ULTRATRACE SPECIATION AND BIOGENESIS OF METHYLTIN TRANSPORT SPECIES
IN ESTUARINE WATERS

F. E. Brinckman, J. A. Jackson, W. R. Blair, G. J. Olson,
and W. P. Iverson

Chemical and Biodegradation Processes Group
National Bureau of Standards
Washington, D.C. 20234 U.S.A.

ABSTRACT

Environmental tin, widely dispersed at low concentrations in
waters, sediments, and biota, is now perceived to be a bioactive
element susceptible to methylation and even hydridization by marine
bacteria. Nonetheless, the redox cycle of tin in natural waters is
poorly understood and recent advances in tin-specific molecular
characterization fail to speciate Sn(II) and Sn(IV) reliably. On
the other hand, such rapid developments in speciation methodology
are now permit growing numbers of studies of organotin distributions in
aquatic systems, raising the question of the "natural" biogeochem-
ical flux of methylstannanes in relation to increased anthropogenic
organotin influx from industry and shipping. New methods for
direct speciation of aquated or involatile organotins by liquid
chromatography are compared with advances in purge-and-trap sampling
of volatile or hydrophobic organotins speciated by gas chromato-
graphy. The work in our laboratory indicates that effective models
for estuarine formation and transport may ultimately be developed,
but that basic roadblocks to progress stem from inadequate descrip-
tive aqueous organometallic chemistry and knowledge of critical
kinetic parameters for the lifetimes of key organotin species in
sea water.

Key words: Atomic absorption detector; biomethylation; Chesapeake
Bay; estuarine organotin transport; flame photometric
detector; gas chromatography; kinetics; liquid chromato-
graphy; methyltin hydrides; purge-and-trap sampling;
redox; tetramethyltin.
ULTRATRACE SPECIATION AND BIOGENESIS OF METHYLTIN TRANSPORT SPECIES IN ESTUARINE WATERS

F. E. Brinckman, J. A. Jackson, W. R. Blair, G. J. Olson*, and W. P. Iverson

Chemical and Biodegradation Processes Group
National Bureau of Standards
Washington, D.C. 20234 U.S.A.

INTRODUCTION

Tin is very widely dispersed in the aquatic environment, appearing as trace amounts in sediments, particulates, and biota (mg kg⁻¹), and at much lower levels in waters (ng L⁻¹) [1]. The concentration of tin decreases notably from estuarine and near short sites to oceanic environments [2], in a manner seen for other metals or metalloids subject to significant anthropogenic inputs and rapid geochemical turnovers and transport [3].

The chemistry of tin in natural waters is generally presumed to be the stannic or tin(IV) species, but its redox chemistry in sea water remains largely unclear, as does its biogeochemistry. This last aspect has recently assumed great importance in understanding the mechanisms by which aquatic tin is mobilized (by atmospheric or aquatic transport) and in questioning both bioavailability and bioaccumulation of tin [4,5]. Several reports on the biomethylation of tin in both inorganic and organometallic forms have now appeared [2,6-9], and ubiquitous distributions of methyltin(IV) species at ultratrace concentrations (ng L⁻¹) in natural waters have been observed [10-12]. This evidence suggests that

such molecules may take part in a global tin cycle. Unfortunately, the matter is obscured by the sharp increase over the past twenty-five years of anthropogenic introduction of both methyl- and other organotins into the environment by diverse commercial materials [13].

Interactions of man-made organotin compounds intrude into the "natural" biogeochemical cycle of tin [14] in special ways. Use of organotins mainly occurs as plastics stabilizers and biocides; these, in turn, find principal applications in ways that involve environmental exposure, especially in fresh and estuarine discharges. We have undertaken studies to speciate tin and to assess its dynamics in the marine environment, but in view of the foregoing situation we are examining the tin concentration gradient from a well-known estuarine source, the Chesapeake Bay, to oceanic sites. In this context, we will discuss progress in current research to solve fundamental problems impeding progress toward resolving the role of tin in the sea. The objectives of this research have been to: (1) establish a firm data base for the aqueous chemistry of organotin(IV), tin(IV), and tin(II) species, including dynamics of their interactions with other trace aquated metal ions; (2) develop and apply reliable ultratrace molecular speciation techniques to the measurement of aquatic tin in its various forms, including key, volatile transport species such as tetramethyltin; and (3) assess the role of marine microbiota in generating or retarding formation of tin transport species, by biosynthesis, biodegradation, and uptake.

BIOAVAILABILITY OF TIN IN RELATION TO OTHER BIOACTIVE ELEMENTS

Several estimates of global transport cycles suggest [15,16] that atmophilic properties of certain elements may, in part, depend upon biological mediation. For a given element, such volatilization processes basically must rely upon release from terrestrial or aquatic sediment reservoirs of hydrophobic gases such as elemental mercury, Hg⁰, or coordinatively saturated methylelements, MeEₙ⁺, where n is the principal oxidation state [5]. In essence, the underlying chemical processes governing retentivity or transport to/from sediments or particulates to/from water columns to/from atmosphere are,

\[ \text{Me}_x E^{(n-x)^+} \rightarrow \text{Me}_x E^{(n-x)^+} \rightarrow \text{Me}_x E^{(n-x-y)^+} \rightarrow \text{Me}_x E^{(n-x-y)^+} \]  

(1)
where \( L = \) some anionic ligand imparting volatility, such as \( \text{H}^-, \text{Me}^-, \text{Cl}^-, \) or \( \text{OH}^- \). Below, we shall review the features of such derivatization chemistry as a means to produce water-air partition coefficients favorable for development of analytical methods employing chromatographic separations coupled with element-selective detection \([17]\). A perspective on mobilization of tin is gained by comparing its non-specific (total) availability in common environmental reservoirs in relation with other bioactive elements known to undergo transformations of the kind implied by equation \((1)\).

Potential Availability of Tin in Environmental Media

Table 1 summarizes data from various sources which suggest that considerable amplification of many such active elements can occur. Sewage sludges from 16 American cities show \([18]\) substantial industrial and microbial accretion of many heavy elements from common sources (cf. cow manure) into forms that may offer enhanced availability to subsequent biota during translocation via stream loadings to estuaries and then to the sea. It is tempting to associate the very high concentrations of biogenic cobalamin (vitamin \( \text{B}_{12} \) derivatives) observed during sewage sludge treatment \((1.9 \text{ to } 27.1 \mu g \text{ g}^{-1})[24]\) with potential involvement of a powerful methylator, methylcobalamin or \( \text{CH}_3\text{-B}_{12} \) \([14]\), in metal release.

Though not yet identified in sewage sludges, presence of \( \text{CH}_3\text{-B}_{12} \), especially in anaerobic sediments, is reasonable. Consequently, the effectiveness with which this exocellular metabolite methylates a broad range of metal ions in solution \([14,25]\) suggests an important new line of investigation for metal release from sewage outfalls. Moreover, Thayer reports \([26]\) that \( \text{CH}_3\text{-B}_{12} \) is also capable of dissolving many refractory metal oxides, including highly insoluble \( \text{SnO}_2 \), to possibly form methylmetal species in aqueous solution.

Similar considerations, doubtless for small chemical concentration gradients, probably are valid for some of the other tin sources listed in table 1. The very high tin concentrations (also associated with heavy loadings of organic matter) in the Baltimore Harbor sites focused the direction of our present work. Moderately high concentrations of total tin in sub-micron materials from the Chesapeake Bay channel represent favorable surfaces for attachment and transformation of metals by marine bacteria \([27]\). In table 1, no
Table 1. Compositional Data* for Tin and Some Other Bioactive Elements in Various Environmental Sources

<table>
<thead>
<tr>
<th>Source</th>
<th>As</th>
<th>Hg</th>
<th>Pb</th>
<th>Se</th>
<th>Sn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow manure b</td>
<td>4.0</td>
<td>0.2</td>
<td>16.2</td>
<td>2.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Sewage sludge b</td>
<td>3.0-10.0</td>
<td>3.4-18.0</td>
<td>136.0-7627.0</td>
<td>1.7-8.7</td>
<td>111.0-492.0</td>
</tr>
<tr>
<td>Urban dust c</td>
<td>115.0</td>
<td>--</td>
<td>0.655</td>
<td>24.0 e</td>
<td>--</td>
</tr>
<tr>
<td>U.S. water supplies f</td>
<td>0.100.0</td>
<td>0.01-10.0</td>
<td>1.0-400.0</td>
<td>0-10.0</td>
<td>0.3-30.0</td>
</tr>
<tr>
<td>Chesapeake Bay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baltimore Harbor sed.</td>
<td>1.4 g</td>
<td>&lt;0.01-0.31 h</td>
<td>&lt;1-1.3e, h</td>
<td>--</td>
<td>1.4 f-240.0 i</td>
</tr>
<tr>
<td>Baltimore Harbor waters</td>
<td>0.29 g</td>
<td>&lt;0.01 f</td>
<td>1.9 g</td>
<td>--</td>
<td>0.19 f-2.0</td>
</tr>
<tr>
<td>Channel sed. i</td>
<td>0.92-10.0</td>
<td>&lt;0.065-0.37</td>
<td>0.54-75.0</td>
<td>--</td>
<td>0.19 f-1.7</td>
</tr>
<tr>
<td>Channel waters</td>
<td>&lt;0.079-0.37</td>
<td>&lt;0.039-0.16</td>
<td>0.10-1.9</td>
<td>--</td>
<td>&lt;0.13-0.47</td>
</tr>
<tr>
<td>Organic colloids l</td>
<td>3.0-4.0</td>
<td>--</td>
<td>--</td>
<td>0.65-2.0</td>
<td>56.0-51.0</td>
</tr>
</tbody>
</table>

*Individual, mean, or range values in pg g⁻¹, dry weight (solids), or pg L⁻¹ (waters).

b Ref. 18

h Given as % Hg

c NBS Standard Reference Material 1638

d Not certified

f Ref. 20, outer harbor station

Ref. 22, S-N Bay axis, 22 stations

Ref. 23
mention of another very important microenvironment for amplification of metals is given. Heavy elements are well known to greatly concentrate in oily surface microlayers [28], but data for total or speciated tin in the Chesapeake Bay are not yet available.

Anthropogenic Introduction of Organotins into Environmental Media

In the foregoing we have discussed the typical total tin content of environmental compartments involved in transmission of this element (and related bioactive elements) from land to sea. The rich chemistry of tin has made this element one of great importance to man’s industries for millennia, and in recent decades its remarkable range of properties as organotin compounds has generated totally new markets and distribution patterns in the environment [13].

The contribution of all anthropogenic tin compounds represents the bulk of the tin discharge system to oceans [16]. Over four percent of this contribution involves organotins of widely varying toxic properties, particularly toward aquatic biota. Table 2 indicates the spectrum of organotin formulations involved. The \( R_3\text{Sn}^- \) class is the most toxic generally, but the \( R_2\text{Sn}= \) species represent still lipophilic moieties that can probably undergo transformations in vivo to create toxic responses within biota, as we shall see later.

It is well known that, in addition to the degree of substitution on tin(IV), the precise molecular structure of the substituent group \( R \) covalently bound to the organotin influences its lethal action in different categories of organisms [29]. Nonetheless, most investigators now agree that biodegradation of even toxic \( R_3\text{Sn}^- \)-containing materials can occur in soils and sediments, presumably caused by bacteria that successively cleave tin-carbon bonds to ultimately produce inorganic tin:

\[
R_3\text{Sn}^- \rightarrow R_2\text{Sn}= \rightarrow R\text{Sn}= \rightarrow \text{Sn(IV)} + .
\]  

In practical fact, these steps are assumed, there having been no clearcut demonstration of the entire sequence in the environment. Moreover, the new evidence for microbial methylation of inorganic tin(IV), tin(II), and common intermediate organotins suggests—just as was shown earlier for the sedimentary cycle for methylmercury [26,30,31]—that a counter mechanism for sustaining methyltin(IV)
Table 2. Production of Organotins and Their Uses\(^a,^b\)

<table>
<thead>
<tr>
<th>Uses</th>
<th>Chemical Form(^c)</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC Plastics (heat stabilization)</td>
<td>(R_2\text{SnL}_2)</td>
<td>66.4</td>
</tr>
<tr>
<td>Catalysts (polyurethanes, silicone elastomers)</td>
<td>(R_2\text{SnL}_2)</td>
<td>20.7</td>
</tr>
<tr>
<td>Biocides (pesticides, antifoulants)</td>
<td>(R_3\text{SnL}) and</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>(-[L]-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{SnR}_3)</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous (research, fiber stabilization, export)</td>
<td>(R_3\text{SnL})</td>
<td>3.3</td>
</tr>
<tr>
<td>Poultry (anthelmintics, coccidiostats)</td>
<td>(R_2\text{SnL}_2)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^a\)Ref. 13  
\(^b\)Represents 4.2\% of 1976 total world production, 2.26 \times 10^{11} \text{ g}
\(^c\)\(R = \text{butyl, cyclo-hexyl, phenyl, octyl, methyl, and propyl; L = F, Cl, laurate, maleate, and thioglycolate.}\)

Species in environmental media is available. The reported \([10-12]\) widespread incidence of highly diluted solvated methyltin(IV) ions, \(\text{Me}_n\text{Sn}^{(4-n)+} (\text{aq})\), in United States coastal waters may therefore simply reflect a steady state concentration. The idea that this situation is reached only through biological mediation is not valid, however. Brinckman has pointed out \([5]\) that among the methyltin species identified in marine waters, \(\text{Me}_3\text{Sn} (\text{aq})\) is itself a potent methylator of many aquatic metal ions at bimolecular rates which might significantly shift the apparent "steady-state" concentration. For example, with aqueous mercuric ion, the reaction

\[
\text{Me}_3\text{Sn}^+ (\text{aq}) + \text{Hg}^{2+} (\text{aq}) \rightarrow \text{Me}_2\text{Sn}^{2+} (\text{aq}) + \text{MeHg}^+ (\text{aq}),
\]

(3)
proceeds to completion at a rate first-order in each metal and dependent on the \([\text{Cl}^-]\) and ionic strength of the solution [32]. Based on available concentrations of free \(\text{Hg}^{2+} (\text{aq})\) and \(\text{Me}_3\text{Sn}^+ (\text{aq})\), along with typical \(p\text{Cl}\), \(p\text{H}\), and ionic strengths for coastal waters, it was estimated [5] that the half-life for leakage of \(\text{Me}_3\text{Sn}^+\) from that compartment was about \(6 \times 10^5\) yr. This residence is in surprising agreement with that estimated for oceanic total tin, \(1 \times 10^5\) yr [33], but must be fortuitous since many other abiotic transmethylation pathways for forming and decomposing methylns are known. We shall consider several of these possibilities in the following section, both in the context of biomethylation and abiotic transmethylation.

**CHEMICAL AND BIOLOGICAL INTERACTIONS OF METHYLTINS WITH ELEMENTS**

Within a microenvironment, concurrent events involving both chemical and biological reduction and methylation of metals may transpire individually or in synergic fashion. Figure 1 illustrates a combination of *in vitro* and environmental experience for three heavy elements of great technological importance that undergo microbial methylation in marine sediments [26]. No evidence is available for common enzymes among the bacterial populations that methylate all three elements, but it is reported that pure strains of genus *Pseudomonas*, for example, can both reduce and methylate \(\text{As(V)}\) to \(\text{As(III)}\) and form \(\text{Me}_n\text{AsH}_{3-n}\) gases [34], reduce \(\text{Hg(II)}\) to \(\text{Hg}^0\) [35], and both methylate and reduce \(\text{Sn(IV)}\) to form \(\text{Me}_n\text{SnH}_{4-n}\) gases [5,12]. Together, these metabolites or their precursor metal species may also interact *in situ*. The case for a mercury-tin "crossover" model has been described [6,36] where combined stresses of \(\text{Sn(IV)}\) and \(\text{Hg(II)}\) (a much more realistic representation of polluted sediments!) were metabolized by a metal-tolerant strain of *Pseudomonas* isolated from the Chesapeake Bay [35]. The metabolic reduction of mercury to \(\text{Hg}^0\) gas and the biomethylation of \(\text{tin(IV)}\), seen to occur for the respective metals as individual stresses, were seen to occur with the combined stress. A new species was formed only during metabolism of the dual metal stress. This species was found to be \(\text{MeHg}^+\), presumably formed by the reaction given in equation (3),
Fig. 1. Associated abiotic chemistries of three elements known to undergo biomethylation in marine sediments are compared.

where it was concluded that both concurrent biomethylation of tin(IV) and its mediation (abiotically) in MeHg⁺ production were kinetically competitive [6].

Other dual metal crossovers are possible in principle. We examined the possibility for Me₃As²⁺ (aq), an isoelectronic analog of Me₃Sn⁺ (aq), for its ability to methylate metal electrophiles, in particular Hg²⁺ [37]. As depicted in figure 1, only a redox reaction in the reverse sense occurred reducing arsenic volatility,

\[ \text{Me}_3\text{As} + \text{Hg}^{2+} \rightarrow \text{Me}_3\text{As}^{2+} + \text{Hg}^{0} + 2e^- \]  

which was highly dependent upon pH and ligands bound to mercury [38]. Such chemistry is important to considerations of microbial production of Me₃As [34] in sediments where high concentrations of both As and Hg are commonly found (cf. table 1). Similar experiments with arsenic and tin species in saline waters at these pH and pCl conditions gave no conclusive reactions.
The abiotic organometallic reactions noted above which readily occur in aqueous solutions represent only a few of many similar redox and transmethylation processes recently described for other metals in this expanding field [5,26]. To aid in understanding our recent results in speciation and dynamics of methyltins in estuarine waters, brief mention of a group of abiotic reactions described for both environmental and laboratory conditions is desirable. In table 3, we summarize some representative reactions which either favor formation of new methylelement species potentially capable of biotic uptake, or elimination of "active" methyl groups from aquatic environments and potential biotic activity. It is clear from the table that ubiquitous coastal production of MeX [15,44] or photo-methylation via metabolic acetates represent possible sources of environmental methylelements, and thus merit further study. All of the aqueous reactions cited in table 3 probably occur at bimolecular (or pseudo first-order) rates of the order, $k \sim 10^{-3}$ M$^{-1}$ s$^{-1}$ or less [5], but like the related transmethylation reaction illustrated in equation (3) probably can kinetically compete with microbial methylation and demethylation reactions [26].

SPECIATION OF ORGANOTINS IN SEA WATER*

Requirements for Nondestructive Methods

Beyond the usual difficulties encountered with determinations of metals at ultratrace (ng L$^{-1}$) concentrations in biological fluids and aquatic media [45], creation of a realistic framework of data for establishing the role of tin in the sea requires molecular speciation as well. We have examined the interdependence of biological and chemical processes for transformation and mobilization of tin and other metals and have noted that rate-determining events depend upon very specific features of molecular architecture and oxidation state of the associated metal ion. Thus, the number and kinds of organic ligands bonded to tin(IV) are not only diagnostic of toxicity, but they also provide a molecularly distinctive analyte for elucidation of trace chemistry, if suitable nondestructive chemical or physical means for isolation, concentration, and unambiguous detection can be applied.

*Complete descriptions of equipment and methods employed at NBS are given in references 12, 51, or 49, 50 for gas and liquid speciation, respectively.
Table 3. Some Non-biological Reactions\textsuperscript{a} Providing Formation or Removal of "Active" Methyl Groups from Aquated Metal Ions

<table>
<thead>
<tr>
<th>Formation Reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Me}^b + \text{Sn}^{2+} \rightarrow \text{Me}_n\text{Sn}^{(4-n)^+} + n\text{X}^-$</td>
<td>7</td>
</tr>
<tr>
<td>($\text{X} = \text{F, Cl, MeCO}_2, \text{SO}_4$) ($n = 1,2$)</td>
<td></td>
</tr>
<tr>
<td>$\text{MeI} + \text{Pb}^{2+} \rightarrow [\text{MePbI}^{2+}] + + \text{Me}_4\text{Pb}$</td>
<td>39</td>
</tr>
<tr>
<td>$\text{Me}^b + \text{Me}_3\text{E} \rightarrow \text{Me}_4\text{E}^+ + \text{X}^-$</td>
<td>40</td>
</tr>
<tr>
<td>($\text{X} = \text{Br, I; E} = \text{As, Sb}$)</td>
<td></td>
</tr>
<tr>
<td>$\text{MeCOO}^{-c} + \text{Hg}^{2+} + \text{hv}^{d} \rightarrow \text{MeHg}^+ + \text{CC}_2$</td>
<td>41,42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Removal Reactions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Me}_2\text{E}^+ + \text{M'}\text{Cl}_4^{2-} \rightarrow \text{M'}^0 + \text{MeCl} + \text{Me-Me} + \text{Me}_2\text{E}^{2+}$</td>
<td>42</td>
</tr>
<tr>
<td>($\text{E} = \text{Sn, Pb; M'} = \text{Pd, Pt}$)</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}_3\text{E}^+ + \text{AuCl}_4^- \rightarrow \text{Au}^0 + \text{MeCl} + \text{Me}_2\text{E}^{2+}$</td>
<td>42</td>
</tr>
<tr>
<td>($\text{E} = \text{Sn, Pb}$)</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}_4\text{Pb} + \text{Cu}^{2+} \rightarrow \text{CuCl} + \text{MeCl} + \text{Me}_3\text{Pb}^+$</td>
<td>43</td>
</tr>
<tr>
<td>$\text{MeHg}^+ + \text{PdCl}_4^{2-} \rightarrow \text{Hg}^{2+} + \text{MeCl} + \text{Pd}^0$</td>
<td></td>
</tr>
<tr>
<td>$42\text{Me}_3\text{E}^+ + \text{TlCl}_n^{(3-n)-} \rightarrow \text{Tl}^+ + \text{MeCl} + \text{Me}_2\text{E}^{2+}$ ($\text{E} = \text{Sn, Pb}$)</td>
<td>42</td>
</tr>
<tr>
<td>$\text{Me}_2\text{Hg} + \text{hv}^{d} \rightarrow \text{Hg}^0 + \text{Me-Me}$</td>
<td>41,42</td>
</tr>
</tbody>
</table>

\textsuperscript{a}In saline solutions
\textsuperscript{b}A common metabolite ($\text{X} = \text{Cl, Br, I}$) from bacteria and algae in coastal sea water, see Ref. 44
\textsuperscript{c}Common bacterial metabolite
\textsuperscript{d}Sunlight or ultraviolet irradiation.

Two basically different approaches are possible, and have been adopted in our present work. We recognize from equation (1) that organotin species of interest to us, $\text{R}_n\text{Sn}^{(4-n)^+}$, may exist in equi-
librium between aqueous solution and complexes on solid substrates, such as inorganic particulates or biological detritus. Apart from those species already in homogeneous or "free" solution, a choice is possible for treatment with selective ligands, L, that can either cause desorption of solid-bound organotins, or, alternatively, form new organotin derivatives possessing necessary volatility (hydrophobicity) for recovery from solution as gases. In both of the methods to be described, we directly speciate aquated organotins--those in "free" solution--without pretreatment. For those organotin molecules of sufficient volatility, e.g., Me₄Sn, a gas chromatographic separation step is employed; for involatile solvated organotins, a direct liquid chromatographic separation process is utilized, and, when appropriate, hydride derivatization can be exploited with the gas chromatograph. Both methods give high precision retention times (RSD < 3 percent) with authentic organotins, a measurement prerequisite for reliable speciation.

**Gas Phase Speciation of Aquatic Organotins**

Braman [10] and Goldberg [11] with their students successfully speciated aquatic organotins (R = Me and Bu), respectively, with flame emission (SnH) and atomic absorption detectors, following gas sparging of large (100 mL) water samples treated with a hydriding agent,

\[ R_nSn^{(4-n)+} + \text{excess } BH_\text{aq}^- \rightarrow R_nSnH_{4-n}^+ \]  

The resulting stannanes (n = 0 to 3) were cryogenically trapped, then distilled into the detector. Though extremely sensitive (< 1 ng L⁻¹) the methods failed to isolate Me₄Sn or other volatile organotins not requiring hydride volatilization. We have modified commercial equipment to provide a system which avoids the above limitations, though it is not as sensitive.

Our system employs a "Tenax-GC" polymeric sorbent [46] in an automatic purge and trap (P/T) sampler coupled to a conventional glass column gas chromatograph equipped with a flame photometric detector (FPD). A schematic of the P/T-GC-FPD assembly with typical operating conditions is depicted in figure 2. Flame conditions in the FPD were tuned to permit maximum response to SnH emission in a H-rich plasma, as detected through narrow band-pass interference filters (610 ± 5 nm) [47]. Two modes of analysis were used: (1) volatile stannanes were trapped directly from sparged 10 to 50 mL
water samples with no pretreatment, and (2) volatilized tin species were trapped from the same or replicate water samples following rapid injection of aqueous excess NaBH₄ solution directly into the P/T sparging vessel immediately prior to beginning the P/T cycle [12].

Direct Speciation of Organotins in Solution

The liquid-solid solvation process illustrated in equation (1) also governs several desirable molecular separation processes offered by high-performance liquid chromatography, HPLC [48]. For either ion exchange resolution of aqueous cations, \( RSn(4-n)^+ \) [49], or their separation as ion pairs, \([RnSn(4^n)n]^- X_4-n \) [50], on reverse bonded-phase columns [50], the method is restricted to "free" tin analytes. Unlike the vigorous hydride derivatization used in the GC-FPD method,
common HPLC solvent combinations or their ionic addends usually will not provide sufficient coordination strength to labilize organotin ions strongly bound to solids in environmental samples. Moreover, the HPLC separations require that injected samples be free of particulates that may clog the column or pumping system.

On the other hand, HPLC, if coupled with a sensitive element-specific detection system such as atomic absorption spectrometry (AA), offers a valuable tool for organotin speciation in complex fluids (especially with high organic loadings) not readily amenable to gas phase derivatization methods. Figure 3 shows a schematic of

![HPLC-GFAA System Diagram](image)

Fig. 3. The HPLC-GFAA system with automated peripherals [50]
our basic HPLC setup coupled to a graphite furnace AA (GFAA) in a manner giving automatic periodic (typically 45 s intervals) sampling of the resolved eluants for tin-specific determination [50]. Injected sample volumes may vary from 10 to 500 µL. Consequently, system sensitivity is broad and samples can be very representative. For example, direct examination of soil or sewage leachates, process waters, solutions exposed to organotin (antifoulant) controlled-release agents, or microbial growth media, only requires separation of particulates (or cells) prior to speciation. This is simply done by decantation, syringe filtration, or ultracentrifugation before direct injection of the solution into the HPLC-GFAA system. Thereby, needed information on the nature and true concentrations of "free," involatile transport or leachate organotin species becomes available without recourse to chemical treatment.

**Comparison of Analytical Results from GC-FPD and HPLC-GFAA**

Before turning to our current organotin speciation studies in the Chesapeake Bay estuary, it is useful to compare the nature of experimental data, their form, and relative reliability, which, in turn, depend on the physico-chemical differences between the two methods. Figures 4 and 5 compare calibration curves for organotins from both anthropogenic and environmental sources. System detection limits, δ (95 percent confidence level)[51], for biocidal triorganotins representative of commercial pesticides in widespread use [13] are shown in figure 4. Mixtures of R₃Sn⁺ compounds (R = n-butyl, phenyl, cyclo-hexyl) were separated by ion exchange HPLC-GFAA [49]. The small spread in calibration slopes in figure 4 signifies similar efficiencies for their separation and column recovery, as well as GFAA sensitivities. The δ values (ng) shown in the figure indicate that with 100 µL sample injections, nominal solution working ranges of 50 to 2,600 µg L⁻¹ may readily be speciated and quantitated. As seen in figure 5, considerably more sensitivity is possible with P/T-GC-FPD speciation of related organotin species known [5,10,11] to occur in environmental media. Much greater divergence in the P/T-GC-FPD system calibration slopes (ratios > 25) is obtained, probably a result of different hybridization rates during the fixed P/T purge time (10 min), different partition coefficients affecting sparging rates of each species [12], or different retentivities on the Tenax-GC sorbent [46]. On the basis of the δ values obtained for the GC method (fig. 5), with 10 mL sample volumes usually employed in our laboratory, nominal working ranges of 10 to 40 ng L⁻¹ organotin are feasible.
Fig. 4. Calibration curves for $R_3Sn^+$ (R = butyl, phenyl, $\gamma$-hexyl) separated by UPLC-GFAA with strong cation exchange (SCX) columns using MeOH/H$_2$O/NH$_4$OAc eluents [40] are shown with respective correlation coefficients ($r$) and system detection limits ($\delta$) (95% confidence level [51]).

Fig. 5. Calibration curves for three aqueous organotins indicated separated by P/T-GC-FPD using the hydride generation mode [12] are shown with respective $r$ and $\delta$ estimates.
Both systems are capable of at least a ten-fold increase in sensitivity with only minor changes in procedure and equipment. For HPLC-GFAA, this can be achieved by both increasing injected sample size and optimizing flow rates with a GFAA thermal program designed to give maximum atomization efficiency for a specific organotin analyte [50,51]. For P/T-GC-GFD, improvements are realized by adjusting purge flow rate and time while altering NaBH₄ additions to optimize evolution of a given organotin analyte [12]. Also, both increasing sample volumes [10,11] and operating the Tenax-GC trap at sub-ambient temperatures [2] will yield lower working ranges.

Ultimately, such concerns for widely different system responses to various organotin analytes are very crucial to reliable environmental speciation and quantitation by coupled chromatographic-element-selective detection methods because: (a) each known species analyzed must have its (same or different) calibration curve established, and (b) unknown species must either be unequivocally identified and calibrated or their calibration curves inferred from similar molecular types or guessed. Under ideal conditions, of course, a perfect chromatograph-detector system will always yield identical calibration curves for any separated molecule containing the diagnostic element of interest [17,51]. It should be recognized that in some circumstances where robust chemical derivatization is called for, as with formation of volatile tin hydrides from sea water for FPD measurements (equation (5)), possibilities exist for unexpected chemical transformations of unknown substrates into forms taken as characteristic of known analytes. In concluding this section, we will present an example for the tin(II)-tin(IV) redox system which we now feel casts uncertainty on the reported speciation and quantitation of tin as Sn(IV) in marine waters [10,11].

The Question of Sn(IV) and Sn(II) Speciation in Aquatic Media

Most workers regard "free" and particulate-bound tin in the sea to be in its +4 oxidation state. Based upon available free-energy data [52] and usual oceanic values of pH = 8.1 and pE = 12 (E° = 0.7394 V) [53], the thermodynamic distributions of supposed principal tin species are given by the following equilibria:

\[ \log_{10} \frac{[SnO_3^{2-}]}{[HSnO_2^-]} = 37, \text{ and } \log_{10} \frac{[SnO_3^{2-}]}{[Sn^{4+}]} = 45. \]
In anaerobic environments (reducing conditions), Sn(II) and other tin(IV) species, including chloro-hydroxy derivatives [5,32], may become important. Additionally, we recognize that the steady-state condition and concentrations of Sn(II) and Sn(IV) in aquatic systems may be kinetically, not thermodynamically, controlled by biological activity. This was found to be the case for observed non-thermodynamic equilibrium concentrations favoring arsenite over arsenate produced by bacterial reduction [54,55] coupled with relatively long lifetimes of As(III) in aerobic waters [56]. We are, therefore, left with the possibility that in some marine waters, especially highly polluted estuarine sites, that Sn(II) species may occur. The question arises: does our current use of hydride generation insure that the SnH$_4$ generated from aquatic samples is a faithful representation of the presence only of Sn(IV) species or can Sn(II) interfere with and obscure the quantitation?

Concern for substantial interference by Sn(II) in the reduction of Sn$^{4+}$ via equation (5) is justified. Schaeffer and Emilius showed [57] that yields of SnH$_4$ as high as 84 percent (based on Sn) were obtained from BH$_4^-$ treatment of stannous chloride in aqueous 0.6 N HCl. Yields were dependent on pH, and smaller yields of distannane were noted. Jolly and Drake subsequently refined this procedure to provide a preferred general method for aqueous generation of Ge, As, Sb, and Sn hydrides [58]. For tin(II) the reaction

$$4\text{HSnO}_2^- + 3\text{BH}_4^- + 7\text{H}^+ + \text{H}_2\text{O} \rightarrow 3\text{H}_3\text{BO}_3 + 4\text{SnH}_4^+,$$

involves oxidation of the metal to Sn(IV) with concurrent redox of H$^-$ and H$^+$ (possibly as H$^-$). The basic problem for our present methods employing borohydride treatments of water samples for Sn(IV) "speciation" is clear. In a recent series of experiments we have attempted to determine the extent of the problem. Our results are summarized in table 4.

Calibration curves were generated by the P/T-GC-PFD method for borohydride reductions of Sn(IV), Sn(II), and Me$_2$Sn$^{2+}$ species to SnH$_4$, SnH$_4$, or Me$_3$SnH$_2$, respectively, in three types of media, as tabulated. All three analytes showed substantial increases in their calibration slopes in going from distilled water to 0.2 M NaCl
<table>
<thead>
<tr>
<th>Species</th>
<th>Medium</th>
<th>Detection Limit, µg L⁻¹</th>
<th>Regression Slope ± S.E.</th>
<th>Number of Runs, N</th>
<th>R</th>
<th>Relative Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sn(IV)</td>
<td>H₂O</td>
<td>19</td>
<td>210.4 ± 16.4</td>
<td>7</td>
<td>0.985</td>
<td>1.00</td>
</tr>
<tr>
<td>Sn(II)</td>
<td>&quot;</td>
<td>26</td>
<td>32.6 ± 4.9</td>
<td>7</td>
<td>0.949</td>
<td>0.16</td>
</tr>
<tr>
<td>Me₂Sn²⁺</td>
<td>&quot;</td>
<td>18</td>
<td>532.0 ± 182.0</td>
<td>7</td>
<td>0.987</td>
<td>25.3</td>
</tr>
<tr>
<td>Sn(IV)</td>
<td>0.7 M NaCl</td>
<td>29</td>
<td>318.5 ± 40.2</td>
<td>10</td>
<td>0.942</td>
<td>1.51</td>
</tr>
<tr>
<td>Sn(II)</td>
<td>&quot;</td>
<td>21</td>
<td>117.9 ± 9.6</td>
<td>11</td>
<td>0.972</td>
<td>0.56</td>
</tr>
<tr>
<td>Me₂Sn²⁺</td>
<td>&quot;</td>
<td>21</td>
<td>10,921.0 ± 1,016.0</td>
<td>10</td>
<td>0.968</td>
<td>52.3</td>
</tr>
<tr>
<td>Sn(IV)</td>
<td>Bay water</td>
<td>30</td>
<td>21.6 ± 2.8</td>
<td>10</td>
<td>0.937</td>
<td>0.10</td>
</tr>
<tr>
<td>Sn(II)</td>
<td>&quot;</td>
<td>10</td>
<td>51.4 ± 2.2</td>
<td>9</td>
<td>0.993</td>
<td>0.24</td>
</tr>
<tr>
<td>Me₂Sn²⁺</td>
<td>&quot;</td>
<td>37</td>
<td>5816.0 ± 589.0</td>
<td>4</td>
<td>0.987</td>
<td>24.2</td>
</tr>
</tbody>
</table>

*Added as solutions of SnCl₂, H₂O, SnCl₂, or Me₂SnCl₂, respectively, to 10 mL P/T samples
*Estimated at 95 percent confidence level by the method in Ref. 51; "Ratio of regression slopes;
*d: In distilled, deionized water (18 MΩ·cm); "In Jones Falls, Baltimore Harbor water, pH ~ 8,
S²⁺/Sn < 1, pre-purged with zero N₂ (1 hr at 50 mL min⁻¹) before spiking with tin species.
solution, the latter approximating the salinity and ionic strength common to estuarine waters. Presumably, these effects could arise from formation of chlorohydroxy tin species favoring more rapid hydridization (cf. equations (5) and (7)) [5,32], as well as the more propitious partition coefficients for dynamic gas stripping of the volatile tin hydrides from saline solutions [12,46]. In the typical laboratory distilled water calibration solutions only 16 percent of Sn(II) was recovered as SnH₄, compared with Sn(IV), though this sensitivity ratio can probably be altered somewhat with pH changes [10,57]. However, in spiking anaerobic pre-purged Chesapeake Bay water with these three tin species, a striking reversal occurred in overall relative sensitivities, i.e., calibration slopes. We found that not only was Me₂SnH₂ generation repressed by 50 percent, but that, very significantly, SnH₄ formation from Sn(IV) was reduced by 15-fold, as compared with the NaCl medium. Production of SnH₄ from Sn(II) was decreased by 50 percent in going from NaCl to Bay waters. Clearly, dissolved or particulate substances in the Bay water, not involving Cl⁻ primarily, were responsible for severe interferences in the P/T-GC-FPD analyses of the three tin species, the most inhibitory effect being seen with the strongest Lewis acid, Sn(IV). Less diminution (about one-half) occurred with the weaker acids, Me₂Sn²⁺ and Sn(II), which either form less stable chlorocomplexes (vide supra) or are less likely to form strong bonds to ligands and substrates present in estuarine waters. The overall effect by estuarine water on the hydridization process is thus one of reducing yields of the three tin species tested. We expect that not only the dissolved and particulate organics and Cl⁻ influence formation of Sn-H bonds, but that other aquated metal ions play an important role, too. Several workers have reported that, for example, As(III), As(V), Cu(II), Co(II), Ni(II), Hg(II), Pb(II), and Ag(I) interfere by unknown means at low concentrations [10,59]. Several of these metals are commonly found in the polluted Baltimore Harbor waters (see table 1), and their presence may explain, in part, our results. In solutions of 0.01M HNO₃, we found that yields for Me₂Sn²⁺ reduction with NaBH₄ were consistent with the values obtained in distilled water and pre-purged Bay water. However, stannane yields from Sn(II) and Sn(IV) in 0.01M HNO₃ diminished considerably as a result of either insufficient trapping from rapid evolution of stannane caused by HNO₃ or
of a repressed reaction caused by HNO₃. In addressing the former consideration, cryogenic trapping would improve recovery of SnH₄ formed by hydride reduction.

In summary, the hydride generation method cannot adequately differentiate between aquated Sn(IV) and Sn(II) which may coexist in certain, especially anaerobic, environments found in marine waters. Previous reports [10,11] of inorganic tin, speciated as "tin(IV)," should probably be regarded as "total reducible inorganic tin" until more discriminatory techniques become available.

**OCCURRENCE AND FATE OF METHYLTINS IN ESTUARINE WATERS**

**Biomethylation of Tin in Sediments**

Recently, three groups have independently reported on the methylation of both inorganic tin and organotin substrates by the mixed populations of microbial flora present in sediments collected from a Canadian fresh water lake [7], and from estuarine sites in San Francisco Bay [8] and Chesapeake Bay [2,9,60]. Biogenesis of Me₄Sn was seen only to occur with additions of Me₃Sn⁺ to incubated sediments [7,8], but the redistribution reactions of intermediate methyltins to form Me₅Sn by non-biological pathways must be noted as competitive events in such experiments [4,8]. The concentrations of tin compounds added to incubated sediments were consistent with values found in polluted sediments (table 1). The influence of other bioactive pollutant metals also commonly found in such sediments was not investigated. We already noted that a pure culture of a metal-tolerant *Pseudomonas* species isolated from the Chesapeake Bay [35] methylates Sn(IV) in the presence of mercury, and subsequent abiotic demethylation of Me₅Sn(4-n)+ (n = 3,4) by Hg²⁺ occurs to give MeHg⁺ [6]. This reaction (equation (3)) and other possible demethylation routes (cf. table 3) may importantly influence methyltin distribution patterns emanating from sediments. It, therefore, becomes very important in future incubated sediment experiments to establish the relationship between biogenic Me₅Sn(4-n)+ distributions and the presence of Hg and other bioactive heavy elements.
Estuarine Degradation, Methylation, and Uptake of Man-made Organotins

Sediment incubation studies implied that commercial organotin biocides, particularly those used prevalently as marine antifoulants, could undergo transformations which replace existing R-Sn bonds with methyl groups [2,7]

$$(Bu_3Sn)_2O \rightarrow Me_3Sn^{aq}, \text{ and } (phenyl)_3SnOAc \rightarrow Me_3Sn^{aq}. \quad (8)$$

No evidence for intermediate products, $R_{3-n}SnMe_3^+$, though plausible, was given. In one sense, this is a desirable environmental biodegradation of spent man-made biocides according to equation (2) if such processes occur in estuaries. On the other hand, the extent (rate) of conversion of $R_3Sn^+$ to $Me_3Sn^+$ is unknown. Hence methyl-tins formed by release of $R_3Sn$ species from widely dispersed vessel antifouling paints cannot be discounted as an active pathway for uptake of physiologically active tin in local food chains. Preliminary evidence points to a bioaccumulation trend for total tin in bottom feeder mussels (Mytilus edulis) from vessel-related activities in Southern California harbors [61]. Thus, we see that bioconcentration of either man-made forms of organotins or biologically transformed organotins may occur to some degree by transfer from one trophic level to the next higher, as has been demonstrated for Hg(II) in a three compartment Chesapeake Bay food web comprising bacteria → ciliated protozoa → copepods [62]. Such studies involve determination of the species and the flux of organotins resulting from anthropogenic releases of organo-tin biocides into estuarine waters, as well as identification and assessment of the microbial flora responsible for uptake of bioactive tins into the primary trophic level of estuarine food webs.

We recently examined the form and extent of organotins leached into water from typical commercial marine antifouling paints [64] and a prototype organotin polymer formulation under development by the United States Navy as a long-term controlled release antifoulant. Such materials are regarded as sources of man-made organotin discharge into navigable waters, especially from ships at dock in harbors. In comparison, we also examined a typical blasting grit from a Chesapeake Bay shipyard where it was used to remove old or "spent" antifouling paint. Here, our aim was to speciate the chief
leachable forms of weathered or degraded organotins discharged into
sluice waters in the shipyard operations or directly into harbor
sediments from ships' hulls. Figure 6 depicts direct speciation of
such leachable organotins by the HPLC-GFAA method. In figure 6a we
see that the organotin polymer (OMP) controlled-release formulation
slowly releases both biocidal triorganotin moieties as free $R_3Sn^+$
species in water. This chromatogram was taken from leachates in
solution during the late, zeroth-order release rate exhibited by
this OMP. At this point, the major release toxicant is $Pr_3Sn$,
implying that mixed formulations of controlled-release agents may
discharge their ingredients at differing rates and thereby yield
variable toxic effects on target microfouling organisms and the
marine environment. Below, we will suggest that steady release of
these triorganotin cations may find rapid uptake on both viable and
dead cells in estuarine waters.

Figure 6b offers direct evidence for degradation of $Bu_3Sn$-
containing antifouling paints following field service on ships
hulls. We cannot ascertain yet if the degradation of original
$Bu_3Sn^+$ to $Bu_2Sn^{2+}$ occurred by purely chemical weathering forces
(light, oxidants, or marine chemicals), or by biological agents
such as bacteria. The obvious important consequence is that such
widespread use of these paints can discharge leachable forms of
organotins into estuarine harbor locales. The tri- and dibutyltin
leachates speciated here were present at 3 and 6 mg L$^{-1}$, respec-
tively. No evidence for other organotin ions, such as methylbutyl-
tin species was found [49].

Tin-resistant bacteria were recently estimated to represent 17
percent of the total bacterial population at nine diverse sites in
the Chesapeake Bay [2,9,60]. We have similarly isolated 11 $Bu_3Sn$-
resistant pure bacterial strains, including Pseudomonas 244 [6,25],
from four stations in Baltimore Harbor and the Bay [63]. All were
gram negative rods which grew on Nelson's agar [35] containing
50 mg L$^{-1}$ tin as $Bu_3SnCl$ or 100 mg L$^{-1}$ tin as Sn(IV). Tin uptake
was measured for nine of the isolates as a function of time: at time
zero $Bu_3Sn^+$ was added (10 mg L$^{-1}$ final concentration) to flasks
Fig. 6. Typical chromatograms obtained by tin-specific HPLC-GFAA employing a strong cation exchange column with isocratic MeOH-H₂O-NH₄OAc mobile phase at 1 mL min⁻¹ show separations of di- and triorganotins following direct injections (100 to 200 μL) of aqueous leachates obtained from: (a) a candidate Navy antifouling co-polymer of tri-n-butyltin and tri-n-propyltin methacrylates (1:1) [64]; and (b) water shaken with shipyard grits used for removal of weathered antifouling paints [49]. Areas under chromatographic "peaks" were accurately estimated by summing all individual GFAA peak heights comprising each HPLC "peak" shown [50]. The vertical-dashed "break" in (b) signifies a flow rate change to 1.5 mL min⁻¹.

containing starved cells in buffer (pH = 7.4) solutions with or without glucose (10 mM). Aliquots of cell suspensions were removed after 1.5 hr, filtered, rinsed, and digested in hot HNO₃ for total
tin analysis by GFAA. For 9 isolates, the total tin uptake as a dry weight concentration factor (mg per g cells/mg per mL medium) was $621 \pm 179$ (no glucose) and $602 \pm 274$ (glucose added). This suggests that Bu$_3$Sn$^+$ accumulation was not an energy-requiring process since glucose did not stimulate starved cells to greater tin uptake. For several isolates, aliquots of cells exposed to Bu$_3$Sn$^+$ solutions were collected at more frequent intervals. Generally, we found the rate of Bu$_3$Sn$^+$ uptake to be rapid, apparently reaching steady state after 2 hr. Cell-bound Bu$_3$Sn$^+$ was substantially removed by $1 \text{ mM}$ EDTA or methanol washes, and this procedure afforded a method for direct solution speciation by HPLC-GFAA of both the organotins taken up by cells and the residual organotins remaining in growth media. Typical results from the speciation work are illustrated in figure 7, where it was seen that virtually no biotransformation of original Bu$_3$Sn$^+$ into a logical degraded product, Bu$_2$Sn$^{2+}$ occurred. We also noted that killed whole cells accumulated almost twice as much Bu$_3$Sn$^+$ as live cells, suggesting that exclusion or volatilization of tin might be a mechanism of resistance and that cellular detritus in estuarine waters may be an important "sink" for organotins [63].

TETRAMETHYLTIN AND METHYLTIN HYDRIDES IN CHESAPEAKE BAY

During 1980 we surveyed unfiltered surface and bottom waters at sites in Baltimore Harbor and the Bay channel, selected for their respective decrease in anthropogenic tin influx (table 1) [2,20,21]. Employing the P/T-GC-FPD system in both modes, e.g., with or without NaBH$_4$ additions to replicate samples, we established [12] for the first time the presence of tetramethyltin in natural waters. We additionally demonstrated the unexpected presence of methyltin hydrides, Me$_n$SnH$_{4-n}$ ($n = 2,3$), also heretofore unknown in the environment. Main features and interpretation of the experimental results are illustrated by the chromatograms in figure 8.

Use of two modes with P/T analyses provides an effective tool for speciating both solvated or involatile Me$_n$Sn$^{(4-n)+}$ and volatile coordinatively saturated Me$_n$SnH$_{4-n}$ according to equation (1).
Fig. 7. HPLC-GFAA chromatograms showing speciation of cell-bound organotin on three examples of Chesapeake Bay tin-resistant bacteria are compared with a calibration run for 200 ng each of Bu3Sn and Bu2Sn species. The isolates were grown in Nelson's broth containing 10 mg L⁻¹ Bu3SnCl, harvested (10 mL) by ultra-centrifugation, and washed 3 times in distilled water. A methanol extract (100 µL) was injected directly into the HPLC-GFAA system.

No significant (95% confidence level) formation of dibutyltin degradation products resulting from cellular extracts (shown) or residual growth solution free of cells was observed. The highest GFAA peaks in the chromatograms represent off-scale values collected with the digitizer. Based on overall system sensitivity, biotransformation to Bu2Sn of 1% Bu3Sn in growth solutions or 3% of cell-bound Bu3Sn would have been detected [63].
Fig. 8. Application of P/T-GC-FPD system for speciation of organotins: (a) replicate calibration solutions containing Me$_2$Sn$^{2+}$ plus Me$_4$Sn run by Mode II (addition of NaBH$_4$) (top) and by Mode I (no addition of NaBH$_4$) (middle) are compared with blank run by Mode II; (b) representative samples from surface and bottom waters at Jones Falls sewage outfall (Baltimore Harbor), fresh (day 1) samples were stored at 2 to 4°C and run (Mode I) within 24 hr after collection, aged (day 7) sample was maintained at 2 to 4°C then run by Mode I.

Figure 8a depicts an example of such clearcut separation, and the future useful fact that Me$_4$Sn is recovered virtually quantitatively after BH$_4^-$ treatment. With Chesapeake Bay waters, several impor-
tant observations were therefore possible. In both surface and bottom unfiltered waters, significant differences in the involatile/volatile methyltin distributions were noted, though highly variable. In figure 8b, for example, one surface water sample from a heavily polluted urban runoff in Baltimore Harbor, comparison of replicate samples by the two P/T modes showed that both Me₄Sn and Me₂SnH₂ were present as volatile, whereas only "reducible inorganic Sn" and some BuSn₃⁺ were present as involatiles. Also in figure 8b, comparison of surface and bottom waters at this station shows characteristic increases in concentrations of Me₂SnH₂ and Me₂S₂ [12], and decreased Me₄Sn in bottom water.

We have noted that reproducible results were obtained only with water samples maintained at 2 to 4 °C and analyzed by P/T methods within 24 hr following collection. As figure 8b illustrates, samples kept refrigerated for seven days undergo substantial alteration with significant increases of Me₄Sn induced by either chemical or biological methylation, or both. These results presage lines of investigation to establish optimum sampling protocols for ultratrace aquatic organotin (organometal) speciation, as well as determining the mechanisms by which continued methylation of tin occurs in vitro.

In Chesapeake Bay, we generally found increasing methyltin concentrations with increased anthropogenic tin influx (table 1), in accord with Hallas' contention [2,60]. Concentration values obtained for individual organotin species often varied more than ten-fold within the water column at a given site and from visit to visit. We found much higher (10 X) organotin concentrations in Baltimore Harbor than in the Bay channel. The highest concentrations observed over nine months (spring to fall) occurred at Harbor locations with greatest organic loadings (Jones Falls), viz. Me₂SnH₂ (0.20 µg L⁻¹), Me₃SnH (0.40), and Me₄Sn (0.48); a maximum value of 0.93 µg L⁻¹ for Me₄Sn was obtained at Colgate Creek which is primarily an urban-industrial run-off site. We conducted a sustained three-week study on Baltimore Harbor in order to compare methyltin levels with those available for other American estuaries. The data are summarized in table 5.
Table 5. Methyltin in Estuarine Waters

<table>
<thead>
<tr>
<th>Site</th>
<th>SnH₂</th>
<th>MeSnH₃</th>
<th>Me₂SnH₂</th>
<th>Me₃SnH</th>
<th>Me₄Sn</th>
<th>Other</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baltimore Harbor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>nd⁴</td>
<td>nd⁴</td>
<td>&lt;0.005-0.02³</td>
<td>nd⁴</td>
<td>&lt;0.01-0.3⁰</td>
<td>0.05-0.10⁴</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>nd-4 ¹</td>
<td>nd⁴</td>
<td>&lt;0.005-0.02³</td>
<td>nd-4 ¹</td>
<td>&lt;0.005-0.01⁰</td>
<td>&lt;0.01-0.3⁰</td>
<td>0.05-0.3⁰</td>
</tr>
<tr>
<td>Bottom</td>
<td>nd⁴</td>
<td>nd⁴</td>
<td>&lt;0.005-0.02³</td>
<td>nd⁴</td>
<td>0.05-0.3⁰</td>
<td>nd-0.10⁵</td>
<td></td>
</tr>
<tr>
<td>San Diego Bay</td>
<td>0.80 ⁷</td>
<td>nd⁴</td>
<td>&lt;0.005-0.10⁴</td>
<td>nd-0.02 ⁷</td>
<td>nd-0.30 ⁷</td>
<td>nd-0.10⁶</td>
<td></td>
</tr>
<tr>
<td>Surface ⁴, ⁷</td>
<td>0.009-0.038</td>
<td>0.002-0.008</td>
<td>0.015-0.045</td>
<td>--</td>
<td>--</td>
<td>0⁸, ⁹</td>
<td>11</td>
</tr>
<tr>
<td>Florida Estuaries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface ⁴, ⁷</td>
<td>0.009</td>
<td>0.002</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>--</td>
<td>--</td>
<td>10</td>
</tr>
</tbody>
</table>

⁴Concentrations in µg L⁻¹ for analytes shown; ⁵Range for three collections during three weeks at two sites; ⁶Node 1, during three weeks at two sites; ⁷Node 1, no addition of NaH₂Sn; ⁸Node 1, addition of NaH₂Sn; ⁹Me₂SnH₂; ¹⁰Range for five sites collected over one day; ¹¹Me₃SnH; ¹²Average of eleven sites collected over various periods of time.
We did not detect MeSnH₃, either as an involatile or volatile species, in Baltimore Harbor. Presumably, from its reported presence in San Diego [11] and Florida [10] estuaries at ranges below 0.008 μg L⁻¹, it may have been present in the Bay waters below our P/T system detection limit (~0.005 μg L⁻¹). Though butyltins were not detected in San Diego Bay, the same workers [11] detected very high concentrations of BuSn³⁺ [aq] (1.22 μg L⁻¹) and Bu₂Sn²⁺ [aq] (1.60 μg L⁻¹) in surface waters of Lake Michigan which they ascribe to atmospheric inputs or possible contamination from PVC water samplers (cf. table 2). We found BuSn species regularly in our three-week study, both as volatile BuSnH₃ and solvated BuSn³⁺ [aq].

We saw a trend toward higher concentrations in surface water (table 5), though not as pronounced as in Lake Michigan. Our P/T methods employed in the present studies, did not reliably speciate Bu₂Sn²⁺ or higher homologs, but it seems more likely to us that such organotins will be anthropogenic and probably introduced from antifouling paints on vessels. More detailed studies on the distribution patterns of the entire sequence BuₙSn⁴⁻[n]⁺ (equation (2)) based on water movements and shipping patterns are now required to resolve this point.

Finally, we can estimate the ratio of organotins to total tin in the suspended particulates found in Baltimore Harbor surface water. Nichols observed [20] that unfiltered surface water from this locale contained 0.19 μg L⁻¹ (with 8.7 μg g⁻¹ Sn in filtered particulates) (table 1). Our estimates of total organotins, including methyl- and butyltins for Baltimore Harbor are represented by the low values of the ranges tabulated in table 5. Thus we derive <0.07 μg L⁻¹ as an upper sum which, in turn, implies that [(<0.07) x 100]/(0.19) = <37 percent of Baltimore Harbor surface water suspensions occur as organotins. This remarkable extent of bioactive tin materials seems large, and may reflect the deficiency of adequate field data at this time. In any event, both the means and needs to further explore this question are at hand [13]. In neither Nichols' [20] nor our present studies has the surface micro-layer been examined. This oleaginous film also supports amplified microbiological activity and amplifies concentrations of heavy metals [5, 28]. "Free" or volatile organotin species would be expected to partition into this lipophilic layer, there to be accumulated, transformed, and possibly released into the atmosphere.
CONCERNING AQUATIC METHYLATION AND HYDRIDIC METAL (TIN) CYCLES

From the foregoing discussion of our recent results and those from other laboratories, we see that tin joins a growing family of bioactive elements for which evidence of biological and chemical methylation supporting global transport cycles widens [5,64]. In large part, substantial advances in ultratrace molecular speciation methodology and accessible commercial equipment [17,45] have spurred many creative efforts to comprehend both the biological and chemical factors that govern the likelihood, forms, and lifetimes of such organotins in the aquatic environment. We can now reasonably ask what will be the shape of future advances, and what are the roadblocks we face?

Though exciting new biogenic organometals, such as the methyltin hydrides in Chesapeake Bay, will continue to be unexpectedly discovered, more rigorous and predictive considerations must guide future progress. Recognition [5,14,15,26,64] that many aquatic organometallic reactions occur by second-order, or at least pseudo first-order, kinetic pathways limits any selection of useful model reactions. Moreover, we cannot presume that the wealth of chemical literature for organometallic reactions, typically obtained at high concentrations in non-aqueous solvents, can be successfully translated to environmental action levels extant at \(<10^{-10}\) M in aqueous saline media (cf. table 5). With current speciation techniques of the sort described in this paper, the limits of detection of reaction products of model reactions is perhaps \(4 \times 10^{-11}\) M, i.e., 5 ng L\(^{-1}\) as MeSn\(^{3+}\). For example, in aqueous oxidative additions of MeI to Sn\(^{2+}\) or Pb\(^{2+}\) (table 3) we estimate \(k_2 < 10^{-3}\) M\(^{-1}\) s\(^{-1}\) [5,7,26,39]. Thus, for this tin methylation, starting with both reactants at \(10^{-6}\) M, kinetic measurements could begin after 11 hr during which time the reaction will have proceeded only 0.004 percent; starting with \(10^{-8}\) M reactants, product MeSn\(^{3+}\) could be detected after 0.4 percent reaction, but this would now require 12.7 years! Clearly, the need for careful selection of reaction conditions is paramount given the constraints of available detection limits for tin species or those of any other metal or metalloid.
We must also consider the thermodynamic bases upon which we assess relative populations or stability domains for aquatic ultra-trace metal-containing molecules. Figure 9 presents a highly simplified two-electron redox cycle for an element E in the chemical or biological synthesis (or degradation) of methyl- or hydrido-derivatives in an aquatic system. Here, either methyl carbanion (Me$^-$) or carbonium ion (Me$^+$) and hydride (H$^-$) or proton (H$^+$) can act as two-electron carriers in both enzymatic [65] or abiotic [15,26] reactions. The relative stability of a given element toward redox is indicated by the usual thermodynamic expression for $E = E_{\text{red}}$ shown as a vertical potential gradient. Approximate equilibrium concentrations for the respective species, viz. As(V) - As(III), Sn(IV) - Sn(II), or Hg(II) - Hg(0), can thereby be estimated, given appropriate environmental parameters of pH, $E_h$, pC1, etc. However, such calculations fall far short of approximating the true nature and concentrations of redox species in natural environments [52,55]. The relative concentrations of organometallic and inorganic species are greatly determined by non-equilibrium, catalytically induced kinetic processes which involve both chemical and biological factors [54].

Fig. 9. Basic features of aquatic synthesis of methyl- and hydrido-derivatives of elements (E), and their possible stabilities are summarized in a two-electron redox cycle.
For a methylation (electrons added, \(+\)) or a demethylation (electrons removed, \(-\)), as well as an analogous hydridization, the crucial knowledge about the stability or lifetime of such species formed is prerequisite to assessing the extent of redox. Beyond this, knowledge about lifetimes of such methylated/hydridized metal species provides guidance for designing relevant approaches to their speciation in active environmental compartments. For example, we expect methylation of mercury(II) to result in formation of relatively long-lived [5] MeHg\(^{aq}\) species which we can (and do) detect in marine waters, whereas any possible HgH\(^+\), HgH\(_2\)\(^0\), or MeHgH\(^0\) species are not expected [66] to survive sufficiently long for analysis (speciation), but eject H\(_2\) or MeH to form Hg\(^2+\) [36].

For many other metals and metalloids, such mobile metabolites can exist in natural waters for periods long enough for environmental speciation. Our discovery of methyltin hydrides, Me\(_n SnH\)\(_{4-n}\) (n = 2,3), in polluted Chesapeake Bay waters [12] is one good example, as was the detection of bacterial formation of methylarsines, Me\(_n AsH\)\(_{3-n}\) in soils [34]. At the concentrations involved, and in the presence of a multitude of potential reactants, we conclude at this point that such hydrides may possess aquatic lifetimes far longer than suggested by the meager literature available. To a great extent, future progress in this new and important area of trace metals in the sea will depend upon careful measurements of such species' lifetimes in saline solutions and characterization of the pathways for their disappearance.

ACKNOWLEDGEMENTS

Contributions from the National Bureau of Standards are not subject to copyright. Major portions of this work were supported by the Office of Naval Research, to whom we are grateful for sustained interest and encouragement. We thank the Chesapeake Bay Institute and Johns Hopkins University for providing ship time on the R/V Ridgely Warfield. Our colleagues, R. R. Colwell, J. J. Cooney, J. M. Belzama, L. E. Hallas, Y. K. Chau, K. L. Jewett, M. Nichols, and W. M. Coleman were generous with ideas and comments, and graciously shared unpublished data with us; we thank them for their invaluable help. Identification of certain commercial equipment or materials in this paper does not imply any endorsement of quality or recommendation by the National Bureau of Standards. Rather, such specifications are made only to adequately detail experimental procedures.
REFERENCES

8. H. E. Guard, A. B. Cobet, and W. M. Coleman, Methylation of trimethyltin compounds by estuarine sediments, submitted for publication.


38. G. E. Parris and F. E. Brinckman, unpublished results.


Ultratrace Speciation and Biogenesis of Methyltin Transport Species in Estuarine Waters.

F. E. Brinckman, J. A. Jackson, W. R. Blair, G. J. Olson, W. P. Iverson

Chemical and Biodegradation Processes Group
Chemical Stability and Corrosion Division
National Bureau of Standards, Washington, D.C. 20234

Approved for Public Release with unlimited distribution. Reproduction is permitted for any purpose of the United States Government.

Distribution of this document is unlimited.


Atomic absorption detector; biomethylation; Chesapeake Bay; estuarine organotin transport; flame photometric detector; gas chromatography; kinetics; liquid chromatography; methyltin hydrides; purge and trap sampling; redox; tetrathyltin.

Environmental tin, widely dispersed at low concentration in waters, sediments and biota, is bioactive element susceptible to methylation and even hydrolysis by marine bacteria. Nonetheless, the redox cycle of tin in natural waters is poorly understood and recent advances in tin-specific molecular characterization fail to speciate Sn(II) and Sn(IV) reliably. Rapid developments in speciation methodology now permit studies of organotin distributions in aquatic systems, raising the question of the "natural" biogeochemical flux of methylstannanes in relation to increased anthropogenic organotin influx from industry and shipping. New methods
Number 20 (continued) - Abstract

for direct speciation of aquated or involatile organotins by liquid chromatography are complimented by advances in purge-and-trap sampling of volatile organotins speciated by gas chromatography. Work from our laboratory indicates that models for estuarine formation and transport may ultimately be developed, but that basic roadblocks to progress stem from inadequate descriptive aqueous organometallic chemistry and knowledge of critical kinetic parameters for the lifetimes of key organotin species in sea water.