Antimony: An Unlikely Confounder in the Relationship between Well Water Arsenic and Health Outcomes in Bangladesh

Kathleen M. McCarty,[1] David B. Senn,[1] Molly L. Kile,[1] Quazi Quamruzzaman,[2] Mahmuder Rahman,[2] Golam Mahiuddin,[2] and David C. Christiani[1]

[1]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; [2]Dhaka Community Hospital, Dhaka, Bangladesh

Recent *in vitro* studies have suggested a potential role for antimony as a confounder in human health studies related to arsenic in drinking water. We measured tube-well water concentrations of antimony and arsenic in the Pabna region of Bangladesh, where arsenic concentrations are known to be elevated and the concentrations of antimony have not yet been thoroughly documented. Two hundred forty-five tube-well water samples were collected from various regions in Pabna, Bangladesh, as part of an ongoing case–control study. Water samples were analyzed for arsenic and antimony concentrations by inductively coupled plasma–mass spectrometry using U.S. Environmental Protection Agency method 200.8. The arsenic concentrations in the tube-well water samples ranged from < 1 μg/L to 747 μg/L. All 245 water samples had antimony concentrations < 1 μg/L. Based on consideration of the concentrations used the *in vitro* studies compared with field-observed concentrations, our results do not support the hypothesis that antimony would be a significant confounder in observed relationships between arsenic exposure through drinking water and potential health outcomes in Pabna, Bangladesh. *Key words*: antimony, arsenic, Bangladesh, drinking water, tube well. *Environ Health Perspect* 112:809–811 (2004). doi:10.1289/ehp.6800 available via *http://dx.doi.org/* [Online 12 February 2004]

Arsenic toxicity is multifactorial, with predisposing factors hypothesized to be sex, age, nutritional status, genetic polymorphisms, and coexposure to other environmental contaminants (Guha Mazumder et al. 1998; Hsueh et al. 1998; Vahter 2000). The precise biologic mechanisms through which As exerts toxicity are currently not well established (Gebel 2000). In addition, there also appear to be differences in susceptibility to As between populations. Gebel (2000) hypothesized that coexposure to antimony may confound the relationship between As exposure and potential health outcomes. As and Sb individually exert toxic effects. However, two *in vitro* studies showed that coexposure to As and Sb can result in less cell damage than expected based on their individual toxicities, suggesting that coexposure to Sb and As may be subadditive. In an *in vitro* study of V79 Chinese hamster cells, Gebel (1998) found that chromosome mutagenicity induced by As(III) was significantly suppressed by Sb(III) in micronucleus tests. In sister chromatid exchange tests using human lymphocytes *in vitro,* Gebel et al. (1997) concluded that the combined effect for As and Sb was subadditive. Gebel and colleagues carried out their experiments using Sb concentrations ranging from 0 to 3,043 μg/L (0–25 μM), which were comparable with or greater than the molar concentration of As (2 μM or 148.9 μg/L). Whether or not Sb has the potential to amplify or attenuate the toxicity of As *in vivo* in humans at environmentally relevant exposure levels has not been explored in epidemiologic studies (Gebel 2000).

Sb and As have been found to co-occur in the environment (Gebel 2000). However, in most regions of the world where there are elevated concentrations of As in the drinking water, the presence and concentrations of Sb have not yet been thoroughly documented. Because of the potential for co-occurrence of As and Sb and confounding effects from concurrent exposure, it has been suggested that Sb concentrations be measured in regions of the world where As concentrations are known to be elevated and to assess the potential impact of Sb on human health (Gebel 1998, 1999, 2000).

Although extensive human health and hydrogeologic studies have documented the scope of the As crisis in Bangladesh (Ashan et al. 2000; Harvey et al. 2002; Nickson et al. 1995; Smith et al. 2000), the importance of Sb as a potential confounder of As exposure has not been adequately addressed. As part of a larger epidemiologic study of health effects of As exposure in Bangladesh, we measured Sb concentrations in a randomized subset of 245 tube-well water samples from our study region of Pabna to assess the potential for Sb to act as a confounder of As exposure.

Materials and Methods

We analyzed 245 water samples collected in 2001–2002 from tube wells in the Pabna district of Bangladesh, located north of Dhaka on the Jamuna River in central Bangladesh, as part of a case–control study conducted by the Harvard School of Public Health in collaboration with the Dhaka Community Hospital Trust. When each 100-mL sample of tube-well water was collected, two drops (0.2 mL) of concentrated nitric acid were added and the sample was sealed. Samples were stored at 4°C until analysis for As and Sb by inductively coupled plasma–mass spectrometry at Environmental Laboratory Services (North Syracuse, NY, USA) following U.S. Environmental Protection Agency (EPA) method 200.8 (U.S. EPA 2001a). The limit of detection (LOD) using this method was 1 μg/L for both As and Sb.

Results

All of the water samples were found to have < 1 μg/L Sb. The results of the water analysis for As are presented in Table 1. The data are presented as samples < 50 μg/L As or ≥ 50 μg/L; the As drinking-water standard is 50 μg/L in Bangladesh. Of the 245 water samples analyzed, 87 samples contained As < the LOD, and 107 samples contained 1–50 μg/L As, with a mean As concentration of 10.2 μg/L. In 51 samples containing ≥ 50 μg/L As, the mean was 299.9 μg/L. The certified reference material we used for Sb and As was QC Standard 1 (catalog no. 140-102-012; SCP Science, Champlain, NY), a multielement standard solution. The mean ± SD recovery rates for Sb and As were 101.1 ± 3.129% and 99.6 ± 3.896%, respectively.

Discussion

Sb and As are individually considered toxic to human health, and each is regulated in drinking water by the U.S. EPA. The U.S. EPA drinking water maximum contamination level goal for Sb is 6 μg/L (U.S. EPA 2002); the

Address correspondence to D.C. Christiani, Department of Environmental Health, Harvard School of Public Health, Building I 1408, 665 Huntington Ave., Boston, MA 02115 USA. Telephone: (617) 432-1260. Fax: (617) 432-3323. E-mail: dchristi@hsph.harvard.edu

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new U.S. EPA As drinking water standard is 10 µg/L (U.S. EPA 2001b). The International Agency for Research on Cancer (IARC) and the U.S. EPA have classified As as a human carcinogen, but IARC and the U.S. EPA have not classified Sb according to its human carcinogenicity (ATSDR 1995; IARC 2003). As and Sb have a shared affinity for binding to sulfhydryl groups (Bailly et al. 1991; Basinger and Jones 1981), and Gebel (1998) has hypothesized that competition between As and Sb for these sulfhydryl groups may be a mechanism by which Sb effects the genotoxicity of As.

Sb and As reside in the same chemical group; therefore, they exhibit some similarities in terms of their behavior in the environment (Smith and Huyck 1999). Sb and As are found primarily in two oxidation states in environmental and biologic samples, trivalent and pentavalent. Sb(III) and As(III) are each generally considered more mobile and more toxic than are Sb(V) and As(V) (Cullen and Reimer 1989; Filella et al. 2002). Sb and As have high affinities for forming complexes with sulfide and coprecipitating as sulfidic minerals (Smith and Huyck 1999). In addition, Sb and As undergo conversions between oxidized and reduced forms in response to changes in redox conditions and pH, and can be immobilized and mobilized by analogous processes in aquatic systems. Therefore, As and Sb may co-occur in groundwater. However, considering their average crustal abundances of 1.8 µg/g for As and 0.2 µg/g for Sb (Smith and Huyck 1999), As might be expected to occur in water at substantially higher concentrations than Sb.

No published epidemiologic studies have reported on the potential confounding effects of Sb and As co-occurrence in Bangladesh or elsewhere. There are, however, some recent data on Sb concentrations in Bangladesh groundwater. Frisbie et al. (2002) surveyed 112 wells throughout Bangladesh and across a variety of geochemical conditions and report that Sb concentrations ranged from 0.0015 to 1.8 µg/L. In that study, As concentrations varied between 0.7 µg/L and 640 µg/L, and interestingly, Frisbie et al. (2002) found no correlation (r^2 = –0.05) between As and Sb concentrations. Data from the British Geological Survey (Kinniburgh and Smedley 2001) also indicate that Sb concentrations are generally well below 1 µg/L across Bangladesh. Because of the extreme spatial variability in groundwater geochemistry in Bangladesh (Harvey et al. 2002) and postulated confounding effects of Sb on As toxicity, we chose to densely sample tube wells in our study region to determine whether Sb concentrations were consistently low in Pabna groundwater, where As concentrations vary more than three orders of magnitude (Table 1). The Sb concentrations < 1 µg/L that we found in Pabna are consistent with other measurements made across Bangladesh by the British Geological Survey (Kinniburgh and Smedley 2001) and Frisbie et al. (2002).

In order to assess whether Sb could measurably confound the relationship between As exposure and associated health outcomes in Pabna, it is important to consider the differences between laboratory and field conditions. In their laboratory studies, Gebel and colleagues directly treated cell cultures with varying concentrations of Sb and As: 1, 5, 10, and 25 µM SB and 2 µM As(III) (Gebel 1998); and 1 and 2 µM Sb and 0.02, 0.2, 1, 2, and 4 µM As(III) [Gebel et al. (1997); note that 1 µM Sb = 122 µg/L and 1 µM As = 75 µg/L]. The concentrations of Sb and As measured in Pabna tube-well water represent an external dose, and these concentrations were at least two orders of magnitude less than the cell-level Sb doses used in laboratory studies (in Pabna the concentration of Sb was < 0.008 µM and the concentration of As ranged from < 0.013 µM to 10 µM). In contrast, the *in vitro* 2 µM As doses were comparable with the external observed field concentrations of As in tube-well water in Pabna. Gebel (1998) found statistically significant suppression of As-induced chromosome mutagenicity by Sb at molar Sb:As ratios that ranged from 1:0.08 to 1:2. Gebel et al. (1997) found statistically significant subadditive genotoxicity at Sb:As ratios ranging from 1:0.01 to 1:2. In Pabna, we found that when As concentrations were greater than provisional guideline values [i.e., > 10 µg/L according to the World Health Organization (WHO 2001) and > 10 µg/L (0.13 µM) by 23 January 2006 according to the U.S. EPA (2001b)], the Sb:As ratio ranged from at least 1:16 to 1:1,200. Gebel (1998) hypothesized that subadditive genotoxicity effects from As and Sb coexposure may occur because of competition for sulfhydryl groups. If competition is the primary mechanism, it is necessary to consider both the absolute concentrations of Sb and As, and the concentration of Sb relative to As when assessing whether Sb is a significant confounder of As toxicity. Because of the lack of correspondence between laboratory conditions and field conditions, it is not possible to rule out a role for Sb in modulating As toxicity at the concentrations observed in the field. However, on the basis of results of published laboratory experiments, it seems unlikely that coexposure to Sb at the low concentrations of Sb and the observed Sb:As ratios in Pabna drinking water could measurably confound the relationship between As exposure and associated health outcomes. In addition, the fact that Sb is present at concentrations that are lower than the WHO and U.S. EPA safe concentrations (by a factor of ≥ 6) suggests that Sb will exert insignificant toxicity on its own.

Although it may be important to measure Sb concentrations in regions with anthropogenic sources of Sb, our results do not support the hypothesis that Sb would be an important confounding factor in the relationship between As exposure through tube-well water and potential health outcomes in Pabna, Bangladesh, and other regions with similar geochemistry.

Table 1. As concentrations in water from tube wells in Pabna, Bangladesh.

As concentration in water sample	No. of water samples	Mean ± SD (µg/L)	Median (µg/L)	Range (µg/L)
< LOD	87	NA	NA	NA
1–50 µg/L	107	10.2 ± 11.9	5.1	1.0–48.3
≥ 50 µg/L	51	299.9 ± 199.5	262.0	57.9–747.0

All 245 water samples were < LOD for Sb.

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