolism and systemic toxicity of arsenic. *Toxicol. Appl. Pharmacol.* 176: 127–144.
24. Cullen, W. R., McBride, B. C. & Reglinski, J. (1984) The reduction of trimethylarsine oxide to trimethylarsine by thiols: a mechanistic model for the biological reduction of arsenicals. *J. Inorg. Biochem.* 21: 45–60.
25. Le, X. C., Ma, M., Cullen, W. R., Aposhian, H. V., Lu, X. & Zheng, B. (2000) Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. *Environ. Health Perspect.* 108: 1015–1018.
26. Styblo, M., Del Razo, L. M., Vega, L., Germolec, D. R., LeCluyse, E. L., Hamilton, G. A., Wang, C., Cullen, W. R. & Thomas, D. J. (2000) Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in human cells. *Arch. Toxicol.* 74: 289–299.
27. Mass, M. J., Tennant, A., Ropp, B. C., Cullen, W. R., Styblo, M., Thomas, D. J. & Kligerman, A. D. (2001) Methylated trivalent arsenic species are genotoxic. *Chem. Res. Toxicol.* 14: 355–361.
28. Lin, S., Del Razo, L. M., Styblo, M., Wang, C., Cullen, W. R. & Thomas, D. J. (2001) Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. *Chem. Res. Toxicol.* 14: 305–311.
29. Del Razo, L. M., Garcia-Vargas, G. G., Vargas, H., Albores, A., Gonesbatt, M. E., Montero, R., Ostrowsky-Wegman, P., Kelsh, M. & Cebrián, M. E. (1997) Altered profile of urinary arsenic metabolites in adults with chronic arsenism. A pilot study. *Arch. Toxicol.* 71: 211–217.
30. Kenyon, E. M., Hughes, M. F., Del Razo, L. M., Edwards, B. C., Mitchell, C. T. & Levander, O. A. (1999) Influence of dietary selenium on the disposition of arsenite and arsenate in the female B6C3F1 mouse. *Environ. Nutr. Interact.* 3: 95–113.
31. National Research Council (1999) *Arsenic in Drinking Water*. 310 pp, National Academy Press, Washington, D.C.
32. National Research Council. (2001) *Arsenic in Drinking Water: 2001 Update*. National Academy Press, Washington, D.C.