Arsenic Speciation in the Environment

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I. Introduction

A. General

Arsenic ranks 20th in abundance in the earth's crust.[1] It is associated with igneous and sedimentary rocks, particularly with sulfidic ores.[2] Natural phenomena such as weathering, biological activity, and volcanic activity, together with anthropogenic inputs, are responsible for the emission of arsenic into the atmosphere, from where it is redistributed on the earth's surface by rain and dry fallout. Arsenic is also mobilized by dissolution in water, with aquatic and soil/sediment concentrations being controlled by a variety of input and removal mechanisms. Quantitative estimates of the relative importance of the pathways comprising the global arsenic budget vary widely.[3-6] There is general agreement that most anthropogenic atmospheric input is due to smelting operations and fossil-fuel combustion, but still unresolved is the extent to which man's activities[7] contribute to the overall arsenic cycle.

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William R. Cullen (left) was born in Dunedin, New Zealand, and is a graduate of the University of Otago (N.Z.) (B.Sc., M.Sc.) and Cambridge (Ph.D.). He has been on the faculty of the University of British Columbia since 1958. His research interests are in the area of organometallic chemistry, one aspect of this being the biogeochemistry of elements such as arsenic, antimony, mercury, and tin.

Ken Reimer (right) was born in Calgary, Canada. He received his B.Sc. and M.Sc. degrees from the University of Calgary and a Ph.D. degree from the University of Western Ontario. After postdoctoral work at the University of British Columbia and a short time teaching at the University of Guelph, he joined the faculty of Royal Roads Military College in 1979. His research background—transition-metal chemistry (with P. M. Boorman, University of Calgary), organometallic synthesis (H. C. Clark, University of Western Ontario), and metalloporphyrin solution chemistry (B. R. James, University of British Columbia)—has served him well in his current research in chemical oceanography. His interest in marine trace element speciation also permits him to indulge his fascination with the mountains and ocean of coastal British Columbia. His life is shared with his friend, spouse, and colleague Deborah. It is also enriched by Sarah (8 years) and Steven (6 years).

However, these inputs are probably significant. Recent estimates have placed the ratio of natural to anthropogenic atmospheric inputs at 60:40.[3]

Despite the quantitative uncertainty regarding arsenic fluxes, it is apparent that arsenic is a ubiquitous element that is found in the atmosphere, in the aquatic environment, in soils and sediments, and in organisms. Because both natural and anthropogenic inputs vary geographically, environmental substrates show wide ranges of arsenic concentrations. Even crustal levels, which are often quoted as 3 ppm, display values from 0.1 to several hundred ppm,[1] depending on the types of rocks being considered. Thus it is quite difficult to establish *typical* arsenic values; therefore one must be cautious about making general comparisons. It is often only possible to establish whether levels for a particular substance are usually in the ppt (ng kg^{-1}), ppb (μg kg^{-1}), or ppm (mg kg^{-1}) range.

There is no shortage of analytical procedures for total arsenic determinations.[8] Colorimetric techniques have been largely replaced by numerous flame and flameless atomic absorption/emission methods. It is also likely that simultaneous multielement ICP determinations will become more common. A consequence of the ease of operation and increasingly large throughput of these methods has been the continued measurement of *total elemental arsenic* in almost every type of environmental sample. Regulatory agencies typically employ these measurements. Drinking-water standards in Canada and the United States, for example, are based on a maximum dissolved arsenic concentration of 50 ppb.[9]

Despite the facility with which total arsenic concentrations can be acquired, there is a growing realization that the environmental behavior of arsenic is dependent on the physical and chemical properties, toxicity, mobility, and biotransformation of *individual arsenic compounds*. In fact, the arsenic biogeochemical cycle can only be properly understood in terms of the dynamic balance of biological, chemical, physical, and geological processes on individual arsenic species. This may appear to be obvious, but the general public, and often even the scientific community, tends to associate arsenic with poison without regard to the widely differing properties of arsenic compounds. This view has, in part, been perpetuated because of analytical limitations. It is only relatively recently that analytical methodologies have been available for comprehensive arsenic speciation at the trace levels frequently encountered.

This review consists essentially of two parts. The first part (sections II–V) deals with the interactions of arsenic compounds with individual organisms ranging from *Methanobacteria* to man. The second part (sections VI–IX) takes a more global view of the flux of arsenic compounds found in the environment.

B. Some History

It is over 40 years since Challenger reviewed his work on the identification of "Gosio gas" as trimethylarsine (Me_3As).[10]

Gosio gas is the volatile, toxic, arsenic species produced by molds growing on wallpaper colored with arsenic-containing pigments such as Scheele's green ("copper arsenite") and Schweinfürt-green ("copper arsenite plus copper acetate").[11,12] In other cases the arsenic had been added to the wallpaper paste to discourage hungry vermin. Gosio gas was responsible for a number of deaths, and the air in the buildings in which it was being produced had a characteristic garlic-like odor. Gosio established the source of the problem and isolated some of the molds capable of metabolizing inorganic arsenicals. Biginelli[13] aspirated the gas from cultures through acidified (HCl) mercuric chloride solution. On the basis of an analysis of the precipitate so obtained, he incorrectly identified the gas as diethylarsine (Et_2AsH). Nonetheless, this was a considerable achievement and established the methodology that Challenger was to use some 30 years later in his classic studies, beginning with the positive identification of the mold metabolite as trimethylarsine.[10,14] Lead arsenate dust from a painted ceiling was the source of the arsenic that caused health problems for Clare Boothe Luce when she was living in Rome as U.S. Ambassador in 1954. No mold action was implicated.[15]

The report by Braman and Foreback in 1973[16] of a hydride generation procedure capable of the individual determination of several inorganic and methylarsenic compounds at low concentrations marked the beginning of comprehensive environmental arsenic speciation. Many techniques have been subsequently developed, and it is now possible to analyze samples with individual arsenic concentrations as low as 1 pM. Speciation techniques are so numerous that a comprehensive review would require a separate article. However, there

TABLE I. Electronegativity Values

element	Pauling	Allred-Rochow
As	2.18	2.20
C	2.55	2.50
O	3.44	3.50
S	2.58	2.44
H	2.1[a]	2.20

[a] Pauling's original value; arsenic was 2.0 on this scale.

is no standard method for arsenic speciation; therefore the technique selected, as well as the way in which it is calibrated, automatically presupposes the arsenic species that might be present in the sample. Even if the same technique is used by several groups, variable detection limits may result in compounds being overlooked in one study but not another. There is also considerable inconsistency in the procedures used to preserve the composition of the sample subsequent to its collection in the field. Thus it does not follow that a particular compound is not present in the environment simply because it has not been detected.

C. Some Chemical Foundations

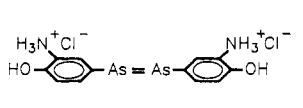
Because there have been a large number of workers in the area from very diverse backgrounds, a miscellany of problems have surfaced concerning the chemical properties of arsenic compounds. Some of these are discussed in the next few paragraphs.

1. Oxidation States

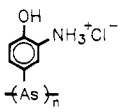
The normal valence states of arsenic are 3 and 5, as in AsF_3 and AsF_5. Coordination numbers for arsenic cover the range 3 (AsF_3), 4 ($As(CH_3)_4^+$), 5 (AsF_5), and 6 (AsF_6^-). The oxidation number of arsenic in arsenic compounds, as for any element, depends on a model of the charge distribution in the bonds. The usual way of estimating this charge distribution is to use the relative electronegativities of the atoms concerned. Listed in Table I are the electronegativities[17] of arsenic and other elements of interest to this review. It is obvious that As–X bonds are polarized $As^{\delta+}$---$X^{\delta-}$ when X = C, O, and S. The situation with respect to As–H is less clear. For the purpose of this review the arsenic will be assumed to be the more electropositive element, and all arsenic oxidation states will be described as positive as follows: As(III) in AsH_3, $As(OH)_3$, and $As(CH_3)_3$; As(V) in $(CH_3)_2AsO(OH)$, $(CH_3)_3AsO$, and $(CH_3)_4As^+$. Failure to observe a self-consistent set of oxidation numbers can lead to confusion,[18-20] a point that has also been made by others.[21]

2. Double Bonds Involving Arsenic

The older literature on organoarsenic compounds is redundant with structures such as that shown for Salvarsan (arsphenamine, 606) (**1a**).[22] This compound,



1a



1b

discovered by Ehrlich, proved to be effective in the treatment of syphilis and other diseases. However, the formulation is incorrect, and Salvarsan is best represented by **1b**, i.e., as a polymeric material containing As–As single bonds.[23,24] In cyclic arsenobenzene ($(C_6H_5As)_6$) the arsenic atoms are in a chair conformation.[25] It is only recently that compounds containing arsenic–arsenic double bonds have been isolated.[26] Similar remarks apply to the so-called arsenoxides RAs=O (also RAs=S). Again, these molecules are oligomeric, e.g., cyclic $(CH_3AsO)_x$ and $(CH_3AsS)_y$, or polymeric, $(-(R)As-E-)_n$.[27]

3. Arsenous Acid

There is little doubt that aqueous solutions of inorganic arsenites contain $As(OH)_3$, $pK_1 = 9.2$, or salts of this.[17,28] The metaacid $HAsO_2$ and its salts are not known in solution.

D. Literature

A number of books and review articles contain material pertinent to this review. Particular mention should be made of two books that appeared about 10 years ago, one from the National Research Council, Washington, DC, Committee on Biologic Effects of Environment Pollutants,[29] and the other from the National Research Council of Canada, Ottawa, Associate Committee on Scientific Criteria for Environmental Control.[30] An American Chemical Society Symposium resulted in a useful volume,[31] as did a Spurenelem symposium[32] and symposia sponsored by the Chemical Manufacturers Association and the National Bureau of Standards[33] and the U.S. Department of Health, Education, and Welfare.[34]

Fowler[35] has edited a book devoted to the biological and environmental effects of arsenic. Other books contain chapters of interest,[36-40] and some useful reviews have appeared.[41-46] The World Health Organization has published a criteria document for arsenic.[47] Challenger continued his interest in the field for a remarkable length of time.[10,48-51]

II. Interaction of Microorganisms with Arsenic Compounds, without Arsenic–Carbon Bond Formation

A. Arsenic Tolerance

Thom and Raper[52] surveyed a range of fungi with the objective of establishing which of these had the ability to "methylate arsenic", i.e., produce ill-smelling arsenical gases. In addition to the organisms that did this (to be described later), a number were found that grew well in Czapek's solution agar augmented with up to 0.2% As_2O_3. These include *Penicillium expansum*, *P. chrysogenum*, *P. roqueforti*, *Aspergillus flavus*, *A. oryzae*, a species of *Helminthosporium*, and a few *Mucor*. Challenger[10] describes how the very common fungus *Cladosporium herbarum* will grow in a solution containing 2% arsenic (but not in 4%); again no garlic odor is produced (this mold grows in Liquor Arsenicalis, a 1% solution of As_2O_3, described in the *British Pharmacopoeia* as late as 1948[53]). In the presence of phosphate, *C. herbarum* will grow in 4.8% solutions of arsenate.[54] Some organisms that will tolerate an arsenite concentration of 0.02 M, without obvious effect, have been isolated from sewage.[55,56] However, only in these last-mentioned studies was it established that the arsenite was not oxidized to arsenate or methylated to

methylarsonic or dimethylarsinic acid (apart from the obvious nonproduction of volatile malodorous arsines).

Chromated copper arsenate (CCA) is a commonly used wood preservative against biological decay. The minimum inhibition concentration (MIC) value is the lowest concentration tested at which growth is prevented. The values for particular fungi (obtained from agar plates) indicate that the effectiveness of the CCA preservative is not simply due to the component with the highest MIC value.[57] Marine fungi seem to be more tolerant than nonmarine. For example, the MIC values for *Dendryphiella salina* are as follows: $CuSO_4$, 1096 mg L^{-1}; $Na_2Cr_2O_7$, 964 mg L^{-1}; As_2O_5, 1220 mg L^{-1}; CCA, 0.4%. The composition of 0.4% CCA is $CuSO_4$, 1046 mg L^{-1}; $Na_2Cr_2O_7$, 1928 mg L^{-1}; As_2O_5, 96 mg L^{-1}. A low concentration of CCA actually stimulates the growth of this fungus. The nonmarine fungus *Botryosporium* sp has the same MIC value of 0.4% for CCA but the value for As_2O_5 is much lower at 224 mg L^{-1}. MIC values for other fungi are available;[58] of special interest are the values of 1722–4305 mg L^{-1} As_2O_5 for *Lenzites trabea* and >4305 mg L^{-1} As_2O_5 for *Poria vaillantii*. The former wood-rotting fungus is known to produce an "arsenic odor" from an arsenic-containing medium; the latter does not.[58,59] Some unidentified bacterial isolates from CCA-treated wood have high MIC values for As_2O_5 (>1220 mg L^{-1}) and CCA (>0.5%).[57]

Green and Kestell[60] found that strains of the bacteria *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* could be accustomed to grow in 0.15 M arsenite without oxidizing it to arsenate. These and other fungi have a greatly increased tolerance to arsenate in the presence of phosphate.[54]

Many bacteria have since been isolated, often from hospital sources, that show a resistance to arsenite and/or arsenate.[61–65] This resistance is coded for by an inducibile operon-like system in both *Staphlococcus aureus* and *Escherichia coli*. This system is also turned on by Sb(III) and Bi(III).

Chromosomally determined arsenate resistance appears to result in reduced accumulation of arsenate; i.e., the cell switches on a phosphate transport system that is more selective for phosphate. The usual transport system is not selective. Thus phosphate can act as a protecting agent against arsenate toxicity (but not arsenite). Plasmid-mediated resistance to arsenate is due to the synthesis of a highly specific arsenate efflux pump that eliminates intracellular arsenate. Arsenite oxidation by an inducible enzyme system is one mechanism of resistance to arsenite. Another plasmid-mediated mechanism seems to exist,[66] but it is not known how this functions, apart from not involving extracellular detoxification.

Similar dual systems for phosphate transport in a yeast have been described.[67]

B. Oxidation of Arsenite to Arsenate

Oxidation of arsenite is one of the protective mechanisms mentioned in the previous paragraph. Arsenite is more toxic than most other forms of arsenic. Penrose[43] gives the order (decreasing toxicity) R_3As (R = H, Me, Cl, etc.) > As_2O_3 (As(III)) > $(RAsO)_n$ > As_2O_5 (As(V)) > $R_nAsO(OH)_{3-n}$ (n = 1, 2) > R_4As^+ > As(0).

The "spontaneous" oxidation of arsenite to arsenate in cattle dipping fluids was first noted in 1909.[68] The suggested relationship of the oxidation to bacterial growth was confirmed in 1918 by the isolation of a bacterium from a dipping fluid that in pure culture could bring about the chemical change.[69] The bacterium, provisionally named *Bacillus arsenoxydans*, was eventually lost. The next observation of this phenomenon was not recorded until 1943,[70–72] when 15 strains (5 species) of arsenite-oxidizing bacteria were isolated. These were provisionally characterized as three *Pseudomonas*, one *Xanthomonas*, and one *Achromobacter*. One of the strains in the genus *Achromobacter* was very similar to the bacterium isolated by Green.[69] Growth of the bacteria can take place in the pH range 6.1–9.4 (the pH drops during growth as a result of the formation of arsenate). There was no evidence that the energy of arsenate oxidation is used for growth. A moderately stable, cell-free, arsenite dehydrogenase is liberated from cells of "*Pseudomonas arsenoxydans-quinque*" after grinding with powdered glass.[73,74] The electron acceptor used in the assay was 2,6-dichloroindophenol. Arsenate is an inhibitor, as is Hg^{2+}. Studies on these cell-free preparations suggest that arsenite oxidation in the intact cell involves a loose association between the enzyme and the cytochromes.

In more recent studies, 34 different strains of arsenite-oxidizing pseudomonads were isolated from sewage[55,56,75] and classified into two main groups: *Pseudomonas fluorescens-arsenoxydans* and *Pseudomonas acidovorans-arsenoxydans*. The properties of these bacteria are similar to those described by Turner and co-workers.[70–74] No methylarsenicals were found in the medium.

Extended studies on one organism, *Pseudomonas acidovorans-arsenoxydans* YE56, have been described.[55,56] It is a strict aerobe, but under anaerobic conditions the organism can use nitrate for the oxidation of various carbon sources; arsenite is not oxidized under these conditions. Sonicated cells are unable to oxidize arsenite in the presence of air; the same preparation is able to couple oxidation of arsenite to arsenate, with the reduction of 2,6-dichloroindophenol. The oxidation of arsenite by the whole organism seems to be associated with the appearance, at the stationary phase, of an enzyme and/or component of the electron transport system; the optimum pH is ~6.6. Phillips has speculated[55,56] that an acquired tolerance to ingested arsenite, the more toxic state, might be due to the enrichment of the arsenite-oxidizing population of pseudomonads in the intestine.

A strain of *Alcaligenes* (*Achromobacter*) isolated from soil also oxidizes arsenite to arsenate. The optimum pH is 7.0 and the enzyme is induced in about three cell generations.[75] *Bacillus cereus* reduces arsenate; there is little arsenic incorporation.[76] The mineral orpiment (As_2S_3) is oxidized by *Ferrobacillus ferrooxidans* to arsenate; arsenite is also present in the medium.[77] Realgar (As_4S_4) is stable under the same conditions; however, arsenopyrite (FeAsS) and enargite (Cu_3AsS_4) are attacked by bacterial action with release of arsenate; arsenite is also present in the medium.[78]

C. Reduction of Arsenate to Arsenite

The reduction of arsenate to arsenite, a more toxic form, has been described. *Pseudomonas fluorescens*, a common aquatic bacterium, carries out this reduction

TABLE II. Action of *Scopulariopsis brevicaulis* on Arsenicals

arsenical	concn, %	medium[a]	time, days	product[b]	yield, %	ref
As_2O_3	0.2	CD	>50	Me_3As	0.78 (av)	95
As_2O_3	0.2	CD + peptone	84	Me_3As	7.55	95
As_2O_3	0.2	B	>365	Me_3As	85[c]	91, 93
As_2O_3	0.2	B + methionine	5–15	Me_3As	≤18	91, 96
Na_2HAsO_4	0.2	B	>58	Me_3As	0.74	95
Na_2HAsO_4	0.37	CD	58	Me_3As	0.74	95
$MeAsO(ONa)_2$	1.0	CD	34	Me_3As	0.88	95
$MeAsO(OH)(ONa)$	1.0	CD	65	Me_3As	1.34	95
$MeAsO(ONa)_2$	≤1.5	B	>21	Me_3As	?	14
$EtAsO(ONa)_2$	0.5	B	>21	$EtMe_2As$	?	14
$PrAsO(OH)(OK)$	0.3	B	23	$PrMe_2As$	*d*	96
$allyl\text{-}AsO(OH)_2$	0.35	B	58	$allyl\text{-}Me_2As$	9	96
$HOCH_2CH_2AsO(ONa)_2$	0.13	B	76	Me_3As	*d*	95
$ClCH_2CH_2AsO(OH)_2$	?	B	?	Me_3As	?	95
$BuAsO(OH)_2$	0.02	CA	?	$BuMe_2As$	?	92
$NaOOCCH_2AsO(OH)_2$	1.0	CD	33	nd		95
$NaOOCCH_2AsO(OH)_2$	0.5	B	33	nd		95
$HOOCCH_2AsO(OH)_2$	1	B	65	Me_3As	0.27	95
$HOOCCH(CH_3)AsO(OH)_2$	0.5	B	50	Me_3As	*d*	95
$PhAsO(OH)_2$	0.2	B		nd		93, 97
$2,4\text{-}Me_2C_6H_3AsO(OH)_2$	0.1	B		nd		93
$PhCH_2AsO(OH)_2$	0.1	B		nd		93
$(MeAsO)_x$	0.05	D	0.5	Me_3As	0.1	94
				$MeAsH_2$		
				$Me_2AsO(OH)_2$	11	
PhAsO	0.2	CD	>150	nd		93
$PhAs(CH(OH)CH_3)_2$	0.3	CD		nd		93
$Me_2AsO(ONa)$	≤0.3	B	>21	Me_3As	?	14
$Et_2AsO(OH)$	0.2	B	27	Et_2MeAs	*d*	96
$Et_2AsO(OK)$[e]	0.4	B	93	Et_2MeAs	*d*	14
$MePrAsO(OH)$	0.2	B	21	$PrMe_2As$	*d*	97
$EtPrAsO(OH)$	0.2	B	80	$EtPrMeAs$	*d*	97
$PhMeAsO(ONa)$	0.2	CD	35	nd		93
$[Me_3AsOH]NO_3$	0.13	B	95	Me_3As	65	95
Pr_3AsO	0.13	B	74	Pr_3As	12	95
$[PhMe_2AsOH]NO_3$	0.17	B	>2	$PhMe_2As$	23	93

[a] B = bread; CD = Czapek-Dox plus glucose liquid medium; CA = liquid medium described by Cox and Alexander;[98] D = Sabouraud's maltose broth (Difco). [b] e.g., Me_3As, product is well characterized; nd, arsine product not detected. [c] The yield depends on the strain of the mold. [d] Trace, but identified. Usually as an $HgCl_2$ adduct. [e] The sodium salt did not afford any arsine.

under aerobic conditions; activated sewage sludge does so under anaerobic conditions.[79] Further transformations are possible in sludge.[80] Another study[81] used an aerated mixed culture of bacteria from sea water. The phosphate concentration steadily decreased during the experiment, but the total arsenic concentration remained constant, indicating no accumulation by the bacteria. Wine yeast also reduces arsenate.[82]

Cultures of mixed flora from the rat stomach reduce arsenate to arsenite. This ability varies from rat to rat.[83] Cecal contents also carry out the reduction rapidly, although some slower methylation is also found. As will be outlined below, most biological methylation reactions proceed without any arsenite (or other possible intermediate) accumulating in the medium.

Forsberg[84] reports that rumen bacteria reduce arsenate to arsenite, although volatile arsine production from this medium is reported by others.[80,85]

An interesting example of arsenate to arsenite reduction by an *Anabaena oscillaroides* bacteria assemblage has been described.[86] The Waikato River in New Zealand normally has a high arsenate content (30–80 mg m^{-3}), but during the spring and summer months arsenite often predominates. It seems that the epiphytic bacteria associated with the cyanophyte algae *A. oscillaroides* are responsible for at least part of the reduction to As(III). The steady-state rate for the assemblage is 12 ng of As 10^6 $cells^{-1}$ day^{-1}. The assemblage also seems to store arsenic.

D. Reduction of Arsenate to Arsine

The addition of sodium arsenate to soils (three diverse samples were studied) enriched with glucose and urea results in the production of arsine (AsH_3) as determined by GC/mass spectrometry (Carbowax 1000 column).[87] Only traces of arsine are produced from unenriched cultures. Two bacteria, *Pseudomonas* and *Alcaligenes*, were isolated from the soil and shown to be arsine producers under anaerobic conditions (He atmosphere; supplementary nitrate or nitrite is essential); cf. Table IV.

E. Conversion of Arsenite to Arsine

Cheng and Focht,[87] who studied the production of arsine from arsenate, also report that some soils and the isolated soil bacteria, *Pseudomonas* and *Alcaligenes*, release arsine from arsenite under anaerobic conditions.

III. Microbiological Methylation of Arsenic

A. Methylation of Arsenic by Fungi

Challenger initially studied the action of four strains of the mold *Scopulariopsis brevicaulis* on arsenicals.[10] This mold is identical with one of the "arsine" gas producers earlier identified by Gosio as *Penicillium brevicaule*. Table II lists some of the results of Challenger's studies and a few more recent findings. The

arsines were identified by Challenger as the mercuric chloride adducts $R_3As{\cdot}2HgCl_2$, as hydroxytrialkylarsonium nitrates or picrates, or as benzyltrialkylarsonium picrates. The arsenical was usually added to sterile bread crumbs in a flask that was then inoculated with the mold. Volatile gases produced during the subsequent growth of the microorganism were swept out of the flask with a stream of sterile air and passed through a solution of $HgCl_2$ in hydrochloric acid (Biginelli's solution[13]), nitric acid, or alcoholic benzyl chloride

The garlic-like odor of the arsines is intense and unforgettable and is a good indicator of reaction (the odor threshold for Me_3As now appears to be 0.002 $\mu g\ kg^{-1}$ in dilute aqueous solution[88]). Because of this, a mycological test for arsenic was developed.[89] Thus *S. brevicaulis* was allowed to act on the suspect substance. If arsenic was present, an odor was detected within 2 h. The test was sensitive to ~1 ppm. Rather remarkably, positive results were obtained from Neosalvarsan, a derivative of 1; probably the compound was impure. Antimony compounds do not give the test and do not inhibit production of the arsine. Pool established in 1912 that *Monilia sitophila* produced Gosio gas from dimethylarsinic acid.[90]

The results of Table II show that *S. brevicaulis* methylates both arsenate and arsenite to Me_3As; arsenate was not studied as extensively as As_2O_3.[10,48] Bread cultures are more productive than cultures in liquid medium, although the rates of arsine production are never great. The second entry shows the maximum yield obtained in liquid medium; peptone seems to stimulate more growth than any of the many other additives studied. High yields can be obtained from bread cultures if they are left for a long time. Methionine and similar compounds do not affect the yield of Me_3As, although methyl transfer is achieved[91,92] (see section III.B).

In general, alkylarsonic and dialkylarsinic acids afford the appropriate methylarsines, RMe_2As and RR'MeAs, respectively. RMe_2As is produced more easily than RR'MeAs, possibly because RR'AsO(OH) (or its salts) is more toxic than $RAsO(OH)_2$. Allyl groups are not reduced during the methylation process; saturated alkylarsines are not obtained from arsonic acids such as $HOOCCH(CH_3)AsO(OH)_2$ (through a decarboxylation step) or from $HOCH_2CH_2AsO(OH)_2$ (through reduction of the *COH* group). This work was done to investigate possible pathways for the biological methylation. The trace of Me_3As sometimes obtained is probably the result of hydrolysis of the arsenical and subsequent methylation, and not biological cleavage. The loss of the chloroethyl group from $ClCH_2CH_2AsO(OH)_2$ is discussed later (section III.G). Trialkylarsenic(V) derivatives are reduced to trialkylarsines, and even $[PhMe_2AsOH^+]$ affords $PhMe_2As$. Arylarsenicals(V) and -(III) are not methylated by *S. brevicaulis*.[93] Arsine (AsH_3) is not a product of these biological reactions of *S. brevicaulis*. Challenger reports[48] that no intermediates from the proposed metabolic pathway (Scheme I) are found in the medium, although no details are available regarding the methodology used to support this statement.

Methylarsenic oxide ($(MeAsO)_x$) is one of the few organoarsenic(III) derivatives studied.[94] These noteworthy recent results show that a primary arsine, $MeAsH_2$, is produced as well as Me_3As and that $Me_2AsO(OH)$ can be isolated from the medium.

TABLE III. Arsenicals as Substrates for Yeasts and Fungi[a,b]

transformation	microorganisms
$As_2O_3 \rightarrow Me_3As$	*Scopulariopsis brevicaulis*;[14,91,95] *Aspergillus glaucus* (trace), *A. versicolor* (trace);[93] *Candida humicola*[98]
$As_2O_3 \nrightarrow Me_3As$	*A. niger, A. fischeri, Penicillium notatum, P. chrysogenum*;[93] *Gliocladium roseum, Penicillium* sp;[98] *Saccharomyces cerevisiae, S. carlsbergensis, S. monacensis*, "Rasse XII"[95]
$H_3AsO_4 \rightarrow Me_3As$	*S. brevicaulis*;[93] *C. humicola*[98]
$H_3AsO_4 \nrightarrow Me_3As$	*G. roseum, Penicillium* sp[98]
$MeAsO(OH)_2 \rightarrow Me_3As$	*S. brevicaulis*;[95] *C. humicola, G. roseum, Penicillium* sp;[98] *P. chrysogenum, P. notatum, A. niger, A. fischeri, A. glaucus, A. versicolor*[93]
$EtAsO(OH)_2 \rightarrow EtMe_2As$	*S. brevicaulis*;[14] *A. niger*[93]
$ClCH_2CH_2AsO(OH)_2 \rightarrow Me_3As$	*P. notatum*[48]
$PrAsO(OH)_2 \rightarrow PrMe_2As$	*S. brevicaulis*;[96] *P. notatum*[93]
allyl-$AsO(OH)_2 \rightarrow$ allyl-Me_2As	*S. brevicaulis*;[96] *P. chrysogenum*[93]
$BuAsO(OH)_2 \rightarrow BuMe_2As$	*C. humicola, S. brevicaulis, G. roseum*[92]
$PhAsO(OH)_2 \rightarrow PhMe_2As$	*C. humicola*[102]
$PhAsO(OH)_2 \nrightarrow PhMe_2As$	*S. brevicaulis*[97,93]
$4\text{-}NH_2\text{-}2\text{-}OHC_6H_3AsO(OH)_2 \rightarrow Me_3As$	*C. humicola*[102]
$4\text{-}NH_2C_6H_4AsO(OH)_2 \nrightarrow ArMe_2As$	*C. humicola*[102]
$Me_2AsO(OH) \rightarrow Me_3As$	*S. brevicaulis*;[14] *P. chrysogenum, P. notatum* (trace), *A. niger*;[93] *C. humicola, G. roseum, Penicillium* sp[98]
$EtPrAsO(OH) \rightarrow EtPrMeAs$	*S. brevicaulis, A. niger*[93]
$PhMeAsO(OH) \rightarrow PhMe_2As$	*C. humicola*[102]
$PhMeAsO(OH) \nrightarrow PhMe_2As$	*S. brevicaulis*[93]
$PhMe_2AsO \rightarrow PhMe_2As$	*C. humicola*,[102] *S. brevicaulis*[93]
$Me_3AsO \rightarrow Me_3As$	*C. humicola*[102]
$Me_3As^+CH_2COO^- \nrightarrow Me_3As$	*C. humicola*[102]
$(MeAsS)_x \rightarrow Me_3As, MeAsH_2$	*C. humicola*[94]
$(MeAsO)_x \rightarrow Me_3As, MeAsH_2, Me_2AsO(OH)$	*C. humicola, S. brevicaulis*[94]
$Me_2AsSR \rightarrow Me_3As, Me_2AsH$[c]	*C. humicola*[94]

[a] Additional transformations using *S. brevicaulis* are listed in Table I. [b] The arsenic species in the medium is dependent on the pH. [c] HSR = cysteine, glutathione.

The arsenic content in the mold *S. brevicaulis* after exposure to As(III) in a liquid culture for 32 days is 0.032%.[95]

The methylating action of *S. brevicaulis* is not confined to arsenic; selenites and selenates are metabolized to Me_2Se, and tellurate is metabolized to Me_2Te.[48] Dialkyl disulfides are converted to alkanethiol and alkyl methyl sulfide by the mold.[48] Trimethylstibine does not seem to be produced from potassium antimonyl tartrate (tartar emetic); the mold consumes the tartrate and Sb_2O_3 is precipitated.[96]

Other fungi methylate arsenicals. Their names and the transformations studied are listed in Table III. Again, a number of the results come from Challenger's studies.[10,48] More modern studies principally involve three fungi, *Candida humicola* (Dazewska) Diddens and Lodder, *Gliocladium roseum* Bain, and a species of *Penicillium*; all were isolated from raw sewage.[98] Cox and Alexander[98] made the first "modern" identification of Me_3As by comparing the gas chromatography retention time of the evolved arsine with that of a known sample (FFAP and Chromosorb 101 columns) and also by using mass spectroscopy. These fungi show a phe-

nomenon of selective methylation first noted by Challenger and co-workers.[10,93] For example, *G. roseum* and *Penicillium notatum* do not methylate inorganic arsenic, but readily metabolize alkylarsenicals. The pH also has an effect on this selectivity: arsenate (0.1% in the medium) is not methylated by *C. humicola* at pH 6 or 7; Me_3As is produced at pH 5, which is the pH for maximum arsine production.[98,99] *C. humicola* grows well in 0.01% phosphate. When excess phosphate (≥0.1% in the medium) is added to the growing culture, production of Me_3As is inhibited from all the substrates but dimethylarsinate.[99] Cox and Alexander[98] speculate that phosphate may suppress Me_3As evolution by blocking the methylation sequence after one methyl group has been added (cf. Scheme I). It seems more likely that some differences in transport are involved because the mechanism of arsenate uptake in *C. humicola* is metabolism linked,[100] and there is a dramatic reduction in arsenate uptake in the presence of phosphate. The uptake of arsenite, methylarsonate, and dimethylarsinate is much slower; entry of these arsenicals into the cell is probably by passive diffusion. Thus arsenate is unique in this regard; however, the difference is not reflected in greater toxicity or faster conversion to Me_3As. At low arsenate concentration (0.01 mM), cells of *C. humicola* rapidly accumulate 100% of the arsenate in solution. This percentage drops off at higher concentrations, e.g., 3.5% at 2.3 mM; nevertheless under the assay conditions the final cellular arsenic concentration is greater than the extracellular concentration.[100]

Cox and Alexander[98] used progressive enrichment techniques to isolate *C. humicola* and the two other arsine-producing fungi from sewage. Preconditioning *C. humicola* with dimethylarsinate enhances the ability of the organism to methylate both arsenate and dimethylarsinate;[101] cells preconditioned with arsenate produce *less* Me_3As from dimethylarsinate. The phenomenon is not due to differences in transport through the wall, as cells preconditioned with dimethylarsinate have the same ability to take up arsenate as cells grown in the absence of dimethylarsinate, as judged from results obtained by using ^{74}As- and ^{14}C-labeled arsenicals.

Growth of *C. humicola* is inhibited at arsenate concentrations of 100 mM, but this concentration of dimethylarsinate has no effect.[101]

C. humicola methylates both $PhAsO(OH)_2$ and PhMeAsO(OH) to $PhMe_2As$;[102] *S. brevicaulis* does not, although it does reduce $PhMe_2AsOH^+$ to this arsine. Arsanilic acid ($4\text{-}NH_2C_6H_4AsO(OH)_2$) is not methylated to a volatile arsine by *C. humicola*, and the aryl group is cleaved from $4\text{-}NH_2\text{-}2\text{-}OHC_6H_3AsO(OH)_2$; the product is Me_3As. A useful technique for trapping volatile arsines was developed for this study.[102] A glass-fiber filter paper soaked in 5% $HgCl_2$ is hung *inside* the growth flask (cf. ref 10). Evolved arsines are trapped, chemofocused, as mercuric chloride adducts. Samples of the filter can be analyzed subsequently as follows: (a) by direct mass spectroscopy, (b) by heating the sample to liberate the arsine, which is then determined by using gas chromatography, etc., and (c) by counting the samples if ^{74}As- or ^{14}C-containing arsines are evolved.

The only arsonium compound studied in pure culture to date, arsenobetaine (3), the principal arsenical found in marine animals (Table V), is not reduced by *C. humicola* to an arsine.[102]

The results of Tables II and III show that arsine oxides R_3AsO are reduced by fungi to the arsines R_3As. In particular, the reduction of Me_3AsO by *C. humicola* has been studied in detail.[103] This reduction is rapid, like the others, and in the case of *C. humicola* requires biologically intact cells. Cell-free extracts do not carry out this reduction; thus Me_3AsO can build up in this reaction medium.[104] The reduction rate is maximum at pH 5.1–5.2, and the optimum temperature is 40 °C. The reaction follows Michaelis–Menten type kinetics and is inhibited by a number of electron transport inhibitors and uncouplers of oxidative phosphorylation such as azide and oligomycin. Arsenate and arsenite are strong inhibitors, whereas methylarsonate is less so; dimethylarsinate and phosphate have little effect. The enzyme system involved in the reduction, either directly or by producing an agent that reduces the arsine oxide in a chemical reaction, seems to be inducible. Cells grown in the presence of Me_3AsO show a dramatic increase in the rate of reduction (ca. fivefold). This effect is counteracted by the protein-synthesis inhibitor cycloheximide.[103]

Only a few organoarsenic(III) derivatives have been studied as substrates for *C. humicola*. The results from $(MeAsO)_x$ and $(MeAsS)_x$ are particularly interesting, as these, or similar compounds, could be intermediates in the methylation pathway (Scheme I). The oxide is metabolized by *C. humicola* and *S. brevicaulis* to Me_3As; smaller amounts of $MeAsH_2$ are also produced. Moreover, $Me_2AsO(OH)$ is found in the medium.[94] As noted above, this is the first observation of the production of an arsenic hydride by a pure mold culture, and it is also the first time that a methylated metabolite has been isolated from the culture medium. The same mixture of arsines is produced by *C. humicola* from $(MeAsS)_x$.[94] This is a particularly facile process and 50% of the available arsenic is volatilized during 3 days. Less than 1% of $Me_2AsO(OH)$, at a similar concentration, would be converted to Me_3As in this time. (As noted above, the reduction of Me_3AsO to Me_3As by *C. humicola* is also a facile process.)

$(MeAsS)_x$ is a potent fungicide and is very toxic to *C. humicola* (MIC < 0.008 mM for 24-h cultures), where the order, $(MeAsS)_x > (MeAsO)_x >$ As(III), is the reverse of the usual toxicity sequence described above and is an example of methylation resulting in an increase in toxicity.[94] An increase in toxicity on methylation is well-known for mercury, where CH_3Hg^+ is much more toxic than Hg^{2+}.[38] Oxidative methylation of arsenic results in a decrease in toxicity.

The production of arsines and $Me_2AsO(OH)$ from $(MeAsO)_x$ by *C. humicola* is not greatly affected by the presence of other arsenicals, apart from $(MeAsS)_x$; when the sulfide is present in the medium, the production of $Me_2AsO(OH)$ from $(MeAsO)_x$ over 24 h is doubled.

A mixture of arsines, Me_3As and Me_2AsH, is obtained from Me_2AsSR (RSH = cysteine or glutathione).[94] These are reasonably facile reactions, especially the latter example; however, the rate of arsine evolution is lower than from $Me_2AsO(OH)$. Other R_2AsSR compounds (RSH = $HSCH_2CH_2OH$, $HSCH_2COOH$) are not transformed to volatile arsines by *C. humicola*.[94] The compounds Me_2AsOR and Me_2AsSR are likely in-

termediates in the methylation sequence shown in Scheme I.

In addition to the fungi listed in Table III, others have been found to evolve an arsine when growing in contact with inorganic arsenic compounds. An early study by Thom and Raper[52] found that while a number of *Penicillium* species are not arsine producers, 2 of the 22 strains of *Aspergillus* studied, *A. fischeri* and *A. sydowi*, are active gas producers and 3 are feeble producers, namely, *A. fumigatus*, *A. glaucus*, and *A. ochraceus*.

The ability of the wood-rotting fungi to metabolize arsenic compounds is of considerable interest since arsenicals are commonly used as wood preservatives (see section II.A). Thom and Raper[52] concluded that the "arsenic fungi" readily attack arsenicals used in wood preservatives. Other detailed studies[59] found "arsine" evolution (presumably Me_3As) from As_2O_3 exposed to two wood-rotting fungi, *Lenzites trabea* and *L. saepiaria*; 65 species were examined. Trimethylarsine has been unequivocally identified as a metabolite from cultures of *C. humicola* in contact with wood treated with the preservative chromated copper arsenate (CCA).[105] The simple chemofocusing technique described above was used to identify the arsine in this case.

The marine yeast *Rhodotorula rubra* reduces arsenate to arsenite, and an unidentified volatile arsine is evolved.[106] Extracts of the cell show the presence of $MeAsO(OH)_2$ (first formed) and $Me_2AsO(OH)$. Phosphate competitively prevents arsenate toxicity.[107]

B. Mechanism of Arsenic Methylation by Fungi

In his 1945 review,[10] Challenger favored the hypothesis that the methylation of arsenic involved the transfer of a methyl group from some already methylated compound such as betaine, methionine, or a choline derivative. His proposed mechanism, involving alternating oxidation and reduction steps, is outlined in Scheme I. Support for this sequence comes from the observation that arsenite, arsenate, methylarsonate, and dimethylarsinate are all substrates for the production of Me_3As by *S. brevicaulis*, and that other $RAs(V)$ and $R_2As(V)$ species are methylated to $RAsMe_2$ and R_2AsMe. Challenger did not speculate on the nature of the reducing agents.

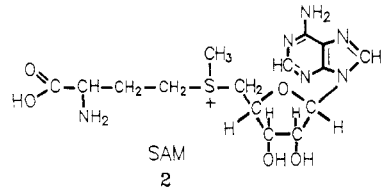
SCHEME I

$$As(OH)_3 \xrightarrow{[CH_3^+]} CH_3AsO(OH)_2 \xrightarrow{2e^-} \{CH_3As(OH)_2\} \xrightarrow{[CH_3^+]} (CH_3)_2AsO(OH)$$

$$(CH_3)_2AsO(OH) \xrightarrow{2e^-} \{(CH_3)_2AsOH\} \xrightarrow{[CH_3^+]} (CH_3)_3AsO \xrightarrow{2e^-} (CH_3)_3As$$

The arsenic(III) intermediates in braces are unknown; $\{Me_2AsOH\}$ is found as $Me_2As–O–AsMe_2$, cacodyl oxide, and $\{MeAs(OH)_2\}$ as $(MeAsO)_x$.[108] (Some arsenicals of formula $RAs(OH)_2$ and R_2AsOH are known, but in these R is usually a substituted aromatic group.[22,108]) Esters $RAs(OR')_2$ and $R_2As(OR')$ are known, but these are not very stable hydrolytically.[108] Thioesters are discussed later in this section.

The work of du Vigneaud and co-workers[109] on transmethylation led Challenger[91] to study Me_3As production in the presence of labeled precursors. Of the three precursors studied in bread cultures of *S. brevicaulis*, only $^{14}CH_3$-labeled methionine was found to transfer its label to arsenite to a significant extent. The methylation percentage [{(activity of the mercuric chloride–arsine adduct) × 33}/(activity of the methionine precursor)] was a maximum of 28% after 5 days. This result was taken as an indication that "active methionine", then recently identified as *S*-adenosylmethionine (SAM)[110] (**2**), is possibly involved in the "transfer of the methionine methyl group to arsenic (or selenium) in mycological methylation".[91]



SAM
2

More recently, Cullen et al.[92] demonstrated that the CD_3 group in L-methionine-*methyl-d*$_3$ is transferred intact to arsenite, arsenate, methylarsonate, and dimethylarsinate by cultures of *S. brevicaulis*. The presence of the label was established by mass spectroscopy, and a very high incorporation of the CD_3 group, 80–90%, was found in the isolated tertiary arsine. Similar results were obtained for *C. humicola*. For example, the arsine from As_2O_3 consisted of 83% $(CD_3)_3As$, 13% $(CD_3)_2AsCH_3$, 3% $CD_3As(CH_3)_2$, and 1% $As(CH_3)_3$. Ethionine, which is an antagonist to methionine,[111] shuts down production of any arsine, including the ethyl species that might be expected from a purely chemical reaction. These results strongly support the idea that SAM is the source of $[CH_3^+]$ in Scheme I. (The fluoromethyl group of $FCH_2SCH_2CH_2CHNH_2COOH$ is not transferred to arsenic by *C. humicola*.[102])

In order to develop these ideas further, broken-cell homogenates of *C. humicola* were examined with respect to their ability to methylate arsenicals.[104] [^{74}As]Arsenate was incubated with the cell preparation, SAM, and NADPH for 8 h. After the cell debris had been removed by centrifugation, the supernatant was applied to a Sephadex G15 column. The elution pattern, fractions 1–3, is seen in Figure 1a. Fractions 1–3 were subsequently eluted from a Dowex AG1-X8-acetate ion-exchange resin as shown in Figure 1b–d, respectively (the species expected are shown under the appropriate elution-fraction numbers). In order to identify fractions I–VI further, their electrophoretic behavior[112] was compared with that of known standards. The derived autoradiogram is shown in Figure 2. It is clear that arsenite, arsenate, methylarsonate, and dimethylarsinate are present, in addition to at least two unidentified compounds. It is not clear why the elution patterns bear no relationship to the fraction contents as revealed by electrophoresis; perhaps a larger molecule is broken down in this final procedure. When the cell preparation is replaced by buffer, there is some reduction of the arsenate to arsenite, but no methylation.

Similar experiments with [^{14}C]methylarsonate and [^{14}C]dimethylarsinate ultimately reveal the formation of trimethylarsine oxide from both and [^{14}C]methylarsonate, a demethylation product, from the latter. Again, complex elution patterns were obtained. These

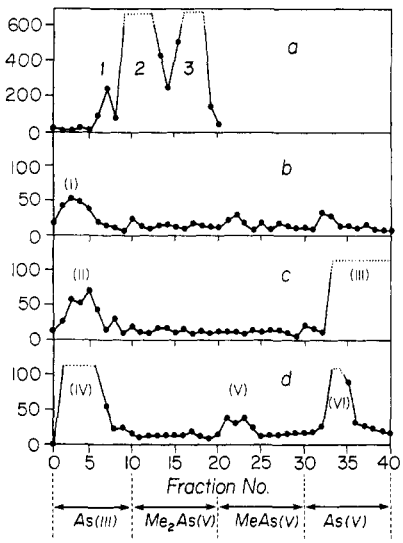


Figure 1. (a) Elution of ^{74}As species derived from incubating ^{74}As-labeled arsenate with cell preparation of *Candida humicola* from Sephadex G15 by 0.02 M NH_4OAc. In b–d the subsequent elution pattern from Dowex AG1-X8-acetate resin of peaks 1–3 is shown. The Dowex columns were eluted as follows: Fractions 1–10, H_2O; fractions 11–20, 0.01 M acetic acid; fractions 21–30, 1 M acetic acid; fractions 31–40, 1 M HCl. The arsenicals normally eluted by each solvent are indicated on the figure.

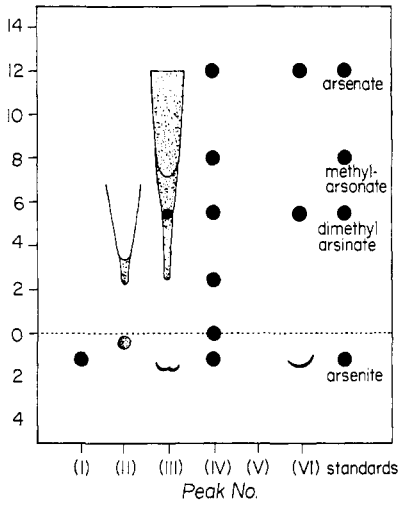


Figure 2. Electrophoresis of fractions I–VI isolated as indicated in Figure 1. The shading corresponds to the degree of darkening of the X-ray film.

findings suggest that definite interactions of the "simple" arsenical with the cell component are necessary for the methylation and electron-transfer reactions. Thus, although all the arsenic(V) intermediates proposed by Challenger can be found in the cell preparations, it seems that in reality the reaction mechanism may be more complex.[104]

This conclusion is reinforced by studies on a model arsenic(III) intermediate, $(MeAsO)_x$.[94] When cell-free extracts of *C. humicola* are incubated with $(MeAsO)_x$, glucose, and NAD, the product is $Me_2AsO(OH)$ (17%); no arsenate is formed. The transformation requires glucose and is pH dependent. The yield of $Me_2AsO(OH)$ is not affected by a range of electron transport inhibitors and methylating agents such as SAM, and a ^{14}C-methyl label on SAM is *not* transferred to arsenic.[94] The reaction cannot be induced by growth of the cells in dimethylarsinate-containing media prior to preparing the cell-free extract, although this is effective for whole cells.[101]

It should be noted that Challenger and Higginbottom[95] tried to prepare an active "press juice" from *S. brevicaulis*, but without success.

C. Model Studies Relating to the Methylation of Arsenic by Fungi

The oxidative addition reaction of methyl iodide or dimethyl sulfate with arsenic(III) in alkaline solution, the Meyer reaction,[108] is one of the classic routes to methylarsonic acid (eq 1). This is the prototype re-

$$CH_3I + NaH_2AsO_3 \xrightarrow{OH^-} NaI + CH_3AsO(OH)_2 \quad (1)$$

action for the biological methylation reactions involving *S*-adenosylmethionine (2) (Scheme I). Models for this sequence would involve transfer of methyl groups from sulfonium or ammonium derivatives to arsenic(III) compounds.

When sodium selenite is heated with betaine or choline chloride, dimethylselenium is produced. Similar experiments involving arsenite do not result in the generation of trimethylarsine.[95] Trimethylsulfonium hexafluorophosphate ($Me_3S^+PF_6^-$) reacts with arsenite (pH 12, 80 °C) to afford methylarsonate.[113] In fact, all the alkylation reactions of Scheme I can be carried out under these conditions, and the reduction reactions of this scheme can be effected by bubbling SO_2 through acidic solutions of the appropriate arsenic(V) compound. Thus the whole stepwise sequence can be modeled.

The closely related methylsulfonium derivative of methionine, $[Me_2^+SCH_2CH_2CH(NH_2)COOH]PF_6^-$, reacts with methylarsonite at a more realistic pH of 5.8 with partial methyl transfer; however, the methylsulfonium derivative of coenzyme M, $Me_2^+SCH_2CH_2SO_3^-$[114] (see section III.F), is inactive under these conditions.[113]

In a search for a better model for the reduction steps of Scheme I, the reaction of arsenic(V) compounds with thiols was studied (arsenic is well-known to have a particular affinity for sulfur). Trimethylarsine oxide is easily reduced to Me_3As by a range of thiols and dithiols, including cysteine, glutathione, and lipoic acid (eq 2). Kinetic studies indicate that the reduction

$$Me_3AsO + 2RSH \xrightarrow{H_2O} Me_3As + RSSR \quad (2)$$

occurs via a two-electron transfer from $Me_3As(SR)_2$.[115] Lipoic acid in particular is an attractive model reductant that also reduces methylarsonate and dimethylarsinate to arsenic(III) species.[116] A range of other thiols and dithiols, including cysteine, glutathione, mercaptoethanol, and dithiothreitol, effect this stoichiometric reduction at neutral pH. Other workers have made significant contributions to studies of this type.[117] The mechanism appears to be as follows:[116]

$$Me_xAsO(OH)_{3-x} + 2RSH \rightarrow Me_xAs(SR)_2(OH)_{3-x} + H_2O \quad (3)$$

$$Me_xAs(SR)_2(OH)_{3-x} \rightarrow Me_xAs(OH)_{3-x} + RSSR \quad (4)$$

$$Me_xAs(OH)_{3-x} + (3-x)RSH \rightarrow Me_xAs(SR)_{3-x} \quad (5)$$

The methylarsenic(III) products would likely be sulfides if a high concentration of reducing thiols was present

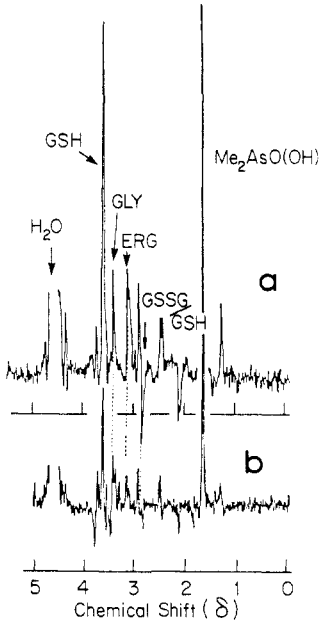
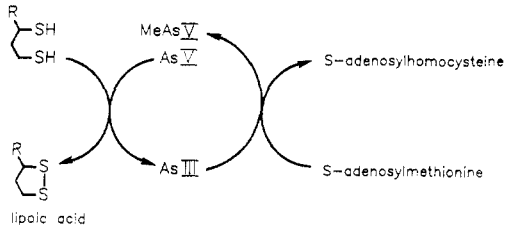


Figure 3. (a) ^1H NMR spectrum of a suspension of human red blood cells in 0.154 M NaCl in D_2O. GSH = glutathione, GLY = glycine, GSSG/GSH = inverted doublet that provides a measure of the degree of oxidation of the glutathione, ERG = ergothioneine. (b) Same sample as in (a) after the addition of $Me_2AsO(OH)$. Both spectra were recorded by using the spin–echo technique to eliminate resonances associated with large molecules.

SCHEME II



in biological systems. If the concentration of thiols was low, the products would be methylarsenic(III) oxo species. A simple and attractive model for the essential steps in Scheme I is shown in Scheme II.[115]

The compounds Me_2AsSR (RSH = cysteine, glutathione) are unstable to oxygen; the products are disulfides and $Me_2AsO(OH)$.[116,117] Thus isolation of alkylarsenic(III) metabolites could be a problem, and a reaction such as this may account for their not being observed.

Results from a "real" system, red blood cells/dimethylarsinic acid, offer further insight into this model.[118,119] A spin–echo ^1H NMR spectrum of a suspension of normal human erythrocytes is shown in Figure 3a. This technique visualizes only the cytosolic components.[120] When oxidative stress in the form of external dimethylarsinic acid is applied to the cells, dramatic changes in the spectrum are observed (Figure 3b). The response of glutathione with time is seen in Figure 4. The change in $Me_2AsO(OH)$ concentration can be attributed to reduction of the arsenical, via glutathione, to a Me_2As^{III}–S– species that is bound to a transmembrane protein and, as a result, is not "seen" in the NMR experiment. Soluble Me_2As–SR′ species are not formed. The response of ergothioneine is harder to explain. Ergothioneine is confined to the inside of the cell, is not synthesized by the cell, and is not likely to act as a reducing agent. Perhaps it acts as a gate for the trans-

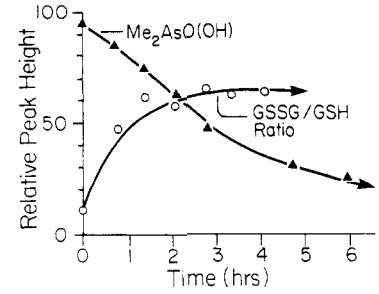


Figure 4. Plot of the variation of the concentrations of $Me_2AsO(OH)$ and the ratio of oxidized to reduced glutathione as a function of time measured from spin–echo NMR spectra of human red blood cells.

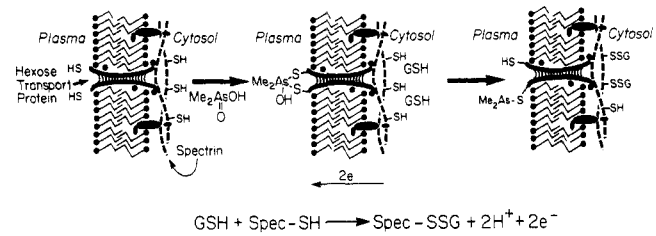


Figure 5. Model for the interaction of $Me_2AsO(OH)$ with human red blood cells.

membrane reduction process on the exofacial surface. A schematic picture of the process is seen in Figure 5.[119]

A number of metalloenzymes, many molybdenum based, are involved in oxygen-transfer reactions: $R_3NO \rightarrow R_3N$; $RS(O)R' \rightarrow RSR'$.[121] It is possible that these enzymes can also reduce Me_3AsO to Me_3As, which might account for the increased number of organisms that are capable of metabolizing this oxide. Model studies are certainly indicated.

D. Methylation of Arsenic by Bacteria

Challenger[10] suggested that bacteria do not methylate arsenic. This conclusion was based entirely on the "odor" test.[88,89] He did concede the possibility that the "garlic breath" of patients undergoing treatment with sodium dimethylarsinate could be associated with the action of bacteria such as *B. subtilis*, which were isolated from their feces.[122] These bacteria could have been induced to produce arsines by extended exposure to the arsenical.

The first well-documented report of an arsine being produced by bacteria was not made until 1971.[20] *Methanobacterium* strain MoH growing anaerobically on H_2 and CO_2 produces a volatile gas with a garlic odor from arsenate. Experiments with cell extracts, to be described later, indicate that the gas is probably dimethylarsine (Me_2AsH); CO_2 is necessary for its production. Cell extracts of *Desulfovibrio vulgaris* also evolve an unidentified arsine when treated with arsenate,[20] so the intact organism probably does the same. Both Me_3As and Me_2AsH are produced from arsenate by cell extracts of *Methanobacterium thermoautotrophicum*.[123]

Since 1971 a number of nonmethanogenic bacteria have been identified as methylarsine producers, notably by Anderson and co-workers.[124-126] The bacteria used in their studies were isolated from the environment and acclimated to grow in arsenate ($\leq$100 μg mL^{-1}). The response to the herbicides sodium arsenate and sodium

TABLE IV. Methylation of Arsenic by Bacteria

bacterium	substrate	products[a]	ref
Methanobacterium MoH	NaH_2AsO_4	Me_2AsH?	20
Corynebacterium sp	NaH_2AsO_4	As(III), Me_2AsH, Me_3As	124
Pseudomonas sp	NaH_2AsO_4	As(III), $MeAsH_2$, Me_2AsH, Me_3As	124
	$MeAsO(Na)_2$	As(V), $MeAsH_2$, Me_2AsH	125
Flavobacterium sp	NaH_2AsO_4	As(III), Me_2AsH	124
	$MeAsO(ONa)_2$	As(V), $MeAsH_2$, Me_2AsH	125
Proteus sp	NaH_2AsO_4	As(III), $MeAsH_2$, Me_2AsH	125
E. coli sp	NaH_2AsO_4	As(III), $MeAsH_2$, Me_2AsH	124
Achromobacter sp	$MeAsO(ONa)_2$	As(V), $MeAsH_2$, Me_2AsH	125
Aeromonas sp	$MeAsO(ONa)_2$	$MeAsH_2$, Me_2AsH_3, Me_3As	125
Enterobacter sp	$MeAsO(ONa)_2$	$MeAsH_2$, Me_2AsH	125
Norcardia	$MeAsO(ONa)_2$	As(V), $MeAsH_2$, Me_2AsH, Me_3As	125
B. coli communis	As_2O_3	no arsine	95
B. lactis aerogenes	As_2O_3	no arsine	95
Veillonella alcalescens, *Streptoccus sanguis*, *Fusobacterium nucleatum*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* K12	Me_3AsO	Me_3As	127

[a] As(III) is arsenite, As(V) is arsenate.

methylarsonate is seen in Table IV. Under *aerobic* conditions, methylation to dimethyl and trimethyl species is found in addition to reduction to methylarsine and demethylation to arsenate. The arsines were separated by gas chromatography (10% OV-1 column) and their rates of formation measured. A feature of the results listed in Table IV is the prevalance of reduced species.

The reduction of Me_3AsO to Me_3As by a wide range of microorganisms not normally associated with arsenic metabolism is particularly interesting.[127] The transformation is immediately obvious if human skin is exposed to Me_3AsO; cultures of two anaerobes and three aerobes isolated from skin were found to produce Me_3As from Me_3AsO. A marine pseudomonad did likewise, as did three organisms, *Veillonella alcalescens*, *Streptococcus sanguis*, and *Fusobacterium nucleatum*, isolated from dental plaque.

All these reductions of Me_3AsO are fast, with the marine pseudomonad showing the highest rate, $d(Me_3As)/dt$ = 585 nmol of Me_3As min^{-1} (g of wet cells)$^{-1}$ from cultures 12 mM in Me_3AsO (standard conditions[103]). Examples of other rates are as follows: *Staphylococcus aureus*, 208 (anaerobic) and 81 (aerobic); *E. coli*, 100; skin organisms, 3–10. If these results are combined with those obtained for fungal reduction of Me_3AsO[103] (Table II), it is apparent that in spite of the relative ease of oxidation of Me_3As to Me_3AsO[128] (and $Me_2AsO(OH)$[10,96,129]), the oxide will be very easily mobilized as the arsine in the environment.

To illustrate this important point further, when Me_3AsO is added to fresh river water, sewage sludge, rumen fluid, and sea sediments, Me_3As can be detected by gas chromatography or by odor.[127] It may well be that the traces of Me_3As found in fresh prawns and other foods[130] actually arise by bacterial action on Me_3AsO. This arsine oxide is found in marine animals (Table V).

E. Mechanism of Arsenic Methylation by Bacteria

As described in section III.B, the methylation of arsenic by fungi probably involves *S*-adenosylmethionine (**2**) as the methyl donor. The question now arises as to whether bacteria might use a different methyl source.

Cell-free extracts of *Methanobacterium* strain MoH produce an arsine when incubated anaerobically with arsenate, H_2, ATP, and a methyl donor (precursor).[20] Either methylcobalamin (Me-B_{12}) or CO_2 served as precursor, but not serine or 5-methyltetrahydrofolate. The arsine was trapped in 2 M HNO_3 or on rubber serum stoppers and was identified as dimethylarsine by radiochemical techniques. The Me-B_{12} was added because at that time it was believed that Me-B_{12} was involved in methane production (and in the methylation of mercury[19]). This conclusion was based on the isolation of a cobalamin-containing protein from a culture of *M. omelianski*.[131] This organism was later shown to consist of two symbiotic bacteria, one of which was the methanogen *Methanobacterium* MoH, which did not contain cobalamin.[132] The true methane precursor in *Methanobacterium* MoH has since been shown to be $HSCH_2CH_2SO_3^-$, coenzyme-M (HS-CoM).[114,133,134] Its function is shown in Scheme III.

SCHEME III

$$(SCH_2CH_2SO_3^-)_2 \xrightarrow{H_2} HSCH_2CH_2SO_3^- \xrightarrow{CH_3-X}$$

$$\underset{\text{MeS-CoM}}{CH_3SCH_2CH_2SO_3^-} \xrightarrow{H_2} \underset{\text{HS-CoM}}{HSCH_2CH_2SO_3^-} + CH_4$$

An enzyme that is a catalyst for the transfer of a methyl group from methylcobalamin (Me-B_{12}) to HS-CoM can be purified from cell extracts of *Methanobacterium* MoH; however, it is not established that Me-B_{12} is a natural carrier in hydrogen-grown *Methanobacterium*, and it is not established that the enzyme methylcobalamin-CoM methyltransferase is important in the reduction of CO_2 to methane.[133,135] Cobalamin-containing enzymes can be isolated from cell-free extracts of *Methanosarcina barkeri*. These enzymes are involved in the methyl transfer from methanol to HS-CoM.[136,137] One of these enzymes also catalyzes the methyl transfer Me-B_{12} to HS-CoM.[136] However, this organism, which belongs to a different group of methanogens from *Methanobacterium* MoH,[135,138] does not seem to have been implicated in the methylation of metals and metalloids.

In spite of these observations relating to the noninvolvement of Me-B_{12} in methylation reactions of *Methanobacterium* MoH, there is no doubt that Me-B_{12} can act as a methyl source for methylarsenical production in cell extracts of this methanogen. The possibilities seem to be as follows: (a) MeS-CoM is produced from Me-B_{12}, and this becomes involved with the methylation of arsenic; (b) Me-B_{12} reacts with the arsenical in a chemical or biological process that is not part of the normal cell response; and (c) Me-B_{12} provides the methyl group to the component in the cell extract, e.g., methionine, which is the ultimate methyl donor to arsenic, again in a reaction that is not a normal part of the whole-cell chemistry. Unfortunately, the available data do not allow any distinctions to be made. In the absence of Me-B_{12}, the cell extracts produce an arsine, presumably Me_2AsH, from dimethylarsinate.

However, an arsine is not produced from methylarsonate.[20]

The only major difference between fungi and bacteria seems to be that reduction of methylarsenic(V) species to arsines, Me_xAsH_{3-x} (x = 0–2), is a more common response by bacteria. This does raise the possibility that reactions such as the one shown in eq 6, which would be analogous to HS-CoM → MeS-CoM (Scheme III), may be involved. The related, well-known reaction of eq 7 and 8 should also be noted;[139,140] reaction 7 proceeds by both B_{12}-dependent and B_{12}-independent mechanisms.[139]

$$\text{SAM} + \text{H–AsRR}' \rightarrow \text{Me–AsRR}' + \textit{S}\text{-adenosylhomocysteine} \quad (6)$$

$$\text{SAM} + \text{H–SCH}_2\text{CH}_2\text{CH(NH}_2\text{)COOH} \rightarrow \text{Me–SCH}_2\text{CH}_2\text{CH(NH}_2\text{)}_2\text{COOH} \quad (7)$$

$$\text{SAM} + \text{CH}_3\text{SH} \rightarrow \text{CH}_3\text{SCH}_3 \quad (8)$$

The methanogens belong to a very ancient group of bacteria, the *Archaebacteria*, with a narrow ecological niche.[135] Most of the transformations of arsenicals described above are carried out by a wide range of bacteria. Until strong evidence is advanced, there seems little need to invoke a different mechanism for arsenic methylation by bacteria from that discussed above for fungi.

F. Model Studies Relating to the Methylation of Arsenic by Bacteria

Because of the similarity of MeS-CoM to methionione (Scheme III), it seemed possible that Me_2^+-$SCH_2CH_2SO_3^-$ (Me_2S-CoM^+) might act as a methyl donor to arsenic. In model studies this sulfonium derivative does not transfer its methyl group to arsenite under conditions that result in transfer from [Me_2^+-$SCH_2CH_2CH(NH_2)COOH$]PF_6^-.[113] This same similarity prompted the synthesis of *S*-adenosyl-CoM and *S*-methyladenosyl-CoM^+ because of the possibility that they might be involved in methane production.[141,142] *S*-Adenosyl-CoM failed to stimulate methane production from cell extracts of *Methanobacterium thermoautotrophicum* when it was substituted for the required ATP.

Although the involvement of Me-B_{12} in the biological methylation of arsenic has yet to be established, a number of model experiments have been conducted with this possibility in mind. Schrauzer and co-workers[144] report that the methylcobaloxime $Me(H_2O)Co(dmgH)_2$, a model for Me-B_{12} ($dmgH_2$ = dimethylglyoxime), reacts with As_2O_3 in the presence of a reducing agent such as dithiothreitol to give low yields of the arsines $MeAsH_2$ and Me_2AsH. The authors speculate that compounds with As–Co bonds could be intermediates. A range of compounds of this type, $RR'AsCo(dmgH)_2(PBu^t_3)$ (e.g., R = R′ = CH_3; R = Ph, R′ = Cl), were subsequently prepared.[145] Reaction of the dimethylarsino derivative with methyl iodide results in methylation at both metal centers without the concomitant formation of iodo derivatives (eq 9). Methyl(pyridine)cobaloxime, a potential source of CH_3^-, like Me-B_{12}, also reacts with the same dimethylarsino derivative to give Me_3As. Thus both nucleophilic attack and electrophilic attack on the Co–As bond result in methylation of arsenic. This appears to be the only documented example of transfer of a methyl group from cobalt to arsenic, although the mechanism is not at all obvious. Methylcobaloximes do not react with arsenic halides such as $AsCl_3$, AsI_3, or $MeAsCl_2$ to afford methylarsenicals; again unexpected chemistry is encountered.[145] Irgolic and co-workers[146] have reported on some experiments attempting to establish methyl transfer from $MeCo(dmgH)_2Py$ to $MeAsX_2$. The limited data available do not support this contention.

$$\text{Me}_2\text{AsCo(dmgH)}_2\text{PBu}^t_3 + \text{CH}_3\text{I} \rightarrow \text{Me}_3\text{As} + \text{MeCo(dmgH)}_2\text{PBu}^t_3 \quad (9)$$

G. Cleavage of Arsenic–Carbon Bonds

The first example of biological arsenic–carbon bond cleavage was described by Challenger.[48] Trimethylarsine is produced when *S. brevicaulis* and *P. notatum* act on $ClCH_2CH_2AsO(OH)_2$, even though heating in alkaline solution is normally required to decompose the arsonic acid to arsenate and ethylene. Because *P. notatum* does not methylate arsenate, it seems likely that loss of the $ClCH_2CH_2$ group takes place after at least one methylation step.

The methylation of $CD_3AsO(OH)_2$ by *C. humicola* and *S. brevicaulis* affords some $(CH_3)_3As$, which presumably is an indication of an initial demethylation step.[92] Likewise $(CD_3)_3As$ can be isolated from biological reactions involving $CH_3AsO(OH)_2$. Demethylation of sodium methylarsonate to arsenate is reported by Anderson and co-workers.[125,126] One organism isolated from soil, *Alcaligenes*, produces only arsenate; others isolated from the same environment produce arsine as well (Table IV). Wine yeast is reported[82] to demethylate dimethylarsinate to methylarsonate.

Methyl cleavage reactions have also been observed in broken-cell homogenates of *C. humicola*.[104] Thus [^{14}C]dimethylarsinate is metabolized to [^{14}C]methylarsonate in the presence of *S*-adenosylmethionine and NADPH. *C. humicola* seems to be able to cleave the aryl group from 2-OH-4-$NH_2C_6H_3AsO(OH)_2$ because trimethylarsine is a product.[102]

IV. Arsenic Compounds in the Marine Environment

A. Mollusks, Fish, and Other Animals

It has been known for many years that the concentration of arsenic species in marine and freshwater animals is considerably above the background concentrations in the surrounding water and that "fish arsenic" is chemically and physiologically different from arsenate and arsenite.[43,147–153] Some arsenic concentrations found in marine animals are given in Table V.

Typical values for arsenic in seawater are ~2 ppb; in fresh water a much wider variation can be encountered, commonly in the range 0.4–80 ppb.[43] Some wells can have concentrations as high as 2500 ppb, as found in Taiwan.[43,154] The water in these wells may be responsible for causing skin cancer and Black Foot disease.[155,156] The latter is a peripheral vascular disorder resulting in gangrene of the extremities, especially the feet. Arsenic is implicated, but other compounds may be responsible.

The arsenic concentrations in Table V range from 0.31 ppm in salmon to a high of 340 ppm in the midgut

gland of the carnivorous gastropod *Charonia sauliae*. Although the arsenic concentrations in crustacea are generally believed to be higher than in fish, samples of plaice taken from the North Sea had arsenic concentrations in the range 3–166 ppm, with 65% of the 255 different samples above 10 ppm.[157] The world's record for arsenic accumulation is probably held by the polychaete worm *Tharyx marioni*.[158] The whole-body concentration usually exceeds 2000 ppm dry weight. Much of this arsenic is concentrated in the palps, which comprise ~4% of the body weight. The concentration in these organs is in the range 6000–13000 ppm, and the bulk of this appears to be in an organic form.

Although substantial contributions to our knowledge about the distribution and nature of arsenicals in the marine environment had been made by Lunde,[149–153] it was not until 1977 that arsenobetaine (**3**) was isolated

$(CH_3)_3As^+CH_2COO^-$ (3) $(CH_3)_4As^+$ (4)

$(CH_3)_3As^+CH_2CH_2OH$ (5) $(CH_3)_3AsO$ (6)

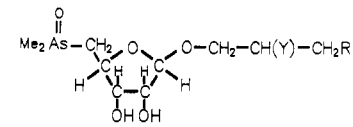
from the rock lobster *Panulirus cygnus*.[159,160] This compound was unequivocally characterized and its structure verified by X-ray crystallography. Since this discovery, arsenobetaine has been shown to be present, and to be the most abundant arsenical, in most marine animals so far investigated; the sea squirt is a notable exception.[161] The carnivorous gastropods can have very high concentrations of this arsenical, reinforcing the thesis[43,162–164] that this compound is an end point of the arsenic cycle in the marine ecosystem. The tetramethylarsonium ion (**4**) has recently been found in the clam *Meretrix lusoria*, a plankton feeder, and is the major arsenical in the gill.[165] Again, arsenobetaine is present in all parts of this clam, in addition to smaller amounts of two unidentified arsenicals. Similar results are obtained for some other bivalves (clams, mussels, scallops), although the relative amount of **4** can vary greatly from 0% in scallops[166] to 48% in the Manila clam.[167] The whelks of northern British Columbia do not seem to produce **4**;[167] however, this ion has been reported in other gastropods.[168]

Relatively high concentrations of as yet unidentified arsenicals are found in a number of these mollusks, especially the gastropods.

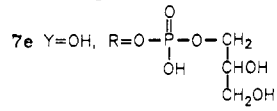
It has been claimed that arsenocholine (**5**) is present in shrimp;[169–171] others[172,173] could not confirm its presence. (Norin and co-workers[171] find that the concentration of arsenocholine in shrimp can be greater than that of arsenobetaine.) Arsenocholine may be a minor constituent in the midgut gland of the gastropod *C. sauliae*.[166] These identifications of arsenocholine have largely been made on the basis of a comparison of HPLC retention times with that of a standard. One study quotes further evidence in the form of a mass spectrum (FAB) with a peak at m/e 165, which is the parent ion of arsenocholine.[169] However, the rest of the mass spectrum shows little resemblance to that of the standard (problems with both fast atom bombardment mass spectroscopy and field desorption mass spectroscopy (FD/MS) have been reported;[172] some matrix effects in FD/MS can be overcome by adding a strong protonating agent such as *p*-toluenesulfonic acid to the matrix); furthermore, the unknown compound elutes from the strong cation-exchange resin AG 50W under conditions that would result in retention of arsenocholine. The elution order from this resin is as follows: arsenical (eluting solvent); inorganic As (0.5 M HCl); $MeAsO(OH)_2(H_2O)$; $Me_2AsO(OH)$, Me_3AsO (**6**), **3** (4 M NH_3); **5** (6 M HCl).[159] The same order is also found by using slightly different concentrations of eluting solvents;[165,167] **4** elutes with 1 M HCl.

In most cases, extraction with methanol is sufficient to remove essentially all the organoarsenicals from marine samples, although some bivalves release only 60% into methanol[167,174] and shark liver releases 40%.[175] (Extraction with chloroform is not effective even though the arsenicals are slightly soluble in this solvent.[169]) A preliminary cleanup of the extract can be achieved by "defatting" with diethyl ether. In the case of the crab, this procedure removes 10–17% of the total arsenic. Higher percentages are extracted from methanol extracts of shark liver and some bivalves, whereas lower percentages are extracted from lobsters. Chromatography of the methanol- and water-soluble fractions on a strong cation-exchange column is a very useful preliminary step for isolating organoarsenicals from biological tissue. The arsenic content of the extracts and fractions can be monitored by plasma emission (ICP) or graphite furnace atomic absorption (GFAA) techniques. Cleanup after ion-exchange chromatography can be achieved by HPLC/ICP or HPLC/AA on ion-exchange,[165,166,175] size-exclusion,[176] or reverse-phase[169,177,178] columns. Matrix effects can affect the chromatogram, so care is necessary;[176,178] a 100-μL injection should contain at least 75 ng of arsenic for detection by HPLC/ICP. (Maher[179] claims success with separating muscle extracts directly on a C-18 column.) Final isolation of the organoarsenical is often accomplished following TLC on silica or cellulose.

The presence of arsenic-containing sugar derivatives in algae had already been established (section IV.B) when two compounds of the same class, **7a** and **7b**, were



7a Y=OH, R=OSO_3H
7b Y=OH, R=OH
7c Y=OH, R=SO_3H
7d Y=NH_2, R=SO_3H



7e Y=OH, R=

found in the giant clam *Tridacna maxima*.[180] A crystal structure determination confirmed that **7a** was formulated correctly. Isolation and separation of the compounds extracted from the kidney were achieved by chromatography on Sephadex LH-20 (size exclusion) and Sephadex DEAE (ion exchange) and by preparative TLC on silica and cellulose. The organoarsenicals from the adductor muscle had chromatographic properties identical with those of **7a** and **7b** from the kidney. The high arsenic content in the kidney (0.02%) should be noted.[180] The source of the arsenic is likely to be the symbiotic, unicellular, green algae living in the clam tissue[180,181] (section IV.B). It is surprising that no

TABLE V. Organoarsenicals Isolated from Marine Animals

animal	organ (As concn, ppm)[a]	arsenical (% of total As)	comments[c]	ref
western rock lobster (*Panulirus cygnus*)	tail (20)	**3** (high)	*d, e*	159, 160, 174
	hepatopancreas (39)	**3** (high)	*f–h*	177
American lobster (*Homarus americanus*)	tail (6.5–26)	**3** (high)	*d, g, h*	*o*
lobster	tail (5.2)[b]	**3** (87)	*f, h*	179
crayfish (*Jasus novaehollandiae*)	muscle	**3** (95)	*f, g*	179
dusky shark (*Carcharhinus obscurus*)	flesh (?)	**3** (high)	*d, g, h*	172
shark (*Prionace glaucus*)	flesh (37.4)	**3** (80)	*d, f, h*	175
	liver (27.7)	**3** (40)	*f, h, i*	175
whitetip shark (*Carcharhinus longimanus*)	muscle (3.1)	**3** (high)	*d, f, h*	*p*
blue pointer (*Isurus oxyrhincus*)	muscle (?)	**3** (high)	*d, f, h*	*p*
octopus (*Paroctopus dofleini*)	muscle (49)	**3** (>90)	*d, g, h*	186
squid (*Sepioteuthis australis*)	muscle	**3** (93)	*f, g*	179
Alaskan king crab (*Paralithodes camtschatica*)	claw (8.6)	**3** (82)	*d, f, g, i*	176
Alaskan snow crab (*Chionoecetes bairdii*)	claw (7.7)	**3** (85)	*d, f, g, i*	176
dungeness crab (*Cancer magister*)	canned (4.0)	**3** (79)	*d, f, g, i*	176
red crab (*Chionoecetes opilio*)	meat (2.1)	**3** (88)	*d, f, g, i*	178
		U (12)		
shrimp (*Sergestes lucens*)	meat (5.5)	**3** (80)	*d, g, h*	173
shrimp (*Pandalus borealis*)	meat (?)	**3** (74)	*d, j*	170, 171
		5 (11)		
shrimp	meat (7.2, 20.8)[b]	**3** (8, 72)	*d, f, h*	169
		5 (76, 15)		
prawn (*Penaeus latisulcatus*)	muscle	**3** (56)	*f, g, i*	179
		U (33)		
		3 (>95)		*q*
giant clam (*Tridacna maxima*)	kidney (200)	**7a** (80)	*d, e, g–i*	180
		7b		
	adductor	**7a**	*d, g*	180
	muscle	**7b**		
clam (*Meretrix lusoria*)	gill (23.8 av)	**4** (74), **3** (5), U_1 (8)	*d, f–i*	180
	midgut gland (6.6 av)	(U_1 + **4**) (4), **3** (48), U_2 (31)		
	siphon (3.9 av)	(U_1 + **4**) (27), **3** (41), U_2 (17)		
	foot (3.6 av)	(U_1 + **4**) (32), **3** (64), U_2 (11)		
	mantle (2.9 av)	(U_1 + **4**) (26), **3** (35), U_2 (9)		
	adductor muscle (2.6 av)	(U_1 + **4**) (17), **3** (72), U_2 (11)		
asari (clam) (*Tapes japonica*)	soft tissue (2.0)	**3** (45), U (50)	*d, f*	165
native littleneck clam (*Protothaca staminea*)	soft tissue (1.8)	U (3), **4** (48), **3** (13)	*d, f, h, i*	166
manila clam (*Verupis japonica*)	soft tissue (1.3)	U (7), **4** (48), **3** (13)	*d, f, h, i*	167
butter clam (*Saxidomus giganteus*)	soft tissue (2.2)	U_1 (2), **4** (21), (U_2 + **3**) (24)	*d, f, h, i*	167
horse clam (*Schizothoerus nuttalli*)	soft tissue (1.8)	U_1 (6), U_2 (39), **3** (12)	*d, f, h, i*	167
soft-shell clam (*Mya arenaria*)	soft tissue (1.2)	U_1 (20), **4** (20), **3** (38)	*d, f, h, i*	167
geoduck (*Panope generosa*)	visceral mass (5.6)	U_1 (8), (**4** + U_2) (62), **3** (16)	*d, f, h, i*	167
	foot (4.1)	U_1 (15), **4** (27), **3** (27)		
	siphon (3.3)	U_1 (4), (**4** + U_2) (67), **3** (11)		
	muscle (2.6)			
hotategai (scallop) (*Patinopecten yessoensis*)	muscle (2.8)	**3** (64)	*d, f, i, n*	166
	midgut gland (4.0)	**3** (29)		
scallop (*Patinopecten yessoensis*)	muscle (0.68)[b]	**3** (88)	*d, f, h, m*	169
scallop (*Pecten alba*)	muscle	**3** (61), U (25)	*f, g, i*	179
ivory shell (gastropod) (*Buccinum striatissimum*)	muscle (38)	**3** (90)	*d, h, i*	162
	midgut gland (18)	**3** (60)		
blue mussel (*Mytilus edulis*)	soft tissue (1.8)	U_1 (10), (U_2 + **4**) (38), **3** (20)	*d, f, h, i*	167
northwest neptune (*Neptunea lyrata*)	soft tissue (28.4)	U_1 (17), U_2 (30), **3** (50)	*d, f, h, i*	167
whelk (*Beringius crebriscotata*)	soft tissue (17.4)	U_1 (12), U_2 (12), **3** (74)	*d, f, h, i*	167
Phoenicean whelk (*Neptunea phoeniceus*)	soft tissue (3.5)	U_1 (5), U_2 (52), **3** (32)	*d, f, h, i*	167
sazae (gastropod) (*Batillus cornutus*)	muscle (5.0)	**3** (58)	*d, f, i*	166
	midgut gland (22.7)	**3** (18)		
boshubora (gastropod) (*Charonia sauliae*)	muscle (116.5)	**3** (58)	*d, f, i*	166
	midgut gland (340.1)	**3** (66)		
bateira (gastropod) (*Tegula pfeifferi*)	muscle (3.1)	**3** (109)	*d, f, i*	166
	midgut gland (6.7)	**3** (51)		
ibonishi (gastropod) (*Reishia clavigera*)	muscle (53.3)	**3** (74)	*d, f, i*	166
	midgut gland (34.5)	**3** (84)		
sea squirt (*Halocynthia roretzi*)	edible parts (25)[b]	**3** (0)	*d, g, i, k*	161
sea cucumber (*Stichopus japonicus*)	muscle (12.4)[b]	**3** (60)	*d, g*	161
flatfish (Limanda *herzensteini*)	muscle (36.0)[b]	**3** (90)	*d, g*	161
school whiting (*Sillago bassensis*)	muscle (7.7 av)	**3** (90)	*d, g, i*	164
fish (*Sillaginodes punctatus*)	muscle	**3** (96)	*f, g*	179
estuary catfish (*Cnidoglanis macrocephalus*)	muscle (0.5)	**3** (52), **6** (35)	*d, g, h*	163
plaice (*Pleuronectes platessa*)	muscle (3–166) (median 14)	**3** (>80), **6** (0.4)	*g, h*	157
			i, h, l	183
"fish meal"		**3** (94)	*d, g, h*	170
haddock	muscle (6.0)[b]	**3** (78)	*d, f, h, m*	169
herring	muscle (1.1)[b]	**3** (89)	*d, f, h, m*	169
halibut	muscle (2.3)[b]	**3** (74)	*d, f, h, m*	169
cod	muscle (5.1)[b]	**3** (80)	*d, f, h, m*	169
mackeral	muscle (0.55)[b]	**3** (80)	*d, f, h, m*	169

TABLE V (Continued)

animal	organ (As concn, ppm)[a]	arsenical (% of total As)	comments[c]	ref
sole	muscle (13.2)[b]	3 (86)	*d, f, h, m*	169
salmon	muscle (0.31)[b]	3 (48), U (41)	*d, f, h, m*	169

[a] Wet-weight basis unless otherwise[b] indicated; av = average value. [b] Dry-weight basis. [c] Organoarsenicals are soluble in methanol. [d] Purified by extensive ion exchange and gel permeation chromatography or chromatography on alumina. [e] Product identity established by a solid-state structure determination. [f] HPLC was used to aid separation. [g] Identification was made by using TLC and/or electrophoresis with standards. [h] Confirmation was made by using a spectroscopic technique such as mass spectrometry, NMR, or infrared spectroscopy. [i] The presence of unidentified organoarsenicals was established; major components are designated U, U_1, etc. [j] Confirmed by use of pyrolysis–gas chromatography/mass spectrometry. [k] The organoarsenical was separated into two basic compounds and one acidic; all are unidentified. [l] Extracted Me_3AsO is reduced to Me_3As with $NaBH_4$. $(CD_3)_3AsO$ is added as an internal standard. [m] Arsenobetaine was confirmed by derivatization to the ethyl ester. [n] Published numbers seem to be incorrect. [o] Edmonds, J. S.; Francesconi, K. A. *Chemosphere* **1981**, *10*, 1041. [p] Hanaoka, K.; Tagawa, S. *Bull. Jpn. Soc. Sci. Fish.* **1985**, *51*, 681. [q] Francesconi, K. A.; Edmonds, J. S. *Comp. Biochem. Physiol. C: Comp. Pharmacol.* **1987**, *87*, 345.

mention of the presence (or absence) of arsenobetaine is made,[180] since this would be expected to be present in some part of the animal.

Benson and Summons[181] had earlier suggested that the arsenicals present in the kidney of the giant clam *Tridacna maxima* are derivatives of lactic acid. Experimental details were not given. It seems reasonable to assume that their conclusions, which are based on their work on the arsenicals found in algae,[182] are in error. This work has been reinterpreted[45] (section IV.B).

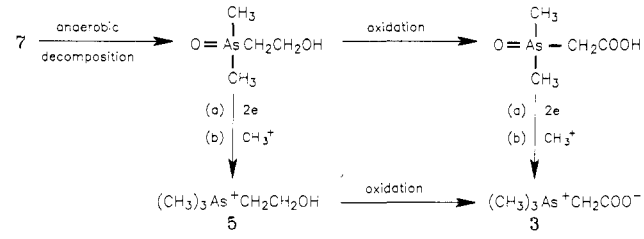
Trimethylarsine oxide (Me_3AsO (**6**)) has been found as a minor component in a number of fish species and may be one of the unknowns found in mollusks.[167] The concentration in fish can be considerably enhanced in samples that have been stored frozen. For example, in fresh perch the concentration is 5.9% of the total arsenic; in frozen perch it can reach 24.2% of the total arsenic.[183] It has been suggested that the Me_3AsO is the breakdown product of some other arsenical already present in the fish. If true, this precursor has yet to be identified.

Apparently, arsenobetaine in fish is not broken down to trimethylarsine oxide on storage,[163] although arsenobetaine is hydrolyzed almost quantitatively by hot alkali to Me_3AsO (arsenocholine and Me_4As^+ yield 6% and 0% Me_3AsO, respectively, on hydrolysis[157,171,172]). The amount of arsenic released from a sample as methylarsenicals by base hydrolysis is reported to depend on the pretreatment of the sample; e.g., cooked shrimp releases more organic arsenic than does uncooked shrimp.[172] Other studies[174] suggest that both Me_3AsO and $Me_2AsO(OH)$, in a 35:60 ratio, are produced from arsenobetaine on hydrolysis; the mixture yields both Me_3As and Me_2AsH on treatment with $NaBH_4$. In fact, some early work used the ratio of Me_3As to Me_2AsH, measured after hydrolysis and $NaBH_4$ reduction, as an indication of the presence of arsenobetaine. Arsenobetaine is stable to cultures of *C. humicola*[102] (Table III). Sugar derivatives such as **7** found in clam kidneys and algae (Table V) are degraded anaerobically to $Me_2As(O)CH_2CH_2OH$[184] (Scheme IV).

Inorganic arsenic appears to be converted to Me_3AsO in the gut of fishes.[152,185] This may be the source of the arsine oxide in the estuary catfish; high concentrations are found as a consequence of its feeding habits. As mentioned in section III.A, reduction of the oxide to Me_3As is a facile process, which may account for the traces of this arsine found in some food.[130]

Generally the concentration of organoarsenicals in freshwater fish is lower than found in the marine environment, e.g., bass (0.12 ppm) and yellow perch (0.053

SCHEME IV



ppm).[43,169] Even so, the arsenic concentrations are higher than found in the surrounding medium; the organoarsenicals do not seem to be the same as found in the marine environment.[169,185] Salmon that spend time in both fresh and salt water contain arsenobetaine and an unidentified arsenical not found in fresh- or saltwater fish.

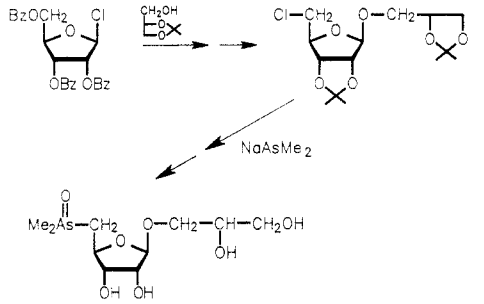
B. Marine Plants and Algae

The most significant work in this area has again been done by the group in Western Australia.[187,188] The arsenic content of the brown kelp *Ecklonia radiata* is ~10 ppm (wet weight) (higher arsenic concentrations have been reported for marine algae; e.g., 95–109 ppm in *Laminaria digitata*[153]). Methanol extraction of one sample followed by chromatography on Sephadex LH-20 and Sephadex DEAE gave two main arsenic-containing fractions. The major arsenicals eventually isolated by using preparative TLC were established to be **7b** and **7c** on the basis of microanalysis and spectroscopic studies. In particular, the ^1H and ^{13}C NMR spectra provided convincing evidence for the suggested structures.[187,188] These two compounds accounted for 81% of the total arsenic present in the kelp; other minor fractions were not characterized. Both compounds afford $Me_2AsO(OH)$ on hydrolysis, acidic or basic, so manipulations were carried out near pH 7.

A second batch of *Ecklonia radiata* was similarly processed 2 years after the first; some lipid-type material (14% of the total soluble arsenic) was isolated but not examined further. The two principal methanol-soluble compounds that were isolated proved to be **7c** again and **7e**. The latter compound, the minor component, was not isolated during the first extraction, and the authors are unable to account for this result.[188]

The identification of **7e** was made on the basis of microanalysis, ^1H and ^{13}C NMR spectra, and a field desorption mass spectrum that produced ions at m/e 527 $(P + 2Na)^+$ and m/e 275 $(P + 3Na)^{2+}$. The stereochemistry of the ribose system in this and the related compounds is assumed to be D, the same as found in

SCHEME V



the solid-state structure of **7a**.[180] As described above, the isolation of **7b** and **7a** from the giant clam *Tridacna maxima* and the determination of the crystal structures of **7a** followed after the initial work on *Ecklonia radiata*. A synthesis of **7c** has been developed by McAdam and Stick.[189] It is outlined in Scheme V.

The structure of **7e** indicates a connection between the water-soluble and lipid-soluble arsenicals, inasmuch as esterification of the two free hydroxy groups on the glycerol side chain could produce a phospholipid analogous to lecithin. This is discussed further below in connection with **8a** and **8b**.

Methanol extracts of the edible brown seaweed *Laminaria japonica* (Makonbu in Japanese) (~4 ppm arsenic wet weight) have also been examined by using Sephadex columns to separate out the arsenicals.[180] In this study HPLC (both ion-exchange and gel-permeation columns) was used effectively to establish the number of compounds of interest. These three proved to be ribofuranoside derivatives that had been previously isolated from *Ecklonia radiata*, namely **7c** (79% soluble arsenic), **7b** (17%), and **7e** (3%). **7c** appears to be present as a diastereomeric pair, epimeric at the CHOH group on the side chain.

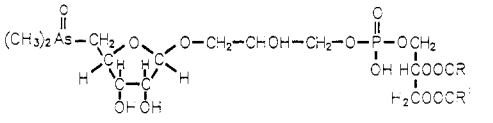
Both the seaweeds described above belong to the order Laminariales. They contain **7c** as the major arsenical and low concentrations of inorganic arsenic.[190,191] Another edible seaweed, *Hizikia fusiforme* (Hijiki in Japanese), which belongs to the order Fucales, has recently been found to contain essentially half its arsenic burden as sugar derivatives and half as arsenate.[192] The major organoarsenical (25% of the total As) was identical with **7a**, and the others (total <5% of extracted arsenic) were characterized as **7c** (again two diastereomers are present), **7e**, and a new ribofuranoside **7d**. The known compounds were characterized on the basis of their spectroscopic and chromatographic properties. The presence of the amine function in **7d** was adduced from pK_a measurements; the value of 7.8 was found for the substituent in this position. All four sugar derivatives, and other trace arsenicals, can be separated on Sephadex DEAE with Tris buffer (0.05 M, pH 8.0) as eluent; arsenate moves in 0.5 M buffer. Similar arsenicals may be present in *Eisenia bicyclis* (Arame in Japanese).[193]

An earlier report[194] claims that the arsenicals extracted from *H. fusiforme* are arsenate and dimethylarsinate, together with smaller amounts of arsenite and methylarsonate. It is likely that the rather harsh extraction conditions and the addition of KI contributed to the degradation of the indigenous compounds.

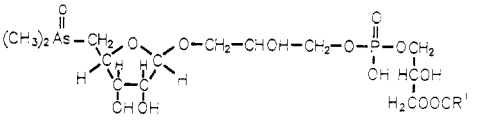
It has been suggested[190] that the difference in order, Laminariales or Fucales, may account for the difference in chemical components in the seaweeds so far investigated, namely arsenate present (in abundance) or absent, **7a** or **7c** as the major organoarsenical. A high concentration of arsenate (38% of total arsenic) is also present in the brown alga *Sargassum muticum*;[191] another member of family Sargassaceae (order Fucales). Maher[195] reports low concentrations of inorganic arsenic in both *Ecklonia radiata* and *Sargassum bracteolosum* (4.0% and 1.7% respectively of the total arsenic).

Recent studies[195a] on *Sargassum thunbergii* (Umitoranoo in Japanese) have revealed the presence of **7a** as the major arsenosugar; however, a minor arsenical proved to be the trimethylarsonium analogue of **7a** (i.e., **7a** with the $Me_2As(O)$ group replaced by Me_3As^+). Clearly more work is necessary.

The marine diatom *Chaetoceros concavicornis* incorporates [^{74}As]arsenate from cultures, and labeled arsenicals can be extracted with water or $CHCl_3$/MeOH. One investigation[182] that concentrated on the lipid fraction established that three main arsenic-containing compounds (33% of the total arsenic) could be isolated by two-dimensional paper chromatography. The initial postulate, that the arsenic-containing moiety in two of these fractions was a derivative of lactic acid, $-OCH(COOH)CH_2As^+Me_3$, has been demonstrated to be without foundation.[196,197] The available data have been interpreted[45] in the light of the knowledge that sugar derivatives **7** are found in other marine algae. The suggested structures for two of the lipids are **8a** and **8b** where R and R′ are alkyl chains (the third originally unidentified lipid fraction[182] remains so). Direct evidence for this conclusion has recently been presented;[197a] the lipid-soluble arsenic compound in the alga *Undaria pinnatifida* (Wakame in Japanese) is probably a diester of palmitic acid (**8a**, R = R′ = $C_{15}H_{31}$).



8a



8b

The water-soluble arsenicals extracted from *C. concavicornis* are probably as follows:[45,182] **7c** (or **7a**) (40% of the total arsenic); **7e** (14%); **7b** (8%); $Me_2AsO(OH)$ (2%). Benson[198] favors **7c** as the principal water-soluble metabolite that can be isolated from cultures of another diatom *C. gracilis* that were exposed to [^{74}As]arsenate. This same sugar is the only excretion product from similarly treated *Macrocystis pyrifera*. Methanol extracts of *C. gracilis* contain **7c**, arsenolipids, **7e**, and a fourth unique arsenical that may be a sugar derivative such as **7c** with the $Me_2As(O)$ group replaced by Me_3As^+ (cf. results from *Sargassum thunbergii*, described above).

C. concavicornis is reported[199] to slowly assimilate arsenate (relative to another unicellular phytoplankton, *Dunaliella tertiolecta*). After a 45-min exposure, extracts of the algae contain a water-soluble arsenical, which was identified as arsenite on the basis of a limited

TLC comparison. An arsenolipid is also produced. Upon exposure to arsenate for the same time period, *D. tertiolecta* produces arsenate, methylarsonate, dimethylarsinate, and three arsenolipids ($CHCl_3$ fraction). These interesting results, which relate to the rate of organoarsenical production, need reinvestigation. *Dunaliella* sp grows in a medium containing 2000 ppm arsenic as arsenate with high uptake of the element.[200]

Other studies on arsenate (and, to a lesser extent, arsenite) uptake by marine plants have established that the arsenic is distributed between the $MeOH/CHCl_3$ fraction and "insoluble" components,[151,199-205] although no individual compounds have been identified. For example, Irgolic and co-workers investigated *Tetraselmis chui*, *Daphnia magna*, and *Hymenomonas carterae*.[146,203-205] *T. chui*, a unicellular green flagellate alga, grows well in arsenate-containing medium, typically 50 ppm, and accumulates arsenic. The uptake and efflux depend on the intensity of illumination. It is possible to adapt *T. chui* to thrive in large-scale cultures (1500 L) containing 1000 ppm arsenic, but the organism does not survive if it is subsequently transferred to an arsenic-free situation. About 50% of the arsenic in the algae can be extracted with $MeOH/CHCl_3$, and extensive chromatographic workup of the lipid fraction afforded two main arsenic-containing fractions. It was suggested that these could be arsenolecithins, i.e., derivatives of arsenocholine (**5**). However, as they are decomposed by base with release of Me_3As- and Me_2As-containing species, they are more likely to be related to the arsenosugars **7**.

The marine macroalgae *Fucus spiralis* (L.) and *Ascophyllum nodosum* (L.) readily assimilate arsenate; arsenite uptake is slower.[206,207] Time-course studies on *F. spiralis* indicate a transition from arsenate, to water-soluble organoarsenical, to lipid-soluble compounds, mainly one, that account for 60% of the label. One possible interpretation of the results is that the lipid-soluble compound is related to **8**; however, it is hydrolyzed to a water-soluble compound that is the principal arsenical found in snails belonging to the food chain *F. spiralis* → *Littorina littoralis* → *Nucella lapillus*.[207,208] This interpretation would identify one of the compounds **7** as the principal arsenical found in the snails—not a comfortable conclusion.

In another study[201] the green alga *Platymonas* cf. *suecica* was found to incorporate arsenate rapidly; the process is not dependent on the phosphate concentration. When the arsenate concentration in the medium was 13 nM, 78% of the label (added [^{74}As]arsenate) was incorporated into the cells after 5 days. Extraction gave the following fractions: insoluble (43% of arsenic content); $H_2O/MeOH$ soluble (8%); $CHCl_3$ soluble (49%). Paper chromatography and TLC were used to show the presence of a number of arsenicals that included traces of arsenate, methylarsonate, and dimethylarsinate. The growth medium contained dimethylarsinate in addition to arsenate.

Similar results were obtained for a number of other plants such as the dinoflagellate *Gonyaulax polyedra* and the diatom *Skeletonema costatum*. The coccolithophorid *Cricosphaera carteri* does not take up arsenate to a detectable extent; however, a substantial amount of the arsenate in the medium is reduced to arsenite.[201]

C. Origins of the Organoarsenicals in Marine Animals and Plants

The presence of organoarsenicals in marine organisms is commonly assumed to be due to the accumulation of compounds that have been synthesized from arsenate at low trophic levels. The basis for this contention is that some species at higher trophic levels do not seem to be able to utilize arsenate for the production of such compounds as arsenobetaine and arsenolipids. However, the work of Klumpp and Peterson[206-208] with mollusks seems to contradict at least the generality of this notion.

In the marine food chain *Fucus spiralis* (L.) → *Littorina littoralis* (L.) → *Nucella lapillus*, the herbivorous snail *L. littoralis* incorporates [^{74}As]arsenate from water or the ^{74}As-labeled macroalga *F. spiralis*. The label is found mainly in the soft tissue of the snail, with some in the shell (9%).[208] (See section IX.F.4 for further results on shells.) The carnivorous snail *N. lapillus* incorporates much less [^{74}As]arsenate from solution, and most of the label (90%) is found in the shell.[208] *N. lapillus* feeds mainly on the soft parts of *L. littoralis* and if ^{74}As-labeled snails are used as food, greater incorporation of the label is achieved; the two snails are equally efficient at assimilating arsenic from the food, and this is their main source of the element.

The majority of the arsenic in the snails, food or water labeled, extracts into water/methanol and appears to be a single water-soluble compound that is not present in the first trophic level, the alga *F. spiralis*. Some of the lipid-soluble arsenical found in *F. spiralis* is present in both snails to the extent of ~10% of the label. These and related results are taken to indicate that the principal water-soluble arsenical in the snails does not come directly from the algae and that the snails can manufacture it from arsenate. Furthermore, the lipid-soluble compound in the snails is neither quantitatively important nor significantly affected by the route of uptake.[207]

Ünlü and co-workers[209-211] in support of the accumulation hypothesis established, for example,[209] that algal arsenic, ^{74}As-labeled and presumably ribose derivatives such as **7**, is readily assimilated by crabs and slowly released, whereas inorganic [^{74}As]arsenate, fed via arsenate-injected mussels, is not readily assimilated, is readily eliminated, and is not converted to organoarsenicals by the crabs. In other experiments[163] very little ingested arsenate is retained by estuary catfish and school whiting. These fish were hand-fed earthworms that had been injected with arsenate. The level of arsenobetaine did not increase over that in the controls; however, the dosed whiting showed the presence of previously absent trimethylarsine oxide, and the catfish showed an increase in the amount of the same oxide relative to the control. The catfish normally has high levels of the oxide (Table V), presumably because it ingests sediment when seeking food. It is argued that arsenate is not converted by the fish to organoarsenicals and that the arsine oxide is the result of bacterial action in the gut tract of the fish.[163]

Some earlier work involving trout[152,185,212] and other fish[153] showed that arsenate is converted to unidentified organoarsenicals, possibly via the gut bacteria. Arsenobetaine was absent in the trout, although, on the basis of recent results, this would not be expected to be

present in a freshwater fish (Table V).

Fish taken from industrially polluted water are reported to have elevated arsenic levels relative to control samples.[213,214] In the species of fish sampled, the inorganic arsenic amounted to 5–10% of the total arsenic present; the rest was organic. The increase in total arsenic in the fish from polluted water is associated with an increase in the relative amount of organoarsenicals. This was taken as an indication that the fish accumulate organoarsenicals via the food chain and do not synthesize these compounds from inorganic arsenic.

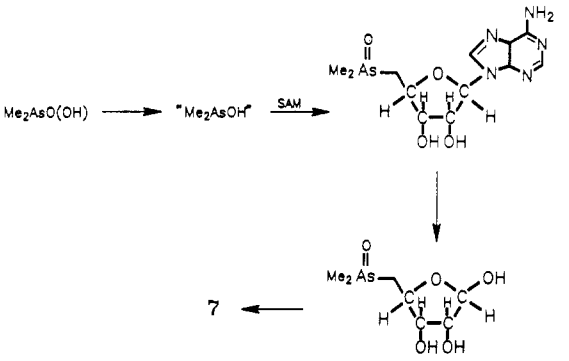
Regarding the proposition that, once synthesized, organoarsenicals are passed along the food chain, it is clear that the arsenic compounds found in algae, for example, are not those found in animals that feed on them. Direct transfer is certainly not a universal phenomenon, as outlined in the following examples.

When the alga *Dunaliella tertiolecta* is cultured with [^{74}As]arsenate and then fed to lobster (*Homarus americanus*), most of the radioactivity is present in the hepatopancreas (38%).[215] The results of a chromatographic study reveal that no arsenobetaine is produced in the time scale of the experiment, 2 days, and that algal arsenic, presumably arsenosugars such as 8, is partially degraded to simple derivatives like **7b**. Thus the lobster apparently is unable to biotransform the arsenosugar derivatives in algae to arsenobetaine, its "natural" organoarsenical (Table V). When [^{74}As]arsenate itself is fed to the lobster, no organoarsenicals seem to be produced.

Arsenobetaine is found in plankton-feeding bivalves such as clams and scallops. The ion Me_4As^+ can also be present in high concentration in addition to other unknown organoarsenicals. Although these arsenic compounds could be present in the food supply, it has been suggested that they are more likely to be the product of the biotransformation of ingested arsenicals.[165] The arsonium ion is found mainly in the gill of the clam *Meretrix lusoria* and could arise through methylation of Me_3As by, for example, SAM. The Me_3As could be produced from arsenocholine, as shown in eq 10, or from ingested $Me_2AsO(OH)$ (Scheme I). In this connection it should be noted that the presence of arsenosugars **7a** and **7b** in the kidney and muscle of the giant clam is attributed to the action of symbiotic algae that live in the mantle of the clam. Here, presumably, the clam does not biotransform the ingested arsenic compounds.[180,181]

The distribution of arsenic in the clam *Meretrix lusoria* is such that the gill contains the highest concentration (21.5 ppm).[165] The distribution in the clam *Merceneria merceneria* is different; the visceral mass has the highest concentration (5.2 ppm).[214] In other species of mollusks the distribution in different organs is more even. Maher has commented[214] that the distribution of arsenic in the organs of marine animals such as clams and crabs may reflect their feeding habits. The clam *Macoma balthica* has been used as an indicator of arsenic contamination;[216] however, the bioavailability of an element can be influenced by the total chemical composition of a sediment and its physical nature. The arsenic content of clams is certainly not dependent on the arsenic content of the sediments in which they are living.[167] For example, the chalky Macoma (*Macoma calcarea*) living in mine-polluted sediment (46.5 ppm arsenic dry weight) contains 3.2 ppm arsenic (wet weight). The same species living in the same region in cleaner sediment (9.4 ppm arsenic) contains 3.9 ppm arsenic.

SCHEME VI



Other marine species such as the gastropod *Reishia clavigera* are carnivores, and these usually have high arsenic levels. It is not surprising that they have a high content of arsenobetaine. However, the herbivorous gastropods, e.g., *Tegula pfeifferi*, also have arsenobetaine as the major arsenical (Table V). In general, herbivorous species have *lower* total arsenic content than the carnivores.[166,167]

The source of the arsenic for the arsenobetaine found in school whiting appears to be the brown kelp *Ecklonia radiata*.[164] If this is so, a likely food chain would be kelp → detritus → detrital feeding worms → whiting. Analytical data for these components indicate that the arsenic-concentrating step in the chain is seawater → kelp, since the worms, whiting, and kelp all have similar arsenic concentrations. In this same ecosystem, the buffalo bream, an algae eater, has a much lower arsenic level, ≤0.6 ppm. The authors suggest that the organoarsenicals in the algae may not be retained by the fish in this case,[164] but the argument needs more support.

It is important to establish at what stage in the food chain interconversion of the organoarsenicals takes place. This can only be done by closely controlled feeding experiments. In the meantime, one possible clue in the puzzle is provided by the observation[184] that anaerobic incubation of *Ecklonia radiata* with seawater and sand results in the production of (dimethylarsinoyl)ethanol ($Me_2As(O)CH_2CH_2OH$) and the corresponding arsine sulfide (Scheme IV).[217] Argon was used to provide an inert atmosphere. The arsenical can be isolated in good yield, based on available arsenic, by using gel chromatography of methanol extracts and can be purified by TLC on cellulose. The fermentation system is complex and little can be said about the mechanism of breakdown of the arsenicals in the kelp. The structure of the product suggests that it might be a precursor for arsenobetaine, and two possible routes to this end product are shown in Scheme IV[184] (cf. Scheme I and eq 10). However, it is not obvious where any of these transformations would occur in a natural ecosystem.

The structure of the arsenosugars **7** with the D-ribose configuration is consistent with their formation from *S*-adenosylmethionine (**2**), as has been noted by Edmonds and Francesconi;[45] their proposed biosynthetic route is outlined in Scheme VI. Here SAM is assumed

to switch its role of CH_3^+ source (Scheme I) to that of a donor of the adenosyl group. It certainly seems reasonable to postulate a sequence starting with $Me_2AsO(OH)$ rather than $MeAsO(OH)_2$; the latter is not a well-defined link in the methylation sequence (section III.B), and the former could be available from the water, where it often reaches high concentrations (section VIII.E.1). Combination of Schemes IV and VI provides a route, albeit rather circuitous, to arsenobetaine and arsenocholine.

V. Arsenic Compounds in the Terrestrial Environment

A. Freshwater Algae

Maeda and co-workers[218-221] sampled algae collected from sites polluted with arsenic. Most of the sites were abandoned Sn, As, Cu, or Au mines in Japan. The organisms were screened for arsenic tolerance; some mixed systems grew well in 50–2000 ppm. The alga *Chlorella vulgaris* was isolated and found to survive in a medium containing 10 000 ppm arsenic. Growth of *C. vulgaris* increased with increase in arsenate concentration up to 2000 ppm, and bioaccumulation of arsenic also increased with arsenate concentration. For example, in 100 ppm medium the arsenic content in the cell at log phase was 20 000 ppm; at stationary phase it was 10 000 ppm. Both values were measured on a dry-weight basis. The amount accumulated, to a maximum of 50 000 ppm (dry weight), depends on the medium used. Arsenite is more toxic to *C. vulgaris*, and growth drops off at concentrations above 10 ppm.

When dried cells of *C. vulgaris* are digested with 2 M NaOH at 90–95 °C, the arsenic is released mainly as inorganic arsenic, which was identified as the hydride AsH_3 following $NaBH_4$ reduction (section VIII.B). Cells were also extracted with $MeOH/CHCl_3$ to give (i) a residue, which contained 79% of the total arsenic, (ii) a water-soluble fraction (4%), and (iii) a lipid-soluble fraction (17%). Digestion (NaOH) of these three fractions, followed by $NaBH_4$ reduction, revealed that some methylation takes place when the cells bioaccumulate arsenic. Most of the arsenic is present in inorganic forms in the residue, where it is bound in some way that prevents easy extraction by, say, BAL (British Anti Lewisite) $HSCH_2CH(SH)CH_2OH$. Similar results were obtained for other algae, namely *Hydrocolium* sp, *Phormidium* sp, *Microchaete* sp, and *Nostoc* sp. The last named bioaccumulates both arsenate and arsenite and the lipid fraction contains only, as yet uncharacterized, methylarsenicals.[219] Optimum culture conditions for arsenic bioaccumulation by *Nostoc* have been established with a view to using the organism for the removal of arsenic from polluted water.[219]

More information about arsenic speciation in the chlorophyte *Rizoclonium* sp is available.[222,223] When these green algae are exposed to [^{74}As]arsenate for 1 week and then extracted with hot ethanol, the principal arsenicals appear to be present as lipid- and water-soluble "lipid-related" compounds, as revealed by two-dimensional paper chromatography. These and related results are given in Table VI. The appearance of the autoradiogram of the products is very similar to that obtained from the extracts of marine algae, so possibly compounds such as 7 and 8 are produced. The charophytes (Table VI) are also algae, and these apparently produce, relatively, more methylarsenicals. Nissen and Benson[222] make the point that these metabolic processes take place in the presence of excess phosphate. As will be seen in the next section, some terrestrial plants seem to require a phosphate deficiency before they methylate arsenic. The cyanophyte *Synechococcus leopoliensis*, like a few other algae, is almost insensitive to arsenate and is unusual in that it shows high discrimination for phosphate; arsenate uptake is low and its fate is undetermined.[224] In other algae such as *Chlorella*[225,226] and *Chlamydomonas*,[227] arsenate decreases phosphate uptake. Thus algae probably have a range of responses to arsenate, as do microorganisms (cf. sections I and II), a point that is made as a result of a study of five strains of taxonomically divergent algae.[227]

TABLE VI. Metabolites Isolated from Terrestrial Plants[223]

plant species	metabolites[a,b] As(III)	$Me_xAs(V)$	as lipid	water soluble "lipid related"
Rizoclonium sp (blue-green alga)	<1	<1	80	20
Chara contraria (charophyte)	3	14	62	21
Nitella tenuissima (charophyte)	5	5	72	18
Azolla filiculoides (water fern)	3	24	55	18
Lemna minima (duck weed)	9	12	53	26
Saggittaria sp (submerged species)	30	11	52	7

[a] Percent of soluble ^{74}As forms. [b] Similar results were also obtained from the water fern *Salvinia rotundifolia*, the duck weeds *Spirodela polyrhiza* and *Wolffia columbiana*, and the water lettuce *Pistia stratiotes*.

The green algae *Ankistrodesmus* sp, *Chorella* sp, *Selenastrum* sp, and *Scenedesmus* sp did not produce volatile arsines when grown in the presence of arsenite; however, the medium contained methylarsonate, dimethylarsinate, and trimethylarsine oxide in all the cultures apart from *Scenedesmus* sp, which did not produce the arsine oxide.[228] It is possible that low phosphate concentrations enhance the ability of these algae to methylate arsenic. The reduction of arsenate to arsenite by *Chorella* had been observed earlier;[226,229] arsenate uptake competes with, and inhibits, phosphate uptake.[225,226] The energy necessary for the reduction of arsenate comes from photosynthesis.[230] Another alga, *Chara fragilis*, seems to cope with the toxicity of arsenate or arsenite by storing the arsenic as an insoluble compound in the thallus,[231,232] a phenomenon that is enhanced in the presence of added calcium or strontium ions.

The alga *Chorella pyrenoidsa* accumulates arsenic on exposure to volatile arsines, but growth is inhibited.[233]

B. Plants

Two rather different reasons have prompted a number of studies on the interaction of arsenic compounds with terrestrial plants. First, arsenicals have seen wide applications in agriculture,[29-33,234] starting with the use of sodium arsenite as a weed killer and soil sterilant. Later, other inorganic arsenicals were employed, e.g., calcium arsenate as a herbicide and lead arsenate as an

insecticide. The herbicidal properties of sodium salts of methylarsonic acid were reported over 30 years ago.[235] Lists of weeds controlled by arsenicals and their application rates have been compiled by Woolson[234] and others.[29-33]

The second reason stems from the possibility that the arsenic content of plants could be used as a biogeochemical indicator, in particular as a pathfinder element for gold.[236,237] Thus the Douglas fir, *Pseudotsuga menziesii*, has the ability to pick up large amounts of arsenic. Ashed samples of the most recent growth of trees situated within 200 feet of mineralization commonly contain in excess of 1000 ppm arsenic. The maximum recorded value seems to be 10 000 ppm (dry-weight basis).

Continuing with the pathfinder theme, the usual arsenic levels found in uncontaminated terrestrial plants are ~0.2 ppm (in vegetables 0.4 ppm[153]). An extensive compilation of arsenic concentrations in higher plants is available;[32,33] examples are given for normal and contaminated situations. Levels are generally less than encountered in the marine environment. In a search for other indicator plants, mine wastes have been studied for arsenic accumulators.[238-240] The foliage of *Agrostis tenuis* plants has arsenic concentrations ranging from 3 to 3170 ppm (dry-weight basis) and the distribution within the plant can range from 2000 ppm in seeds to 160 ppm in stems.[240] In another study,[238,239] it was found that samples of little bluestem, *Andropogon scoparius*, taken from an arsenic mine exhibit a wide *evolved* tolerance to arsenic, which nonmine members of the species lack. Some plants grow in soil containing 43 000 ppm arsenic (dry-weight basis), and most of the arsenic is accumulated in the roots. One other possible indicator plant, the ox-eye daisy, *Chrysanthemum leucanthemum*, accumulated more arsenic into the leaves than the roots. Differences in distribution are considered to be the result of different tolerance mechanisms evolved by the plants.[238,239] Some species that contain high arsenic concentrations also contain high gold concentrations.[241]

Very little is known about the biochemical behavior of arsenicals in terrestrial plants. In general, it seems that cleavage of As–C bonds does not take place,[242,243] although some [^{14}C]CO_2 evolution has been observed following treatment with ^{14}C-labeled arsenicals.[244] The formation of As–C bonds has only recently been established.[222,245] It seems that following uptake of [^{74}As]arsenate via the roots, arsenite (more toxic?) can be extracted from the roots and tops of the following plants: pine seedlings *Pinus halopensis*, *P. pinea*, *P. radiata* (all 4 weeks old); corn *Zea mays*; melon *Cucumis melo*; pea *Pisum sativum*; and tomato *Lycopersicon esculentum*.[222] However, when the same plants are allowed to grow in nitrate- and/or phosphate-deficient conditions prior to exposure to [^{74}As]arsenate (2 days), methylation is found, as judged by two-dimensional paper chromatography of ethanol extracts of the plants, particularly of the leaves.[222] Similar results were obtained for corn, melon, and pea. The rationale for these experiments was the observation that marine algae appear to metabolize arsenate better under phosphate- and nitrate-deficient conditions.[223]

The methylarsenicals in the leaves of tomato plants are distributed as follows (percent relative activity): $Me_2AsO(OH)$, 76%; $MeAsO(OH)_2$, 22%; and an unknown compound, 2%, which is tentatively identified as "$MeAs(OH)_2$" (section III.B) on rather limited evidence.

The results of similar studies on freshwater plants (and algae) are shown in Table VI. Methylation and the production of compounds related to **7** and **8** seem to be reasonably facile even under eutrophic conditions.

The difference in response to arsenate between freshwater plants and other plants has been tentatively attributed to differences in [P]/[As] ratios in water and soil.[223,252] However, as described above, some plants do adapt to living in soils that have a high arsenic concentration. Recent work[245] has established that tissue cultures of the periwinkel, *Catharanthus roseus*, will grow in a medium containing up to 7 ppm arsenate. Most of the arsenate is rapidly taken up by the culture. Extracts of the cells show the presence of arsenate, arsenite, and low levels of methylarsonate and dimethylarsinate. Thus methylation of arsenic by higher plants growing under normal conditions does appear to be a possibility. Arsenite is less toxic to these cultures of *C. roseus* and has a pronounced effect on growth only above 10 ppm. In contrast, the response of *C. roseus* to methylarsonate is almost linear over the range 1 ppm (no effect) to 15 ppm (no growth).

Monosodium methylarsonate (MSMA) is a widely used herbicide.[31-33,234,246] The annual application to roadside rights of way in the United States was estimated to be 3.3×10^6 pounds in 1977.[247] It is especially effective against Johnsongrass, *Sorghum halepense* (L.).[31,234] An early study[248] showed rapid uptake of [^{14}C]MSMA from nutrient solution and rapid translocation of the arsenical into all parts of the plant. Foliar application also resulted in wide translocation. Chromatographic studies of methanol extracts of the plant indicate that the MSMA is complexed in the plant. The authors[248] suggest that the arsenic may be combined with histidine or one of its analogues. As another example, [^{14}C]MSMA can be extracted from beans, *Phaseolus vulgaris* (L.), following treatment with the arsenical. However, the extract also contains a complex that may be similar to that isolated from Johnsongrass.[243,249] Some MSMA can be extracted with methanol from wheat, *Triticum aestivum* (L.), as the unmodified arsenical, but small amounts of the arsenical remain in some bound insoluble form that still retains the As–C bond.[244] Vascular plants such as the water hyacinth, *Eichhornia crassipes*, are able to take up MSMA from the water and serve as a sink for concentrating the arsenical from the environment.[250,251]

Dimethylarsinic acid, a nonselective herbicide, is less widely used than methylarsonic acid and its salts. Very little is known about the metabolism of this organoarsenical and inorganic arsenic. In one study involving beans, unchanged dimethylarsinic acid was extracted with water. The residue contained a small amount of arsenic, ~5% of the extractable amount.[243]

The herbicidal action of MSMA on Johnsongrass has been attributed to a photoreduction of the acid to the arsenic(III) state, which then inhibits the activity of the $NADP^+$ malic enzyme, resulting in a buildup of malic acid.[252] MSMA itself does not inhibit the function of a preparation of this enzyme. The relationship between this work and that described above[244] is not obvious.

C. Man and Other Terrestrial Animals

Following the development of hydride generation techniques for the determination of inorganic arsenic and methylarsenic species,[16,254-256] the method was applied to a number of biological fluids, establishing, for example, the presence of arsenite, arsenate, methylarsonate, and dimethylarsinate in human urine.[16] It is assumed that these arsenicals are the precursors to the arsines $AsMe_xH_{3-x}$ (x = 3–0) liberated by the pH-selective reduction of the fluids by borohydride. The arsines are best separated by GC techniques, although there is enough difference in their volatility to permit separation from a frozen mixture on warming. A number of workers have confirmed these observations,[257] but there is only one report[258] of the presence of Me_3AsO in human urine. This last result needs confirmation since arsines have been found to undergo redistribution reactions;[256] this phenomenon is more likely to occur in the presence of oxygen and takes place during initial arsine generation.

Ion-exchange procedures (AG 50W-X-8) have also been used to separate out arsenicals in human urine.[257,259-261] The arsenic in these fractions can then be measured by hydride generation techniques (e.g., HG/AA) or, if radioactive, by counting. A survey of 148 subjects who had no occupational exposure to arsenic compounds and who had not eaten fish or shellfish within the preceding week gave the following results for arsenic species in urine (mean values, ppb): inorganic As, 1.9; $MeAsO(OH)_2$, 1.9; $Me_2AsO(OH)$, 2.1; total As, 17.2.[260] These data require that ~70% of the total is present in an undetected (non-hydride producing) and unidentified form. The total urinary excretion of arsenic per day is of the same order of magnitude (10–50 μg of As) as would be expected to be ingested from a normal diet.[262] The same determination for 12 subjects who had eaten seafood prior to the analysis gave the following data (mean, ppb): inorganic As, 2.1; $MeAsO(OH)_2$, 1.7; $Me_2AsO(OH)$, 1.8; total As, 132.3. Thus the inorganic arsenic and methylarsenic concentrations are essentially unchanged, while the total arsenic is considerably increased because of the increase in the undetected arsenic (127 ppb). This is further confirmation of the results indicating that "fish arsenic" is not metabolized to any great extent by humans.[147,148,153,263,264] Experiments involving arsenobetaine and arsenocholine are described below.

A number of studies have been made that involve the monitoring of arsenic species in urine following ingestion by humans. For example, Buchet et al.[265] found that sodium arsenite (maximum dose = 1000 μg of As per day for 5 days) is methylated to monomethyl- and dimethylarsenic species when it is eliminated in the urine; no other organoarsenical seems to be produced. The methylation process is not stimulated by prior treatment with potential methyl donors such as methionine, choline, or vitamin B_{12}, and is not reduced when liver function is impaired, although the process can become saturated with large doses of arsenite (>100 mg of As).[266]

Similar results have been obtained from ingested arsenate. For example, in one study a dose of [^{74}As]-arsenate was monitored with the aid of a whole-body counter for a period of up to 103 days.[267] In the first 7 days 62% of the dose was recovered in the urine and 6% in the feces. The pooled data from six subjects can be represented by a three-component exponential compartment as follows (the compartment assignments are acknowledged to be very speculative): 65.9% $t_{1/2}$ 2.09 days (kidney?); 30.4% $t_{1/2}$ 9.5 days (liver?); 3.7% $t_{1/2}$ 38.4 days (muscle?). In a complementary study,[268] 1 day after ingestion 73% of the total arsenic eliminated was in the form of dimethylarsenic acid. After 5 days when 58% of the dose had been excreted in the arsine, the composition of the sample was 51% dimethyl, 21% monomethyl, and 27% inorganic arsenic species.

Workers who are exposed to airborne arsenic compounds, particularly smelter workers who can inhale As_2O_3, also seem to eliminate this arsenic in the urine, principally in the dimethylated form.[269] In high-exposure groups the level of this metabolite can be 6 times that of the control.

The concentration of monomethylarsenic species in human urine is much higher than found in other animals so far studied. Some representative results for urine analysis follow:[270] rabbit, dimethylarsinic acid (79.4%), methylarsonic acid (3.9%), inorganic arsenic (17.7%); 18.5% of the total dose ([^{74}As]arsenite, 50 μg/kg of body weight, intraperitoneal injection) was eliminated in the urine in this period (24–48 h after injection) and 3.3% in the feces. On the same basis the corresponding figures for the rat are 81.7% dimethylarsinic acid, 0.2% methylarsonic acid, and 18.3% inorganic species; however, the total eliminated dose was only 0.6% in the urine compared with 7.5% in the feces. Results such as these confirm the well-established dogma that the rat is not a very good model for human exposure to arsenicals.[29,30] In rats, arsenic compounds tend to accumulate in the red blood cells, predominantly as dimethylarsinate.[271,272]

Mice excrete ingested arsenite and arsenate rapidly,[273-276] mainly as dimethylarsinate; methylation of ingested arsenite appears to be more facile than arsenate.[273] Dimethylarsinate is the principal arsenical found in the urine of dogs,[277,278] pigs,[279] cows,[277] and monkeys,[279] although the marmoset monkey seems to lack the ability to methylate arsenic at all.[280]

Because of the presence of arsenobetaine, arsenocholine, and other arsenicals in marine species, there has been considerable interest in the biochemistry of these species. Some studies will now be described.

Arsenocholine has a marked lipotropic activity in rats and mice and has the effect of promoting growth in chicks and preventing perosis.[281,282] The arsenocholine fed to rats is found to be associated with the choline, and it has been suggested that arsenocholine may replace choline in the synthesis of lethicin. The exhalation and tissue of chicks and rats during treatment have a garlic-like odor, possibly associated with the production of Me_3As. The addition of arsenocholine to fresh chicken or rat liver extracts results in the liberation of a volatile oxidizable gas;[283] oxygen is consumed in the process that can be provisionally represented by eq 10.

$$(CH_3)_3As^+CH_2CH_2OH \rightarrow (CH_3)_3As^+CH_2CHO \rightarrow (CH_3)_3As^+CH_2COO^- \rightarrow (CH_3)_3As(?) \quad (10)$$

Gas is also evolved under anaerobic conditions. Choline oxidase seems to be involved in these oxidations, as do cytochromes; the arsenical has a lower affinity for the enzyme than choline.[283] Arsenocholine is an alternative

substrate for choline acetyltransferase.[284]

Contrary to what would be expected on the basis of eq 10, chicken liver extracts do not liberate a gas from arsenobetaine. However, when the arsenical is administered orally to rats and chickens, the exhalations and tissues also have a garlic-like odor, which perhaps indicates that the arsenical is broken down in the digestive tract. (Betaine is converted to trimethylamine by adapted species of *Pseudomonas*.[285])

In most recent studies, arsenocholine and arsenobetaine were fed to mice, rats, and rabbits.[274,275,286] Almost all the arsenobetaine given orally to rats and mice (4 mg of As kg^{-1} of body weight) was rapidly excreted in the urine within a few days of ingestion. Excretion of the same dose by rabbits is slower, and the compound has a longer residence time in the muscle. In one study no significant breakdown of the arsenical was detected; however, it should be noted that this was a single-dose study employing ^{73}As-labeled arsenicals. This isotope has advantages over ^{74}As in terms of a longer half-life and lower γ emission. The synthesis of $^{73}AsMe_3$ and its conversion to arsenobetaine and arsenocholine are straightforward.[287]

In the case of [^{73}As]arsenocholine[275] the same dose was eliminated somewhat slower, but again mainly in the urine of the same animals. The main urinary metabolite was *arsenobetaine*. The rate of elimination of the label seems to depend on the rate of oxidation of arsenocholine to arsenobetaine (cf. eq 10), which will vary from species to species, and also on the extent of incorporation of the arsenocholine in the phospholipids.

Intraperitoneal injection of a high dose of arsenobetaine, 500 mg kg^{-1}, into mice does not seem to have any noticeable effect, and no volatile metabolites were obtained from mice injected with lower doses of arsenobetaine.[177] The LD_{50} value for arsenobetaine in mice is higher than 10 g/kg (cf. As_2O_3 LD_{50} 34.5 mg/kg).[274]

Arsenobetaine was isolated from the urine of human subjects who had eaten cooked lobster tails[159] and plaice.[157] The latter experiment involved eight volunteers, and within 5 days 69–85% of the ingested arsenic was excreted.

Arsenobetaine gives negative test results in the Ames *Salmonella typhimurium* system.[177] Other genotoxicity tests also indicate that the potential risks associated with the consumption of food products containing arsenobetaine seem to be of very minor importance.[288]

Tam and co-workers[289] fed cooked witch flounder fillets (*Glyptocephalus cynoglossus* (L.)) to 15 human volunteers and found that, after the one dose, the arsenicals in the meal were excreted, unchanged, in the urine (76% over 8 days), with essentially none in the feces. Similar results had been obtained earlier by others.[290] The arsenic compound(s) in the flounder was not identified,[289,291] although evidence against it being arsenobetaine was presented. This now seems unlikely because the same arsenical was isolated from sole and shrimp by these investigators. The arsenical in prawns, presumably arsenobetaine, is similarly excreted in the urine, 50% in the first 6 h and 90% after 60 h; the urine content peaks 2 h after ingestion, and the blood content peaks at the same time.

All these studies relate to the early work of Chapman[147] and Coulson[148] and reinforce the conclusion regarding the nontoxic nature of "fish arsenic" to humans. A word of caution has been expressed concerning the presence of Me_4As^+ in shellfish because of the known toxicity of Me_4N^+.[165] Little is known about the physiology of this ion, although by the assessment of Penrose[43] (section II.B) it should be harmless.

The high level of arsenic in seaweeds has prompted a number of feeding studies.[292,293] Commercially available preparations of *Hizikia* contain arsenic in the range 19–172 ppm (dry-weight basis) with a mean of 111.8 ppm. The arsenic is now known to be in the form of arsenate (~50%) and arsenosugars 7 (~50%).[192] When these preparations are fed in diluted form to rats, the effects are more similar to those of inorganic arsenic than "fish arsenic". For example, *Hizikia* arsenic has a long biological half-life; "fish arsenic" does not.[292]

Arsanilic acid (4-$NH_2C_6H_4AsO(OH)_2$) and roxarsone (4-OH-3-$NO_2C_6H_3AsO(OH)_2$) are two arsenicals used as food additives to promote the weight gain of swine and poultry and to control diseases.[29–33,36] They are excreted essentially unchanged,[294–296] although their presence in animal wastes affects the performance of anaerobic and aerobic waste digesters by diminishing methane production. Some arsenic is lost from aerobic digesters, possibly as volatile arsines.[295] There is some evidence that arsanilic acid fed to rats is partly converted to arsenate, although the bulk of the arsenical is not biotransformed.[297] The possibility of arsenic–carbon bond cleavage occurring in both swine and rats has been recorded.[294,295,297]

Dimethylarsinic acid does not seem to be metabolized to any great extent in the rat;[298,299] only traces of [^{14}C]CO_2 (<0.2% of administered dose) have been released from the ^{14}C-labeled arsenical 24 h after administration.[298] Most of the arsenical is found in the red blood cells, as is the case for administered arsenate. The question as to whether arsenicals are in or on the cells remains unanswered (cf. section III.C). Dill and co-workers[300] report that phenyldichloroarsine can enter guinea pig red blood cells and interact directly with glutathione and hemoglobin. This study employed spin–echo ^1H NMR spectroscopy (cf. Figure 3); the conclusions are at variance with those of others[301] who suggest that this arsenical, like $Me_2AsO(OH)$ (Figure 5), does not enter the cell.

The results outlined in this section so far, and others, reveal little information relating to the mechanism and site of the metabolism of arsenicals by terrestrial animals. Indeed, the possibility that some of these processes take place in the gut of the animals, with the involvement of the associated microorganisms, cannot be entirely ruled out. Preparations made from mouse ceca, sites of high microbiological activity, methylate methylarsine oxide to dimethylarsinate and demethylate it to arsenate;[94] rumen fluid also methylates the same oxide.[94] Rowland and Davies found that preparations of rat ceca can reduce arsenate to arsenite and methylate arsenate,[83] although gut flora do not seem to contribute significantly to the biotransformation of arsenic in vivo.[272] Likewise, there is no difference in the methylation abilities between normal and germ-free mice, as judged by urine analysis.[273] Germ-free mice have no bacteria in their gut.

Preparations of rat liver methylate arsenate, and it seems that two different enzymes may be involved, one for each methylation step.[302] The enzyme responsible

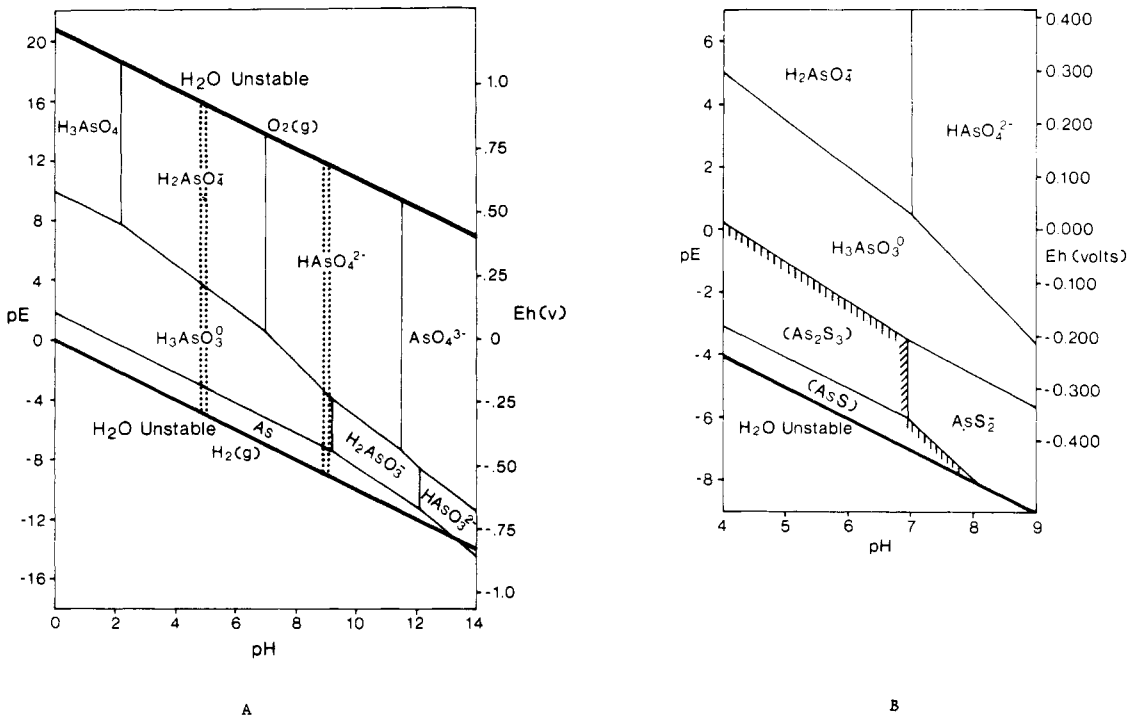


Figure 6. (A) pE–pH diagram for the As–H_2O system at 25 °C. Total dissolved As species set at 50 ppb. The area within the vertical bars represents the common pE–pH domains for natural water. (B) pE–pH diagram for the As–S–H_2O system at 25 °C with total dissolved As and S species set at 50 ppb and 32 ppm, respectively. The area within the hatched lines denotes that the solid phases are predominant (i.e., total dissolved As species < 5 ppb). Reprinted with permission from ref 306; copyright 1979, Elsevier Science Publishing B.V.

for the first methylation may occur in the liver in both a soluble and particulate-bound form. Hepatocytes isolated from rat liver convert arsenite to dimethylarsinic acid, and toxic effects occur at arsenite concentrations >0.012 mM, probably when the methylation capacity of the cells is exceeded.[303] Arsenate is not affected under the same conditions.

VI. Predictions of Environmental Arsenic Speciation

A. General Thermodynamic Considerations

Chemical and physical processes operate in concert with the biological transformations described above in order to collectively determine the overall arsenic biogeochemical cycle. Geochemical modeling is a useful means of identifying the major controls on the distribution and speciation of arsenic in the environment. This approach is obviously limited by the scope and quality of the thermodynamic data base and, in the strictest sense, applies only to systems at equilibrium. Nevertheless, it provides useful predictions of the occurrence, absence, or fate of various dissolved and solid arsenic species under different environmental conditions. Deviations in the expected distribution of arsenicals can often be traced to biological intervention or to anthropogenic sources. It is also possible to predict the direction, but unfortunately not the rate, that the system will move if perturbed.

Thermodynamic data have been compiled[44,304-309] and used for predictions of arsenic speciation[44,304-307,310-314] by several authors. A recent report by Crecelius et al.[307] describing a model for the geochemical behavior of arsenic in coal fly ash ponds is particularly informative regarding the general concepts, and includes a useful tabulation of relevant thermodynamic parameters. In the following sections we have adapted this approach to a consideration of the factors controlling the oxidation state, solution species, and solubility of inorganic arsenic over a range of environmental conditions. As thermodynamic data for organoarsenicals are limited, the response of these compounds to various environmental conditions has been predicted on the basis of known chemical properties.

This separation into inorganic and organic arsenic compounds is typical in environmental chemistry but can cause confusion among interdisciplinary researchers. Some workers interpret organic arsenic as only those compounds found within biological tissue. In the following sections, inorganic and organic refer to the absence or presence of As–C bonds, respectively.

B. Inorganic Arsenicals

1. Aqueous Complexes

Geochemical systems are commonly interpreted in terms of their response to pH and E_H—the thermodynamic redox potential. pE, the hypothetical electron activity at equilibrium, is used interchangeably with E_H. These parameters are simply related by $pE = (F/2.3RT)E_H$, where T is the absolute temperature, and F and R are the Faraday and gas constants, respectively.[306] Thus, at 25 °C, $2.3RT/F = 0.059$ V mol^{-1} and $pE = E_H/0.059$.

The equations linking arsenic speciation to pH and pE are readily available,[306,311,313] but E_H versus pH diagrams, which indicate the predominant soluble species and relevant solids, are the most concise way of presenting this information. Most such stability diagrams have been prepared[44,304,306,307,310] for systems including oxygen, water (Figure 6A), and sulfur (Figure 6B). The domains of each species are defined by the solid lines.

Two species have equal activities at the boundaries, while one species becomes increasingly dominant toward the interior of its domain.[306] These diagrams differ slightly depending on the conditions selected, but the major conclusions are consistent.

The pE range for aquatic systems is restricted by the stability of water and extends (for pH 4–10) from approximately 17 to −10; beyond these limits water is oxidized to O_2 or reduced to H_2, respectively.[315] Various values have been cited for the redox levels in air-saturated natural waters. Turner et al.[305] proposed a simple equation, pE = 20.6 − pH, for the calculation of the electron activity in oxic systems; similar results are obtained by other approaches.[306,311,313] However, the calculated pE values are often higher than those that are experimentally determined. For example, Wagemann[304] quoted a pE range of 5–8 for natural water in contact with air. Crecelius et al.[307] suggested an upper limit of 6.8 for fresh water. These discrepancies are probably due to limitations in the behavior of the electroactive species at the electrode surface, which results in lower and variable readings.[313] Regardless of the specific pE, it is apparent (Figure 6A) that in oxygenated waters, arsenic acid (As(V)) species—H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-}—are stable. Under slightly reducing conditions and/or lower pH arsenous (As(III)) acid becomes stable, mainly as neutral H_3AsO_3.

The range of arsenic species is more restricted, however, when the pH domain of natural waters is considered. Freshwater systems rarely exceed a pH range of 5–9[307] and the maximum pH distribution in seawater is even narrower (7.5–8.3).[316] Thus As(V) should strongly dominate over As(III) in oxygenated waters—at least on thermodynamic grounds. For example, As(V)/As(III) ratios of 10^{15}–10^{26} have been calculated[317] for seawater, depending on the choice of pE (8.0[317] or 12.5,[311] respectively). Furthermore, As(V) should mainly consist of $HAsO_4^{2-}$ in oxygenated seawater (calculations show 98% $HAsO_4^{2-}$ and 1% each of $H_2AsO_4^-$ and AsO_4^{3-} at pE = 12.4[305]). In fresh water of pH 6 $H_2AsO_4^-$ becomes dominant (89% versus 11% $HAsO_4^{2-}$ at pE = 14.6[305]). Inorganic As(III) species should mainly be neutral, as H_3AsO_3, but it has been suggested[305] that hydrolysis can result in the formation of a significant (13%) quantity of $H_4AsO_4^-$ (i.e., $As(OH)_4^-$) at the higher pH of seawater.

The solution properties of arsenic acid (H_3AsO_4) closely resemble those of phosphoric acid (H_3PO_4), with the structure of both acids better represented by $EO(OH)_3$. Successive pK_a values (25 °C) for $AsO(OH)_3$ are 2.2, 6.9, and 11.5 as compared to 2.2, 7.2, and 12.4 for $PO(OH)_3$.[312,318] Unlike phosphorus acid (H_3PO_3), which has both H–P and H–O bonds, arsenous acid species are derived from $As(OH)_3$ with pK_a values of 9.2, 12.1, and 13.4.[318] In fact, the ionization behavior of $As(OH)_3$ more closely resembles that of boric acid.[318] There is no support in the Raman spectra[28] of arsenous acid solutions for $HAsO_2$ or AsO_2^- species.

Detailed calculations[305] of the equilibrium speciation of 58 trace elements in oxygenated fresh water (pH 6 and 9) and seawater (pH 8.2) indicated that, unlike many elements, arsenic forms few additional aqueous complexes with typical water constituents. Other calculations have confirmed this prediction. It has been suggested[307] that $HAsO_3F^-$ and AsO_3F^{2-} could account for up to 10% of the total As(V), but only at high fluoride concentrations (1.58 mg L^{-1}). In seawater, arsenate may undergo significant ion pairing with magnesium and calcium.[312]

In anoxic water systems, bacterial action will result in lower pE values, as dictated by the reduction of SO_4^{2-} to S^{2-} and HCO_3^- to CH_4.[305] Turner et al.[305] defined a lower limit for pE in natural waters as pE = [34 − 9 (pH)]/8. Thus at a pH of 7.5, which is typical[313] of anoxic marine environments, pE = −4.2. However, the concentration of sulfide shows wide variations, especially where it can undergo precipitation with iron, and larger values (pE = −2.9) are frequently reported.[313,314] Nevertheless, under the influence of these reducing conditions As(III) will become more thermodynamically important (Figure 6B). In the presence of dissolved sulfur, arsenic sulfides will be formed. Under acidic conditions orpiment (As_2S_3) and/or realgar (AsS) will precipitate (Figure 6B). More soluble thioarsenite species will be important at neutral or alkaline pH.[17,44,304,306] The formulation of thioarsenite as AsS_2^- and sometimes[44,304] $HAsS_2$ (depending on the pH) is questionable in view of the nonexistence of AsO_2^- and $HAsO_2$. It is more common in inorganic chemistry[17] to write this ion as AsS_3^{3-}. Spectroscopic information regarding the solution structure of thioarsenite would be useful. In the absence of such data, we will use AsS_3^{3-} in this article. In doing so, no implication regarding the degree of protonation at a given pH is intended.

It therefore appears that the range of water-soluble inorganic arsenic compounds is quite limited and that pH is the major factor controlling the differences in aqueous arsenic speciation in the freshwater and the marine environments.[304,305,307] For a given oxidation state, there is no reason to doubt this thermodynamic prediction. There is a tendency, however, to simply refer to the aquatic inorganic arsenic species as arsenate (or simply As(V)) and arsenite (As(III)), without regard to the degree of protonation. This simplification is convenient and does not present any difficulty as long as it is recognized that there are other compounds in the environment with arsenic in the +5 oxidation state and that in the pH domain typical of natural waters the dominant arsenate and arsenite species will be differently charged. Indeed these differences may be largely responsible for the differences in mobility, and perhaps biological uptake, reported for these species. Statements that arsenate is isoelectronic with phosphate in the marine environment, for example, should really compare[312] $HAsO_4^{2-}$ to HPO_4^{2-}. More serious errors result from attempts to predict As(V)/As(III) distribution by means of E_H–pH diagrams.

It has already been noted that at redox potentials typical of aerobic soils and oxygenated aquatic systems As(V) should dominate As(III). Inspection of Figure 6 suggests that at these potentials a decrease in pH should increasingly favor the more toxic arsenite over the less toxic arsenate. However, thermodynamically predicted As(V)/As(III) ratios are rarely observed, and experimental evidence clearly indicates that a multiplicity of factors influences the relative concentrations of these species. Paramount among these are biologically mediated redox reactions. For example, As(V)/As(III) ratios of 0.1–250 (versus 10^{15}–10^{26} predicted

above) are common in marine systems due to algal/bacterial transformations (section VIII). Bacteria have been observed to both oxidize and reduce inorganic arsenic in a variety of environmental systems (section II). Even redox reactions that appear to be strictly chemical have caused considerable controversy.[306,319]

Crecelius et al.[307] have suggested that, for natural waters, a thermodynamic equilibrium between the As(III) and As(V) oxidation states does not exist; instead a steady state may be achieved. Cherry et al.[306] proposed that even in the absence of thermodynamic equilibrium, the ratio of As(III) to As(V) may still be reflective of the apparent redox level. To be useful, the As(III) $\rightleftharpoons$ As(V) change would have to be rapid relative to the biogeochemical processes controlling the redox level, but sufficiently slow so that sample collection and analysis could be carried out.[306] To examine these conditions the kinetic stability of the As(III)/As(V) ratio was investigated. It was found[306] that 1:1 (50 ppb each) arsenite:arsenate solutions remained unchanged in deoxygenated buffer solutions (pH 2–10) for up to 3 weeks. The addition of relatively high concentrations of environmentally important redox agents did cause appreciable (10–20%) oxidation (by O_2 and Fe^{3+}) and reduction (by H_2S), especially at low pH (for Fe^{3+} and H_2S), but over periods of tens of hours. It was therefore concluded that over long time frames the As ratio in natural water would adjust to a value indicative of the redox level established by these agents and that it could be used as a measure of the prevailing E_H. These kinetic effects were verified[319] over a larger concentration range (1–100 ppb) in response to an observation by Feldman[320] of a rapid, complete conversion of As(III) to As(V) (~4 days at 1–10 ppb, ~7 days at 100 ppb, and ~18 days at 1 ppm) even in the absence of added oxidants. Ascorbic acid stabilized the As(III) but the reasons for the differences in these studies were not established.

Others have noted loss of As(III) on storage[321] (e.g., 50% loss at 20 ppm in 33 days), and in some instances there was an inexplicable disappearance of total arsenic from solution over extended time periods.[306] Crecelius et al.[307] found that deionized water had an oxidizing capacity whereas filtered (0.4 μm) river water spontaneously reduced As(V) to As(III). Some changes can be explained by temperature effects, but not all. For example, As(III) was dominant in geothermal sources when sulfide was present but was rapidly converted to As(V) at elevated temperatures (30–40 °C) once all of the S^{2-} was oxidized.[307,322] In at least one study[307] freezing of freshwater samples (except for rapid freezing in liquid nitrogen) surprisingly caused the oxidation of As(III) to As(V). In another case, the As(III)/As(V) ratio was found[317] to be unchanged in seawater samples stored at 4 °C for 10 days but was unstable with respect to freezing unless dry ice was used.

These observations suggest a complex redox behavior for inorganic arsenic. Bacteria are capable (sections II.B,C) of facile redox transformations (As(III) $\rightleftharpoons$ As(V)) and may be responsible for a great deal of the observed behavior. Crecelius et al.[307] attributed the reducing properties of river water to the presence of unidentified dissolved organic compounds, but both reducing[81] and oxidizing[323] bacteria have been found in natural waters and can probably pass through 0.4-μm filters.[323] We have noted that arsenic solutions prepared in deionized water that has not been sterilized by autoclaving or UV irradiation are more susceptible to speciation changes.[324] Abiological reactions are also possible, and the photochemical oxidation (254 nm) of arsenite to arsenate has been employed synthetically.[325] Enhanced oxidation rates have also been observed with the application of sunlight.[326] Further work is needed to clarify the situation, but it seems likely that numerous discrepancies can be attributed to bacterial and/or photochemical effects. Heterogeneous redox reactions involving manganese oxide solids and particulate matter are also possible.[327] In the rare absence of such factors, arsenate/arsenite redox changes are likely to be slow.[323,326]

2. Solubility Controls

In the preceding discussion the only limitation on the solubility of dissolved inorganic arsenic was the formation of arsenic sulfides or elemental arsenic. Over 245 arsenic-containing minerals are known in nature,[1] and it is possible that the formation of such solids could limit environmental arsenic concentrations. Solubility product constant data are limited, but this point has been examined by two independent groups.[304,307] Wagemann[304] considered the total dissolved arsenic concentrations allowed by orpiment and 14 metal arsenates in equilibrium with freshwater solutions representing a range of metal ion and anion concentrations. Crecelius et al.[307] recalculated the behavior of 8 of these minerals as well as some arsenic oxides and sulfides for fresh water containing low and high amounts of dissolved solids. The calculations of both groups were in essential agreement.

Orpiment (As_2S_3) was found to be oversaturated in the presence of sulfide at low E_H and low pH ($<2 \times 10^{-7}$ M at $\leq$pH 7 as $HAsS_2(aq)$) with slightly greater solubility ($\sim 1 \times 10^{-5}$ M as $AsS_2^-(aq)$) at higher pH[304] (but see section VI.B.1). Thus orpiment may provide a solubility control on dissolved arsenic at low pH and E_H and in the presence of high sulfide (0.1–10 μg L^{-1}, depending on the dissolved solid—i.e., other ion concentrations).[304,307] However, sulfide activity may be limited by other elements (e.g., Fe^{2+}), and arsenic sulfide may not reach saturation.[306] The conversion of orpiment to AsS_3^{3-} in neutral or alkaline solution will also increase arsenic solubility.[17] Reaction of As_2S_3 with sulfides or polysulfides to give AsS_3^{3-} has been employed in qualitative analysis.[17] Under similar conditions, carbonate is reported to produce both AsS_3^{3-} and AsO_3^{3-}. These reactions need to be investigated further, as they may dramatically influence the behavior of arsenic in anoxic environments.

Wagemann[304] and Crecelius et al.[307] both found that $Ba_3(AsO_4)_2$ was oversaturated over a wide range of E_H, pH, and dissolved solid values. Wagemann[304] suggested that the formation of this material severely limits dissolved arsenic concentrations but Crecelius et al.[307] questioned this conclusion. They pointed out that there is no evidence for the geologic occurrence of this material and, if it did occur, dissolved barium would be analytically undetectable even in waters with very low arsenic concentrations. They attributed this unreasonable result to a major error in the thermodynamic data.[308] It is likely, though, that metal arsenates are

important in soils[328] and, by analogy, sediments.

The limited thermodynamic parameters available preclude further considerations of solubility controls on dissolved arsenic concentrations. It should be noted that even if a solid is found to be important (such as As_2S_3), calculations do not provide insight into its rate of formation or, conversely, its dissolution. However, at present, there appear to be few thermodynamic limitations on the solubility of inorganic arsenic compounds in nature.[306,307] Aquatic arsenic concentrations probably reflect availability from geological materials and direct input, versus removal via adsorption processes (sections IX.D,F.1).

C. Organoarsenic Compounds

Organoarsenic compounds are widely distributed in the environment, having been detected in the atmosphere, aquatic systems, soil and sediments, and biological tissue. A number of alkyl- and aryl-arsenicals are synthesized for commercial use—usually as biocides in agriculture and forestry. However, except for localized, often accidental, discharges, the quantities of these materials that are anthropogenically introduced into the environment are generally small. Of greater relevance to this article are the organoarsenicals that are produced in the environment and form part of the global biogeochemical cycle.

A review dealing with organometallic compounds in the environment has appeared and in it, Craig[329] points out that there is little evidence for other than methyl species. He further argues that for most elements the production of such compounds is best described as *environmental methylation*, i.e., a chance abiotic reaction between an available metal/metalloid and a biologically formed methylating agent outside the cell. However, he notes that arsenic is an exception, as there is little evidence that As–C bonds can be formed in the environment by purely chemical means. Thus the synthesis of organoarsenic compounds requires the involvement of a living organism and, presumably, the intervention of arsenic within the metabolic pathways of the cell. It is therefore appropriate to refer to *biomethylation* when discussing the origin of organoarsenicals. Although biologically produced organoarsenicals with more complex organic moieties are also known, these also possess methyl groups. A consequence of this essential biological involvement is the increased difficulty in making a priori predictions of those compounds most likely to be found in the environment. It is possible to construct a list of likely candidates by examining the results of biomethylation studies, but the observation that a species is formed does not imply that it is stable under normal environmental conditions. In this section we examine the likely fate of biologically produced organoarsenicals. In sections III–V, the mechanisms of formation and biotransformation are presented.

No stability diagrams have been reported for organoarsenicals due to the limited thermodynamic data that are available for such compounds. Earlier suggestions[44] that these compounds were unstable except at extremely low E_H are inconsistent with their occurrence in the environment. As–C bonds, like most metal/metalloid–carbon bonds, lie in the normal range of chemical bond energies.[329] All organometallics are *thermodynamically unstable* with respect to both the constituent elements and to the decomposition products, but many are *kinetically stable* and are thus assured of more than a transient existence under normal environmental conditions.[329] Main-group alkyls tend to be more stable than alkyl–transition metal compounds because they have access to fewer decomposition pathways. This is particularly true of arsenic derivatives. Cleavage of As–C bonds is probably rare in the presence of normal environmental reagents. Intense UV irradiation of aryl- and alkylarsenicals under oxidizing conditions results in the quantitative formation of arsenate;[330,331] similar behavior has been observed for organophosphates.[332] This has implications for the fate of organoarsenicals in the upper atmosphere, but there are no data to indicate that photochemical cleavage is an important degradative pathway at the earth's surface. Indeed, bacterial demethylation (section III.G) is probably the most important route for the loss of organoarsenicals in the troposphere.

The effective stability of the As–C bond does not preclude the chemical transformation of one organoarsenical to another. Oxidation, reduction, hydrolysis, and reactions with sulfur compounds can significantly alter the distribution, volatility, and mobility of biologically produced compounds. This has two related implications that are usually overlooked. First, compounds capable of rapid reaction with environmental reagents may not be present in significant concentrations in certain substrates even though they are being biologically produced. Second, conclusions regarding the products of biomethylation may be in error if they are based only on the analytically observed species. The fate of dimethylarsine illustrates these points. Soil microorganisms produce $(CH_3)_2AsH$ under culture conditions and this compound has been detected[333] in air samples collected directly above appropriate soils. With time, dimethylarsine reacts with oxygen to produce dimethylarsinic acid ($(CH_3)_2AsO(OH)$). This species is air and water stable, but it is likely to be ultimately adsorbed on the surface of atmospheric particulates where it can react with radicals or be photochemically demethylated to arsenate. The resulting product, probably As_2O_5, is also directly introduced into the atmosphere by other routes, including natural erosion. A correct interpretation of the role of soil microorganisms in the arsenic biogeochemical cycle requires careful attention to this chemistry.

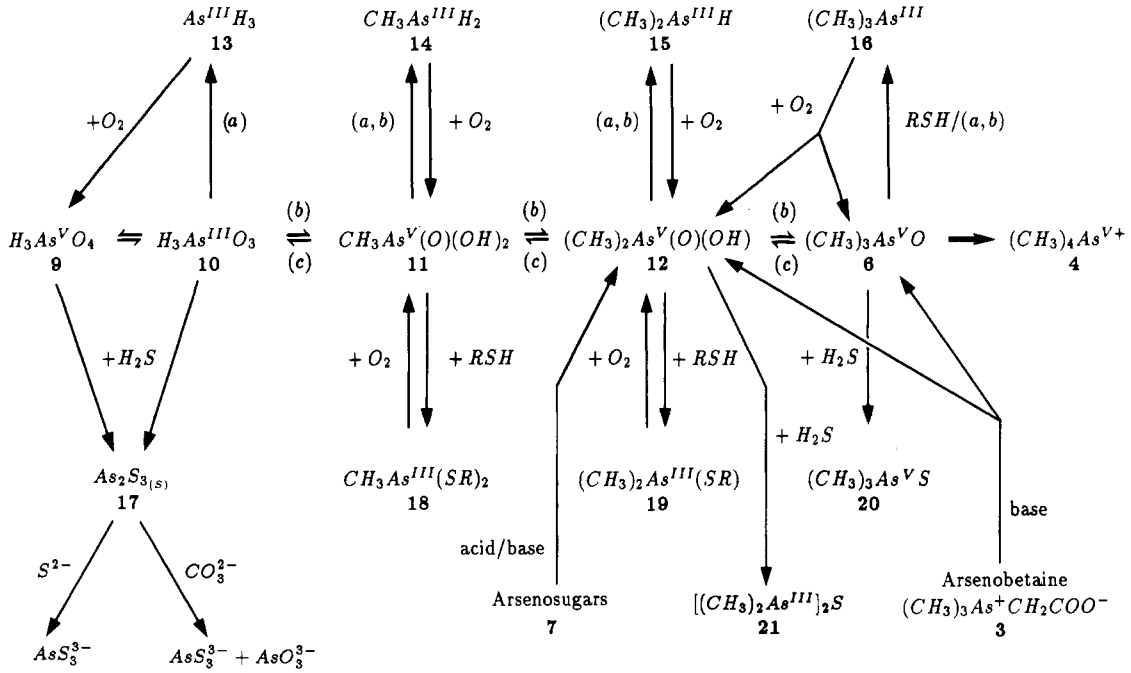
Table VII is a compilation of known biomethylation products. Included is an indication of the air and water stability of these compounds, together with the likely products of the more reactive species. The interrelationships of these compounds are also displayed as Scheme VII. For simplicity the arsenic acids are written as fully protonated. It is common in the literature to make no distinction between 11, monomethylarsonic acid (or methylarsonic acid; abbreviated MMAA), and (mono)methylarsonate, $CH_3AsO(OH)(O^-)$, the predominant species in most natural waters; similarly, dimethylarsinic acid (DMAA) will usually be present as dimethylarsinate, $(CH_3)_2AsO(O^-)$. These conventions are reasonable as long as it is recognized that the environmental behavior of these compounds will be that of the negatively charged species or their corresponding salts. Other abbreviations in this article are more obvious: TMAO, MMA, DMA, and

TABLE VII. Distribution and Properties of Environmentally Important Organoarsenicals

compd[a]	endocellular (+) or exocellular (-)	reactive[b] to O_2	reactive[b] to H_2O	comments
$(CH_3)_4As^+$ (4)	+	no	no	has only been found in tissues to date, but is likely to be more widely distributed
$(CH_3)_3AsO$ (6)	+, -	no	no	limited detection in environment may be due to facile reduction to $(CH_3)_3As$; reacts with H_2S to give $(CH_3)_3AsS$
$(CH_3)_3As$ (16)	-	yes	no	easily oxidized to $(CH_3)_3AsO$ and $(CH_3)_2AsO(OH)$; may escape detection by some analytical procedures
$(CH_3)_2AsO(OH)$ (12)	+, -	no	no	stable and water soluble, easily detected by conventional methods, can be reduced to $(CH_3)_2AsH$, and reacts with RSH to give $(CH_3)_2As(SR)$; pK_a = 6.2[318]
$(CH_3)_2AsH$ (15)	-	yes	no	easily oxidized to $(CH_3)_2AsO(OH)$
$CH_3AsO(OH)_2$ (11)	+, -	no	no	stable and water soluble, easily detected, can be reduced to CH_3AsH_2, and reacts with RSH to give $CH_3As(SR)_2$; pK_a = 3.6, 8.2[318]
CH_3AsH_2 (14)	-	yes	no	easily oxidized to $CH_3AsO(OH)_2$
AsH_3 (13)	-	yes	no	oxidized to $AsO_{4(aq)}^{3-}$ or $As_2O_5(s)$; limited observation in environment
$(CH_3)_2AsSR$ (19)	not yet observed	yes	no	may be formed in anoxic soils and sediments; reacts with O_2 to give $(CH_3)_2AsO(OH)$
$CH_3As(SR)_2$ (18)	not yet observed	yes	no	may be formed in anoxic soils and sediments; reacts with O_2 to give $CH_3AsO(OH)_2$
arsenosugars (7)	+	no	no	found mainly in algae, stable at neutral pH, and reacts with acid/base to give $(CH_3)_2AsO(OH)$
$(CH_3)_2As(O)CH_2CH_2OH$	-	no	no	(dimethylarsinoyl)ethanol
$(CH_3)_3As^+CH_2CH_2OH$ (5)	+	no	no	arsenocholine; easily converted to arsenobetaine
$(CH_3)_3As^+CH_2COO^-$ (3)	+	no	no	arsenobetaine; can give $(CH_3)_3AsO$ and $(CH_3)_2AsO(OH)$ on reaction with base

[a] Numbers are those used for identification throughout this article. [b] A "yes" entry indicates reactivity under normal environmental conditions.

SCHEME VII



TMA for **6**, **14**, **15**, and **16**, respectively.

Compounds **9–12** are the most frequently observed exocellular arsenicals. They are also the easiest to detect as they undergo facile pH-selective reactions, indicated as (a), with borohydride to give volatile products that can be analyzed by GC/AAS. The arsines, **13–16**, have also been found in varying amounts as products of biomethylation (b) but their distribution is limited by reactivity toward oxygen. Trimethylarsine oxide (**6**) has been found less often than **11** and **12**, probably because it is rapidly reduced to trimethylarsine (**16**) by RSH and by a number of aerobic and anaerobic organisms.[127] Oxidation of **16** gives partial conversion to DMAA.[129] Both **12** and **6** can be produced by hydrolysis of endocellular compounds and may be found as a consequence of the degradation of organic matter. To date, $(CH_3)_4As^+$ has only been found in tissues, but this may be an analytical limitation as it is undetectable by hydride generation procedures.

Anoxic environments commonly produce hydrogen sulfide (this is particularly true of marine systems as they contain higher concentrations of sulfate than fresh waters). A variety of other sulfur compounds are also present, and it has recently been demonstrated that thiols (RSH) are produced by the reaction of H_2S with sedimentary organic matter.[334] During periods of high productivity, RSH concentrations are known to approach those of the inorganic sulfur compounds.[335]

Little is known about the arsenic species present in anoxic waters and sediments, but it is likely (Scheme VII) that a variety of arsenic sulfides will be produced by reaction with these environmental reagents. Reference to the relevant inorganic chemistry provides insight into the possibilities especially with respect to organoarsenic compounds. The literature on the inorganic arsenic sulfides is quite old and needs to be reexamined, but it is useful to speculate on the possibilities.

The reaction of arsenous acid (**10**) with hydrogen sulfide to give insoluble arsenous sulfide (**17**) will be important at pH <7. The formation of more soluble thioarsenite salts (tentatively formulated as AsS_3^{3-}) by further reaction of **17** with sulfides may be especially important in anoxic sediment pore waters. It is not known if AsS_3^{3-} reacts with borohydride to give AsH_3, but the AsO_3^{3-} that is formed by the reaction of **17** with carbonate does. This process alone would explain the detection (section VIII), by typical analytical procedures (hydride generation GC/AAS), of significant quantities of As(III) in anoxic samples. Arsenate is also found in such samples, despite the low redox potentials that accompany environmental sulfide production. Arsenic acid (**9**) reacts with H_2S. The product depends on the conditions. At pH >7, As_2S_3 is slowly precipitated as the As(V) is first reduced to As(III), possibly via a thioarsenic acid intermediate.[17] Arsenic pentasulfide (As_2S_5) is rapidly formed as an insoluble precipitate under acidic conditions. Like the trisulfide, As_2S_5 forms soluble thioarsenate (possibly AsS_4^{3-}) and arsenate (AsO_4^{3-}) salts. Presumably, the distribution of As(III)/As(V) in the environment will depend on the mechanism of formation. These possibilities need to be modeled.

The supporting literature for the reaction of organoarsenicals with sulfur compounds is more recent. It is known that $(CH_3)_2As(O)(OH)$ can be converted[108] to **21**, and a similar reaction is possible for $CH_3As(O)(OH)_2$. Thiols will promote the formation of $(CH_3)_2AsSR$ and $(CH_3)As(SR)_2$.[116] Trimethylarsine oxide gives **20** or **16**, or both,[115,116] depending on the relative amounts of H_2S and RSH. None of the species **18**–**21** have been identified in environmental samples but it is likely that the variety of organoarsenicals in soils and sediments is much greater than reported to date. Depending on the as yet unknown response of these compounds to the usual analytical procedures (i.e., can $CH_3As(SR)_2$ be detected as CH_3AsH_2?), their presence may cause a significant underestimation of the extent of biomethylation.

The evidence presented for the aerobic and anaerobic biological methylation of arsenic and the likely chemical fate of the products of these reactions leads to the conclusion that the environmental distribution of organoarsenic compounds is quite different from that originally proposed by Wood.[336] This is in fact the case.

VII. Arsenic in the Atmosphere

A. Fluxes

Arsenic enters the atmosphere from natural sources that include volcanic activity, wind erosion, sea spray, forest fires, and low-temperature volatilization (mainly biological formation of volatile arsenicals). Smelting operations and fossil fuel combustion contribute to anthropogenic sources[7] of arsenic. These inputs are balanced by removal processes such as dry deposition and rainfall. There has been considerable attention directed toward calculating global atmospheric fluxes for arsenic, but the results have not always been in agreement.[3]

In estimating the atmospheric fluxes of arsenic, Lantzy and MacKenzie[4] found that known inputs from anthropogenic and natural sources could not account for the large output of arsenic from the atmosphere by precipitation. Mass balance could be achieved by postulating[4,6] a considerable biological volatilization of arsenic from the surface of the ocean, a process that had been suggested by Wood.[336] However, their calculations were based on earlier data[337,338] for the concentration of arsenic in rain (1.6 ppb As in rain on land; 0.6 ppb in rain at sea), and more recent results[339] identified much lower levels (0.019 ppb As: 0.007 ppb As(III) and 0.012 ppb As(V); no methylarsenicals) in samples collected from unpolluted areas. These data provided a reasonable mass balance without invoking oceanic biological volatilization, a conclusion that was supported by the results of marine studies.[317,340,341] Using the lower arsenic content of rain, Walsh et al.[5] determined that 75% of the yearly global emissions of arsenic to the atmosphere were from pollution sources. However, it was recently suggested that much of the early data on arsenic concentrations and emission factors are outdated with regard to existing environmental control strategies and analytical techniques.[3] Using current information, Chilvers and Peterson[3] estimated the ratio of natural to anthropogenic inputs as 60:40. Natural sources of arsenic were largely comprised of low-temperature volatilization from soils (60%), with most of the remainder due to volcanic activity. Anthropogenic emissions were dominated by metal production,[342] especially copper smelting (40%).[3] It was noted that stricter regulations and increased world demand for arsenic might encourage industry to capture arsenic-rich effluents and reduce anthropogenic contributions. These calculations were considered to be preliminary and there is agreement that better data bases are required, especially regarding the importance of biomethylation.[343] Complicating the picture is growing evidence[344] of significant spatial and temporal variability of atmospheric elemental concentrations. In fact, Buat-Mênard and Duce[344] have suggested that the concept of constant background concentrations of trace elements in air, rain, and dry deposition is itself erroneous.

B. Analytical Considerations and Speciation

There are few reports of atmospheric arsenic speciation. Most anthropogenic emissions, such as smelting and fossil fuel combustion, consist of As_2O_3.[342] It was suggested that sea spray will mainly contribute arsenate[339]—the dominant species in seawater. However, arsenic speciation studies[339] of rain and snow samples suggest that the ratio of inorganic oxidation states is not reflective of the arsenic source but is governed instead by redox changes in the atmospheric environment. Consistent with this, thermodynamic calculations[339] indicate that in the condensed-phase reactions expected for atmospheric particulates, low

concentrations of reducing (e.g., SO_2) or oxidizing (H_2O_2, O_3) agents can increase or decrease the As(III)/As(V) ratio, respectively.

Speciation is also complicated by the fact that most of the arsenic in the atmosphere is in the form of particulate matter. Less than 10% is present in the vapor phase or on particles smaller than 0.2 μm.[5,6,344] Analysis of these solids has revealed that they are often considerably enriched (10–1000 times) in arsenic in comparison to the continental crust.[6] This is probably due to gas–particle reactions such as adsorption and/or complexation that capture volatile arsenicals.[333] It is common practice to trap these particles on filter paper but care must be taken in the subsequent extraction of adsorbed arsenic in order to preserve the speciation.[345] Waslenchuk[341] digested particulate atmospheric samples collected off the coast of the southeastern United States (Georgia Bight) in concentrated nitric acid. Subsequent speciation analysis of the digest gave, not surprisingly, only As(V). Nitric acid solutions can decompose organoarsenicals, whereas (depending on the concentrations used) hydrochloric acid usually does not.[346]

Johnson and Braman[333] found that organoarsenicals could be removed, without dealkylation, from particulates collected on glass fiber filters by treatment with dilute base. Vapor-phase arsines—AsH_3, CH_3AsH_2, $(CH_3)_2AsH$, and $(CH_3)_3As$—and gaseous As_2O_3 could be quantitatively trapped on silver-plated glass beads and removed similarly. Because the extraction procedure converted alkylarsines to alkylarsenic acids, it was not possible to identify which of these two types of arsenic compounds was originally associated with the particulate matter or trapped on the beads. However, di- and trimethylarsenic species were found in both vapor-phase and particulate samples collected in a variety of indoor and outdoor environments. No monomethyl species were detected above 0.1 ng/m^3. The original identity of the inorganic arsenic was not revealed by the analytical procedure, but it was only found on the filters. The similarity of the inorganic arsenic fraction in all of the samples and the high alkylarsenic levels associated with plants was interpreted in terms of two different general sources for airborne arsenic: nonbiological processes, both natural and anthropogenic, producing inorganic species, and biological processes that release volatile alkylarsenic compounds.[333] These authors made one of the early suggestions of the importance of biomethylation in the global arsenic budget.

It is now known that communities of microorganisms possess the collective ability to produce a full range of volatile arsenicals (section III)—MMA, DMA, and TMA—and all of these, as well as AsH_3, have been detected in samples collected close to their source.[234] Parris and Brinckman[128] calculated a second-order rate constant (10^{-6} M^{-1} s^{-1}) for the gas-phase oxidation of $(CH_3)_3As$ by O_2, suggesting that this compound can travel considerable distances without undergoing chemical change. Eventually, however, each of the methylarsines will be oxidized to MMAA, DMAA, and TMAO or undergo other reactions,[347] possibly on the surface of particles.

The concentrations of atmospheric methylarsenic species have been shown to be subject to seasonal variation.[348] Sampling of airborne particulate matter was carried out over more than a year at two sites, representing polluted and rural areas, in Japan. Both dimethyl and trimethyl species were extracted from quartz fiber filters by treatment with 1 M HCl and 0.02 M EDTA (9:1). The ratio of the dimethyl to trimethyl form was usually 0.15–0.34, indicating that $(CH_3)_3As$ was the dominant species produced by biomethylation. A similar order of concentrations was reported by Johnson and Braman.[333] At both Japanese sampling locations,[348] the concentrations of methylarsenicals were high in summer and low in winter, in accordance with the observed temperature changes. Trace amounts of a monomethylated species were detected in the summer but its presence was attributed to contamination by a pesticide, iron methylarsonate, used near the sampling site.[348] A calculated activation energy of ~12 kcal mol^{-1} was considered to be within the range of normal biological reactions (8–18 kcal mol^{-1}). Although the ratio of methylarsenic to inorganic arsenic compounds was <10% in the airborne particulate matter, their overall concentration was up to 18 ppm. Since this in itself represents a significant enrichment over crustal arsenic concentrations (~3 ppm), these data provide strong support for a biomethylation contribution to the atmospheric arsenic cycle.

It is therefore clear that methylated arsenic compounds are present in the atmosphere in association with particulate matter, but more work is needed to evaluate the vapor-phase distribution of these compounds. Maher[46] has stated that As(V) and DMAA are found in rain, but this does not seem to be substantiated by more comprehensive work in which Andreae[339] indicates that only As(V) and As(III) are present. It is notable that particulate samples containing methylarsenicals were collected[333,348] over land masses where biomethylation is more likely to produce *volatile* arsenicals (MMA, DMA, and TMA). The rain and snow samples analyzed by Andreae[339] may be more representative of arsenic speciation over the Pacific Ocean or of arsenic that has spent a longer time in the atmosphere. Although methylarsenicals, like DMAA, are quite stable, they can be demethylated by UV irradiation.[330,331] This may only be significant at altitude, as MMAA and DMAA have been found to be unaffected by exposure to sunlight at ground level.[348] Additional precipitation data and information on the residence time and stability of these compounds are needed in order to clarify the situation.

VIII. Arsenic in Aquatic Systems

A. Arsenic Concentrations

The concentration of arsenic in fresh waters shows considerable variation with the geological composition of the drainage area and the extent of anthropogenic input. Andreae has compiled data for dissolved arsenic in some European as well as North and South American rivers.[349,350] The geometric mean is 1.4 ppb but the range is large, approximately 0.1–75 ppb.[349] The greater density of industrial activity appears to be reflected in slightly higher average arsenic concentrations in Europe, 3.5 ppb, as compared to North America, ~0.5 ppb, but this comparison is no doubt prejudiced by the sample selected.[350] For example, 16 California and Florida rivers averaged 2.3 ppb, but a value as high as

43 ppb was also observed.[340] Geothermal waters have particularly high levels of dissolved arsenic (e.g., 1275 ppb in Old Faithful, Yellowstone National Park),[351] but freshwater lakes can also reach these values. Woolson[234] has tabulated results showing ranges from trace amounts to 276 000 ppb! It is therefore extremely difficult to suggest typical arsenic levels, but most values are in the ppb range. Identification of a particular site as arsenic polluted, therefore, is probably best accomplished by comparing the results obtained for samples collected from similar watersheds.[350]

Oceanic constituents tend to be less variable than their freshwater counterparts.[316] Accordingly, a narrower arsenic concentration range, 1.0–1.8 ppb, has been observed for deep Pacific and Atlantic waters.[317,340,352,353] Surface water arsenic concentrations are subject to some seasonal variations due to biological uptake;[317,340] these changes will be most important in highly productive coastal waters. Cycling models typically suggest that arsenic enters the ocean from the atmosphere and via rivers as dissolved species and as part of suspended particulate matter. These inputs are offset by removal to the atmosphere as sea salt spray and by precipitation/adsorption to the sediments. The balance may not be perfect and it has been suggested that the concentrations of arsenic in the world's oceans will increase by 1–2% by the year 2000—mainly as a consequence of increased anthropogenic activity.[3–6,46] These predictions will be tested as more current data (especially with respect to anthropogenic emissions[3]) become available and as input/removal mechanisms are examined in greater detail.

Of particular interest to land/ocean fluxes is the behavior of arsenic in estuaries—at the interface between fresh- and saltwater environments. Most models[3–6] emphasize the riverine transport of arsenic both as dissolved species as well as that bound to suspended matter. However, it is now recognized[46] that most particulate matter is deposited in estuarine and coastal sediments.[341,354,355] The open ocean contains little arsenic in this form.[317,340,341]

In many estuaries, and within a continental shelf water body, dissolved arsenic has been found to be conservative;[354,355] i.e., arsenic concentrations are controlled by simple physical mixing of the fresh- and seawater masses and vary linearly with salinity. Deviations from conservative behavior can be related to the action of particulate matter or to anthropogenic inputs. In principle, river-borne particulates transported from regions of higher (or lower) arsenic content can alter dissolved arsenic concentrations by desorption (or adsorption),[349,355] but most of this material has been found to be inactive.[46,341,354] Of much greater importance is the precipitation of soluble Fe^{3+} and coagulation of iron colloids at the saltwater interface in regions of increasing ionic strength and pH.[356–359] Coprecipitation of arsenic with iron can dramatically reduce the flux of arsenic to the sea. In some regions, this removal can be offset by input from pollutant sources.[349,356,357] These processes are considered later (section IX). This section focuses on the interplay between the dissolved arsenic compounds.

In this article, arsenic concentrations are quoted in parts per billion (ppb), because most of the cited articles have employed this format. There is also a growing tendency to use nM (nanomolar). Regardless of the units, the concentration refers to the amount (as micrograms or nanomoles) of elemental As per liter of solution. Environmental data are often calculated in this manner because knowledge of the specific compound is not required. Biological scientists frequently employ units such as μg-atom L^{-1} (microgram-atom per liter; equivalent to μM) to indicate this. The convention is usually implied, not stated, and confusion can easily arise.

B. Analytical Considerations

It is only in the past 10 years that most data have been acquired for the distribution and speciation of arsenic in natural waters. A summary of information up to 1965 gave an average seawater concentration of 2 ppb and an average value of As(III)/[As(III) + As(V)] of 0.2.[337] Subsequent work, usually employing the molybdenum blue spectrophotometric method, suggested a greater range (10^{-1}–10^{1}) for this ratio,[81] but due to the absence of a suitable analytical procedure, essentially no data were available regarding the presence of organoarsenicals. The first such information was reported in 1973 by Braman and Foreback,[16] who found monomethylarsonic acid and dimethylarsinic acid, together with the usual inorganic arsenicals, in a variety of freshwater and seawater samples. Almost all aquatic arsenic speciation studies carried out since have employed either Braman's original technique or a closely related adaption.[360–362] These utilize selective hydride generation of volatile arsines (reaction a in Scheme VII), which are concentrated by cryogenic trapping. Separation of these evolved arsines, plus any that were present in the original sample, is effected by successive boiling point evolution and/or gas chromatography. DC discharge emission spectroscopic detection is used in the original Braman procedure,[16] whereas Howard and co-workers[360] employ atomic absorption spectroscopy (AAS). Detection limits of ~0.02 ppb have been regularly obtained with both techniques by a variety of groups. Andreae originally[361] used two separate detection systems, flame ionization detection and AAS, for the determination (detection limits 0.1–2 ppt) of organoarsenicals and inorganic arsenic, respectively. More recently,[362] a single quartz cuvette AAS system has been used with consistently impressive detection limits (0.1 ppt; ~1 pM). As will be noted below, these differences in detection limits may account for some of the discrepancies regarding the distribution of organoarsenicals.

To date, monomethylarsonic acid and dimethylarsinic acid are the only organoarsenicals that have been found as dissolved species in natural waters. In part, this may be reflective of analytical limitations as the "Braman" and "Howard" procedures are typically only calibrated for the detection of CH_3AsH_2 and $(CH_3)_2AsH_2$ evolved from the hydride generator. Of the techniques employed for arsenic speciation in aquatic samples, only Andreae has specifically described[361] standard curves for trimethylarsine (it is not clear if he used TMAO to produce the TMA). In an early report[317] describing a study of arsenic speciation in the northeast Pacific (California coast), a description was provided of the precautions taken to prevent the loss of volatile species (MMA, DMA, and TMA) from water column and in-

terstitial water samples collected at one station. No methylarsines or TMAO were found. It is not apparent if these precautions were employed in later work but Andreae has subsequently stated that no such species are present in the marine environment.[36,363] This may not be surprising in view of the chemical reactivity of these compounds (section VI.C). In the absence of sufficient biological production, these compounds may only be present at low steady-state concentrations, if at all.

A further caveat regarding analytical limitations has been noted by Howard et al.[364] The hydride generation procedure is only capable of discriminating between nonmethylated arsenic(III) and -(V) compounds, monomethylarsenic species, and dimethylarsenic species. They point out that despite the many reports of monomethylarsonate and dimethylarsinate in natural waters, such species have not, and cannot be, positively identified by borohydride-based systems that generate methylarsines from any methylarsenic compound. This is an important point, and one that can only be completely clarified by the application of analytical procedures that identify individual dissolved species directly (e.g., HPLC–GFAA) or separate them (e.g., ion exchange, HPLC) prior to hydride generation.[8,36,365] Indeed such techniques are necessary for a more comprehensive examination of aquatic arsenic speciation. Unfortunately, they have not yet found widespread application in environmental studies.

Howard's criticism can be partially addressed by an examination of the behavior of other organoarsenicals under the conditions typical of hydride generation. The most likely candidates are the endocellular compounds (Tables V and VII) such as the arsenosugars, tetramethylarsonium ion, and arsenobetaine found in marine organisms. Some of these compounds may be released into the water column as exocellular metabolites during the life, or upon the death, of the organism. Andreae has observed[36,363] that endocellular algal compounds, isolated by extraction with methanol or chloroform, undergo subsequent acid or base hydrolysis to give species that are detected as di- and/or trimethylarsenic species by hydride generation. In order to determine if these organoarsenicals constituted a significant fraction of the arsenic content of natural waters, he subjected samples for which the arsenic speciation had previously been determined to rigorous base hydrolysis and then reanalyzed them. The similarity in the results obtained before and after was interpreted as strong evidence that these species were not present in river or ocean waters at levels higher than 1–2% of the total arsenic content.[363] This conclusion appears to have been verified by digestion of seawater with methods capable of mineralizing all of the organic matter (e.g., magnesium nitrate ashing aid). No significant amounts of arsenic were observed that could not be accounted for by the original inorganic arsenic, MMAA, and DMAA.[36,363] It was not clear if the samples were obtained from the surface waters of the open ocean or from more productive coastal waters (preferably during a bloom), where (when) the largest concentrations of potential exoceullular metabolites might be present.

An alternative source for the methylarsines generated by hydride generation is the microorganisms (e.g., phytoplankton) themselves. It is not likely that the bound organoarsenicals would react with borohydride, but they could be hydrolyzed under the acidic conditions (pH 1) often used for the storage of the samples. Abiological particulate matter could also release arsenicals under these conditions, but in most cases water samples have been filtered after collection. In one study, no difference was found in the results obtained for filtered and unfiltered samples.[340] Preservation of unfiltered samples, collected in highly productive waters, by acidification should be avoided. Of course, the correct speciation of all arsenicals is dependent on how the sample is handled prior to analysis. Unfortunately, no universal preservation procedure has been found.[365] It is therefore quite likely that postsampling changes have influenced the results presented in the literature, but it is not possible to determine the extent of this problem.

It therefore appears, at least on the basis of available data, that the only sources of the mono- and dimethylarsines produced by hydride generation of environmental water samples are the dissolved species monomethylarsonic acid and dimethylarsinic acid. Furthermore, considering the analytical and sampling limitations that have been imposed in all studies to date, these are the only simple methylarsenicals that could have been found. It remains to consider how they, together with the inorganic species, are distributed in the aquatic environment. Most speciation work has focused on marine systems.

C. Arsenate

It has been previously noted (section VI.B.1) that arsenate, or, more rigorously, $HAsO_4^{2-}$ (ocean) or $H_2AsO_4^-$ (fresh water), is the thermodynamically stable form of arsenic in oxygenated water. In view of the large calculated As(V)/As(III) ratio, 10^{15}–10^{26}, it should be the only detectable species in most natural waters. However, significant amounts (~10% of total As) of arsenite (H_3AsO_3) have been found even in uncontaminated surface and deep ocean waters. Conversely, even under anoxic (reducing) conditions, arsenate was still present. Andreae has used these observations to suggest that the redox pair arsenate/arsenite is rarely in thermodynamic equilibrium anywhere in the marine environment.[363]

Total dissolved arsenic concentrations have shown only slight increases with depth in the deep Pacific and Atlantic oceans. Maher et al.[352,353] contrasted this behavior to that of phosphate, implying that the two elements are subject to different influences. However, Andreae was the first to demonstrate[317,340] that the profiles of the isoelectronic arsenate are at least qualitatively similar to those of HPO_4^{2-}. Phosphate undergoes depletion in photosynthetically active surface waters (euphotic zone). The falling biomass is degraded by bacterial activity, and nutrients are returned to solution. This results in a sharp increase in phosphate concentrations just below the thermocline—the layer separating the less dense surface waters from the more dense, typically nutrient-rich, deeper waters. Arsenate concentrations also showed this increase with depth, but the gradients were much less pronounced.[317,340] This is not surprising as the surface water depletion of arsenate was found to be less (typically 20–30%) than that of phosphate (up to 100%). These observations

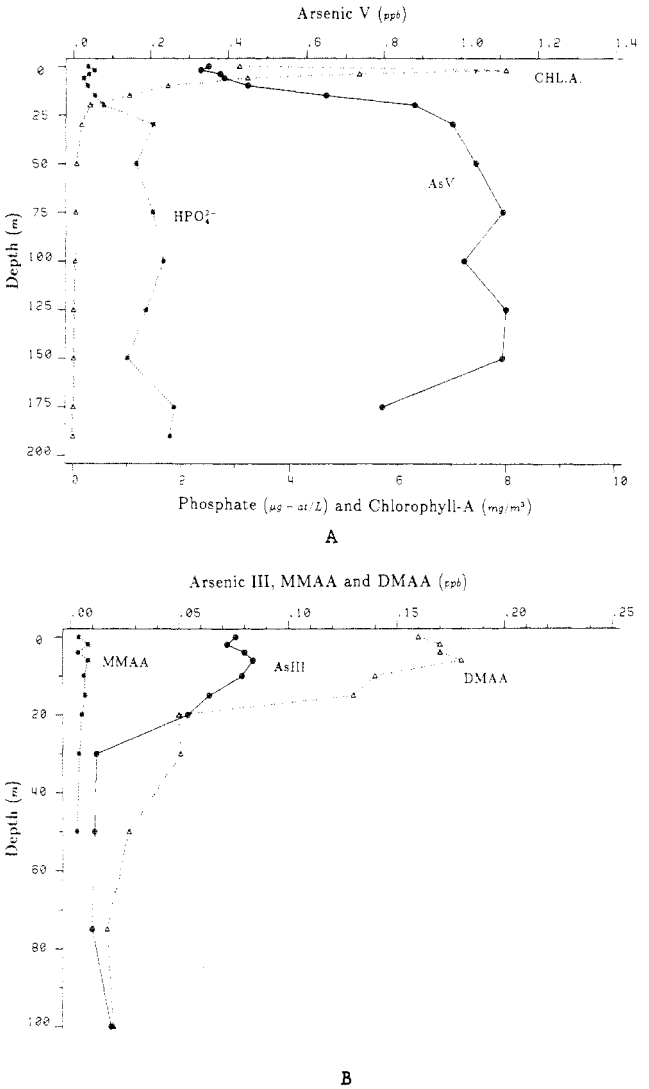


Figure 7. Water column profiles of $HAsO_4^{2-}$ (As(V)), H_3AsO_3 (As(III)), $CH_3AsO(OH)_2$ (MMAA), $(CH_3)_2AsO(OH)$ (DMMA), HPO_4^{2-}, and chlorophyll *a* (CHL.A) concentrations during a bloom in Alice Arm, BC.

suggest that arsenate is a biointermediate element[316] that is actively taken up, principally by phytoplankton during a bloom. This is further supported by the striking coincidence between surface water arsenate minima and photosynthetic activity maxima determined by carbon uptake and/or chlorophyll *a*. In the absence of biological activity (e.g., winter conditions), arsenate (together with a small amount of As(III)) account for essentially all of the dissolved arsenic. The lack of a significant transport of arsenic to deeper waters with the descending biomass indicated that only a small proportion of the total available arsenic was incorporated into the biota.[366] These points have found support in laboratory culture studies.[367,368]

Related arsenate behavior has been found in shallower coastal waters. Typical relationships are illustrated in Figure 7 using data that Reimer and Thompson[369] obtained from a study of arsenic speciation in British Columbia coastal waters. The surface 100 m of the water column was much more depleted (~75%) in As(V) than the waters first studied by Andreae.[317,340] The extent of this depletion was dependent on the sampling date, relative to that of the maximum photosynthetic activity; not shown is the rapid return of arsenate as the bloom ended. It therefore appears that the $HAsO_4^{2-}$ concentration in the surface waters is particularly dependent on the *nature and degree of biological activity at the time of sampling*. Marked differences are expected (also see section VIII.E.1) for the open ocean in comparison to more highly productive coastal areas. The loss of As(V) at depth (Figure 7) is indicative of its incorporation into these, but not necessarily all, sediments (section IX).

Thus the aquatic distribution of arsenate is seasonally dependent—accounting for most of the dissolved arsenic in the absence of photosynthetic activity and undergoing depletion in the presence of a rapidly growing bloom. Some arsenic is incorporated into the biota, but what happens to the rest of it? A considerable body of evidence indicates that a major influence of biological processes on the distribution of *exocellular* arsenic occurs in the euphotic zone and involves the cycling of arsenite, MMAA, and DMAA. Speciation changes also take place in deeper waters but these are generally distinct from surface processes. Indeed the two regions are usually considered separately since, when the thermocline is present, there is little mixing of surface and deep water masses except for the downward movement of particulate matter. Even in shallow water where such stratification is absent or ephemeral the photic zone can usually be considered separately from deeper water.

There are two exceptions to these otherwise general observations regarding the response of arsenate to biological activity. Howard and co-workers twice investigated the behavior of arsenic during a spring diatom phytoplankton bloom.[370,371] The studies were conducted in a closed ecosystem (bag) deployed in Loch Ewe, Scotland. In both experiments classic spring conditions led to rapid changes in phytoplankton biomass and correspondingly rapid depletion of nutrients; phosphate eventually reached zero concentration. In the first study[370] only total inorganic arsenic was measured (the concentrations are incorrectly cited as $\mu g\ L^{-1}$ instead of μg-atom L^{-1}, i.e., μM) and no methylated species were detected (the detection limit, 0.2 ppb, appears to be an order of magnitude higher than reported in other work from this group). Despite the rapid depletion in phosphate, there was no change in inorganic arsenic. Arsenite and arsenate concentrations were individually monitored in the second study, which displayed similar bloom activity. Neither arsenical underwent significant variation throughout the course of the experiment. These results were interpreted in terms of either rapid uptake and subsequent excretion of unchanged arsenate or a high degree of phosphate/arsenate discrimination. Surprisingly, the dominant bloom diatom, *Skeletonema costatum*, has been the subject of several other investigations in which ready assimilation and methylation of arsenate was observed at a variety of phosphate concentrations.[201,367,368] The only difference between these studies and the ones being considered here is temperature. The spring bloom in Loch Ewe took place at water temperatures between 6 and 7 °C whereas arsenic uptake and methylation has been observed at higher temperatures. Howard et al.[371] suggest that either warm and cold water algae ecotypes exist or arsenic metabolism is a fluid process that is modified in response to an external stimulus.

The details of the uptake mechanism for arsenate by marine algae are still poorly understood. In particular, it is not clear if $HAsO_4^{2-}$ and HPO_4^{2-} are competitive or if algae are capable of discriminating between them.[201,367,368] Culture experiments that measure the response of these systems to temperature might be informative, but care must be taken in extrapolating the results of culture studies to the natural environment. Natural phytoplankton assemblages have been found to respond differently to arsenic stress than single-species cultures.[372] Arsenate may be differentially inhibiting to some plankton, resulting in a change in species composition and in the succession of dominant species.[373] It may turn out that most arsenic is adventitiously absorbed.[45] Whatever the mechanism, uptake results in profound changes in the composition of arsenic compounds in the water column.

D. Arsenite

1. Production and Introduction of Arsenite

Andreae and co-workers have conducted studies of arsenic distributions in the eastern north Pacific Ocean, in the Baltic Sea, and off the California coast,[317,340,350] all of which show elevated levels of H_3AsO_3 (5–10% of total As) in surface waters coincident with the zone of primary productivity. These observations are consistent with the increases in arsenite concentrations found in the media of phytoplankton cultures, especially during periods of rapid growth.[367] However, it is unlikely that plankton were responsible for all of the As(III) production, since arsenite maxima were not strictly correlated with regions of maximum photosynthetic activity. In one of the two studies of the deep waters off the California coast, increases in As(III) occurred near the base of the photic zone,[340] and in the other,[317] arsenite maxima were generally indistinct and varied with depth from one location to another. These observations suggested that arsenite production may also be connected with bacterial and zooplankton activity.

It turns out that the ability of microorganisms to reduce arsenate to arsenite is widespread[36] (section II.C). Among aquatic organisms (section IV), this property has been reported for a coral species[374] (and/or the symbiotic zooxanthellae), by *Sargassum* sp communities,[375,376] and by freshwater phytoplankton *Chlorella* sp cultures.[229,377] Johnson[81] demonstrated the ability of a mixed-population bacterial culture from Narragansett Bay to reduce As(V) to As(III) in seawater medium. Johnson and Burke[378] followed the change in As(III)/As(V) during a winter–spring diatom bloom. An increase in this ratio correlated with the increase in total cell numbers and was inversely related to the phosphate–arsenate ratio. As part of the same study, arsenite was monitored in unialgal cultures of *Skeletonema costatum* containing 4 ppb arsenate and a variety of phosphate concentrations. Arsenite production was delayed in cultures grown at high phosphate. Bacteria were present in both experiments, but the profiles of the lowest phosphate experiment were very similar to results reported by Sanders and Windom[367] for axenic cultures of the same algae. Algae appear to differ in their tendency to produce arsenite. Andreae and Klumpp[201] found considerable arsenite after mild acid digestion of the coccolithophorid *Cricosphaera carterae*. This same organism was isolated in field studies of the North Central Pacific Gyre, where it was associated with high concentrations of dissolved arsenite.[317] An *Anabaena oscillaroides* bacteria assemblage, isolated from the arsenic-rich Waikato River (New Zealand) was found[86] to reduce arsenate to arsenite at a rate of 12 ng of As 10^6 cells^{-1} day^{-1}.

It is therefore reasonable that arsenite should be present at nonthermodynamic concentrations, particularly when conditions support high microbial populations. As will be discussed shortly, methylarsenic species are usually good indicators of active phytoplankton growth, even when direct evidence such as cell counts or chlorophyll *a* measurements are not available. However, since arsenite production is not limited to algae, it is difficult to identify *typical* As(III) concentrations in oxic waters. In a study of arsenic species succession during a spring phytoplankton bloom in a British Columbia fjord (Saanich Inlet), Reimer et al.[379] observed increases in H_3AsO_3 surface (0–50 m) concentrations from 1% at peak photosynthetic activity to 12% upon the death of the bloom. Even in oxic deep waters, high As(III) levels have been observed. Andreae found normal total arsenic levels at 100–200 m at a station in Santa Monica Bay located near a sewage outfall and sludge dumping site, but 84% of this was arsenite.[340] Elevated arsenite levels, but of lower magnitudes, were found in other, uncontaminated deep (200 m) waters that were, as a consequence of oceanic circulation/mixing patterns, rich in organic matter and hence suitable for heterotrophic activity.[317]

In addition to biological activity, arsenite can also be introduced anthropogenically. Chemical and biological oxidation of arsenite, together with dilution effects, will reduce the impact of anthropogenic emissions far from shore. Consequently, arsenite pollution is most evident in estuaries receiving As(III)-rich river input. Klumpp and Peterson[202] found that arsenic which was up to 42 ppb in the Carnon River (Great Britain) consisted predominantly (95%) of arsenite due to the influence of long-term mining activity in the region. Marine waters principally contained arsenate. No methyl species were detected above 0.03–0.05 ppb. Arsenite in the river water entering the estuary was either converted to arsenate or diluted when mixed with saline waters. Consequently, the As(V)/As(III) composition of the estuary varied with the tide.

The Tejo River Estuary (Portugal) is the recipient of large amounts of arsenic, both directly and by atmospheric emissions.[349] A nearby pyrite roasting plant is largely responsible for emissions of 1000–2000 tons of arsenic each year. These inputs are reflected in nonconservative behavior, i.e., strong positive deviations of arsenic/salinity from that expected on the basis of simple mixing of the fresh and marine waters. The composition of the emissions was not determined but it was likely that arsenic trioxide was predominant. Since the transport time between the emission source and the estuary was too short for redox changes to occur, it was assumed that most of the atmospheric arsenic deposition was in this form. At the time of sampling, arsenate was most abundant followed by arsenite. Only traces of MMAA and DMAA were observed. Arsenite was between 2.9% and 20% of the total inorganic arsenic in the estuary. The highest percentages were observed nearest the atmospheric emission source,

suggesting that the most recent input of arsenite emissions did not have time to oxidize in the surface waters. Other factors such as mixing were also important as the maximum in As(III) did not coincide with the maximum in total arsenic.

Arsenite can also be produced in response to redox changes in the water. This has been observed in water column studies of two areas—Saanich Inlet (Canada)[380] and the Baltic Sea.[350] In both cases, the water circulation is such that the water column becomes stratified and the deep water anoxic. For the Baltic Sea, replacement of the bottom waters occurs only during unusual meteorological conditions, on the average every 2–5 years. Saanich Inlet is an intermittently anoxic fjord in that oxia–anoxia in the bottom water usually follows an annual cycle. The water becomes stratified in late winter and spring, but in the late fall coastal water with a high density and oxygen content displaces the anoxic bottom water. In the periods between deep-water renewal, bacterial sulfate reduction takes place and H_2S concentrations can reach 20 μM. When SO_4^{2-}/HS^- is the controlling redox couple, the presence of HS^- requires a pE of approximately −3. This should result in a conversion from As(V) to As(III).

Peterson and Carpenter[380] studied Saanich Inlet twice—once when complete anoxia prevailed, and a second time when there was a partial intrusion of oxygenated water. Only As(III) and As(V) were determined, but at the stations sampled there was no (station A) or slight (station E) evidence of arsenate depletion in surface waters, and it is unlikely that photosynthetic activity was high on either occasion. Total dissolved arsenic was determined as the sum ($\sum$As) of As(III) + As(V) as determined by hydride generation and independently by neutron activation analysis of samples in which arsenic was coprecipitated with ferric hydroxide (TAs). Oxic samples tended to give positive values of $\sum$As − TAs and anoxic samples negative differences. The authors favored the accuracy of the TAs results and made little comment on the difference except to suggest that negative values could reflect the presence of other arsenic species such as MMAA and DMAA. Methylarsenicals have been found[350] in the anoxic zone of the Baltic Sea. Also possible is the formation of arsenic sulfides that are insensitive to hydride generation detection. Reimer[324] has found that hydrogen sulfide interferes with hydride generation GC/AAS analyses. Regardless of the differences in $\sum$As and TAs, this study demonstrates the response of the As(V) $\rightleftharpoons$ As(III) couple to a redox gradient in the water column.

The arsenic concentration profiles (Figure 8) for station A (oxic) are typical of nonproductive ("winter") conditions. Arsenate concentrations were constant at 1.45 ± 0.02 ppb to a depth of 70–100 m and increased steadily below this depth to 2.39 ± 0.17 ppb. The arsenite profile was uniform with depth, averaging 0.14 ± 0.04 ppb. The enrichment of total arsenic within the bottom layer was consistent with the release of arsenic from the decomposing organic matter. The TAs profile for station E (anoxic) was similar to that of station A in that concentrations were generally less in the upper part of the water column. This was the extent of the similarity. The authors attributed the minimum at the surface to mixing. Surprisingly, they did not consider biological activity. A second minimum at 95 m corresponded to the top of a 40-m-thick manganese- and bacteria-rich particulate layer (coprecipitation of arsenic with manganese and iron oxides is an important factor in its aquatic distribution; section IX). The particulate layer decreased with depth, as did TAs, at the O_2/H_2S interface at 135 m. The most dramatic feature of arsenic speciation at station E was the essentially complete speciation reversal across the redox front at 135 m. As(III) was responsible for only 5% (0.10 ppb) of the dissolved arsenic content above this front but increased to 87% (1.58 ppb) below it.[380]

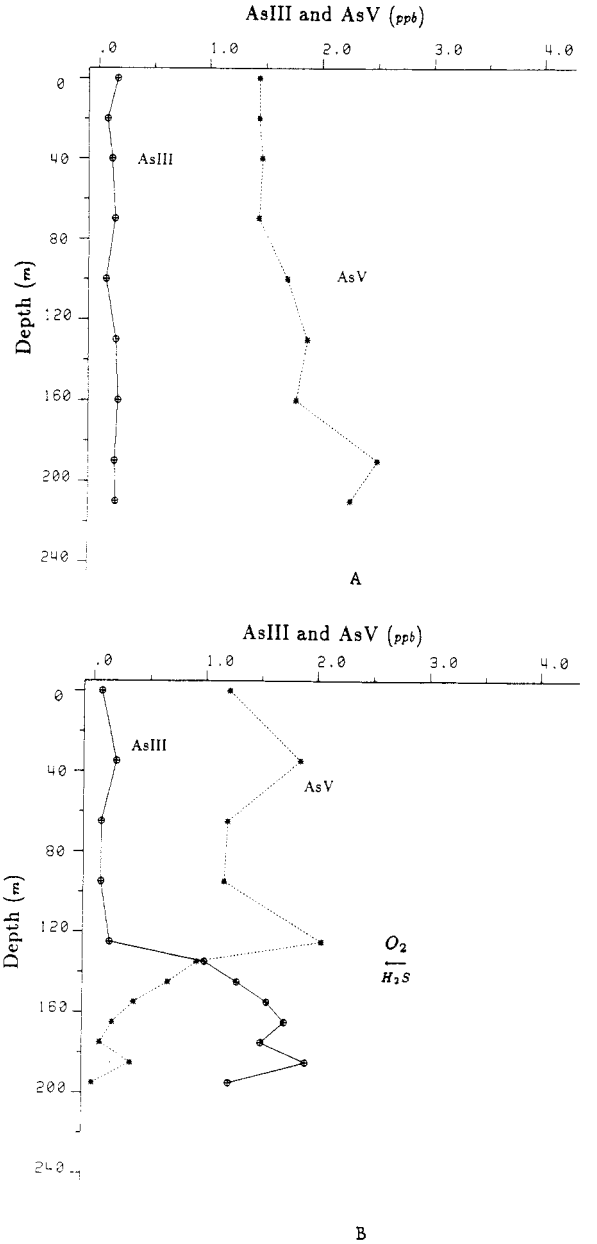


Figure 8. Water column profiles of arsenate (As(V)) and arsenite (As(III)) concentrations in Saanich Inlet, BC, at (A) an oxic station and (B) an anoxic station. The arrow indicates the transition from oxic to anoxic conditions (plotted from data presented in ref 380).

Andreae and Froelich[350] reported similar reduction of As(V) to As(III) in anoxic and even oxygen-deficient (no H_2S; oxygen levels 30–40 μM) deep waters of the Baltic Sea. The in situ production of arsenite in waters containing over 30 μM O_2 and no H_2S is curious. Peterson and Carpenter[380] observed no change in the As(V)/As(III) ratio at O_2 concentrations >1.5 μM, and Andreae[317] has reported a similar insensitivity in the

arsenate/arsenite couple in oxygen-depleted waters in the Santa Catalina Basin. However, the reducing influence of H_2S is clear elsewhere in the Baltic Sea study. In the oxic waters arsenate was the predominant species; in the anoxic zones arsenite accounted for 56–76% of the total inorganic arsenic. Although the percentage of arsenic(III) is high, both groups have suggested that, on the basis of thermodynamic calculations, arsenic should be completely in the reduced state under anoxic conditions. Peterson and Carpenter[380] have suggested that the observed disequilibrium may be a kinetically controlled condition. An alternative possibility for the lower than expected As(III) levels would be the formation of an arsenic–sulfur complex that would not be detected by hydride generation, but their thermodynamic calculations suggested that "AsS_2^-" (section VI.B.1) concentrations would be insignificant. Andreae and Froelich[350] approached the problem from the opposite perspective, i.e., why arsenate levels were higher than expected. They suggested that thioarsenates might be present but provided no supporting evidence. Alternate explanations for these observations were considered in section VI (Scheme VII), but further work on the nature of arsenic–sulfur compounds in anoxic water and the response of these species to conventional analytical procedures is clearly essential.

2. Oxidation to Arsenate

The production of arsenite, as H_3AsO_3, appears to be controlled by the prevailing biological, anthropogenic, and chemical influences. Presumably the observed concentrations of As(III) are a balance of these factors and those controlling its loss. However, comparatively little work has focused on the oxidation of arsenite to arsenate.

Johnson and Pilson[326] found the oxidation rate of As(III) to be generally slow, increasing with initial arsenite concentration, temperature, and salinity. No effect was found over the range of O_2 concentrations that were used (0.15–15 ml L^{-1}). Particularly interesting was a photochemically induced arsenite oxidation. Samples contained in Vycor and Pyrex flasks showed normal rates in the dark and in the presence of diffuse laboratory light. A marked increase in rate (5–10 times) was observed on exposure to sunlight on the roof of the laboratory building. This effect could be switched on or off by covering and uncovering the flasks. Obviously, this may increase the rate of conversion of arsenite to arsenate in surface waters. It may also have important implications for the storage of arsenite standards in analytical laboratories.

Scudlark and Johnson[323] remeasured the linear rate for the chemical oxidation of As(III) and found reasonable agreement with the previous report. By extrapolation to the "real world" from the experimental conditions, they estimated that an As(III) concentration of 6 nM (0.45 ppb) would be chemically oxidized at a rate of 0.3 nmol L^{-1} day^{-1}. In the presence of bacteria, the oxidation, which was pseudo first order, was considerably faster: 1.5 nmol L^{-1} day^{-1}. Water samples containing bacteria capable of this oxidation were not ubiquitous but were found in freshwater and marine regions of high productivity. Curiously, much of this quantitative experimental work made use of the Narragansett Bay (NB) water that had been stored for several months before use. Fresh NB water did not display this activity. Johnson also used NB water in one of the first examples of arsenate *reduction* by marine bacteria.[81] The reasons for these differences are not clear and it would be particularly useful to know what controls the relative populations of reducing and oxidizing organisms in a given region. Arsenite oxidizing bacteria are common (section II.B) and the bacteria cultured by Scudlark and Johnson[323] were similar to a *Pseudomonas arsenoxydans* Quinque described by Turner and Legge.[73] A comparison of the overall rates of oxidation (chemical and biological ~2 nmol L^{-1} day^{-1}) to an estimated rate of reduction by phytoplankton suggested an As(V)/As(III) ratio of ~20. Considering the variability in biological factors and the accuracy of the calculation, this is remarkably consistent with the values that have been described in this section.

E. Organoarsenicals

1. Production

The methylarsenic oxyacids, monomethylarsonic acid, and dimethylarsinic (cacodylic) acid were first detected in aquatic systems by Braman and Foreback in 1973.[16] They reported the arsenic speciation for a number of freshwater and some saline water samples. Arsenate and usually arsenite were also present, but in some fresh waters the methylarsenicals comprised up to 70% of the total arsenic. More typically this was 10–20%, but in all cases the dominant organoarsenic compound was DMAA. Andreae[340] has also determined the speciation of arsenic in some freshwater samples; similar results were obtained, but on average, MMAA and DMAA concentrations did not exceed 10% of the total dissolved arsenic. Quantitative measurements of primary productivity were not made in these studies, but from qualitative observations Andreae suggested a correlation between the sites with the highest densities of planktonic algae and the highest concentrations of methylarsenicals. Apart from these reports, little is known about the distribution of organoarsenicals in freshwater environments. In several cases where riverine input has been examined in conjunction with a study involving the adjacent marine environment, arsenate was the only species detected. This may be an analytical limitation. In most examples of this type the total arsenic concentration was <0.5 ppb, putting the individual MMAA and DMAA concentrations near the usual detection limits.

Methylarsenicals are used as herbicides and it might be expected that they would find their way into natural waters. However, this does not appear to be a general phenomenon, probably because of high retention by soils.[234] Direct introduction of methylarsenicals did occur in the Menominee River (Wisconsin) in the form of effluents from the Ansul Co. Demethylation was observed in response to this contamination (section IX.F.2).[381]

Much more is known about the distribution of MMAA and DMAA in the marine environment. Waslenchuk[341,354] observed DMAA and arsenite in the continental shelf waters of the southeastern United States and attributed their presence to unidentified biological mediation. Together these species comprised up to 20% of the total arsenic. This grouping together

of arsenite and methylarsenic compounds is common, especially in early papers in which authors referred to $H_3As^{III}O_3$, $CH_3As^V(O)(OH)_2$, and $(CH_3)_2As^V(O)OH$, all as *reduced* arsenic compounds. This can be particularly confusing for those who correctly (section I.C.1) identify the oxidation state of arsenic in both MMAA and DMAA as +5.

Andreae reported[317,340] the first detailed concentration profiles for the distribution of MMAA and DMAA with depth in deep (up to 5 km) northeast Pacific and Californian coastal waters. The depletion of arsenate and increase in arsenite in surface waters discussed above was accompanied by the appearance of both monomethylarsonic and dimethylarsinic acids. The methylarsenic concentrations dropped to the detection limit (0.002 ppb) of the analytical technique near the bottom of the photic zone, and neither species was detected at more than occasional trace amounts in deeper waters. These results suggested that these compounds were produced by phytoplankton or by closely associated heterotrophs. Supporting this proposal was a significant correlation between typical indicators of primary productivity, i.e., chlorophyll concentrations,[340] ^{14}C uptake,[317] or both.[340] It was concluded[36,317,340,363] that arsenate was taken up by phytoplankton in the euphotic surface waters together with phosphate and subsequently converted, probably as part of a detoxification mechanism, to arsenite, MMAA, and DMAA and released back into the water column. The sharp decrease in the methylarsenic profiles, unlike the broader arsenite distribution, supported release by live algal cells as bacterial decomposition of organic matter would be expected to distribute both compounds to greater depth. From these studies, Andreae has concluded[36,363] that $CH_3AsO(OH)_2$ and $(CH_3)_2As(O)(OH)$ are ubiquitous in the euphotic zone of the oceans, accounting for ~10% of the total dissolved arsenic. In the studies described above, the concentration of MMAA was much less ($^1/_{10}$) than that of DMAA and often below the detection limits of the "Braman" and the "Howard" techniques.

Similar distributions of arsenic species were found by Reimer and Thompson[369] for a bloom in British Columbia coastal waters. These are illustrated in Figure 7B. The depletion of arsenate, coincident with that of phosphate, in surface waters (Figure 7A) has already been discussed. The DMAA concentration maxima (15% of total dissolved arsenic) was coincident with arsenate uptake and the chlorophyll *a* maxima (Figure 7B; note different depth scales for A and B). MMAA concentrations were low, but detectable, and were consistent with the production of this compound in surface waters. Arsenite mimicked the DMAA distribution near the height of the bloom, but other work[379] has shown that arsenite concentrations increase and spread over a greater range of depths as photosynthetic activity and DMAA levels both decrease. These rapid changes in the amounts of the individual arsenicals in response to changing biological activity emphasize the difficulties in establishing *typical* concentrations for aquatic arsenic species. Andreae's suggestion that methylarsenicals account for ~10% of the total dissolved arsenic is also subject to other factors.[317,340]

Sanders,[382] for example, found high concentrations of methylarsenicals during a summer bloom in the highly productive estuarine environment of Chesapeake Bay. In some cases, MMAA and DMAA accounted for 60% of the total dissolved arsenic. The quantity of arsenic present in reduced or methylated form was highly correlated to phytoplankton cell densities. Monomethylarsonic acid was present in amounts equal to, or greater than, that of DMAA, and its distribution corresponded almost precisely with the presence and abundance of a summer dominant algal species *Chroomonas* sp. In contrast to these results, only arsenate was found during the winter, despite cell densities that were comparable to the high summer values but dominated by a different species, *Katodinium rotundatum*. Sanders interpreted these observations in terms of the differing abilities of these algae to transform arsenate. More generally, he proposed that[382] the differences in estuarine and oceanic arsenic biogeochemistry could be due to differences in the nutrient strategies of the phytoplankton species that typify these regions. Oceanic phytoplankton communities are dominated by species with a high affinity for nutrients (suitable for the low nutrient conditions in the open oceans) and may be able to better discriminate between arsenate and phosphate. He suggested that the greater amounts of arsenic that are processed in the estuarine environments are due to less discriminating phytoplankton growing in a nutrient-rich environment.

Froehlich et al.[383] have also speculated on the relationship between nutrient and methylarsenic concentrations. They attributed the very high MMAA and DMAA levels in estuaries like Chesapeake Bay to high productivity in nitrogen-rich, but phosphorus-poor waters. Supporting this argument were the much lower concentrations (approximately 10% of total arsenic) of methylarsenicals found during an intense bloom in Charlotte Harbor (Florida)—a site of considerable phosphorus enrichment (>2 μM). However, no specific organisms were identified, so it is not known if the bloom was dominated by plankton that are poor methylarsenic producers.

A temperature dependence has been suggested for the appearance of methylated arsenic species in the estuaries of the River Beaulieu (England). In two related papers,[359,364] Howard et al. described the results of a 2-year study that provided evidence for a transition temperature range of 9–12 °C below which methylation did not occur. The coproduction of arsenite was evident at moderate temperatures but was less frequently observed at higher temperatures (15–18 °C), because of either a reduced rate of production or an enhanced rate of oxidation. Unfortunately, no measure of the extent or timings of biological activity or identification of dominant algal species was reported, but it was indicated that no methylarsenic species were observed in the Beaulieu estuary during the initial spring phytoplankton blooms. It would be interesting to know if these blooms were characterized by algal species that did not uptake or transform arsenic even under conditions of phosphate deprivation, similar to the results of the ecosystem study in Loch Ewe, or if in both cases cold temperatures (6–7 °C in Loch Ewe) were the controlling factor. In partial contradiction to the temperature argument, Andreae[36] has detected methylarsenicals, albeit at low concentrations (≤0.02 ppb) in seawater samples collected from below the ice in the

McMurdo Sound region, Antarctica. The responsible organisms were not identified.

Suggestions that methylarsenicals might be produced by species other than growing phytoplankton have been reported by Andreae and Froelich[350] and are based on the results of an investigation of arsenic speciation in the Baltic Sea. The arsenate and arsenite concentration profiles, discussed elsewhere (sections VIII.C,D), were largely consistent with known arsenic behavior. What was surprising were the unusually high concentrations of methylated arsenic species in surface waters. Total arsenic surface concentrations in these brackish waters (9‰ salinity as compared to ~35‰ in the open ocean) were low (0.5–0.65 ppb) compared to most marine waters, yet DMAA concentrations reached 0.5 ppb, 83% of the total dissolved arsenic. Moreover, the distribution of MMAA and DMAA concentrations across the surface of the Baltic Sea was opposite to that of the phytoplankton biomass. It was not obvious why this was the case, but the authors suggested that this might reflect the relative rates of methylation by algae and demethylation by bacteria. Also of interest was a secondary maximum in the concentration of methylarsenicals in the deeper anoxic zone. The mobilization of these species from the sediments was discounted, but it could not be determined if the methylarsenicals were produced by bacteria in situ or released as a consequence of the decomposition of algal matter sinking from the surface.

In summary, it can be seen that $CH_3As(O)(OH)_2$ and $(CH_3)_2As(O)(OH)$ are widely distributed in the marine environment, mainly as a consequence of phytoplankton activity. Accordingly, they are most frequently found in surface waters, but their appearance, concentration, and relative proportions are related to the type and abundance of algal species and/or temperature. It is consequently impossible to assign a typical concentration value. It is interesting that these methylarsenicals are almost exclusively found in oxic waters whereas Wood in an early, but still frequently quoted, paper[336] proposed that they (and the methylarsines CH_3AsH_2, $(CH_3)_2AsH$, and $(CH_3)_3As$) would only be found under anoxic conditions.

2. Demethylation

Both MMAA and DMAA are stable in the aquatic environment. Andreae has reported successful long-term storage under sterile conditions.[36] The abrupt loss of these compounds in regions below zones of productivity must, therefore, be due to bacterial demethylation. Sanders[384] found that mixed bacterial cultures from an estuarine system demethylated $(CH_3)_2As(O)(OH)$ to $HAsO_4^{2-}$ at a rate of 1 ng L^{-1} day^{-1}, comparable to estimated rates of production.[385] It remains to be determined if a corresponding (like the inorganic arsenic oxidizers/reducers) methylating bacteria will be found in aquatic systems. Such species are common in the terrestrial environment (section III.D).

3. Biochemical Origins

Phytoplankton are, directly or indirectly, primarily responsible for the presence of MMAA and DMAA in aquatic systems. There is some debate regarding the routes by which these compounds find their way into the water column.

Arsenate, together with phosphate, is usually assimilated by growing plankton cells. It is not clear if these compounds compete for the same transport system or if there are separate routes of entry into the organism. Part of the confusion may be because this varies from one species to another. Once inside the cell arsenic compounds such as 7 and 8 are formed, probably as part of the detoxification process. Andreae[36,363] argues that the more complex organoarsenic products are enzymatically converted, within the cell, to MMAA and DMAA, which are then excreted. Alternatively, hydrolysis to give these compounds could occur outside the cell, but thus far analysis of water samples before and after digestion has provided no evidence for significant concentrations of other dissolved arsenic compounds.

Regardless of the endo- or exocellular nature of the process, these arguments seem somewhat circuitous. MMAA and DMAA are precursors (Schemes I and VI) of the compounds from which they are purported to be derived, and a more direct route into the water would be their excretion after biosynthesis.

Howard et al.[359] reported that there was a time delay after the onset of the spring phytoplankton bloom before MMAA and DMAA were found in the water column. They suggest that MMAA and DMAA are released from decaying plankton by bacterial and abiotic degradation and by grazing by zooplankton. Most other studies[317,340,369,379] have shown a close correspondence between the extent of photosynthetic activity and depth at which it occurs with the distribution of $CH_3As(O)(OH)_2$ and $(CH_3)_2AsO(OH)$. If other organisms are involved, their activity would appear to be restricted to the highly productive euphotic zone.

Additional biochemical data are needed in order to resolve these issues.

IX. Arsenic in Soils, Sediments, and Fossil Fuels

A. Focus

The use of inorganic and organic arsenicals as pesticides and herbicides has diminished in recent years. Previously, they were used in agriculture, prompting many studies of the factors influencing the behavior of arsenic in soil ecosystems. Several reviews[31,234,386] have described the results of these investigations. Therefore this section will focus primarily on sediments but will also discuss the adsorption, desorption, redox, and biological transformation reactions that influence arsenic cycling in both soils and sediments. The processes that influence arsenic distribution in sediments are also at least partly responsible for the control of aquatic arsenic concentrations. The redistribution of arsenic by fossil fuel combustion has important environmental consequences. Accordingly, section IX.G will deal with arsenic speciation in oil shales and the impact of coal fly ash. The influence of acid rain on arsenic in soils and sediments is also considered (section IX.H).

B. Arsenic Concentrations

Tabulations of natural arsenic concentrations in rocks, soils, and sediments as well as the influence of anthropogenic activity on these levels are available from several sources.[1,2,234] Arsenic, with average concentra-

tions of 3 ppm in the continental crust, is relatively scarce (20th in elemental abundance) but ubiquitous. It is a constituent of numerous minerals and is found most frequently in association with sulfur—especially as arsenopyrite, FeAsS. Igneous and sedimentary rocks therefore contain varying amounts of arsenic—Woolson[234] has listed values of 0.4–100 ppm. A similarly wide range, with an average of 5–6 ppm, has been reported for soils that contain arsenic at concentrations that are reflective of the parent rock material from which they were formed. Some soils contain particularly high levels of arsenic. For example, African soils associated with gold deposits or reefs contain between 300 and 5000 ppm As.[234] This association of arsenic with valuable elements has led to suggestions of its use as a geological prospecting marker.[2,234] An unfortunate consequence of this association is the emission of arsenical gases and particulates (typically As_2O_3) during smelting and mining operations.[342] Arsenic is also present in coal, in petroleum, and especially in oil shales[234] (section IX. G.1). It is therefore introduced into the atmosphere as a consequence of fossil fuel utilization. These anthropogenic atmospheric inputs play a role in the global redistribution of arsenic (section VII.A), but the largest accumulations are generally in soils and/or sediments close to the source. The ASARCO smelter (Tacoma, WA) has been the subject of many studies, and these provide a useful perspective on the marine[387,388] and lacustrine[389] distribution of arsenic originating from an industrial point source. A comparison can be made to arsenic cycling in relatively pristine areas.[390,391]

Pesticides have been applied in amounts as large as 2690 kg of As/ha, resulting in occasionally high residual levels, but Woolson has suggested that 100–200 ppm is more common.[234] Concern about potential arsenic phytotoxicity to subsequent crops, accumulation in foodstuffs, and mobility has focused attention[31,234,386] on the processes affecting the distribution and speciation of arsenic in soils rather than simply total elemental arsenic concentrations. For example, it is now known that biological volatilization accounts in large part for the eventual loss of arsenic from treated soils. Adsorption also restricts the mobility and availability of the arsenicals that are present.

Arsenic concentrations in freshwater, estuarine, and pelagic sediments have been summarized.[1,2,234] These levels are much higher in sediments (ppm) than those of the overlying waters (ppb). Most current effort is directed at a better understanding of how arsenic accumulates in sediments and of the factors that control its postdeposition remobilization, migration, and biological availability. It is reasonable to expect that these processes will be speciation dependent. There are, however, certain analytical restrictions on species determination in both soils and sediments.

C. Analytical Considerations

The water contained in sediments or soils (section IX.E) can be removed and analyzed by the same techniques used for aqueous samples. The solid fraction presents an interesting challenge to those who are interested in total elemental content. Speciation information is even more difficult to acquire. Techniques such as neutron activation analysis (NAA) readily provide totals but are not useful in speciation studies.[8] More common procedures, such as atomic absorption spectroscopy, require that the sample be dissolved before analysis, but soils and sediments are resistant to complete dissolution. Fusion, or the application, at least in part, of hydrofluoric acid will destroy the silicate lattice and liberate all of the constituent elements.[392,393] Total, but not speciation, information can be aquired in this way. Other chemical treatments[392-394] provide different degrees of extraction but do not completely dissolve silicates; these can be selective in that they attack individual sediment fractions.[392] Depending on the nature of the extraction, some subsequent speciation may be possible.

The degree to which an element is bound can provide useful information. For example, sediments originate as biologically formed particulates, as precipitates from overlying waters, and as products of continental erosion (lithogenous precipitates).[316] The material that is dissolved by the less rigorous (no HF) chemical extractions is referred to as nonresidual (or detrital). Further treatment with hydrofluoric acid releases the remaining, or residual (nondetrital) elements—those that reflect the geological origins of the substrate.

Chemical extractions of various types can be used to categorize further the detrital material. Care must be taken in interpreting the results of these so-called selective chemical extractions. The reagents might not be as selective as they are thought to be, and it is possible that an element may be liberated from one phase only to be readsorbed on another before it is analytically determined.[395] Nevertheless, selective extraction provides a type of speciation that is important to the development of a biogeochemical cycle.

D. Selective Chemical Extractions

1. Association of Arsenic with Other Elements

Maher[396] investigated the distribution of arsenic in some marine sediments by application of two selective extraction procedures and by complete digestion using a mixture of nitric, perchloric, and hydrofluoric acids. The approach and the results are illustrative of the type of information that can be obtained. Total arsenic was determined by hydride generation/carbon furnace AA detection analysis of the solutions from the complete digest. The arsenic concentrations in pelagic (deep ocean) sediments collected off the coast of Africa ranged from 13.9 to 17.9 ppm, similar to the values (6–32 ppm) obtained by other groups for sediments collected in an analogous region[397] and to values (1.8–17.7 ppm) for other Atlantic samples.[398] Sediments from the Beaulieu River and Southampton estuaries in England had an As content of 16.4–28.7 ppm, intermediate between those (3–15 ppm) reported for other uncontaminated inshore sediments[387] and the much higher concentrations (up to 10 000 ppm) found for sediments that have been influenced by mining and smelting operations.[387,388] All total arsenic concentrations were reported on a carbonate-free basis. This gives the amount of arsenic/dry weight of sediment after removal of carbonate by treatment with dilute HCl. Although common in oceanography, it is not always obvious that this practice has been followed.

Maher's extraction schemes[396] separated the fractions into exchangeable (adsorbed ions), carbonates (shells, inorganic debris), amorphous Fe–Mn oxides, organics,

and residual (primary/secondary minerals). The proportion of As bound to the carbonate, organic, and Mn–Fe oxide phases accounted for 68–84% of the arsenic in pelagic sediments and 66–70% of the arsenic in estuarine sediments. Most of the arsenic (42–58%) was associated with the Fe oxide (sesquioxide) phases. Other extraction schemes, although employing slightly different reagents, indicate that arsenic is distributed among similar fractions in both freshwater and marine sediments.

Lum and Edgar,[399] using an extraction scheme developed by Tessier et al.,[393] found that arsenic in a sediment core from Moira Lake (Ontario), which drains an area of extensive gold, silver, and arsenic mining and smelting, was present at elevated levels (up to 807 ppm). The most important solid phases binding arsenic were Mn–Fe oxides, organic matter/sulfides, and, to a lesser extent, carbonates. Huang and Liaw[400] found that the bulk of sedimentary arsenic in several Canadian prairie lakes resided in the sesquioxide and carbonate (apatite) components. An evaluation of the geographical distribution of arsenic suggested that land erosion and agricultural runoff were the main contributors to the arsenic concentration in the lake sediments.

The consistent appearance of arsenic in the Mn–Fe oxide fraction of sediment extracts has led to suggestions that coprecipitation with these oxides may be involved in the control of dissolved arsenic concentrations in the overlying water column. For example, Seydel[401] noted that geological considerations would be expected to give higher arsenic concentrations in Lake Superior relative to Lake Michigan, but the opposite was observed. Lake Michigan was found to contain 3 times as much arsenic in the water as Lake Superior (average: 1.6 vs 0.5 ppb) and about 4 times as much arsenic in the sediments (15.2 vs 4.1 ppm). The difference was attributed to greater anthropogenic input to Lake Michigan, but this did not explain the arsenic distribution in both lakes, and indeed the basic question relating to aquatic systems—i.e., if the arsenic source (natural watershed, pollution) was outside the lakes, why did the water contain so little and the sediments so much arsenic? Seydel suggested that arsenic did not stay dissolved in the water under oxidizing conditions as long as there was iron present with which it could coprecipitate. Thus arsenic and iron coprecipitated in oxic surface waters, and these precipitates were deposited in the sediments. If bottom waters were anaerobic, iron was reduced, and both elements were redissolved. Ferguson and Gavis summarized similar data and presented an arsenic cycle for a stratified lake in which the association of arsenic and iron is clearly emphasized.[44]

An alternate explanation for the association of arsenic with Mn–Fe oxides is its preferential adsorption to this phase after the sediments have accumulated. As will be seen shortly (section IX.F.1), this process does occur, and it plays an integral role in the postdepositional remobilization of arsenic. However, it is equally clear that arsenic and iron interact in the overlying waters. For example, Aggett and Roberts[402] found that laboratory simulations and core sample analysis were consistent with the incorporation of arsenic (and phosphate) into the sediments of Lake Ohakuri (New Zealand) by coprecipitation at the time of formation of the hydrous oxides rather than by adsorption of existing surfaces.

Coprecipitation can be particularly important in estuaries. The flocculation of iron compounds at the saltwater interface to give amorphous precipitates (interchangeably referred to as hydrous oxides, oxyhydroxides, and hydroxides and presented variously as Fe_2O_3, $Fe(OH)_3$, and FeOOH) has been found to be an important process for the removal of a variety of dissolved elements,[395] including arsenic.[359,403] Under such circumstances, the dissolved arsenic concentrations will not be those calculated by simple mixing of the fresh- and saltwater components. Alternatively, conservative arsenic behavior may be indicative that coprecipitation has been precluded by low iron concentrations or as a consequence of competition from humic/fulvic-type compounds.[403] Arsenic–humic acid interactions have been demonstrated[404] and at certain pH values may be more important than adsorption to hydrous oxides. Waslenchuk and Windom attributed the conservative behavior of arsenic in the estuaries of several southeastern U.S. rivers to the association of arsenic with low molecular weight organic matter, the blocking of iron colloids by such matter, or both.[354,403]

The absence of significant quantities of arsenic in association with particulate matter in the open ocean[317,340,341] makes it more difficult to identify the sources of arsenic in pelagic sediments. Neal et al.[397] have shown that a majority of the arsenic in North Atlantic deep-sea sediments is associated with iron and little is of detrital origin. The coprecipitation of dissolved arsenic in the seawater with hydrothermal iron oxyhydroxides was suggested as a likely source. Other enrichments are also possible.[46]

2. *Speciation and Biological Availability*

It is rare for studies of arsenic removal from the water column and deposition to sediments to consider individual arsenic species. However, Knox et al.[357] proposed an estuarine cycle for inorganic arsenic based on the distribution of As(III) and As(V) in the water column and sediments of the Tamar estuary in southwest England. The area is characterized by high arsenic levels, especially of arsenite, from inputs of disused mine workings at the head of the estuary and by a tidally energetic system that periodically injects sedimentary As(III), As(V), and dissolved manganese into the overlying waters. In a 13-month investigation it was shown that dissolved As(III) was effectively removed at the freshwater/brackish water interface by heterogeneous oxidation (catalyzed by hydrous manganese dioxide) and adsorption onto iron oxyhydroxide. Budgets of both arsenite and arsenate agreed well with the proposed model and with the expected rates of oxidation of As(III) to As(V) by both chemical and microbiological processes.

The relationship between arsenic and iron in sediment extracts may be indicative of at least one of the factors influencing biological availability. For example, Langston[403] found that for a variety of England and Wales estuaries, arsenic concentrations in a bivalve, *Scrobicularia plana* (de Costa), were significantly correlated (correlation coefficient 0.82) to sediment concentrations normalized to iron content (i.e., were proportional to As/Fe) than to arsenic sediment concen-

trations alone (correlation coefficient 0.64). It was suggested that the presence of proportionately larger amounts of a strongly binding substrate such as iron oxides and hydroxide resulted in reduced availability to organisms. This relationship was not found for the polychete *Nereis diversicolor* and the seaweed *Fucus vesiculosus*, for which there were relatively poor correlations (correlation coefficient 0.44 and 0.52, respectively) even between tissue levels and arsenic sediment concentrations. Variation in the uptake of arsenic between biological species is not uncommon,[405] and it is to be expected that other factors may also determine arsenic availability.

One such factor may be arsenic speciation, but analysis of sediment extracts for individual arsenic compounds has rarely been reported. In part, this may be due to the loss of speciation information as a consequence of the chemical extraction itself. Maher[396] sequentially extracted the African coast and English estuarine sediments discussed above with 6 M hydrochloric acid and a 0.1 M sodium hydroxide–1 M sodium chloride solution. The arsenic species in this extract were separated by solvent extraction and ion-exchange chromatography and determined by atomic absorption spectroscopy.[406] Only 80% of the arsenic so extracted from pelagic sediments could be accounted for as inorganic As, but there was no evidence of methylated species. All of the arsenicals could be characterized in the estuarine sediment extracts as either inorganic (45–90%), monomethyl (4–39%), and dimethyl (4–22%) derivatives. The presence of the methylated arsenic compounds raises the question of whether these species are due to material entering the sediments in this form or to their production by chemical reactions within the sediment. Maher[396] could not distinguish between these possibilities since the estuarine sediments could have received anthropogenic (e.g., agricultural) inputs of methylated As compounds. Plankton or other organic remains that may have entered the sediments could have also been the source of these compounds. Hydrolysis of endocellular arsenicals under the extraction conditions would have given MMAA and DMAA. Maher indicated that if this was the case the methyl species should also have been found in the organic-rich pelagic sediments. However, only one of the three pelagic sediments had an organic carbon content (3.08%; others 0.42 and 0.12%) comparable to the three estuarine samples (1.83, 1.17, and 1.72%). There was no suggestion of in situ biomethylation, which is common in soils.[234,386]

E. Interstitial Water

1. General

The problem of chemical alteration of arsenic speciation during extraction can be eliminated if sediment pore water (interstitial water) is examined. This is the water that is trapped in the sediment pores or interstitial sites during accumulation of sediment particles. Hence, the terms pore water and interstitial water are used interchangeably. It can be separated from the sediment by several methods, but the most common of these employ devices that pressurize a diaphram in order to squeeze the sediment against a 0.45-μm filter. Different filter sizes can be used but 0.45 μm reflects an operational definition of particulate versus dissolved. Sometimes filtration is preceded by centrifugation. In intertidal or shallow coastal areas, dialysis bags have been buried in the sediment in order to obtain pore water. Regardless of the technique, it has been found to be essential to the integrity of the speciation that the sediment temperature be maintained at ambient levels throughout the isolation procedures. Interestingly, such precautions are rarely observed in chemical extraction experiments. It might be expected that the exchangeable fraction, obtained in extraction studies by agitation of the sample with a neutral salt solution,[393,396] would isolate the interstitial water speciation equally well. However, leaching and rapid ion-exchange phenomena are known to cause large compositional changes. Presley and Trefry[407] have reviewed these concepts as well as the general geochemistry of pore water.

In view of the association of arsenic predominantly with the Mn–Fe oxide phase, it might be expected that the interstitial water arsenic would represent only a minor part of sedimentary arsenic. This may be true in terms of absolute concentrations, but it is because of this concentration gradient that the pore water is a sensitive indicator of the chemical interactions between the water and the surrounding solid phases of the sediment. Furthermore, interstitial water provides the main pathway for the diffusion of dissolved species and fluxes across the sediment–water interface. Thus it is the fraction most related to postdepositional remobilization and, possibly, biological availability. Surprisingly, there are few reports of the porewater behavior of arsenic species. This is a subject of increasing importance.

2. Inorganic Arsenicals

Andreae[317] investigated the arsenic concentration profiles with depth in the pore waters of three sediment cores collected in the deep northeast Pacific and the continental borderland off southern California. The sedimentary environments spanned a wide range of redox potential and biological activity. The northeast Pacific sediments were brown clays, low in organic matter, whereas green mud, oxidized to 60 cm and anoxic below, was obtained from the Santa Catalina Basin. The Santa Barbara Basin provided sediments with high organic content and biological activity, intense sulfate reduction, and low redox potential. Despite these differences the arsenic distributions were remarkably similar. Arsenate dominated arsenite even in the reducing sediments, where a maximum of 20% of the arsenic content was As(III). The profiles were generally featureless, showing slight increases in concentrations with depths up to 85 cm. Andreae suggested that arsenic was released to the pore water during organic matter degradation or iron and manganese dissolution. Adsorption or coprecipitation must have also been important as the total interstitial arsenic concentrations were lower than in the overlying waters. The balance between addition to and removal from the pore water differed for arsenate and arsenite. The As(V) concentrations in the uppermost portion of the sediment were lower than those of the overlying waters (e.g., 1.13 vs 1.69 ppb for the Santa Barbara Basin), suggesting that there was a diffusion of arsenate from the water column to the sediments. Arsenite displayed an opposite gradient (0.25 to 0.15 ppb upward across

the sediment–water interface), indicating a possible net flux of arsenite from the sediments. Andreae found no methylarsenicals (he attempted detection of MMAA, DMAA, TMAO, and the corresponding methylarsines) in the oxic and anoxic sediments. This observation has often been taken as evidence for the absence of in situ biomethylation in aquatic sediments.

Quite different results were obtained by Peterson and Carpenter,[408] who examined dissolved As(III), As(V), Mn^{2+}, Fe^{2+}, HPO_4^{2-}, NH_4^+, and H_2S in 12 sediment cores from Puget Sound, Lake Washington, the Washington coast (U.S.A.), and Saanich Inlet (British Columbia, Canada). The pore water arsenic concentration profiles exhibited subsurface maxima with magnitudes of 26–120 ppb, 10–60 times greater than in overlying bottom waters. Below these maxima, the concentrations in marine interstitial waters decreased rapidly, indicative of solid-phase incorporation, whereas the lacustrine pore waters displayed an increase in arsenic concentrations with depth. The surficial arsenic maxima was attributed to the dissolution of solid-phase arsenic in response to the redox gradient between well-mixed surface and the deeper, stagnant, sediments. The magnitude of the dissolved arsenic maxima was correlated (correlation coefficient 0.85) with the solid-phase arsenic concentrations for the marine, but not the lacustrine, sediments. The greater coincidence of arsenic with Fe^{2+} porewater maxima rather than Mn^{2+}, indicated that arsenic release to pore water was closely associated with the dissolution of iron oxyhydroxide phases. Peterson and Carpenter dismissed any relationship to organic matter degradation but they did note that factors such as rate of diffusion, adsorption, and precipitation must be involved in restricting the arsenic porewater maxima to narrow depth ranges. Redox controls on arsenate and arsenite were not clear. The As(III)/As(V) ratio was variable: <1.0 for Washington coastal environments, and between 1.0 and 4.0 for Puget Sound and Lake Washington. Arsenate was not detectable in Saanich Inlet when bottom waters were anoxic, but As(III)/As(V) equaled 2.8 when slightly oxic conditions prevailed. The difficulties in interpreting this ratio were ascribed to a delicate positioning of the redox equilibrium. A pE range of only −2.9 to −3.5 was calculated for the observed range of As(III)/As(V) values (4.0–0.2). Unfortunately, the equations used to calculate the pE values did not take into account the dominant forms[306] of As(III) (H_3AsO_3) and As(V) ($HAsO_4^{2-}$) at the likely interstitial water pH (7.5–8.0).[407]

Edenborn et al.[409] reported a relationship between the interstitial water distributions of arsenic and iron and the redox potential for some Laurentian Trough sediments. The surficial (3–4 cm) oxidized zone of a sediment core was characterized by positive to slightly negative E_H and low porewater concentrations of Mn^{2+}, Fe^{2+}, and arsenic. Below 4 cm bacterial sulfate reduction began. Coincident with the associated drop in E_H, there were large increases in the amounts of dissolved manganese, iron, and arsenic. Only total arsenic and arsenite were determined, but at a 12–15-cm depth maximum, As(III) accounted for 65–70% of the dissolved arsenic pool (~40 ppb). Laboratory experiments indicated that manganese dissolved at higher E_H than iron and arsenic. The release of the last two was simultaneous and occurred at a sediment redox potential characteristic of the anoxic degradation of organic matter. Chemical alteration of the redox potential in pH-buffered samples of the oxidized surficial sediment samples confirmed the concurrent release of iron and arsenic in response to dropping E_H, but sulfate reduction and the production of sulfide ions were not solely responsible for the release of arsenic to pore waters. However, it was suggested that sulfate reduction was necessary in order to create and maintain a highly reducing environment conducive to rapid iron dissolution. Thermodynamically, iron(III) should be reduced at higher E_H than sulfate. The simultaneous onset of these processes was attributed to slow reduction kinetics of the Fe(III) mineral phases, resulting in iron dissolution at redox values lower than expected.

Redox potentials in sediments are controlled by the bacterial degradation of organic matter in which available oxidants (O_2, NO_3^-, Mn(IV), Fe(III), SO_4^{2-}, etc.) are consumed in a stepwise manner according to the decreasing free energy availability.[407,410] In sediments with a high rate of sedimentation and high organic content, the organic matter will be rapidly consumed, making it difficult to distinguish the depths at which reduction of the specific oxidants begins. For sediments of low organic content, Reimer and Cornish[411] found that the reduction of insoluble iron(III) phases to the more soluble iron(II) species was accompanied by arsenic dissolution at a depth well above the sulfate reduction zone. The E_H values calculated[306] from the observed As(III)/As(V) ratios were consistent with the expected, bacterially controlled, redox zones in these, and several different, sediment samples. In contrast, Edenborn et al.[409] estimated that only 0.1% of the As(V) should have been reduced to As(III) at the E_H values observed in their experiments. Much higher arsenite concentrations were found; this was attributed to bacterial reduction of free or bound arsenate. These calculations appear to be in error. At an E_H of −170 mV, the appropriate equation[306] yields an As(III)/As(V) value of ~60 000. It is therefore more pertinent to question why *any* As(V) is present. A similar question has been raised[350,380] regarding the relative concentrations of arsenite and arsenate in anoxic water column samples. Explanations based on the involvement of arsenic sulfides, and secondary reactions with carbonate, discussed earlier (sections VI and VIII.D.1) are increasingly attractive. This is especially true in view of the excellent agreement between observed and expected redox values in sediments with little free sulfide.[411]

3. Methylarsenicals

The involvement of sediment bacteria in arsenic speciation has been demonstrated by Reimer and Thompson,[412] who presented the first evidence for in situ biomethylation of arsenic in marine interstitial water. Subsurface arsenic porewater maxima were found for ten sediment cores collected at two British Columbia coastal sites (Rupert Inlet and Alice Arm). The range of dissolved arsenic concentrations was 3–24 ppb in natural sediments. Mine tailings from two different mining operations gave both the lowest (<3 ppb) and the highest (>50 ppb) arsenic levels. In contrast to the correlation reported by Peterson and

Carpenter,[408] these values displayed only a general relationship with solid-phase arsenic concentrations and were found to be more dependent on the redox nature of the substrate. Although arsenate and arsenite constituted >90% of the dissolved species, every porewater sample contained mono-, di-, and trimethylarsenicals (hydride generation GC/AAS). There was a strong correlation (correlation coefficient >0.9) between the total dissolved arsenic concentrations and ΣMeAs—the sum of the MMAA, DMAA, and TMAO concentrations—indicating that methylation was dependent upon the amount of dissolved arsenic in the interstitial waters. One equation was found to be characteristic of anoxic sediments, while another defined the behavior of ΣMeAs and total dissolved arsenic for the remaining samples. Thus both the dissolution and speciation of arsenic were related more to the nature of the sedimentary environment than simply to solid-phase concentrations.

Methylarsenic compounds have also been recently found in the interstitial water of estuarine sediments, but it was equivocal if they were present as a consequence of in situ microbial methylation or from an alternate source. Ebdon et al.[413] collected pore water at five Tamar estuary sites over a 4-month period. Porewater samples were obtained by burying cellulose acetate dialysis bags containing a known volume of deoxygenated, purified water into the surficial sediments at low tide and allowing them to equilibrate for 7 days. Subsequent analysis for total inorganic arsenic, $(CH_3)As(O)(OH)_2$, and $(CH_3)_2As(O)(OH)$ was accomplished by hydride generation GC/AAS. The total inorganic arsenic concentrations of 5–60 ppb compared favorably to those of Knox et al.[357] Of particular interest was the presence of MMAA and DMAA in all samples.[413] Although the concentrations were low, 0.04–0.70 ppb, accounting for 1–4% of the total dissolved arsenic concentration, this was the first report of methylated arsenic compounds in estuarine pore waters. There was no relationship between the total or relative amounts of either MMAA or DMAA with time of sampling (March–June), seasonal changes in river flow (9.5–24.9 $m^3 s^{-1}$), pH (7.0–8.2), temperature (8–15 °C), sediment carbon content (3.2–4.5%), or total arsenic (solid phase) concentration (35–78 ppm). However, the proportion of methylated arsenic did increase from the upper estuary to the seaward sites (2–4%), as did the MMAA/DMAA ratio. The source of the methylarsenicals was attributed to either in situ microbial methylation or degradation of organic debris. Ebdon et al.[413] concluded that phytoplankton and macroalgae would be concentrated seaward and would contain primarily DMAA, opposite to the observed MMAA/DMAA distribution. This conclusion may be debatable in view of the varying proportions of mono- and dimethylarsenic compounds that are released by different algal species (sections IV and VIII.E). Greater support for the proposal of in situ biomethylation comes from the significant amounts of methylarsenicals that were present at the beginning of the study, even when biological activity in the overlying waters was at a low level.

Other examples of methylarsenicals in sediments are quite limited. Crecelius[389] found DMAA (1 ppb), but no MMAA in Lake Washington interstitial water but did not indicate if this sample was obtained from natural sediments or those influenced by input from the ASARCO smelter. Wong et al.[233] demonstrated that biomethylation can occur in lacustrine sediments. Sediments from a polluted watershed (Moira River, Ontario) that contained high arsenic concentrations (200–550 ppm) as well as sediments amended with arsenate and arsenite were incubated at 20 °C until a garlic odor signaled the production of methylarsines. MMAA, DMAA, and sometimes TMAO were found in the culture media. Some samples also gave mono-, di-, and trimethylarsines, as did pure bacterial cultures isolated from Lake Ontario. Andreae[36] questioned the generality of these results in view of the elevated arsenic levels at which the experiments were conducted and suggested that bacterial methylation in these cases was a response to extreme arsenic stress. He cited the absence of methylarsenic species in the interstitial water of three northeast Pacific and Californian coast sediment cores and in a marine mud culture experiment conducted by McBride et al.[80] On the basis of these observations, Andreae[36] concluded that excretion by algae and decomposition of deposited algal material were the only important processes leading to the presence of organoarsenicals in natural waters.

The idea that methylarsenicals will only be found in interstitial water when the total arsenic concentrations are elevated has some support from a soil pore water study. MMAA, but not DMAA, was found in soil pore waters of the Tamar valley (the watershed area feeding the same area studied by Ebdon et al.[413]) and other southwest England sites.[414] Arsenate (~90%) and arsenite (59–77%) were the dominant species present in aerobic soils and anaerobic soils, respectively. This is in accordance with previous soil studies.[234] Much lower levels of dissolved arsenic, 16–56 ppb, were found in unmineralized aerobic soils, as compared to 80–215 ppb in mineralized areas. With one exception, no MMAA was found in unmineralized areas. From the relative amounts of As(III) and MMAA in the remaining samples, the authors suggested that there was a threshold concentration of arsenite at which monomethylarsonic acid was microbially produced. However, there was no clear relationship between the absolute concentrations of the various arsenic species.

Andreae's conclusion[36] regarding biomethylation in the sedimentary environment must also be counterbalanced by the fact that, although the number of studies is small, in every other case where sediment interstitial water has been analyzed for methylarsenic compounds, they have been found.[389,412,413] Also, the sediments sampled by Reimer and Thompson[412] did not contain elevated solid-phase arsenic levels (9.9–34.1 ppm; average 22.4 ppm); yet methyl species were present in all cases. Decomposed organic matter may be a source of organoarsenicals, but bacterial methylation of inorganic arsenic is equally possible. It would be interesting to know if pore water methylarsenic concentrations are controlled by a balance between methylation and demethylation—similar to what has been found for methylmercury compounds.[329] Clearly additional work is required.

F. Cycling of Arsenic Species

1. *Inorganic Arsenic*

Aggett and O'Brien[415] and Edenborn et al.[409] recently

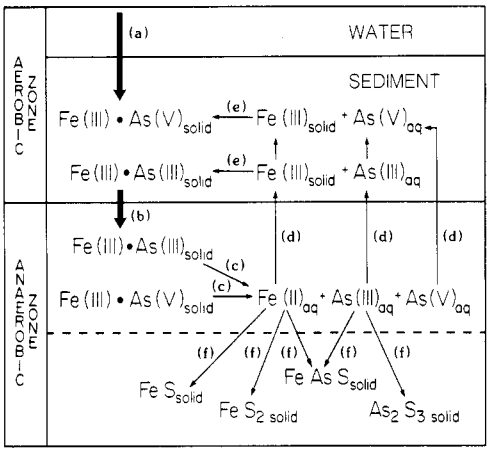


Figure 9. Proposed model for the biogeochemical cycling of arsenic in coastal marine sediments. The dashed line indicates sediment below which free sulfide favors formation of arsenic and iron sulfides. Solid iron species above the line are presumed to contain oxide and hydroxide groups. (a) Sediment deposition; (b) burial; (c) dissolution; (d) diffusion; (e) coprecipitation; (f) diffusion and reaction with sulfide. (Adapted from ref 409 and 415.)

presented essentially identical models to explain the distribution of inorganic arsenic within lacustrine and coastal marine sediments, respectively. The concepts have been extended by Maher[46] to include the water column. These models illustrate the factors that are known to influence the mobilization and speciation of arsenic within sediments (and soils), as well as the flux of arsenic at the sediment–water interface. These factors have important implications with respect to the biological availability and impact of arsenic. The following discussion provides an overview of the adsorption, homogeneous and heterogeneous redox, and biotransformation reactions that are pertinent to an assessment of the proposed models.

The model for the biogeochemical cycling of arsenic in coastal marine sediments is illustrated in Figure 9, adapted from Edenborn et al.[409] In both models[409,415] it is presumed that arsenic is deposited on sediments mainly as iron(III) oxyhydroxide–arsenic complexes and that subsequent reactions are closely tied to the behavior of the iron phase. Selective extraction experiments have demonstrated that arsenic is indeed mainly associated with the manganese–iron oxide extracts, but they rarely distinguish between manganese and iron. However, studies[408,409,411] of the pore water distributions of dissolved arsenic, Mn^{2+}, and Fe^{2+} have shown that arsenic maxima are more closely related to Fe^{2+} than Mn^{2+}. Thus even if significant quantities of arsenic were adsorbed onto, or incorporated into, manganese oxides during early sediment diagenesis,[407,410] Mn(IV) would be reduced and the manganese phase dissolved (as Mn^{2+}) before the iron(III). Release of arsenic during this process would likely be followed by rapid readsorption by iron oxyhydroxides. Thus the iron phase would still control the ultimate release of arsenic to pore waters.[408,409] This is further supported by experiments with birnessite (δ-MnO_2) that indicate that manganese oxide sorbs little As(V).[327]

Similar associations occur in soils as reviewed by Woolson.[234] All of the arsenicals, As(V), As(III), MMAA, and DMAA, form very insoluble complexes in soil systems by interaction with hydrous oxides coating clay particles.[31,234,386] Adsorption is related to the concentration, the pH, and the clay type and texture, but generally, the higher the iron content, the greater the adsorption. Once adsorbed, arsenicals are not easily removed. Leachability is limited and movement depends on the rate of application and volume of water passing through the soil. In sediments, compaction forces an upward movement of pore water, but for the most part, redistribution of dissolved arsenic species is controlled by diffusion.[407]

According to the proposed sediment models,[409,415] the dissolution of the iron phase and its associated arsenic occurs as a consequence of the continued burial of this phase and its eventual response to the gradient from aerobic to anaerobic conditions (Figure 9). The depth of this transition is controlled by the sedimentary redox zones. These are defined by bacterial degradation of organic matter and depend on the rate of sedimentation, the organic carbon content, and the diffusion of oxygen from overlying waters.[407,410] The simultaneous release of dissolved arsenic and iron into the pore waters is followed by diffusion both upward and downward along concentration gradients. It is assumed that at depth, arsenic reacts with sulfide ions produced in the sulfate reduction zone to give distinct arsenic sulfides, arsenopyrite, or both. Pyrite is undoubtedly involved in the sedimentary cycling of arsenic.[416] However, suggestions[46,417] that arsenic sulfide compounds are not formed require further consideration. It is possible that there is a balance between insoluble (e.g., As_2S_3) and soluble (e.g., AsS_3^{3-}) species (sections VI.B,C). Upward diffusion results in escape into the overlying water column or coprecipitation in the aerobic zone with the reoxidized iron(III) phase.

The models are consistent with the subsurface maxima that have been observed for the arsenic concentration profiles in interstitial water. Less obvious is an explanation for the distribution of As(III) and As(V). Aggett and O'Brien[415] found that in cases where the aerobic–anaerobic transition began near or above the sediment–water interface, arsenite was the major pore water constituent, accounting for up to 90% of the total dissolved arsenic. Surficial sediment samples that had been in contact with well-oxygenated lake water contained 17–27% As(III). In principle, the arsenite/arsenate profile with depth should be governed by the redox gradient. In fact, Cherry et al.[306] suggested that measured concentrations of As(III) and As(V) would be a useful indicator of redox conditions in groundwater and would avoid the difficulties of existing methods such as potential-sensing inert electrodes or analytical determination of other redox-indicator species. The E_H would be simply calculated from the appropriate equations using the observed arsenate and arsenite concentrations. In sediments with low organic carbon and free sulfide ion content, this simple relationship to E_H has been observed[411] (section IX.E). However, a number of other factors might also contribute to the relative distribution of arsenite and arsenate in porewater.

Oscarson and co-workers have reported[327,418] that the oxidation of As(III) to As(V) in lake sediments is essentially an abiotic process, with microorganisms playing a relatively minor role. Experiments with lacustrine sediments and with synthetic Mn(IV) oxide

indicated that manganese was the primary electron acceptor in the conversion of arsenite to arsenate. Although thermodynamically favorable, no reaction was observed between As(III) and Fe(III), presumably because the kinetics of this redox process are comparatively slow.

Arsenate and arsenite also differ in their adsorption characteristics and this will certainly influence porewater mobilizations and subsequent distributions. Pierce and Moore[419] determined isotherms for the adsorption of As(V) and As(III) on amorphous iron hydroxide over the concentration range 10^{-7}–10^{-3} M (7.5 ppb to 75 ppm) and the pH range 4–10. Significant adsorption of both species (~35% at pH 4 at 50 ppm added or 50 mmol As/g of absorbent) was observed with rates increasing with concentration and with arsenate greater than arsenite. Although adsorption was rapid, the time scale was on the order of hours rather than seconds, which suggested formation of a specific interaction between the arsenic species and the adsorbent rather than a simple electrostatic process. Nevertheless, it was pointed out[419] that even in fast-flowing rivers, a significant amount of arsenic would be adsorbed as soon as the arsenic species came into contact with the oxide. The adsorption processes followed Langmuir equations, indicating monolayer coverage of the adsorbent up to maximums of 0.5 mmol g^{-1} As(III) adsorbed at pH 7 and 1.5 mmol g^{-1} (As(V) adsorbed at pH 4 (13.3 μM; 1000 ppb initial As concentration). The greater adsorption of arsenate than arsenite and the pH dependence (pH 4 optimum for As(V); pH 7 for As(III)) was attributed to the differing pK_a's and resulting charges of the adsorbing species. The wider pH range over which As(V) is charged and adsorbed, whereas As(III) is not, is consistent with the results of an investigation of the removal of arsenic from geothermal fluids with colloidal ferric hydroxide.[420] The adsorption of arsenicals by sediments/soils and suspended particulates and by soils have been reviewed by Hart[395] and Woolson,[234] respectively.

The effects of adsorption on the mobility of arsenic in groundwater were considered by Gulens et al.[421] They considered the fate of arsenate and arsenite in a confined aquifer with a sequence of redox zones similar to those found in sedimentary environments. It was found that the extent of adsorption varied with the oxidation state of arsenic, the redox environment, and/or the pH of the eluting water. In laboratory elutions through sand columns, it was found that in an oxidizing environment, As(III) was eluted faster (5–6 times) and in greater quantities (~8 times) than As(V). Both species were eluted similarly under reducing conditions. The weak retention by the sand of As(III) was considered indicative of a weaker interaction between As(III) and Fe(III) as compared to that between As(V) and Fe(III). The increase in mobility of As(V) under more reducing conditions was attributed to an increase in pH in the water used, to reduction of the higher oxidation state species (Fe(III) to Fe(II); As(V) to As(III)), or to both.

2. Methylarsenicals

The models proposed for the biogeochemical cycling of sedimentary arsenic do not include methylarsenic compounds. Early studies suggested that these species are absent from sediments, but more recently, mono-, di-, and occasionally trimethylated arsenicals have been found in marine and estuarine pore waters (section IX.E). It is not yet clear if in situ biomethylation is a general phenomenon. However, it might be expected that the major controls on sedimentary methylarsenicals will be similar to those that dictate the behavior of arsenic compounds in soils.

The production and fate of organoarsenicals in soils have been studied more extensively because of the agricultural application of these compounds. The salts of methylarsonic and dimethylarsinic acids are employed as herbicides and pesticides, and inorganic compounds, principally metal arsenates, were also widely used. Woolson[31,234,386] has summarized much of the relevant literature and several points have emerged. For example, when organoarsenicals are applied to soils, they adsorb to, and leach from, clay surfaces (hydrous oxides) in much the same manner as arsenate, the extent of fixation decreasing with increasing numbers of methyl groups bonded to As. Demethylation, methylation, and reduction reactions are all important in controlling the distribution of arsenic compounds. The promotion of these types of transformations by individual organisms is described in section III, but it is important to recognize that in the mixed microbial community of soils these processes can occur concurrently. Typically they are slow and depend on the conditions and the soil type. For example, Woolson and Kearney[422] reported that for aerobic soils, 41% of the added DMAA (100 ppm) was converted to arsenate in 24 weeks. A volatile organoarsenical was also formed and was the sole product under anaerobic conditions.[234,422] In other work, up to 10% of ^{14}C-labeled MSMA, $CH_3AsO(OH)(O^-)Na^+$, was degraded in 60 days to $^{14}CO_2$ and arsenate.[234,423] Cheng and Focht[87] found both DMA and AsH_3 in the head space of flasks containing flooded enriched soils treated with MSMA; dimethylarsinic acid was reduced to DMA and AsH_3. No trimethylarsine was detected in contrast to an earlier report[424] that sodium [^{74}As]arsenate, [^{14}C]-MMAA, and [^{14}C]DMAA spiked soils each gave DMA and TMA under both aerobic and anaerobic conditions. In this study, the evolved gases were trapped in KI/I_2 solutions prior to identification, and no AsH_3 or MMA was found. The principal arsenical remaining in the soil appeared to be trimethylarsine oxide. Between 1% and 18% of the activity was transferred to the trapping solutions in 160 days, with slightly greater volatilization observed under aerobic conditions. Even though the amount of volatile arsenicals produced in such reactions is small, the process is presumably continuous and is believed to be an important part of the global arsenic cycle (section VII.A).

The similar, balancing, processes of methylation and demethylation have been found in sediment studies. Wong et al.[233] reported the release of methylarsines from some sediments incubated with arsenate or arsenite, but the main products were MMAA, DMAA, and, in one instance, TMAO (section IX.E). These compounds have also been detected in pore waters. Demethylation was found to dominate in Menominee River sediments, where improper disposal of industrial wastes led to severe arsenic pollution. Concentrations were as high as 8.0×10^{-2} M in groundwater, 1.6×10^{-5}

M in river water, 8.0×10^{-2} M in sediment pore water, and 4000 ppm in solid sediments. Between 10% and 40% of the pore water arsenic was present as MMAA and DMAA, the balance being inorganic arsenic. Incubated sediment samples spiked with methylarsenicals showed decreases of these species concurrent with increases in inorganic arsenic concentrations. These changes were microbially related as no demethylation of MMAA and DMAA was observed for sterilized controls. It was further demonstrated that the rate of demethylation and the transport of arsenic were affected by adsorption (As(V) > MMAA > DMAA). The interstitial water distribution of arsenic species was successfully modeled[381,425] by using first-order kinetics and Langmuir adsorption of the reactants and products.

Abiotic methylation of metals and metalloids by reaction with naturally occurring methylating agents has been reported.[426,427] Methyl iodide formed by the reaction of I^- with dimethyl-β-propiothetin (a dimethylsulfonium compound prevalent in marine algae) is widely distributed in the ocean.[427,428] Thayer et al.[426,427] reported increased dissolution of arsenic from the reaction of CH_3I with enargite (Cu_3AsS_4) and arsenopyrite (FeAsS), but no methylarsenic compounds were formed. This is not surprising as abiotic methylation of arsenic is unknown (section VI.C). The increased solubility of these arsenic minerals appears to be due to the formation of methyl derivatives of sulfur. Dimethyl sulfide is a common product of the decomposition of algal material[428,429] and has been associated[430] with the characteristic "smell of the sea". The role of sulfur compounds (H_2S, RSH) in altering apparent organoarsenic concentrations has already been discussed (section VI.C).

3. Fluxes at the Sediment–Water Interface

The sediment models for the cycling of arsenic (Figure 9) predict that it will be internally cycled in sediments with an oxic surficial layer[409,415] and in general act as a trap for dissolved arsenic in the water column. In a laboratory study the toxicity of arsenite to *Daphnia magna* was found to be reduced when sediment was added, presumably due to arsenite adsorption.[431] However, Edenborn et al.[409] have noted that diffusion can occur from sediments, via the pore water, into the overlying water column. High sedimentation rates of easily metabolized organic matter will rapidly consume oxygen, resulting in an oxic layer too thin to trap all of the upward-diffusing arsenic. Polluted sediments, which enhance the concentration gradient between sediments and the water column, will also promote such an upward flux of arsenic. In these cases the sediments can act as a source of arsenic to the water column, to benthic organisms, or to both. There is considerable support for this proposal.

Aggett and O'Brien[415] found that seasonal variations in the arsenic concentrations in Lake Ohakuri (New Zealand) were related to changes in the surficial layers of the sediment. During periods of intense stratification, the anoxic region of the sediments rose to the vicinity of the sediment–water interface, resulting in diffusion of arsenic, principally as As(III), to the overlying water. This caused a temporary increase of 10–20% in the arsenic concentration in the hypolimnion. The extent to which both As(V) and As(III) entered the water column was dependent on the length of the stratification period. Much of the "extra" arsenic was reburied upon the intrusion of oxygenated waters. The concurrent formation of an oxic surficial sediment layer once again contained the interstitial arsenic by adsorption on hydroxyiron(III) species.

In related work, Brannon and Patrick[432] have investigated the processes influencing the fixation, transformation, and mobilization of arsenic in dredged sediments—those that are removed, transported, and discharged to land and water. Indigenous arsenic and As(V) added to sediment samples were found to be largely associated with the Mn/Fe/Al oxide phase. Anaerobic incubation of amended sediments resulted in considerable interstitial water enrichments of As(V), As(III), and unidentified organic arsenic compounds. The latter were determined by the difference between the total arsenic content and that calculated after passing the effluent of the hydride generation apparatus through a concentrated sulfuric acid trap to remove alkylarsines. Enrichment factors (amended concentration/unamended concentration) were as high as 72 for As(V), 3350 for As(III), and 1542 for organic As. Similar trends were observed in the exchangeable phase. Arsenite was also the predominant dissolved arsenic species in unamended sediments. It was concluded that when anaerobic sediment is in contact with anaerobic water, releases of both As(III) and organic As should occur. Short-term leaching experiments indicated that when anaerobic sediments are physically mixed such as by dredging operations or by turbulence from passing ships, As(III) would be released in proportion to the amount of arsenic in the interstitial water and exchangeable phases. This is consistent with the assumption that arsenic in these phases is the most mobile. Long-term leaching experiments indicated that releases of arsenic will persist for 6 months under agitated, aerobic conditions. Particularly interesting were the substantial releases of organic arsenic throughout the leaching period—indicative of continued biomethylation. The extent of the long-term arsenic releases were related to sediment total iron, extractable iron, or $CaCO_3$ concentrations, but *not* to the total sediment arsenic concentrations nor to the arsenic concentration in any of the chemically extracted phases.

Very similar results were obtained by Clement and Faust[433] in laboratory experiments with arsenic-contaminated sediments obtained from Tulpehocken Creek (Pennsylvania). Adsorption/desorption equilibria greatly influenced the amount of dissolved arsenic in simulated reservoirs, with anaerobic conditions giving about 10 times more arsenic (principally as arsenite) than aerobic systems. Faust et al.[434] confirmed these observations in a field study of the bottom sediments and waters of the Maurice River, its tributaries, and Union Lake (New Jersey). The major transport and distribution of arsenic occurred from arsenic-contaminated sediments. Seasonal variations showed that arsenite dominated in the warm months when anaerobic conditions prevailed in the hypolimnetic waters. As(V) was found in the highest concentrations in both sandy and organic aerobic sediments. There was also evidence of in situ methylation, but inorganic species accounted for up to 88% of the total arsenic in the sediments.

4. Biological Availability

The above results are consistent with the proposed biogeochemical models. Adsorption to sediments appears to play a major role in the control of aquatic arsenic concentrations, but remobilization of sedimentary arsenic can occur. Arsenic is principally associated with the bound phase but the interstitial water offers an avenue for the input of arsenic into the overlying water column. The extent of this diffusive input and the speciation of the arsenic involved will depend on the degree of bottom water oxygenation, the sedimentation rate, and the organic content of the sedimenting material. It seems reasonable that the uptake of arsenic by benthic organisms will be related to the levels of arsenic in the surficial pore waters and perhaps show preferential assimilation of individual arsenic species. However, only total arsenic concentrations have been used to evaluate the impact of sedimentary arsenic, and the results are confusing. It has been discussed elsewhere in this article (sections IV.C and IX.D.2) that there is only a rare correspondence between the total arsenic content of sediments and those of tissues. The factors controlling the incorporation of arsenic into shells are also unclear.

Braman and Foreback[16] were the first to note that methylarsenicals are present in sea shells and even in sedimentary rock. Others have reported a high incorporation of [^{74}As]arsenate in the shell of the snail *Nucella lapillus*[208] (section IV.C). Recent studies on the shells of clams and whelks reveal a surprising variation in arsenic content.[167] For example, for the soft-shell clam (*Myna arenaria*) the range to date is 0.4–26.3 ppm (dry weight), with the maximum value found in clams from a pristine wilderness area with total sediment arsenic concentrations of ~16 ppm. An intermediate value of 4.4 ppm was found for the same clam living on the edge of a mine slag heap (50 ppm As in sediments). These values also do not correlate with the arsenic content of the soft tissue of the living animal. The major arsenical present in the shell is arsenate, although there are some methylarsenicals.[435]

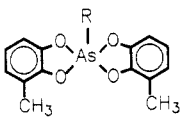
Arsenic is also found elsewhere in bivalves. Mussels such as *Mytilus galloprovincialis* incorporate arsenic in the byssus,[167] the threads that attach the mollusk to rocks and other stationary objects. Perhaps this incorporation serves as a mechanism of detoxication, related to the well-known human capability to incorporate arsenic from all types of exposure into growing hair.[436,437] Speciation studies would be valuable.

G. Arsenic Compounds in and from Fossil Fuels and Oil Shales

1. Concentration and Speciation

Arsenic is found in fossil fuels such as coal; the mean arsenic content of U.S. bituminous coals is 150 ppm,[438] and the average arsenic content of 799 coals of varying rank is 15 ppm.[439] An average value of 0.015 ppm is given for oil,[440] although higher average numbers of up to 0.263 ppm are quoted.[439] Speciation studies have been made only on oil shales and shale oils.[441-445] However, there appears to be renewed interest in the processing of oil shale,[446] so the work described below is environmentally important.

Extraction of an oil shale, specifically the NBS standard reference material from the Green River formation, with methanol removes ~20% of the total initial arsenic content of 20 ppm (most of the arsenic in the shale is associated with pyrite[439]). The presence of methylarsonic acid and phenylarsonic acid ($C_6H_5AsO(OH)_2$), arsenate, and an unknown neutral organoarsenical in the extract was established by using HPLC/GFAA with an anion-exchange column. The organoarsenicals were derivatized with 3-methylcatechol to afford **22**, which are volatile enough for GC/mass



R=CH_3, C_6H_5

22

spectrometric identification.[444,447] A related derivative of ethylene glycol has been used for the determination of methylarsonate by gas chromatography.[448] Similarly, arsenate was verified as the volatile tris(trimethylsilyl) derivative.[442] (There may be some problems with applying this procedure[449] to other arsenicals.[448]) Another report describes the presence of arsenate, $CH_3AsO(OH)_2$, $(CH_3)_2AsO(OH)$, but no $PhAsO(OH)_2$ from a similar sample.[445] It has been suggested[444] that this discrepancy may be due to differences in geological site and sample maturity.

Shale oil is recovered by controlled pyrolysis of the oil shale; surface (retort) and in situ techniques are employed. The oil from these two processes is accompanied by so-called "retort water" and "process water", respectively. This waste water contains arsenic in the concentration range 5–15 ppm.[450] HPLC/GFAA studies indicate that the same arsenicals found in the oil shale are found in the water. Typical results are as follows:[443] retort water, $CH_3AsO(OH)_2$ (0.10 ppm), $PhAsO(OH)_2$ (0.02 ppm), arsenate (0.32 ppm), together with arsenite and unidentified arsenicals; process water, $CH_3AsO(OH)_2$ (0.36 ppm), $PhAsO(OH)_2$ (0.10 ppm), arsenate (0.44 ppm), and unidentified arsenicals.

A sample of shale oil (from the Paraho surface retort; 23 ppm total arsenic) was extracted with aqueous bicarbonate or benzene. The extracts contained inorganic arsenicals and $CH_3AsO(OH)_2$. HPLC studies of organic extracts on size-exclusion columns reveal that the arsenic compounds coelute with the iron and have high molecular weights.[440] It is believed that oil shale, actually a kerogen, was formed from the accumulation of algae, plant, and animal material deposited at the bottom of freshwater lakes over 50 million years ago. Thus it is not unexpected to find some methylarsenicals in this fossil fuel precursor. It is interesting that methylarsonate rather than the more common dimethylarsinate is found in extracts of shale oil, in waste water, and in extracts of a sample of oil shale.[444] This may be because the dimethyl species is harder to extract from these complex matrices.

The origin of the phenylarsonic acid is more intriguing. Biological phenylation of arsenic, or any other element, is unknown, and it is possible that this arsonic acid is produced by a chemical reaction between inorganic arsenic and a benzene derivative during the formation of the oil shale. A reaction of the type outlined in eq 11 would be a possible candidate. This is the

$$ArBr + As(OH)_3 \xrightarrow{OH} ArAsO(OH)_2 + HBr \quad (11)$$

Rosenmund reaction, analogous to the Meyer reaction of eq 1,[108] but it is not a route of great practical utility.

X-ray absorption spectroscopy using synchrotron radiation appears to be useful for the in situ analysis of the arsenic species in oil shales.[451] The As absorption edge was used to distinguish between As(V) species with oxygen and carbon bonds and more reduced compounds with As–S or As–M ligation. The relative amounts of these types of arsenic varied with the type of shale. Rundle Munduran and Brazilian Iratic shales primarily contained oxidized and reduced arsenic species, respectively. Green River oil shale included both species. Concentration of the kerogen in this shale by treatment with HCl and HF indicated selective removal of the oxidized arsenic species. X-ray absorption promises to be useful in the analysis of other solid-phase samples.

Arsenic can be volatilized from retorted oil shales in what has been shown to be a microbially mediated process.[452,453] Experiments with soils and oil shales demonstrated different responses to added nutrients. In soil, arsenic volatilization showed a direct relationship to nutrient levels and microbial growth, whereas a threshold response to available nutrients was evident for shale. The addition of retort process water to shale gave arsenic volatilization without other nutrients. Surprisingly, the presence of retort process water resulted in decreased volatilization from shales amended with DMAA and MMAA but not sodium arsenate. The extent of volatilization was quite small (<3% in 15 weeks) even in the presence of nutrients at higher than ambient levels. However, it was suggested that continuous low-level volatilization from organic-rich microenvironments might have disposal implications. The volatile arsenicals were not identified, but in view of this concern it is instructive to compare the similar, or higher, levels of arsines that are lost from soils.[234]

2. Behavior of Coal Fly Ash

Coal produces up to 30% of its weight as fly ash after combustion, and increased coal usage presents problems regarding the disposal of this material.[454] Arsenic is enriched on fly ash particles as a consequence of the condensation of volatile arsenic species onto the surfaces of the particles. There is concern that leaching of this arsenic will result in contamination of aquatic disposal sites. While studies have shown that adsorption, dissolution, and precipitation are important in controlling desorbed arsenic concentrations, there is considerable variation among fly ash samples.

Turner[455] found that pond effluents from 12 coal ash disposal systems contained variable quantities of dissolved arsenic (0.5–130 ppb), but there was no relationship between these values and the arsenic content (30–222 ppm) of the fly ash. In a leaching experiment with one sample, arsenic was initially released mainly as As(III), but even though the dissolved arsenic concentration continued to increase, arsenite decreased. Adsorption, or, more likely, catalytic oxidation, appeared to be responsible. Other work suggested that As_2O_3 should be a predominant component of fly ash, but this could not be confirmed as a general property of the samples investigated by Turner. Breslin and Duedall[454] found that both As(V) and As(III) leached from a different fly ash, but arsenate was predominant (77%) in this case. There was a tendency in both studies for increased arsenic dissolution with increasing pH, but exceptions were found. The fly ash samples themselves demonstrated acidic or basic characteristics when added to distilled water, probably as a result of different surface coatings. Considering these differences, it is not surprising that inconsistent behavior was observed.

Despite variable initial behavior, there is evidence that the ultimate fate of leached arsenic will be controlled by E_H, pH, and adsorption to hydrous oxides, i.e., the same factors that control arsenic from any source. Crecelius et al.[307] found that the distribution of arsenic species in a reservoir contaminated by arsenic discharged from a coal fly ash pond was consistent with a geochemical (thermodynamic) model. However, equal amounts of As(III) and As(V) were present in interstitial water. Further work is required to evaluate the influence of sedimentary deposits of fly ash material which may become acidic or basic, possibly altering normal diagenesis.

H. Influence of Acid Rain

Acid precipitation, which is due in part to coal combustion and smelting operations, is of increasing concern to environmentalists. The responsible anthropogenic processes are also the main contributors of atmospheric arsenic. Increased acidity in the atmosphere, the aquatic environment, and soils may influence the environmental impact of arsenic, but the nature and extent of this influence are largely unknown. In principle, knowledge of the pH dependence of the major controls on arsenic speciation would suffice, but it is recognized that there is a multiplicity of ways in which these factors can interact.[456–458]

Wood[458] has suggested, on the basis of standard reduction potentials, that acidification will result in differences in the reactivity of arsenic oxyanions. However, this approach does not take into account the kinetic aspects of interconversion nor does it include the response of microbially induced redox transformations.[457] Mushak[457] has presented a useful overview of the current knowledge on this subject. Increased solubilization of the airborne, particulate form of arsenic, changes in the microbial composition of water and soils, and alterations of the adsorption behavior of arsenic species will all influence arsenic concentrations and speciation. The importance of iron oxides in the control of aquatic arsenic concentrations and in the cycling of arsenic in soils and sediments has been described elsewhere in this review. Mushak points out that increased acidity will result in decreased stability of amorphous $Fe(OH)_3$ and thus changes in the binding efficiency for arsenicals. This could result in major changes in arsenic distributions. However, as our understanding of the various biological, chemical, and geological processes that constitute the arsenic biogeochemical cycle grows, so will our ability to predict the response of this cycle to changes like acid rain.

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