Environ. Sci. Technol. **2005**, *39,* 248−254

X-ray Absorption Near-Edge Structure Analysis of Arsenic Species for Application to Biological Environmental Samples

PAULA G. SMITH,[†,‡] IRIS KOCH,[‡] ROBERT A. GORDON,[§,#] DINA F. MANDOLI,[∥] BRANDON D. CHAPMAN,[⊥,○] AND KENNETH J. REIMER*[,†,‡]

Biology Department, Queen's University, Kingston, Ontario, Canada K7L3N6, Environmental Sciences Group, Royal Military College, P.O. Box 17000, Station Forces, Kingston, Ontario K7K7B4, Canada, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, Canada V5A1S6, Pacific Northwest Consortium Collaborative Access Team, Advanced Photon Source, Argonne National Laboratory, 9700 South Cass Avenue, Building 435E, Argonne, Illinois 60439, Department of Biology, University of Washington, P.O. Box 351800, Seattle, Washington 98195-1800, Department of Physics, University of Washington, P.O. Box 351560, Seattle, Washington 98195-1560, and NSLS, Brookhaven National Laboratory NSLS, Building 725D, P.O. Box 5000, Upton, New York 11973-5000

Arsenic is an element that is ubiquitous in the environment and is known to form compounds with toxic, even carcinogenic properties. Arsenic toxicity is a function of its chemical form (species). Identification of arsenic species is necessary to accurately determine the transformation and fate of arsenicals as well as the actual risk posed by arsenic contamination. We report X-ray absorption near-edge structure (XANES) measurements of 16 biologically important arsenic compounds. Solid and aqueous standards were studied for differences in XANES spectral features, white line positions, stability during exposure to the beam, and stability between two beam exposures separated by 48 h. Samples containing As(III) (11870.0−11871.7 ± 0.5 eV) and As(V) (11872.6−11875.3 ± 0.5 eV) were easily distinguished by white line energies and could be further subdivided into a total of seven groups. Valuable examples include As(III)−sulfur compounds (11870.0 ± 0.5 eV), arsenobetaine and arsenocholine (11872.6 ± 0.5 eV), and a dimethyl arsinyl riboside (11873.3 ± 0.5 eV). A growing number of environmental and biological studies use X-ray absorption spectroscopy (XAS) results to complement their more traditional analyses. Results provided here are intended to help make XAS more accessible to new users interested in the study of arsenic in the environment.

* Corresponding author phone: (613)541-6000, ext 6161; fax: (613)541-6596; e-mail: reimer-k@rmc.ca.
[†] Queen's University.
[‡] Royal Military College.
[§] Simon Fraser University.
[#] Advanced Photon Source.
[∥] Department of Biology, University of Washington.
[⊥] Department of Physics, University of Washington.
[○] Brookhaven National Laboratory.

Introduction

Arsenic (As) is ubiquitous in the earth's crust in a variety of minerals including arsenopyrite (FeAsS), orpiment (As_2S_3), and realgar (As_4S_4). It can then be released into the environment through natural weathering. Mining, industrial activities, and to a lesser extent, agricultural practices, are common anthropogenic sources of arsenic compounds. Once introduced, the chemical form of arsenic is altered by a variety of biogeochemical processes (*1*−*3*).

The two most common soluble forms of inorganic arsenic in environmental samples are arsenate (with arsenic in the +5 oxidation state, i.e., As(V)) and arsenite (with arsenic in the +3 oxidation state, i.e., As(III)). In aerobic environments arsenate is thermodynamically predicted to occur as an oxyanion ($H_2AsO_4^-$ and/or $HAsO_4^{2-}$), while arsenite (as $As(OH)_3$) dominates under reducing conditions (*4*−*6*). Organic arsenic compounds produced from the biotransformation of these inorganic arsenicals are widely distributed in biological tissues and to a lesser extent in soils and water. Figure 1 displays the chemical formula of 16 environmentally important arsenic compounds. Each of these varies in toxicity and potential for accumulation in organisms (*1*). It is therefore important to identify these compounds in samples to better understand and predict the risks they may present to human, wildlife, and plant populations.

While much has been learned about the distribution of inorganic and organic arsenic compounds in the environment, there are still a number of unanswered questions (see Supporting Information). Inorganic arsenic species are known to be converted to methylated arsenicals via biochemical pathways (*5*, *7*), and methylated compounds are common in the terrestrial environment. The presence of simple methylated compounds such as MMA(III and V) (monomethylarsonous acid and monomethylarsonic acid), DMA(III and V) (dimethylarsinous and dimethylarsinic acid), TMAO (trimethylarsine oxide), or Tetra (tetramethylarsonium ion) indicates metabolism of arsenic either by microbes or more complex organisms (*1*, *8*). Methylated arsenic compounds formed or taken up by organisms occupying a lower trophic level (e.g., mushrooms, algae, earthworms) are thought to be the precursors for more complex organoarsenicals such as AB (arsenobetaine) or the arsenosugars that have been identified in these organisms (*1*, *9*, *10*), but the actual pathways of, for example, arsenobetaine synthesis have yet to be elucidated. In some cases the presence of more complex organoarsenicals in organisms occupying higher trophic levels in the marine environment (e.g., fish) might indicate accumulation from an outside source, most likely through ingestion (*1*, *11*). Conversely, some compounds (e.g., TMAO) may result from catabolism of more complex arsenicals (*1*).

Traditionally arsenic species are identified using time-consuming, indirect methods, that determine the identity of the compound through chemical manipulation. Pretreatment can involve physical homogenization and drying of samples, processes that may alter the chemical form of the arsenic. Strong acid is used to determine the total amount of arsenic in a sample but cannot distinguish particular arsenic species. Extraction methods have therefore been designed to extract only specific arsenic species (*12*). For example, water or methanol/water is typically believed to extract the mobile component of arsenic in the sample (*12*, *13*), whereas hydrogen peroxide extracts arsenic bound more tightly by organic matter. Extraction methods using oxalic acid and sodium dithionite are used to release arsenic bound by metal

10.1021/es049358b CCC: $30.25
© 2005 American Chemical Society
Published on Web 11/18/2004

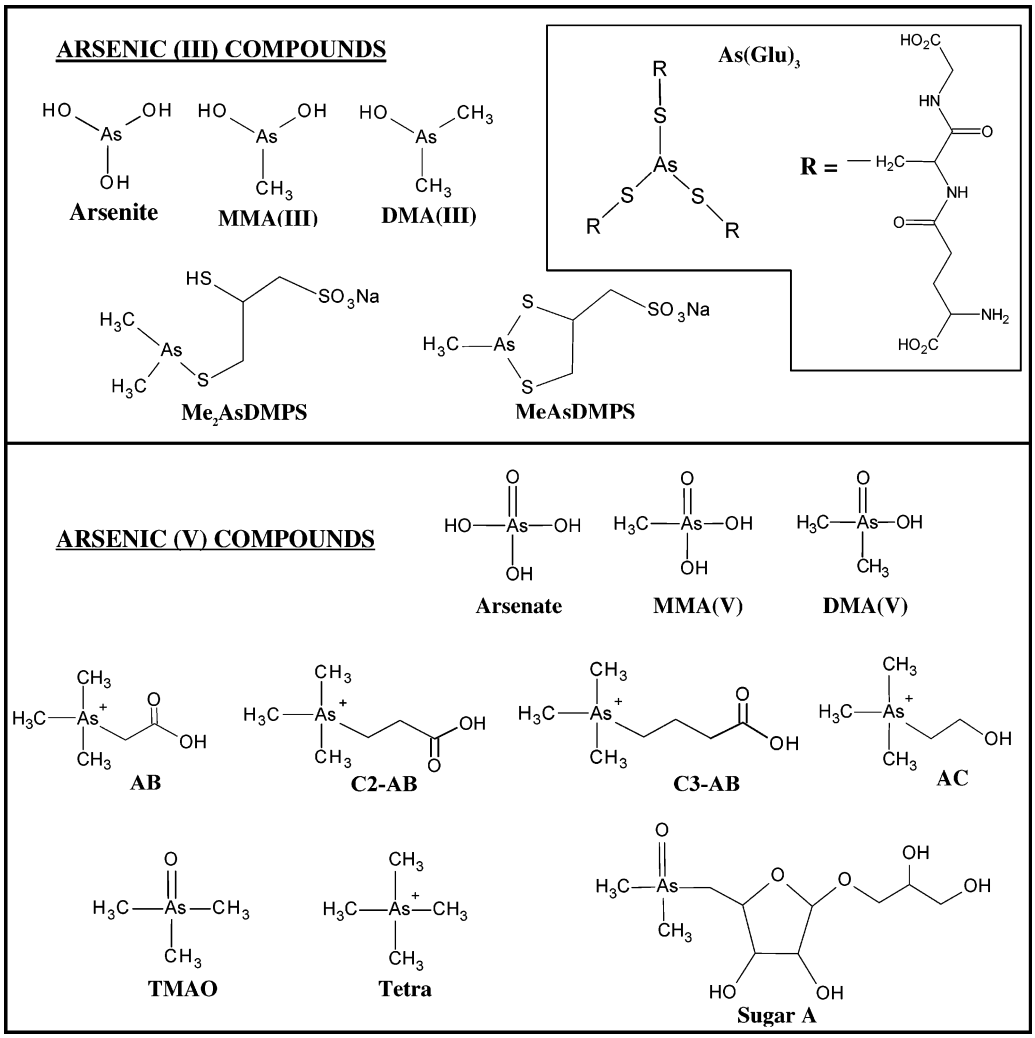

FIGURE 1. Chemical structures of 16 model arsenicals most relevant to biological systems. MMA(III), monomethylarsonous acid; DMA(III), dimethylarsinous acid; Me_2As, dimethylarsenic;, MeAs, monomethylarsenic; DMPS, 2,3-dimercapto-1-propane sulfonic acid; $As(Glu)_3$, arsenic glutathione; MMA(V), monomethylarsonic acid; DMA(V), dimethylarsinic acid; TMAO, trimethylarsine oxide; Tetra, tetramethyl arsonium; AB, arsenobetaine; C2-AB, arsenobetaine 2; C3-AB, arsenobetaine 3; AC, arsenocholine; sugar A, a dimethylarsinyl riboside.

oxyhydrides, and diethyl ether removes arsenic bound by lipids. Once extracted, arsenicals are commonly separated using ion exchange, size exclusion, and reverse-phase high-performance liquid chromatography (*12*). Different arsenic compounds can be measured by element-specific detection methods (e.g., atomic absorption spectrometry or inductively coupled plasma mass spectroscopy) (*12*, *13*). Although these techniques have the advantage of low (i.e., parts per billion) detection limits, they are often limited by incomplete extraction; this is particularly true for biological samples from the terrestrial environment (*13*). X-ray absorption spectroscopy (XAS) is a quick means of sample analysis that minimizes sample manipulation, is indifferent to solution or solid-phase samples, is element and oxidation state specific, and is sensitive to parts per million (*14*, *15*).

By convention, XAS is divided into two regions: X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). XANES analysis focuses on the absorption-edge region of the XAS spectrum from a few electronvolts below an element's K-edge to approximately 30 eV above the edge. The K-absorption edge (K-edge), or ionization threshold, is defined as the energy required to remove an electron in a 1s orbital and eject it into the continuum. The white line energy is defined as the energy required to transfer a 1s electron to a bound orbital below the ionization threshold. Features identified in XANES analysis reveal the most probable chemical environment around the arsenic, through the identification of oxidation state, and atom type and arrangement. Higher K-edge energies generally indicate a higher oxidation state because the greater positive charge of these compounds makes it more difficult to photoionize the 1s electron. These data in turn can be used to identify and quantify phases present in a sample (*16*). The EXAFS part of the spectrum has a typical range of 30 eV up to 1000 eV above the K-edge and provides direct structural information (e.g., near-neighbor type, distance, and coordination number).

In environmental and biological studies, XAS has been used to study the interaction of metals with surfaces of major mineral phases (*6*, *17*–*19*), the oxidation state and coordination of metals to soils and proteins, and the local structural environment of metals in living organisms (*15*). For the study of arsenic, XAS has been used to examine arsenic speciation in mine tailings (*16*, *20*) and in arsenic-tolerant plants and earthworms (*21*, *22*). XANES is used to assign chemical identity empirically (compare sample spectra to known compounds) and not quantitatively through simple mathematical formula. Inappropriate comparisons of, for example, pure crystalline structures to molecular structures in biological samples may make it difficult to determine the composition and/or relative concentrations of the compounds present in the sample of interest. To maximize the usefulness and

accuracy of XAS for the identification of arsenic species within biological samples, the scientific community needs the spectral fingerprints of a comprehensive, expanded list of model compounds. To this end we have studied 16 individual organic and inorganic arsenic compounds (Figure 1) that are frequently detected in a variety of organisms. We have characterized their XANES for use as standards relevant to samples taken from the environment.

Experimental Approach

Standards Preparation. Standards were classified as containing arsenic in either the V or the III oxidation state. Starting materials containing As(V) included arsenate (obtained as KH_2AsO_4, Fluka reagent grade), monomethylarsonic acid [MMA(V), Pfaltz and Bauer reagent grade], dimethylarsinic acid [DMA(V), Aldrich reagent grade], trimethylarsine oxide (TMAO, synthesized), tetramethylarsonium iodide (Tetra, synthesized; *23*), arsenobetaine (AB, synthesized; *24*), arsenobetaine 2 (C2-AB, synthesized; *11*), arsenobetaine 3, (C3-AB, synthesized; *11*), arsenocholine (AC, synthesized; *24*), and (*R*)-2,3-dihydroxypropyl-5-deoxy-5-dimethylarsinyl-β-D-riboside (sugar A, synthesized; *25*). Starting materials containing As(III) included arsenite (obtained as As_2O_3, Fluka reagent grade), monomethylarsenic(III) diiodide (MMA(III), synthesized; *26*), dimethylarsenic(III) iodide (DMA(III), synthesized; *27*), dimethylarsenic 2,3-dimercapto-1-propane sulfonic acid sodium salt (Me_2AsDMPS, synthesized; *28*), monomethylarsenic DMPS (MeAsDMPS, synthesized; *28*), and arsenic glutathione ($As(Glu)_3$, synthesized; *29*). Synthesized compounds were prepared by Drs. H. Sun and W. R. Cullen using standard methodologies. All such compounds were provided in a pure (>97%) state as determined by nuclear magnetic resonance (NMR), elementary analysis (EA), and mass spectroscopy (MS). Sugar A, DMA(III), and TMAO were only available as aqueous solutions; the remaining arsenic compounds were originally obtained as solids.

Both solid samples and aqueous solutions (dissolved in distilled deionized H_2O) of the arsenicals were analyzed. Solid arsenic standards were mixed with silica and sealed into 3 mm deep wells of an aluminum sample holder with Kapton tape on either side. Silica was chosen as a neutral material for diluting standards (Table 1); however, biological matrixes are predominantly water; therefore the XANES of the arsenic compounds in aqueous solution are probably most relevant. Aqueous preparations were absorbed into a piece of Kimwipe, and then the saturated samples were mounted between two strips of Kapton for XANES analysis (Table 1). During analysis, samples prepared in this fashion were observed by camera and did not leak. These samples were visually moist after analysis. Aqueous arsenic standards mounted in the aluminum sample holder and/or a wet cell during an independent beamtime session (Figure 2 open circles) had similar XANES spectral shape to samples absorbed into Kimwipe. Undiluted standards (arsenite, arsenate, DMA(V), MMA(V)) were mounted as a thin layer of powder between two pieces of Kapton tape.

Spectroscopic Techniques. XANES and EXAFS spectra were collected at the bending magnet beamline of the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT), Sector 20 at the Advanced Photon Source (APS), Argonne National Laboratory (*30*). A silicon (111) double-crystal monochromator (bandwidth ~ 5000 $\Delta E/E$ at 12 keV, detuned to 85% of maximum intensity for harmonic rejection, with 1 mm slits 51 m from the source) and a rhodium-coated harmonic mirror for further planar rejection provided X-rays for measurement. The monochromator was calibrated using the first inflection point of the gold L_{III} absorption edge (11919.7 eV; *31*) for measurements at the arsenic K-edge (11868 eV). A slit of 1 mm vertical by 4 mm horizontal was used after the mirror to define the size of the X-ray beam incident on upon the sample. Nitrogen-filled transmission ionization chambers were present before and after the samples for normalization to the incident intensity and transmission measurements, respectively. Fluorescence data for aqueous preparations were collected using a solid-state Ge(Li) detector (Canberra model GL0055PS) or an argon-filled fluorescence ionization chamber, and no self-absorption was observed for the compounds studied (*32*). Transmission measurements were collected for the undiluted samples, and some silica diluted samples using a nitrogen-filled parallel plate transmission ion chamber. Typically, 5 scans were collected and averaged (0.5 eV step size over the edge) before background removal and normalization-to-edge jump. The program, WinXAS (*33*), was used for processing the spectra.

TABLE 1. List of Arsenic Standards for Sample Comparisons[a]

As standard	solution concn (ppm)	solid concn (ppm)	pH
arsenite	1000	102271	9.0
MMA(III)	50	7635	0.5
DMA(III)	immiscible	na	1.0
MeAsDMPS	10052	10408	4.0
Me_2AsDMPS	9495	10056	5.0
$As(Glu)_3$	10294	3766	na
arsenate	10408	10936	5.0
MMA(V)	870	10284	9.0
DMA(V)	968	10 643	6.5
TMAO	100	na	na
Tetra	10540	10000	5.0
arsenobetaine	100	10009	2.0
arsenobetaine 2	~1000	10000	na
arsenobetaine 3	~1000	10000	na
arsenocholine	12128	10817	4.0
sugar A	~100	na	4.5

[a] Acronyms are defined in terms of the starting material: arsenite, As_2O_3; MMA(III), monomethylarsenic(III) diiodide; DMA(III), dimethylarsinic(III) idodide; Me_2As, dimethylarsenic; MeAs, monomethylarsenic; DMPS, 2,3-dimercapto-1-propane sulfonic acid; $As(Glu)_3$, arsenic glutathione; arsenate, KH_2AsO_4; MMA(V), monomethylarsonic acid; DMA(V), dimethylarsinic acid; TMAO, trimethylarsine oxide; Tetra, tetramethylarsonium iodide; AB, arsenobetaine; C2-AB, arsenobetaine 2; C3-AB, arsenobetaine 3; AC, arsenocholine; sugar A, a dimethylarsinyl riboside. [b] Standards selected have the most relevance to biological systems. Standards were prepared as aqueous solutions (diluted with distilled deionized H_2O) and as solids (mixed with silica). pH values correspond to aqueous standards (na = not available).

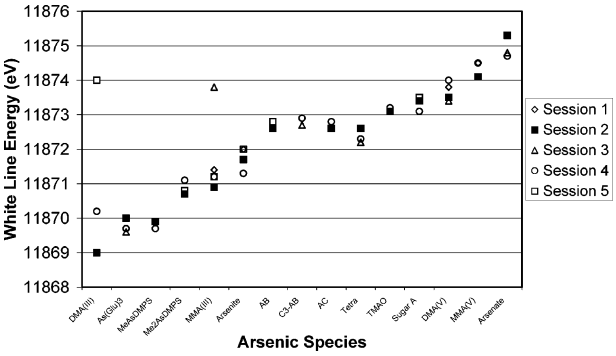

FIGURE 2. White line (peak of the absorption edge) energies for arsenicals in aqueous arsenic solution analyzed during independent sessions at the beamline. Generally, white line energies were reproducible within 0.5 eV. DMA(III) was not reproducible. Session 5 DMA(III) and session 3 MMA(III) may have oxidized to DMA(V) and MMA(V), respectively. The experiment testing both aqueous and silica standards took place during session 2.

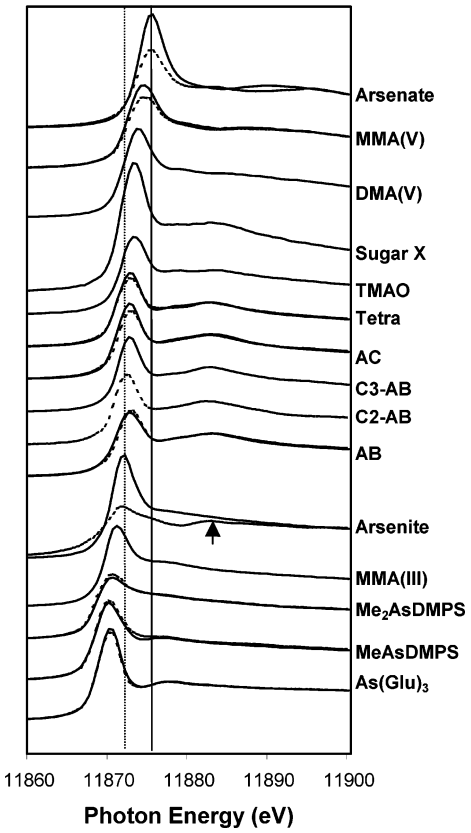

FIGURE 3. XANES spectra for 14 aqueous (solid line) and 10 solid mixed with silica (dotted line) arsenic compounds. Solid vertical line = arsenate white line position (11875.3 eV). Dotted vertical line = arsenite white line position (11871.7 eV). Arrow indicates spectral feature present in arsenite + silica sample but not in aqueous arsenite samples.

Results and Discussion

Analysis of standards was conducted to determine (a) if solid samples and aqueous solutions of the same arsenic compound had similar white line energies and XANES spectral features, (b) if standards suffered radiation-induced changes due to beam exposure, and (c) if aqueous arsenic standards were stable over a period of 48 h. XANES spectra for the arsenic compounds analyzed are given in Figure 3. The white line energy for arsenate was observed to be 3.6 eV greater than that of arsenite. The greater positive charge of arsenate causes the core electrons to be bound more tightly by the nucleus; therefore, more energy is required to remove one of these electrons. Generally for every electron lost there is a 2−3 eV increase in observed white line energy (*34*). Group 1 compounds have lower white line energies than arsenite due to the difference in electronegativities of oxygen and sulfur. Core electrons of arsenic atoms in an As−S bond are more easily photoionized than the 1s electrons of arsenic in an As−O bond. For both arsenate and arsenite, peak height for the silica mixture was lower than the aqueous preparation (Figure 3). This result has been reported previously for the sodium salts of arsenic versus those salts in solution (*21*). To accurately fit and quantify the amount of arsenic compounds in a sample, appropriate measurements for white line height to post-edge height are needed and are usually obtained in transmission mode. The XANES profile for solid arsenite, both undiluted and mixed with silica, had a unique peak feature at 11882.8 eV (Figure 3). Our studies examined solid arsenite as arsenic trioxide (As_2O_3), and the observed peak is attributed to the crystal structure. In comparison, solid sodium arsenite XANES spectra do not have the observed feature (*6*).

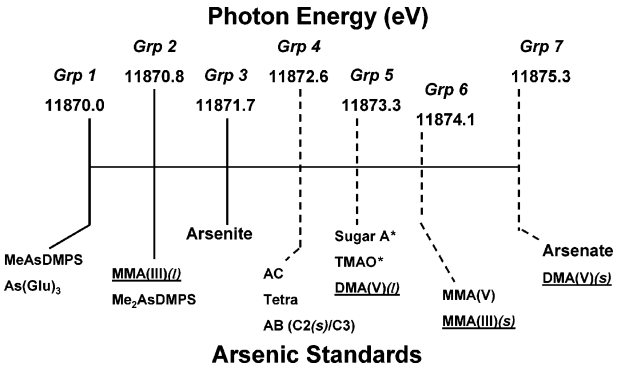

FIGURE 4. Mean white line (peak of the absorption edge) energies in eV for arsenical groups (Grp). Underlined standards had different white lines for aqueous preparations and solid samples mixed with silica. Asterisked (*) arsenicals were only available as aqueous solutions. For all other arsenicals, white line energy was independent of the phase. Dotted vertical lines represent pentavalent species (except MMA(III)(s)), solid vertical lines represent trivalent species. (s), solid mixed with silica; (aq), dissolved in distilled deionized H_2O; AB, arsenobetaine; C2, arsenobetaine 2; C3, arsenobetaine 3.

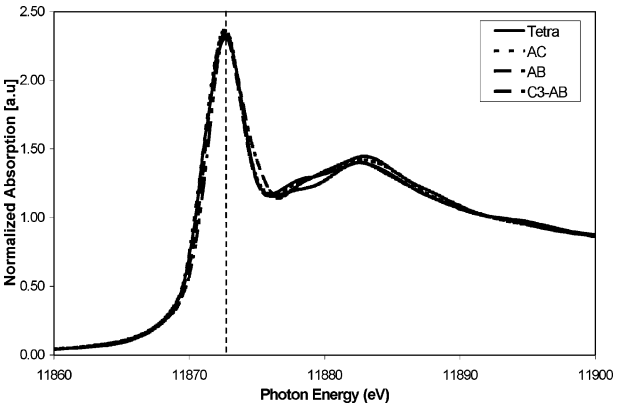

FIGURE 5. XANES spectra of aqueous solutions of group 4 arsenic standards could not be distinguished by their white line energies (vertical dashed line = 11872.6 eV). AC, arsenocholine; AB, arsenobetaine; C3-AB, arsenobetaine 3; Tetra, tetramethyl arsonium.

White Line Comparisons. A schematic of the mean white line energy positions for the arsenic compounds is shown in Figure 4. White line peak positions (in eV) were obtained from the point where the first derivative of each averaged near-edge spectrum crossed zero. Arsenic species with similar white lines (<0.5 eV difference) were assigned to groups (Figure 4), an example of which is group 4 (Figure 5). The usefulness of using EXAFS for distinguishing arsenic species with similar XANES spectra (e.g., AB, C2-AB, C3-AB, AC) is currently being studied. White line positions did not distinguish between sulfur-containing arsenic(III) compounds in our group 1. Data from a previous study indicated that white line energy could not be used to distinguish between FeAsS (arsenopyrite), and arsenian FeS_2 or between AsS (realgar) and As_2S_3 (orpiment) (*18*). The white line energies we report for arsenite (11871.7 eV), arsenate (11875.3 eV), and group 1 compounds (11870.0 eV) fall within the values reported in the literature (Table 2). Spectra for a selection of aqueous standards were replicated during an independent session at APS (Figure 2). White line energy values for a given arsenic compound generally varied by less than 0.5 eV between beamtime sessions. DMA(III) (session 5) and MMA-(III) (session 3) appear to have oxidized to DMA(V) and MMA-(V), respectively (Figure 2).

To determine if white line energies for each arsenic compound depended on the physical state of the samples,

TABLE 2. Summary of White Line Energies for Arsenate, Arsenite, and Arsenic−Sulfur Compounds Reported in Literature

white line energy (eV)			
arsenite	**arsenate**	**arsenic−sulfur**	**ref**
~11871	~11874	~11869 (AsS)	*17*
11871	11874	11869 (AsS)	*18*
*	11875	11869 ($As(Glu)_3$)	*22*
11872	11876	*	*34*
11872	11876	*	*35*
*		*	*36*
~11872		~11870 (AsS)	*37*
*		*	*38*
11871		11870 ($As(Glu)_3$)	this paper

*, no data available. ~, Data estimated from graph.

we compared XANES spectra of solid state and aqueous standards. With the exception of MMA(III) and DMA(V), arsenic standards had similar white line energy positions when dissolved in water and when mixed with silica. MMA(III) and DMA(V) had higher white line energies in the solid (i.e., mixed with silica) state than in the aqueous state (Figure 4). One interpretation of this result is that the solid MMA(III) standard may have oxidized during preparation. This interpretation is consistent with the observation that the peak position of the shifted MMA(III) solid mixed with silica (11874.1 eV) corresponds to both the peak position for solid MMA(V) mixed with silica and undiluted solid MMA(V). Similar reasoning does not seem plausible for DMA(V) since the arsenic is already present in the +5 oxidation state. Further analysis revealed that the white line energies of undiluted solid DMA(V) and aqueous DMA(V) match, both occurring at a lower energy than the white line of solid DMA(V) in silica. It is possible that this difference reflects how the hydrogen-bonded dimer form of DMA(V) in solid phase (*39*) is altered by bonding to silica. Further analysis using boron nitride in place of silica may help to resolve this issue.

Beam Damage. Factors influencing beam damage include arsenic sample species, concentration, preparation (e.g., state), analysis conditions (e.g., temperature, pressure), and experimental parameters (e.g., beam flux level, run time). Arsenic compounds studied here were examined at room temperature and ambient pressure, as both solids and as aqueous solutions, with exposure times from 30 to 50 min. No alterations in XANES spectra, for solid or aqueous arsenic preparations at high concentrations were observed. Several other studies have reported no beam damage for aqueous and salt standards of both sodium arsenate and sodium arsenite as well as arsenic oxide, As_2O_5 (*6*), either using similar experimental conditions or higher photon flux beams with shorter scan time per sample (i.e., similar total number of photons per time) (*40*).

At lower concentrations, damage attributed to total beam exposure was observed for aqueous arsenic standards. An increase in white line energy was the most commonly observed form of beam damage. We found that for selected aqueous arsenic standards at concentrations of 10000, 1000, 100, and 10 ppm over ~1 h the arsenite (100 ppm) and MMA(III) (10 ppm) spectra showed obvious shifts to higher photon energies, consistent with oxidation to arsenate and MMA(V), respectively (MMA, Figure 6).

Beam damage was also observed on the higher flux undulator beamline. The arsenite spectrum developed a peak feature consistent with oxidation to arsenate after only three scans. Bostick et al. reported oxidation from beam exposure (~180 min) for arsenite adsorbed to PbS using a high flux wiggler beamline (*17*). Their data indicated that the majority of oxidation occurred as a result of exposure to the X-ray

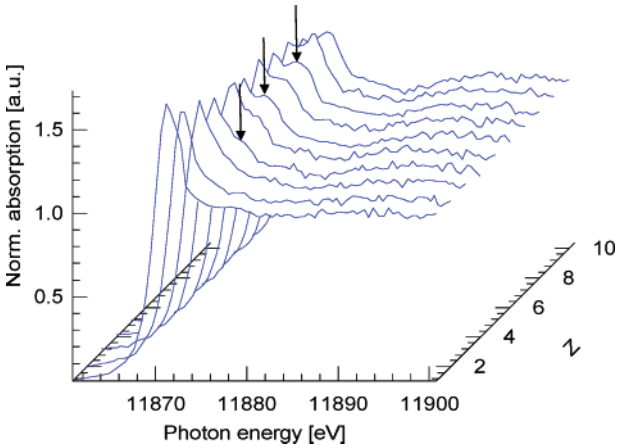

FIGURE 6. Oxidation of aqueous 10 ppm MMA(III) was marked by the growth of a spectral feature (indicated by arrows) at 11874.2 eV over successive scans. This new feature has a similar white line position to that of aqueous MMA(V) (11874.1 eV). Z = scan number.

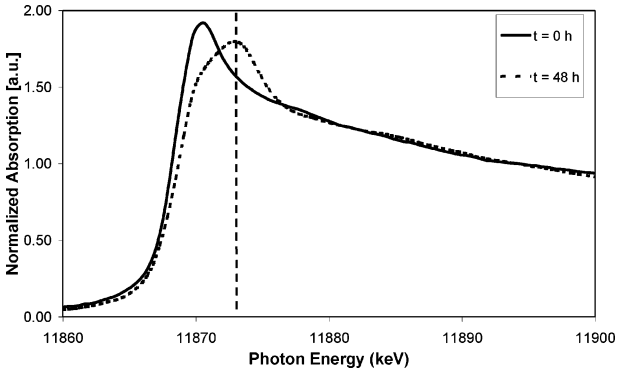

FIGURE 7. Oxidation of $Me_2AsDMPS$ was marked by the appearance of a spectral feature at 11872.9 eV (vertical dashed line) which was not present during initial XANES analysis 48 h earlier. The original $Me_2AsDMPS$ standard remained sealed in Kapton at room temperature and ambient pressure for the intervening 48 h period.

source rather than atmospheric oxygen; in part because the samples were stored in an anaerobic glovebox and had limited exposure to an aerobic environment before analysis (*17*). The literature suggests cooling samples to reduce the destructive effects of the high energy X-rays (*15*). In our studies, samples analyzed at −20 °C on the undulator beamline experienced decreases in signal as well as beam damage. The high energy of the undulator beam melted the sample, which was hypothesized to flow (convection) to the edge of the beam carrying the absorber with it. This loss of signal was also observed in the study of proteins using a high flux beamline (*41*).

Standard Stability. To determine the stability of arsenic preparations over time, under ambient conditions, the same aqueous arsenic standards were analyzed twice with 48 h between analyses. Samples remained mounted in Kapton tape between analyses. With the exception of $Me_2AsDMPS$, which appeared to oxidize over the intervening 48 h (Figure 7), no degradation of other aqueous preparations was observed. The behavior of $Me_2AsDMPS$ may reflect both oxidation during preparation (*40*) and lower stability under these experimental conditions.

To understand the risks posed by arsenic contaminated environments to human, wildlife, and plant populations, it is important to understand how arsenic occurs, how it is taken up, and how it is subsequently transformed and stored or accumulated by organisms (*8*). Traditional speciation

analysis can result in potentially erroneous and/or incomplete identification of arsenic species. XAS identifies arsenic compounds in situ, which circumvents the limitations of sample preparation required with conventional methods, but it is sensitive only in the ppm range. Combining XAS with conventional analyses and the use of the most appropriate standards available will provide a more accurate and complete picture of arsenic speciation in the environment.

Acknowledgments

This work was supported by an NSERC Strategic Projects Grant (STPGP235041-00) at the Royal Military College, Environmental Sciences Group. PNC-CAT is supported by the U.S. Department of Energy, Basic Energy Sciences, under Contract DE-FG03-97ER45628, the University of Washington, and grants from the Natural Sciences and Engineering Research Council of Canada. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract W-31-109-Eng-38. Thanks are extended to Dr. W. Cullen and Dr. H. Sun of the University of British Columbia for the arsenic standards they provided and to Dr. Gerald Seidler and Lynne Townsend of the University of Washington.

Supporting Information Available

Summary of As species detected in marine, terrestrial, and freshwater organisms. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Cullen, W. R.; Reimer, K. J. Arsenic speciation in the environment. *Chem. Rev.* **1989**, *89*, 713.

(2) Burlo, F.; Guijarro, I.; Carbonell-Barrachina, A. A.; Valero, D.; Martinez-Sanchez, F. Arsenic species: effects on and accumulation by tomato plants. *J. Agric. Food Chem.* **1999**, *47*, 1247.

(3) Francesconi, K.; Visoottiviseth, P.; Sridokchan, W.; Goessler, W. Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. *Sci. Total Environ.* **2002**, *284*, 27.

(4) Manning, B. A.; Fendorf, S.; Bostick, B. C.; Suarez, D. L. Arsenic(III) oxidation and arsenic(V) adsorption reactions on synthetic bimessite. *Environ. Sci. Technol.* **2002**, *36*, 976.

(5) Cullen, W. R.; Hettipathirana, D.; Reglinski, J. The effect of arsenicals on cell suspension cultures of the Madagascar periwinkle (*Catharanthus roseus*). *Appl. Organomet. Chem.* **1989**, *3*, 515.

(6) Arai, Y.; Elzinga, E. J.; Sparks, D. L. X-ray absorption spectroscopic investigation of arsenite and arsenate adsorption at the aluminum oxide−water interface. *J. Colloid Interface Sci.* **2001**, *235*, 80.

(7) Challenger, F. Biological methylation. *Environ. Int.* **1945**, *36*, 315.

(8) Meharg, A. A.; Hartley-Whitaker, J. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytol.* **2002**, *154*, 29.

(9) Langdon, C. J.; Piearce, T. G.; Feldmann, J.; Semple, K. T.; Meharg, A. A. Arsenic speciation in the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus*. *Environ. Toxicol. Chem.* **2003**, *22*, 1302.

(10) Lai, V. W.-M.; Cullen, W. R.; Harrington, C. F.; Reimer, K. J. The characterization of arsenosugars in commercially available algal products including a Nostoc species of terrestrial origin. *Appl. Organomet. Chem.* **1997**, *11*, 797.

(11) Francesconi, K. A.; Gailer, J.; Edmonds, J. S.; Goessler, W.; Irgolic, K. J. Uptake of arsenic-betaines by the mussel *Mytilus edulis*. *Comp. Biochem. Physiol. C* **1999**, 131.

(12) Gong. Z.; Lu, X.; Ma, M.; Watt, C.; Le, C. Arsenic speciation analysis. *Talanta* **2002**, *58*, 77.

(13) Koch, I.; Hough, C.; Mousseau, S.; Mir, K.; Rutter, A.; Ollson, C.; Lee, E.; Andrewes, P.; Granhchino, S.; Cullen, W.; Reimer, K. Sample extraction for arsenic speciation. *Can. J. Anal. Sci. Spectrosc.* **2002**, *47*, 109.

(14) Cutler, J. N.; Jiang, D. T.; Remple, G. Chemical speciation of arsenic in uranium mine tailings by X-ray absorption spectroscopy. *Can. J. Anal. Sci. Spectrosc.* **2001**, *46*, 130.

(15) Parsons, J. G.; Aldrich, M. V.; Gardea-Torresdey, J. L. Environmental and biological applications of extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES) spectroscopies. *Appl. Spectrosc. Rev.* **2002**, *37*, 187.

(16) Foster, A. L.; Brown, G. E.; Tingle, T. N.; Parks, G. A. Quantitative arsenic speciation in mine tailings using X-ray absorption spectroscopy. *Am. Mineral.* **1998**, *83*, 553.

(17) Bostick, B. C.; Fendorf, S.; Manning, B. A. Arsenite adsorption on galena (PbS) and sphalerite (ZnS). *Geochim. Cosmochim. Acta* **2003**, *67*, 895.

(18) Bostick, B. C.; Fendorf, S. Arsenite sorption on troilite (FeS) and pyrite (FeS_2). *Geochim. Cosmochim Acta* **2003**, *67*, 909.

(19) Farquhar, M. L.; Charnock, J. M.; Livens, F. R.; Vaughan, D. J. Mechanisms of arsenic uptake from aqueous solution by interaction with goethite, lepidocrocite, mackinawite, and pyrite: an X-ray absorption spectroscopy study. *Environ. Sci. Technol.* **2002**, *36*, 1757.

(20) Paktunc, D.; Foster, A.; LaFlamme, G. Speciation and characterization of arsenic in Ketza River mine tailings using X-ray absorption spectroscopy. *Environ. Sci. Technol.* **2003**, *37*, 2067.

(21) Webb, S. M.; Gaillard, J.-F.; Ma, L. Q.; Tu, C. XAS speciation of arsenic in a hyperaccumulating fern. *Environ. Sci. Technol.* **2003**, *37*, 754.

(22) Langdon, C. J.; Mehrag, A. A.; Feldmann, J.; Balgar, T.; Charnock, J.; Farquhar, M. L.; Piearce, T. G.; Semple, K. T.; Cotter-Howells, J. Arsenic-speciation in arsenate-resistant and non-resistant populations of the earthworm, *Lumbricus rubellus*. *J. Environ. Monit.* **2002**, *4*, 603.

(23) Auger V. Méthodes nouvelles de préparations de quelques dérivés organiques de l'arsenic. *C. R. Hebd. Seances. Acad. Sci.* **1906**, *142*, 1151.

(24) Minhas, R.; Forsyth, D. S.; Dawson, B. Synthesis and characterization of arsenobetaine and arsenocholine derivatives. *Appl. Organomet. Chem.* **1998**, 635.

(25) Stick, R. V.; Stubbs, K. A.; Tilbrook, D. M. G. An improved synthesis of (R)-2,3-dihydroxypropyl 5-deoxy-5-dimethylarsinyl-beta-D-riboside, a common marine arsenical. *Aust. J. Chem.* **2001**, *54*, 181.

(26) Quick, A. J.; Adams, R. Aliphatic arsonic and arsinic acids, and aliphatic−aromatic arsinic acids. *J. Am. Chem. Soc.* **1922**, *44*, 5.

(27) Roberts, E.; Turner, E. E.; Bury, F. W. As-chlorotetrahydroarsinoline and some quaternary arsonium compounds. *J. Chem. Soc* **1926**, 1443.

(28) Adams, E.; Jeter, D.; Cordes, A. W.; Kolis, J. W. Chemistry of organometalloid complexes with potential antidotes: structure of an organoarsenic(III) dithiolate ring. *Inorg. Chem.* **1990**, *29*, 0.

(29) Serves, S. V.; Charalambidis, Y. C.; Sotiropoulos, D. N.; Ioannou, P. V. Reaction of arsenic(III) oxide, arsenous and arsenic acids with thiols. *Phosphorus, Sulfur Silicon Relat. Elem.* **1995**, 109.

(30) Heald, S. M.; Brewe, D. L.; Stern, E. A.; Kim, K. H.; Brown, F. T.; Jiang, D. T.; Crozier, E. D.; Gordon, R. A. XAFS and micro-XAFS at the PNC−CAT beamlines. *J. Synchrotron Radiat.* **1999**, *6*, 347.

(31) Kraft, S.; Stumpel, J.; Becker, P.; Kuetgens, U. High-resolution X-ray absorption spectroscopy with absolute energy calibration for the determination of absorption edge energies. *Rev. Sci. Instrum.* **1996**, *67*, 681.

(32) Jiang, D. T.; Crozier, E. D. Glancing -angle XAFS of buried ultrathin films. *Can. J. Phys.* **1998**, *76*, 621.

(33) Ressler, T. WinXAS: a new software package no only for the analysis of energy-dispersive XAS data. *J Phys. IV* **1997**, *7*, C2−269.

(34) Deschamps, E.; Ciminelli, V. S. T.; Weidler, P. G.; Ramos, A. Y. Arsenic sorption onto soils enriched in Mn and Fe minerals. *Clays Clay Miner.* **2003**, *51*, 197.

(35) Manning, B. A.; Fendorf, S.; Suarez, D. L. Arsenic(III) complexation and oxidation reactions on soil. *Biogeochem. Environ. Imp. Trace Elem.* **2003**, *835*, 57.

(36) Wang, H. C.; Paul Wang, H.; Peng, C. Y.; Liu, S. H.; Yang, Y. W. Speciation of As in the blackfoot disease endemic area. *J. Synchrotron Radiat.* **2001**, *8*, 961.

(37) Hansel, M.; La Force, M. J.; Fendor, S.; Sutton, S. Spatial and temporal association of As and Fe species on aquatic plant roots. *Environ. Sci. Technol.* **2002**, *36*, 1988.

(38) Bull, D. C.; Harland, P. W.; Vallance, C.; Foran, G. J. EXAFS study of chromated copper arsenate timber preservative in wood. *J. Wood Sci.* **2000**, *46*, 248.

(39) Parris, G. E.; Brinckman, F. E. Reactions which relate to environmental mobility of arsenic and antimony. II. Oxidation of trimethylarsine and trimehtylstibine. *Environ. Sci. Technol.* **1976**, *10*, 1128.

(40) Strawn, D.; Doner, H.; Zavarin, M.; McHugo, S. Microscale investigation into the geochemistry of arsenic, selenium, and iron in soil developed in pyretic shale materials. *Geoderma* **2002**, *108*, 237.

(41) Heald, S. M. PNC−CAT, Argonne, personal communication, 2003.

Received for review April 27, 2004. Revised manuscript received September 21, 2004. Accepted September 22, 2004.

ES049358B