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Project Summary

Speciation of Arsenic Compounds in Water Supplies

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The objectives of this project were to develop and test analytical methods that would allow the chemical form (i.e. valence state or compound) of arsenic in drinking waters to be determined, and to use the methods to analyze samples of drinking water from sources where adverse health effects in consumers had been attributed to arsenic. Analytical techniques were developed for the determination of arsenate (differential pulse polarography), for inorganic and organic arsenic compounds (high pressure liquid chromatography with graphite furnace atomic absorption spectrometry as element-specific detector) and for the detection of arsenocholine, arsenobetaine, and iodoarsines (mass spectrometry). These techniques, inductively coupled argon plasma emission spectrometry, and hydride generation/DC-helium arc emission were used for the characterization of water samples from Utah, Alaska, Antofagasta, Taiwan and Nova Scotia. The total arsenic concentration ranged from 18 ppb to 8 ppm with arsenite/arsenate ratios between 0.007 and 3.4. No organic arsenic compounds were detected in any of the water samples. The trace elements Al, B, Ba, Ca, Cu, Fe, Li, Mg, Mn, Na, P, S, Si and Sr were present in most of the water samples. The results show that the various physiological effects observed in populations exposed to the arsenic-containing water supplies could not be caused by arsenic compounds other than arsenite or arsenate. Other trace elements acting in concert with arsenite and/or arsenate might produce these symptoms. However, sufficient data are not yet available to evaluate these hypotheses.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Arsenic is an element possessing a rich chemistry Inorganic and organic arsenic compounds may contain trivalent arsenic The trivalent arsenic compounds are generally more toxic than the pentavalent derivatives. Many inorganic and organic arsenic compounds are linked in a cycle with chemical and biologically mediated reactions changing the compounds into each other The input of arsenic into this cycle is supplied by weathering of arsenic-containing rocks, human use, and disposal of various arsenic compounds

Since arsenic is ubiquitous, man consumes small amounts of arsenic compounds with the food he eats and the water he drinks. Life developed in the presence of arsenic. Therefore, organisms are expected to tolerate a certain, not yet clearly defined, dosage of arsenic. Certain geographically limited groups of people have taken arsenic compounds into their systems over extended periods of time. These arsenic compounds are present in their drinking water supplies. The most famous localities where arsenic-containing waters have been consumed are certain regions in Taiwan and the city of Antofagasta in Chile. Hyperpigmentation, skin cancer, vascular problems and other ailments have been attributed to the arsenic present in the drinking water. Other groups, such as the people in Fallon, Nevada, have been exposed to similar arsenic levels in their drinking water without any ill effects. This project was undertaken in order to determine the arsenic compounds and other trace elements present in arsenic-containing water supplies and to check whether these drinking water supplies contain the same or different arsenic compounds.

Thus far, arsenite, arsenate, methylarsinic acid, dimethylarsinic acid, methylarsines, arsenobetaine, trimethylarsoniolactic acid and arsenic-containing lipids have been identified in environmental samples.

Preservation of Arsenic Compounds in Aqueous Solutions

Water samples generally cannot be analyzed for trace elements immediately after collection. Several hours or even several days elapse between collection and analysis. During this time the chemical nature of a trace element, such as arsenic, can change. Trace elements can be lost by volatization and/or can be adsorbed on container walls. The absorption of many metal ions and of phosphate ions has been studied, but arsenic was rarely included.

Disagreements exist in the literature as to the extent of loss of arsenic from solutions stored in various containers. Even less certainty exists about the conditions under which various arsenic compounds can be preserved. Experiments have shown that arsenite, arsenate and dimethylarsinic acid are not adsorbed from 1 ppm solution on the walls of Cubitainers* (soft polyethylene containers manufactured by Kimberly) Walls of Pyrex containers removed approximately one percent of the arsenic from the solutions. Ascorbic acid at a concentration of 1 mg/mL has been found to prevent the oxidation of arsenite to arsenate in distilled water solutions at room temperature. On the basis of these results most water samples were collected and stored in Cubitainers and some of the samples were preserved by adding ascorbic acid.

Development of Analytical Techniques for the Determination of Arsenic Compounds

Whereas adequate methods for the determination of total arsenic concentrations do exist, the choice of techniques for the estimation of arsenic compounds is limited. All the methods available for the speciation of arsenic compounds at the time this project was initiated had severe limitations. The methods were applicable only to the determination of arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, ethyl-, propyl- and butylarsonic acid, and the arsines obtainable from these compounds. Non-volatile arsenic compounds and arsenic compounds not reducible to volatile arsines could not be determined with the existing methods. Therefore, an analytical system with an element-specific detector had to be developed that was capable of separating volatile and non-volatile arsenic compounds in complex matrices.

The development efforts produced a high pressure liquid chromatography-Hitachi Zeeman graphite furnace atomic absorption system, a differential pulse polarographic method for the determination of arsenite and arsenate and the elucidation of the mass spectral behavior of organylarsenic acids, organyl iodoarsines, arsenocholine and arsenobetaine.

Hitachi Zeeman Graphite Furnace Atomic Absorption Spectrometer as an Element-Specific Detector for High Pressure Liquid Chromatography

Liquid chromatography and specially high pressure liquid chromatography (HPLC) with the great resolving power of its microparticulate columns are potentially the best techniques for the simultaneous detection and determination of arsenic compounds. A water sample may contain many substances in addition to arsenic compounds. The common detectors will not respond specifically to arsenic compounds. The identification of arsenic-containing fractions is, therefore, difficult if not impossible unless element-specific detectors with high sensitivity are available. A graphite furnace atomic absorption spectrometer (GFAA) combines the advantage of element-specificity with high sensitivity for many elements. An HPLC-GFAA analytical system was developed employing a Hitachi Zeeman GFAA with a sample valve, an injector, and associated electronics to control the analysis sequence.

The HPLC-GFAA system has functioned almost flawlessly during the past three years. Aliquots of the column effluent are automatically transferred into the graphite cup of the GFAA for analysis. The time interval between consecutive analyses can be made within 30 seconds. The Hitachi Zeeman GFAA Model 170-70 has a detection limit for arsenic of 10 picograms. This sensitivity is, of course, retained in the HPLC-GFAA system for each injection. Upon migration through the chromatographic column the arsenic compounds are separated and spread out into bands. Aliquots of 40 μL are withdrawn from the effluent. The 40 μL aliquots taken from the center of the band must each contain at least 10 picograms of arsenic. The detection limit of the HPLC-GFAA system is, therefore, strongly dependent on the degree of band spreading.

Conditions have been found which allow the separation of inorganic arsenic (arsenite and arsenate), arsenocholine and arsenobetaine employing a C-18 reverse phase column, organylsulfonates as counterions and mixtures of water/acetonitrile/acetic acid as the mobile phase. Arsenite, arsenate, methylarsonic acid and dimethylarsinic acid were similarly separated using water/methanol mixtures saturated with tetraheptylammonium nitrate as the mobile phase. The HPLC-GFAA system, of course, is neither limited to the analyses of the arsenic compounds listed above nor to compounds containing only arsenic.

*Mention of tradenames or commercial products does not constitute endorsement or recommendations for use

Differential Pulse Polarographic (DPP) Determination of Arsenate and Arsenite

Arsenite is reducible at the dropping mercury electrode and can be determined polarographically at concentrations as low as 0 3 ppb. Arsenate is polarographically inactive under these conditions. Addition of polyhydroxy compounds to an acidic solution of arsenate makes arsenate reducible Among 11 polyhydroxy compounds, *D*-mannitol at 0.5 *M* concentration in 2 0 *M* aqueous perchloric acid produced the largest reduction peak for arsenate The DPP curve of arsenate under these conditions is characterized by maxima at -0 55 V and -0 75 V Above an As (arsenate) concentration of 500 ppb a current maximum appears at -0 59 V, which increases in intensity with increasing concentration The peak at -0 55 V merges into the current maximum and becomes a shoulder at As(arsenate) concentrations of approximately 5 ppm The rather low intensity peak between -0 7 V and -0.8 V might be obscured at low arsenate concentrations by the solvent breakdown and at high arsenate concentrations by the current maximum The arsenite reduction wave in 2 0 *M* perchloric acid solution shifts from -0.425 V to -0 34 V upon addition of mannitol

When arsenite and arsenate are present in solution, the arsenate reduction peak at -0 55 V can be used for the determination of arsenate with some confidence only when the concentration of As(arsenite) is between 100 ppb and approximately 500 ppb, the current is not lower than 2 μÅ and the arsenate concentration is equal to or higher than the arsenite concentration If these conditions are not fulfilled, arsenite must be oxidized to arsenate by cerium(IV) ammonium nitrate. Excess cerium(IV) must be reduced with hydroxylamine hydrochloride Arsenate is then determined in the presence of mannitol using the peak at -0 55 V. Arsenite is determined in another aliquot of the sample in the absence or presence of mannitol. The arsenate concentration in the sample is obtained as the difference between the total arsenic concentration and the As(arsenite) concentration The detection limits for arsenate under these conditions are 6 ppb at the 95 percent confidence level

Mass Spectrometry of Organylarsonic Acids, Diorganylarsinic Acids, Organyliodoarsines, Arsenocholine and Arsenobetaine

Organic arsenic compounds could perhaps be determined by mass spectrometry in the residues obtained by evaporation of the water samples Therefore, the mass spectral behavior of several organic arsenic compounds was studied

At probe temperatures between 110°C and 250°C required to obtain satisfactory mass spectra, organylarsonic acids, $RAsO_3H_2$, and diorganylarsinic acids, R_2AsOOH, formed anhydrides and decomposed The products of these thermal reactions were then ionized and fragmented yielding complicated mass spectra with many peaks at m/e values higher than those expected for the molecular ions A detailed investigation of the spectra of five arsonic acids and nine arsinic acids indicated that mass spectrometry was of little value for the identification of arsonic acids, but can be used to establish the presence of diorganylarsinic acids in the residues from water samples Exact mass measurements by high resolution mass spectrometry might be necessary to distinguish arsenic-containing from arsenic-free ions

Organyliodoarsines, R_nAsI_{3-n} (n=1,2), are much more volatile than arsinic or arsonic acids and are easily prepared by treating these acids with hydriodic acid All of the 14 organyliodoarsines investigated gave intense molecular ion peaks Fragmentation proceeded by loss of alkyl groups, iodine and hydrogen abstraction Organyliodoarsines are well suited for the mass spectrometric identification of organic arsenic compounds which can be converted to iodoarsines

Arsenocholine chloride, $[(CH_3)_3AsCH_2CH_2OH]Cl$, and arsenobetaine chloride, $[(CH_3)_3AsCH_2COOH]Cl$, produce rich mass spectra which do not contain molecular ion peaks The highest mass peaks correspond to $(CH_3)_3AsCH_2CH_2O$ and $(CH_3)_2AsCH_2COOH$, which were formed by thermal cleavage of HCl and CH_3Cl from the arsonium salts In spite of the absence of molecular ions, mass spectrometry can provide an indication of the presence of arsenocholine and/or arsenobetaine in a sample.

Analysis of Water Samples

Samples of arsenic-containing drinking water supplies selected by the EPA project officer were collected and shipped to College Station as quickly as possible Total arsenic concentrations and the concentrations of arsenite and arsenate were determined by several methods Each water sample was checked for the presence of methylated arsenic compounds and other organic arsenic derivatives Water samples from Hinckley, Utah, Delta, Utah, Barefoot Site, Alaska, Mauer Site, Alaska, Antofagasta, Chile, Yenshei, Taiwan; Hartlin Site, Nova Scotia; and Sullivan Site, Nova Scotia were investigated

Graphite furnace atomic absorption spectrometry, differential pulse polarography, high pressure liquid chromatography with a GFAA as an element-specific detector, the hydride generation technique with a DC-helium arc detector, and inductively coupled argon plasma emission spectrometry were employed for the determination of concentrations of total arsenic, and trace elements The samples were collected and stored in Cubitainers or Pyrex glass containers Unpreserved samples and samples preserved with ascorbic acid or nitric acid were analyzed The analyses were carried out as soon as possible after receipt of the samples

The water samples had total arsenic concentrations in the range of 18 ppb to 8 ppm The arsenite/arsenate ratios were in the range of 0 007 to 3 4 (Table 1) No indications of the presence of methylated arsenic compounds, which are reducible to methylarsine or dimethylarsine, have been found Experiments with the high pressure liquid chromatograph/graphite furnace atomic absorption spectrometer system, which would provide information about the presence of organic arsenic compounds not reducible to methylarsines, detected only arsenite and arsenate Comparison of total arsenic concentrations with the sum of the arsenite and arsenate concentrations placed an upper limit on the concentrations of any other arsenic compounds which might be present These upper limits were in most cases in the low ppb range

The other trace elements found in these water samples by ICP are also listed in Table 1. There were no significant concentrations of these elements

Table 1. *Summary of Total Arsenic, Arsenite, Arsenate and Trace Element Concentrations in Drinking Water Samples†*

	Hinckley	Delta	Barefoot††	Mauer††	Antofagasta Untreated	Antofagasta Treated	Yenshei I	Yenshei II	Nova Scotia 1	Nova Scotia 2
Total As	0 18	0 02	3 1	4 5-6 0	0 75	0 41**	0 85	1 1	8 0	0 63
Arsenite	0 010	0 010	2 4	0 35-4 6	0 016	0 003	0 023	0 024	4 5	0 31
Arsenate	0 18	0 010	0 7	0 1-4 3	0 74	0 41	0 84	1 08	3 5	0 32
Arsenite/ Arsenate Ratio	0 06	~1 0	3 4		0 02	0 007	0 03	0 02	1 3	1 0
Al	—	—	0 06-0 50	0 06-0 54	0 08	3 1	—	—	*	*
B	1 2	0 01	—	—	2 8	2.8	—	0 87	*	*
Ba	0 026	0 045	0 17	0 29	0 008	0 008	—	—	*	*
Be	—	—	—	—	7 4	—	—	—	*	*
Ca	3 24	15 4	180-291	200-309	20 0	20 3	4 3	17 5	*	*
Cu	0 08	0 26	—	—	0 003	0 007	—	—	0 008	0 21
Fe	—	0 007	29-53	25	0 11	0 30	0 46	0.50	<0.1	<0 1
K	—	—	12	13-118	13 3	13 2	1 0	13 6	*	*
Li	—	—	—	0 007	0 62	0 64	0 007	0 01	*	*
Mg	1 83	6 65	52-113	55-139	7 4	7 5	8 6	23	*	*
Mn	—	—	0 42	0 56-0 76	0 002	0 006	—	—	2 4	<0 1
Na	233	65	37-47	35-50	102	103	280	196	60	50
P	—	—	0 27	—	0 34	0 26	4 9	2 3	*	*
Pb	—	1 17	—	—	—	—	—	—	*	*
S	*	*	28	17 5	*	*	0 5	1 5	*	*
Si	13 4	15 3	11 0	11 0	38 7	36 3	3 4	3 4	*	*
Sr	0 10	0 52	0 47	0 60	0 28	0 29	0 08	0 20	*	*
Ti	—	—	0 008	0 009	—	—	—	—	<0 1	<0 9
V	—	—	0 15	0 17	—	—	—	—	*	*
Zn	—	—	0 14	0 30	0 10	0 10	—	—	*	*

*Not determined
**As in treated water is normally less than 100 ppb See text for discussion
†The concentrations are given in ppm
††Results from two different samples collected one year apart

in these water supplies with the exception of beryllium found in the untreated Antofagasta sample.

The various physiological effects observed in populations exposed to these arsenic-containing drinking water supplies (Table 2) could have been caused by the presence of varying amounts of arsenite and arsenate It is also conceivable that one or more of the trace elements present in the water supplies acted in concert with arsenic to cause the observed effects More samples need to be analyzed and the results of these analyses correlated with epidemiological studies before a definite statement can be made about the interactions of trace elements with arsenite or arsenate

The chromatographic work on the fluorescent compounds in the Taiwan well waters strongly suggests the presence of alkaloids, such as *D*-lysergic acid, ergometrine and calciferol Additional experiments (preparative chromatography, mass spectrometry) could not be carried out because of insufficient samples

Table 2. *Arsenic-Containing Water Supplies and Their Physiological Manifestations in Man*

Sampling Location	Type of Water and Range of the Total Arsenic Concentration	Symptoms Observed in the Population
Taiwan (37 villages)	Artesian well waters used for 45 years, arsenic leached from geologic deposits, 0 017-1.097 ppm; median 0 5 ppm	Melanosis, keratosis, skin cancer 15% prevalence among males over age 60, normal incidence 2-3%
Chile (Antofagasta)	Drinking water supply since 1958, 0 8 ppm before water treatment, 0 1 after water treatment	Melanosis, hyperkeratosis, vascular manifestations: myocardialischemia, hemipleglia with occlusion of the carotid artery, mesenteric arterial thrombosis, pneumonia
Bakersfield, CA.	Community drinking water supply, 0.3-0 7 ppm	No adverse effects reported *
Fallon, NV	Drinking water, 0.1 ppm.	No known adverse physiological effects.*

*In a study of the arsenic exposure of populations in Bakersfield, Ca , and Fallon, Nv., by a questionnaire designed to elicit information about arsenic related symptoms and diseases, very few symptoms were reported The incidence of these symptoms was not significantly different from control populations not exposed to arsenic

Recommendations

The use of methods such as the hydride generation technique; differential pulse polarography, high pressure liquid chromatography with sensitive, element-specific detectors and colorimetric methods for determining concentrations of total arsenic and arsenic compounds, and the availability of simultaneous, inductively coupled argon plasma emission spectrometers for trace element determinations and of ion chromatography for anion analyses make the thorough characterization of water samples a relatively easy and not too-time-consuming task. Additional arsenic-containing water samples must be analyzed in support of or in preparation for epidemiological studies. Experience has shown that analysis by one method is not sufficient to produce reliable results. At least two independent techniques should be used for the determination of arsenic compounds.

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The complete report, entitled "Speciation of Arsenic Compounds in Water Supplies," (Order No. PB 82-257 817; Cost: $12.00, subject to change) will be available only from:
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