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Interactions of Tin(IV) and Monomethyltin Cation in Estuarine Water–Sediment Slurries from the Great Bay Estuary, New Hampshire, USA

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This study describes experiments on sediment–estuarine water slurries originating from a *Spartina alterniflora* salt marsh. We investigated the fate of tin(IV) or monomethyltin cation ($MeSn^{3+}$) chlorides after their additon to slurries under anaerobic and aerobic conditions. We did not observe methylation of tin in anaerobic or aerobic slurries with and without added tin(IV). $MeSn^{3+}$-amended samples occasionally formed small amounts of Me_2Sn^{2+} or Me_3Sn^{+} after extended periods of time, particularly when $MeSn^{3+}$ remained in solution. The stability of $MeSn^{3+}$ in slurries demonstrates that the absence of net methylation of tin(IV) is not due to rapid demethylation of $MeSn^{3+}$ or its further methylation. Inorganic tin concentrations in the aqueous phase of anaerobic slurries spiked with $MeSn^{3+}$ and unspiked slurries decreased by about 85% in 21 days and remained relatively constant until the end of the 59-day experiments. In similar anaerobic experiments about 25% of the $MeSn^{3+}$ spike was adsorbed to sediment within 1 h and about 75% was adsorbed within 10 days. The lack of methylation and demethylation reactions in our aerobic and anaerobic slurries, which contrasts with two previous reports, undoubtedly reflects the absence of added nutrients and low concentrations of added tin(IV) in our experiments. We believe that our model experiments more accurately reflect conditions in salt marshes than do previous studies. We conclude that future model studies on methylation of inorganic tin should include *S. alterniflora* because it is so prominent in observations of methyltin compounds in the estuary.

Keywords: inorganic tin; monomethyltin cation; model studies; salt marsh; sediment; *Spartina alterniflora*

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INTRODUCTION

Methyltin compounds are very common in freshwater and marine environments.[1,2] For example, we have determined methyltin compounds in the Great Bay Estuary, NH, USA, in water,[3] eelgrass,[4] porewater[5] and *Sparina alterniflora*, which had the highest concentrations.[5,6]

The source of estuarine methyltin compounds is not anthropogenic, but otherwise their origin is not known. It is not even clear whether methylation of inorganic tin is biotic, abiotic or a combination of both processes. Model studies show inorganic tin can be methylated by methyl iodide in porewater,[7] simulated estuarine water[8] or 0.1 M KCl.[9] Decaying macroalgae,[10] estuarine water[11] and live, hydroponically grown *S. alterniflora*[12] methylate tin compounds even in the absence of added methyl donors. Two studies in nutrient-and tin-amended estuarine sediment slurries[13,14] resulted in conversion of inorganic tin to monomethyltin cation ($MeSn^{3+}$) in low yields.

This study used sediments form an *S. alterniflora* salt marsh for several reasons. First, salt marshes, which have the fourth greatest area of any habitat within the Great Bay Estuary, are important as habitats, feeding areas and breeding grounds for many organisms.[15] Second, we have considerable experience and data on the Chapman's Landing area of the salt marsh with respect to its biogeochemistry[16,17] and the methyltin content of its *S. alterniflora* and porewater.[5,6] Third, although macroalgae are an important bio-

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mass in the salt marsh, healthy macroalgae adsorbed, but did not methylate or demethylate, tin compounds.[18]

This research tested aerobic and anaerobic salt marsh sediment slurries, composed of estuarine water and sediment, for methylation of $SnCl_4$ and demethylation of $MeSnCl_3$. Loss of inorganic tin and $MeSn^{3+}$ from the aqueous phase and adsorption on the sediment phase occurred over several days. We observed no methylation or demethylation reactions despite studies as long as 59 days, even after addition of inorganic tin at double the ambient concentration in one experiment. Methylation reactions in previous studies[13,14] are probably due to addition of nutrients to slurries. We believe that our studies are more representative of conditions in the salt marsh because only natural amounts of nutrients were in the slurries and we added less inorganic tin than researchers in previous studies.[13,14]

EXPERIMENTAL

Collection of marsh sediment and estuarine water

Sediment used in these experiments was collected in February (via a hole in the ice), April and June 1994 from Chapman's Marsh in Stratham, NH, USA. The April sampling (3 h before high tide) was before the spring growth of *Spartina alterniflora*, but it was 1 m high at the June sampling (2 h after high tide).The sampling site is located approximately 150 m downstream from the bridge in an area where the tall form of *S. alterniflora* is prevalent. Sediment of *ca* 900 cm^2 area and 18 cm depth was sampled with a shovel. A representative sample was transferred to a polyethylene container and transported back to the laboratory. The sediment was placed in a Waring stainless steel commercial blender (Waring, Model 31BL92) and homogenized on 'HIGH' for 1 min. The resulting sediment was homogeneous in both texture and color.

Estuarine water was collected on the same days as sediments from Chapman's Marsh. The collection site was about 10 m downstream from the bridge. A high-denisty polyethylene carboy was placed under the surface of water about 1 m deep, uncapped and allowed to fill. The water was collected during flood tide about 2.5 h before high tide. The salinity of the estuarine water was 8 ppt (parts per thousand) in April and 20 ppt in June.

Determination of inorganic tin and methyltin compounds (MeSn)

Concentrations of tin compounds were determined by hydride formation, chromatographic separation after trapping at liquid-nitrogen temperature, and atomic absorption spectrophotometric (ASS) detection. The method was developed by Donard *et al.*[3] and further modified by Francois and Weber[4] and by Weber *et al.*[6] One major difference from our previous papers was that the column was silanized with 5% (v/v) tri(isopropyl)chlorosilane (Farchan Laboratories Gainesville, FL, USA) in toluene[19] rather than with dimethyldichlorosilane. The new silanizing agent was superior because it gives excellent results for more runs than the original one. The second difference was that an air filter of silanized glass wool was placed in the outlet of the hydride generation flask to prevent uncentrifuged particles from sediment extracts from reaching the column and reducing sensitivity. The air filter was changed on a daily basis.

A brief description of the procedure follows. Approximately 40 ml water, 0.5 ml glacial acetic acid and an aliquot of standard or extract were added to the hydride generation flask. Acidic extracts were neutralized with Merck Suprapur 10 M NaOH in the hydride generation flask prior to the addition of $NaBH_4$. Tin compounds were volatilized as hydrides by addition of 2.5 ml of 6% $NaBH_4$ and trapped in a liquid-nitrogen-cooled Pyrex U-trap. As the trap was warmed, eluted compounds were atomized using an electrothermal quartz furnace, and detected with a Perkin–Elmer 503 atomic absorption spectrophotometer. Quantitation was based on standards run after about every six samples. Each extract was run at least twice on the AAS. Limits of detection (LODs) were based on background $+3\sigma$. LODs for inorganic tin and MeSn were 0.8 ng in anaerobic sediment extracts and 3 ng in aerobic sediment extracts. LODs in the aqueous phases were 0.6 ng for inorganic tin and 0.4 ng for $MeSn^{3+}$.

The concentration units of aqueous samples are ng Sn ml^{-1} and are listed as ng ml^{-1}. Sediment samples are in the units ng Sn per g dry sediment and will be written as ng g^{-1}.

Sediment ratios

Ratios of wet weight to dry weight and centrifuged wet weight were determined for later use in calculations of concentration of tin compounds.

The ratio of wet uncentrifuged sediment weight to dry sediment weight ($R_{uw/d}$) was determined in quadruplicate in the following manner. About 12–14 g (weighed exactly) of homogenized wet sediment samples were placed in preweighed aluminum weighing boats and wet weights were obtained. The sediments were dried for three days in an oven at 105 °C and dry weights obtained. The $R_{uw/d}$ value of 3.660 ± 0.0409 (1.12% RSD) was obtained for April sediment. The corresponding value for the June sample was 4.948 ± 0.0256 (0.517% RSD).

The ratio of centrifuged sediment wet weight to uncentrifuged sediment wet weight ($R_{cw/uw}$) was obtained in quadruplicate. Homogenized sediment samples were placed in preweighed polystyrene 15-ml conical centrifuge tubes and uncentrifuged wet weights (about 1–2 g, weighed exactly) were obtained. The tubes were centrifuged in an ICE clinical centrifuge at 3200 rpm for 15 min, the porewater was removed, and centrifuged sediment wet weights were obtained. A $R_{cw/uw}$ value of 0.800 ± 0.0451 (5.6% RSD) was obtained for the April sediment. Similar experiments for the June sediment gave a value of 0.5984 ± 0.0075 (1.25% RSD).

Slurry preparations

Slurries for all but one experiment were prepared in an approximate ratio of 6 g wet, homogenized sediment to 60 ml of estuarine water. (One experiment had a ratio of 0.6 g wet sediment per 60 ml estuarine water.) For April sediment samples about 13–16 g wet sediment (weighed exactly), depending on the amount of vegetative matter observed, was sieved through a size 40 mesh sieve with the help of 60 ml of estuarine water as a rinse and was collected. Since the roots, rhizomes, etc, removed during the sieving step weighed about 6–10 g, the net wet weight of sediment going into the slurry was approximately 6 g. The exact weight of the wet sediment was determined by subtracting the mass of residual vegetative matter from the orignal weight. Because some organic matter broke up during previous homogenization, some of it remained in filtered slurries. The resulting slurry was transferred to 100 ml glass serum bottles. (All glassware was cleaned by soaking at least overnight sequentially in two baths of 7% nitric acid.) Because the June sediment had very little vegetative matter, only *ca* 6 g (weighed exactly) was sieved.

The headspace of anaerobic slurries was flushed with nitrogen and then the bottles were quickly capped and sealed with Teflon-lined butyl-rubber septa and crimped with aluminium seals. These bottles were placed in a BBL Gas-Pak System airtight chamber that was flashed with nitrogen before closing it. In aerobic experiments the bottles were plugged with cotton. Serum bottles plus slurries were weighed in order to replace evaporated water with distilled water in the open aerobic slurries.

All slurries were shaken at 200 rpm and covered with a thick, black cloth to prevent photolysis of carbon–tin bonds. Slurries for anaerobic experiments were shaken for two days to allow them to become anaerobic before $MeSn^{3+}$ spikes and collection of Day-0 samples. Aerobic samples were also shaken for two days before spiking.

At Day 0, exactly 1830 ng per g dry sediment of $MeSn^{3+}$ was added to three of six anaerobic slurries. (The remaining slurries remained unspiked.) The slurries were vortexed immediately following injection in order to minimize bacterial shock. The Day 0 sampling was conducted immediately following this initial MeSn spike. At Day 49, a 1830 ng g^{-1} $MeSn^{3+}$ spike was injected into *all six* slurries based on the amount of sediment remaining at that time. Day-49 samples were collected after shaking for 1 h.

Slurry sampling and separation of aqueous and sediment phases

Anaerobic slurries were removed from the gastight chamber and were shaken vigorously to homogenize them. A volume of 4 ml nitrogen was added to the slurry via a sterile 5 ml plastic syringe with a 23-gauge 1.5-inch (3.8 cm) sterile needle and a 4-ml water–sediment aliquot was removed. The nitrogen is used to replenish the sample volume to be removed and prevent a partial vacuum in the vials. It was necessary occasionally to shake the bottle during sampling in order to maintain homogeneity. A new sterile syringe and needle were used for each slurry. The slurry aliquot was then transferred to a weighed centrifuge tube and the slurry sample weight was obtained by difference.

Slurry samples were centrifuged at 3200 rpm for 15 min. The supernatant aqueous phases were carefully removed using glass Pasteur pipettes, transferred to glass scintillation vials, and immediately acidified to 1 M H^+ concentration with 12 M HCl. The samples were refrigerated until

determination of tin compounds by AAS. The centrifuge tube plus centrifuged sediment was weighed and the weight of the centrifuge tube subtracted to obtain the weight of the centrifuged sediment, which was then extracted as described below.

In most respects aerobic slurries were treated in the same way as anaerobic slurries. One difference was that distilled water was added to replace evaporated water. In addition, aerobic slurry samples were capped to allow vigorous shaking and homogenization before sampling with a syringe having a larger (20-gauge) needle.

Extraction of inorganic tin and methyltin compounds from centrifuged sediments and estuarine water.

Ten milliliters of extractant (MeOH–1.5 M HCl, 2:1 v/v) was added per gram of centrifuged sediment. The samples were shaken violently to resuspend the centrifuged wet sediment and then vortexed for 30 s. The centrifuge tubes were placed horizontally on the shaker set at 250 rpm for 1 h to allow mixing of sediment and extractant. The samples were sonicated with a Branson sonicator (Model no. B-22) at 50/60 Hz and 40–50 °C for 1 h and were centrifuged at 3200 rmp for 15 min. The aqueous phase was transferred to glass scintillation vials using Pasteur pipettes and frozen until measurement of tin compounds by AAS.

Ambient concentrations of inorganic tin and MeSn in estuarine water were determined in quadruplicate. Samples of 5 ml were placed in glass scintillation bottles and acidified to 1 M H^+ concentration using 12 M HCl. Concentrations of tin compounds were determined immediately by AAS.

Recovery experiments for $MeSn^{3+}$ from estuarine water and sediment slurries

$MeSn^{3+}$ spikes of 1, 5, and 10 ng ml^{-1} were added in duplicate to 10-ml volumes of centrifuged estuarine water in scintillation vials. The samples were vortexed for 30 s, left in the dark for 1 h, and acidified to 1 M H^+ concentration with 12 M HCl. Linear regression analysis of added $MeSn^{3+}$ concentration vs measured $MeSn^{3+}$ concentration gave a $104 \pm 9\%$ recovery. No correction was made to measured aqueous-phase concentrations in later calculations.

$MeSn^3$ from sediment slurries was recovered on Day 0 from anaerobic slurries containing spikes of 1830 ng g^{-1}. Slurry aliquots were sampled, centrifuged and extracted as described above. Recoveries of $MeSn^{3+}$ were $54.9 \pm 3.2\%$. A consistent recovery of *ca* 55% $MeSn^{3+}$ was obtained from several slurries at different slurry ages. Sediment concentrations of $MeSn^{3+}$ in future calculations were corrected by the 54.9% factor.

Calculations

Step 1
Integrator areas were converted to ng by daily calibration curves for each tin compound analyte and divided by AAS sample valume to calculate ng ml^{-1} in extracts.

Step 2
Correction of aqueous-phase volume for dilution by HCl gives ng ml^{-1} in the aqueous phase. No corrections for extraction efficiency were necessary for aqueous phases.

Step 3
Total uncorrected ng of tin compounds in the sediment of 4-ml aliquots were obtained by multiplying the concentration in the extract (Step 1) by the volume of the HCl–MeOH extractant. The corrected mass of $MeSn^{3+}$ in wet centrifuged sediment was obtained by dividing the uncorrected mass by the 0.549 extraction efficiency. No correction was made for inorganic tin in the sediment phase.

Step 4
Dividing the mass (sometimes corrected) of tin compound in the sediment by the mass of the centrifuged wet sediment gave the concentration, in ng per g of centrifuged wet sediment.

Step 5
This concentration was multiplied by the sediment ratio values $R_{cw/uw}$ (centrifuged wet mass/uncentrifuged wet mass) and $R_{uw/d}$ (uncentrifuged wet mass/dry mass) to calculate ng per g of dry sediment.

RESULTS

Ambient concentrations of inorganic tin and methyltin compounds (MeSn)

In April samples, the inorganic tin concentration was 1660 ± 351 ng g^{-1} in sediments and was not measurable in estuarine water. The samples had

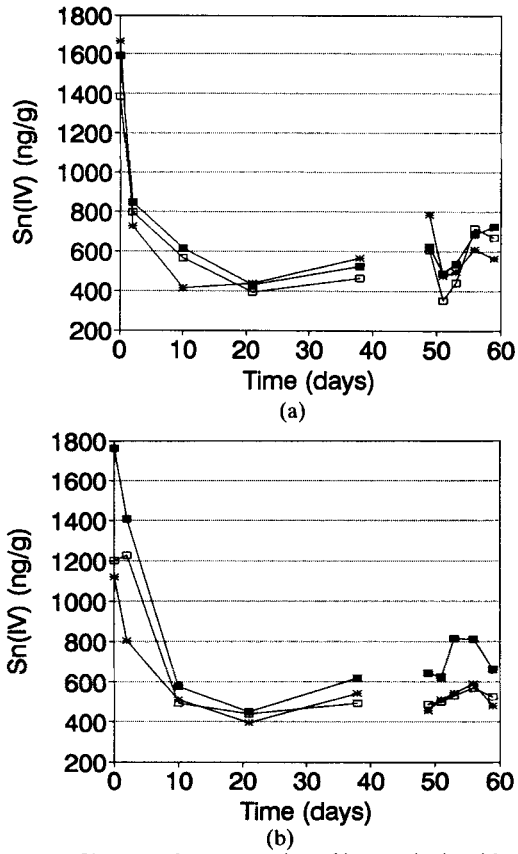

Figure 1 Changes of concentration of inorganic tin with time in sediment phases of anaerobic slurries (a) spiked with $MeSn^{3+}$ on Days 0 and 49, and (b) control slurries spiked with $MeSn^{3+}$ only on Day 49. Slurry code: no. 1 (■), no. 2 (□) and no. 3 (*).

no MeSn in the sediment and a very low 0.32 ± 0.20 ng ml^{-1} concentration of $MeSn^{3+}$ in the estuarine water. In June samples, the inorganic tin concentration was 1120 ± 200 ng g^{-1} in sediment and 7.6 ± 0.65 ng ml^{-1} in estuarine water. We did not observe $MeSn^{3+}$ in the sediment and its concentration was 1.57 ± 0.12 ng ml^{-1} in estuarine water. The February sediment sample had 3960 ± 176 ng g^{-1} inorganic tin. We did not find Me_2Sn^{2+} or Me_3Sn^{+} in any sample.

Anaerobic experiment with $MeSn^{3+}$ spikes

The figures show changes in concentrations of inorganic tin in sediment (Fig. 1) and aqueous (Fig. 2) phases and of $MeSn^{3+}$ in the sediment (Fig. 3) and aqueous (Fig. 4) phases of six slurry samples (April sediment). The (a) figures represent triplicate slurries spiked with $MeSn^{3+}$ at 1830 ng g^{-1} on Days 0 and 49, while the (b) figures represent triplicate slurries that were unamended until spike with $MeSn^{3+}$ on Day 49. All concentrations given in the following description of the figures are *average* concentrations of triplicate slurry samples.

Figures 1(a) (slurries spiked at Days 0 and 49) and 1(b) (slurries spiked only on Day 49) describe inorganic tin concentration changes in sediment phases over the 59-day experiment. The spiked experiment and slurries unamended until Day 49 showed decreasing inorganic tin concentration in sediment phases from 1450 ng g^{-1} at Day 0 to 531 ng g^{-1} at Day 10. From Day 10 to Day 39 the inorganic tin concentration in sediment phases was approximately constant. The second 1830 ng g^{-1} $MeSn^{3+}$ spike in all six slurries at Day 49 did not cause appreciable change in inorganic tin concentration in any sediment phase up to the final sampling at Day 59.

Figure 2 shows changes in aqueous-phase inorganic tin concentration in the same six slurries described in Fig. 1 for sediment phases. Figures 2(a) (twice-spiked slurries) and 2(b) (once-spiked slurries) show that inorganic tin concentration decreases in the aqueous phase from an average of 18 ng ml^{-1} at Day 0 to 2.4 ng ml^{-1} at Day 21. The inorganic tin concentration was nearly constant from Day 21 to Day 38 and, despite some outliers, was little changed by the second $MeSn^{3+}$ spike from Day 49 to Day 59.

Figure 3 describes changes in $MeSn^{3+}$ concentrations in the sediment phases of six slurries described for Fig. 1. Figure 3(a) from Day 0 to Day 38 shows that 1340 ng g^{-1} of the 1830 ng g^{-1} $MeSn^{3+}$ spike quickly went to the sediment phase and remained there. The initial part of Fig. 3(b) reflects the absence of significant $MeSn^{3+}$ in the original sample. The second 1830 ng g^{-1} $MeSn^{3+}$ spike on Day 49 increased the $MeSn^{3+}$ sediment phase concentration by about 1400 ng g^{-1} in both the slurry samples, i.e. spiked (Fig. 3a) and unspiked (Fig. 3b) at Day 0. Thus the sediment phase rapidly adsorbed about 73% of the first $MeSn^{3+}$ spike and about 76% if the second one.

Figure 4 describes the fate of $MeSn^{3+}$ in the aqueous phase. Figure 4(a) shows that for the first $MeSn^{3+}$ spike the Day 0 concentration of $MeSn^{3+}$ in the aqueous phase was 15 ng ml^{-1}, which was 24% of the average 61.1 ng ml^{-1} (1830 ng g^{-1}) spike added 1 h earlier. The average $MeSn^{3+}$ concentration decreased to 4.1 ng ml^{-1} at Day 2 and to 1.9 ng ml^{-1} at Day 10. From Day 21 to Day 38, $MeSn^{3+}$ concentration was 0.6 ng ml^{-1},

which is similar to the $MeSn^{3+}$ concentration of *ca* 0.3 ng ml^{-1} in unspiked samples (Fig. 4b) from Day 0 to Day 38. The second $MeSn^{3+}$ spike (Fig. 4a), averaging 59.6 ng ml^{-1}, gave a concentration of 29 ng ml^{-1} at Day 49 1 h after the spike. Thus 49% of the second spike remained in the aqueous phase for 1 h. In both the previously spiked samples (Fig. 4a) and previously unspiked samples (Fig. 4b) about 85% of the spike disappeared from the aqueous phase in two days. (The high concentration for the Day 49 spike in slurry no. 3 (Fig. 4b) results from basing the amounts of spikes on g dry weight of sediment; sample 3 has more sediment than the other five samples.) In Fig. 4(a) the previously spiked sample had a residual $MeSn^{3+}$ concentration of *ca* 4.9 ng ml^{-1} at Day 59. In the sample spiked for the first time (Fig. 4b), two of three samples had residual 0.6 ng ml^{-1} $MeSn^{3+}$ concentrations, while slurry no. 3 had a 2.3 ng ml^{-1} $MeSn^{3+}$ concentration.

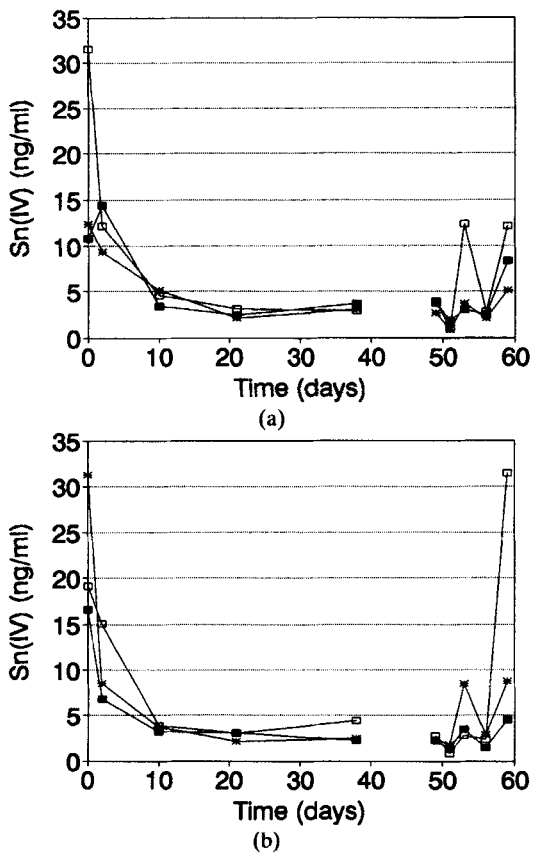

Figure 2 Changes of concentration of inorganic tin with time in aqueous phases of anaerobic slurries (a) spiked with $MeSn^{3+}$ on Days 0 and 49, and (b) control slurries spiked with $MeSn^{3+}$ only on Day 49. Slurry code: no. 1 (■), no. 2 (□) and no. 3 (*).

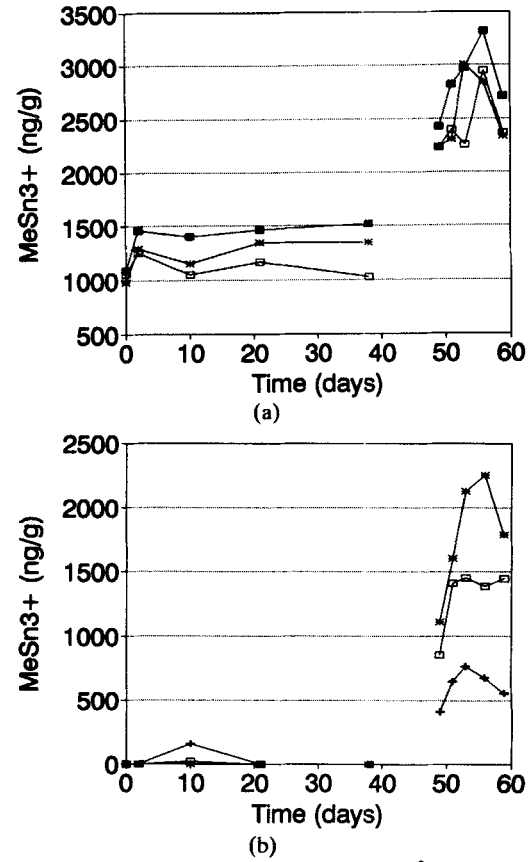

Figure 3 Changes of concentration of $MeSn^{3+}$ with time in sediment phases of anaerobic slurries (a) spiked with $MeSn^{3+}$ on Days 0 and 49, and (b) control slurries spiked with $MeSn^{3+}$ only on Day 49. Slurry code: no. 1 (■), no. 2 (□) and no. 3 (*).

Although neither Me_2Sn^{2+} nor Me_3Sn^{+} occurred in ambient estuarine water of sediment samples, Me_2Sn^{3+} appeared sporadically in anaerobic slurries spiked with $MeSn^{3+}$ as described above. For example, Me_2Sn^{2+} had a concentration of 9.2 ± 4.6 ng ml^{-1} in two of three once-spiked Day 53 sediment phases, but was below the level of detection in the four other slurries. On the same day Me_2Sn^{2+} had a concentration of *ca* 0.2 ng ml^{-1} in the aqueous phases of five of six slurries.

Anaerobic experiment with low sediment/estuarine water ratio

This experiment with July sediment contains a 10-fold lower sediment/water ratio than all other experiments. Three of six slurries were spiked

with $MeSn^{3+}$ at 30 ng per ml estuarine water. Samples taken at Day 0 (1 h after spiking), Day 1 and Day 2 showed that about 90% of the $MeSn^{3+}$ was in the sediment phase and that total $MeSn^{3+}$ was constant.

Aerobic experiments

We designed an aerobic experiment (June sediment) that consisted of three unspiked slurries with rapid sampling over two weeks. Total inorganic tin remained constant throughout the experiment, but only 25% of the inorganic tin initially in the aqueous phase remained there at Day 13.

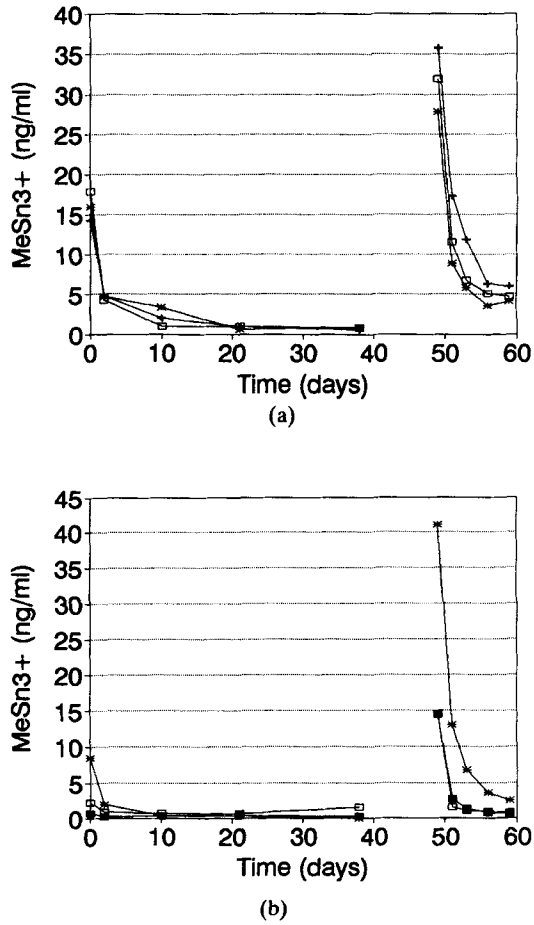

Figure 4 Changes of concentration of $MeSn^{3+}$ with time in aqueous phases of anaerobic slurries (a) spiked with $MeSn^{3+}$ on Days 0 and 49, and (b) control slurries spiked with $MeSn^{3+}$ only on Day 49. Slurry code: no. 1 (■), no. 2 (□) and no. 3 (*).

DISCUSSION

Adsorption and reactivity of monomethyltin in sediment slurries

$MeSn^{3+}$ added to sediment slurries was rapidly lost from the aqueous phase (Figs 4a, 4b). For example, loss of $MeSn^{3+}$ from solution at Day 0 (Fig. 4a) was extremely rapid (76% removal in 1–2 h) while the bulk of the remaining $MeSn^{3+}$ was removed in fewer than 10 days. Donard and Weber[20] reported that 83–100% of $MeSn^{3+}$ was adsorbed in 12 h from simulated estuarine water by hydrous iron oxides and by hydrous iron oxides coated with fulvic acid. Under the same conditions Me_2Sn^{2+} (28–66%) and Me_3Sn^{+} (15–28%) were considerably less effectively adsorbed. In the current study the *ca* 100% recovery of Me_2Sn^{2+} and Me_3Sn^{+} spikes from sediment slurries, compared with the approximately 55% recovery of $MeSn^{3+}$, reflects the strength of $MeSn^{3+}$ adsorption to estuarine sediments and parallels our previous results with hydrous iron oxides.[20] Dai *et al.*[21] also showed in model experiments that Me_2Sn^{2+} was adsorbed onto sedimentary particles. Its percentage adsorption was highest at pH 6 and decreased with increased salinity. These adsorption phenomena are important for determining the bioavailability of tin compounds for biota, and for their biological or chemical transformation in the environment.

Our experiments clearly showed that $MeSn^{3+}$ adsorbed to sediments where it remained virtually inert for at least several weeks. Concentrations of $MeSn^{3+}$ adsorbed to sediment did not appreciably change after the first spike (Fig. 3a). Slurries that were initially spiked on Day 49 (Fig. 4b) exhibited behavior that was similar to those which were spiked at Day 0; $MeSn^{3+}$ was removed almost completely from solution in about 10 days and the bulk of that $MeSn^{3+}$ was found on the sediments (Fig. 3b). Hence, aged slurry sediments adsorbed $MeSn^{3+}$ as readily as fresh material did. In slurries that received a second spike of $MeSn^{3+}$, a portion of the $MeSn^{3+}$ remained in the aqueous phase even after 10 days (Fig. 4a). The probable reason is that the first spike partially occupied adsorption sites on sediments, resulting in a higher concentration of $MeSn^{3+}$ in the solution phase after the second spike.

We rarely observed Me_3Sn^{+} in slurries, but sporadically found Me_2Sn^{2+} in aqueous phases. Slurries spiked with $MeSn^{3+}$ on Day 0 formed no Me_2Sn^{2+} before the second spike, and slurries

initially spiked on Day 49 formed measurable Me_2Sn^{2+} in only four of fifteen aqueous phases over the next 10 days. Three of four aqueous phases containing Me_2Sn^{2+} were in slurry no. 3, in which low concentrations of $MeSn^{3+}$ persisted in solution for the duration of the experiment (Fig. 4b). Behavior was different with the second $MeSn^{3+}$ spike at Day 49. Concomitantly with the presence of $MeSn^{3+}$ in the aqueous phase (Fig. 4a), low concentrations of Me_2Sn^{2+} occurred in 14 of 15 aqueous-phase samples. It seems clear that the small extent of methylation of $MeSn^{3+}$ to Me_2Sn^{2+} required that $MeSn^{3+}$ remain in solution where it was more biologically and/or chemically available, and that the nearly quantitative removal of $MeSn^{3+}$ by sediments prevented this activity. Either biological or chemical processes could explain the formation of $MeSn^{3+}$. Makkar and Cooney[22] demonstrated methylation of $MeSn^{3+}$ by a co-culture of bacteria isolated from estuarine sediments. A chemical redistribution process could also convert $MeSn^{3+}$ into Me_2Sn^{2+} (Eqn [1]),

$$2\,MeSn^{3+} \rightarrow Me_2Sn^{2+} + Sn(IV)^{4+} \qquad [1]$$

but this reaction did not occur during model studies in simulated estuarine water[8] or in estuarine porewater.[7] Any ligand that binds aqueous tin(IV) or an anion that precipitates it would drive Eqn [1] to the right and increase demethylation of $MeSn^{3+}$.

Me_2Sn^{2+} occasionally appeared in sediment phases in our experiments. However, adsorbed Me_2Sn^{2+}, like $MeSn^{3+}$, did not appear until Day 38 or later, indicating that Me_2Sn^{2+} was not sigificantly produced early in the incubation. Formation of Me_2Sn^{2+} probably rquired that $MeSn^{3+}$ remain in solution for a certain period of time, a simulation that occurred after the second spike.

Total corrected recovery of $MeSn^{3+}$ spikes from aqueous plus sediment phases at three times during the experiment was $103 \pm 21\%$. In addition the Me_2Sn^{2+} formed was less than 0.5% of the $MeSn^{3+}$ added. It is, therefore, unlikely that significant demethylation or methylation of $MeSn^{3+}$ occurred. Clearly the bulk of the $MeSn^{3+}$ added to slurries was removed by adsorption onto sediments (Figs 3a, 3b, 4a, 4b). We would, however, have had difficulties in directly measuring minor $MeSn^{3+}$ decomposition due to errors in its measurement. In addition, indirect measurement of minor $MeSn^{3+}$ loss by increases in inorganic tin concentration would have been difficult because of our inability to determine all inorganic tin present as the incubations progressed (Figs 1a, 1b) in anaerobic slurries (discussed below).

We know of no examples of aerobic or anaerobic demethylation of $MeSn^{3+}$, but results of $MeHg^+$ studies in sediment slurries both agree with and dispute the lack of demethylation of $MeSn^{3+}$ in our experiments. $MeHg^+$ is demethylated anaerobically in sediments.[23] In contrast, recent work on freshwater slurries[24] confirms that $MeHg^+$, like $MeSn^{3+}$, is adsorbed quickly on sediments, but not demethylated.

Absence of methylation of inorganic tin

Tin-amended (twice the ambient concentration) anaerobic slurries as well as anaerobic and aerobic slurries without added inorgaaic tin failed to accumulate detectable quantities of $MeSn^{3+}$ or other methyltin species. This lack of net methylation differs from previous studies by Gilmour *et al.*[13] and Hallas *et al.*[14] in which anaerobic estuarine sediment slurries and cultures isolated from sediments produced methyltin compounds, especially $MeSn^{3+}$. A first major difference between the experiments reported here and those of others is that other researchers amended sediments with culture media to stimulate bacterial activity. In contrast, our slurries were simply sediments diluted in estuarine water. Even though nutrient media were added to sediments from Baltimore Harbor, more than 40 days were required to convert 0.007–0.019% of added inorganic tin to $MeSn^{3+}$ (*ca* 0.5–8 ng ml^{-1}).[13] It is possible that the salt-marsh slurries in our experiments were not capable of producing measurable methyltin without nutrient supplements. However, our slurries were extremely bacterially active, as evidenced by the rapid accumulation of sulfide. In addition, rates of sulfate reduction in these sediments are very rapid.[16,17] A second reason is that other researchers added at least 100-fold more inorganic tin than our approximately 100 ng Sn ml^{-1} (as $SnCl_4$); in most experiments we did not add any inorganic tin. It is clear that our experiments more closely represent estuarine marsh conditions than those of past workers.

Our data from experiments with added $MeSn^{3+}$ indicate that the vast majority of added $MeSn^{3+}$ was transformed only by adsorption to sediments (Figs 4a, 4b). Therefore, it is possible that only small amounts of methyltin produced in our slur-

ries could be transient due to rapid demethylation. The active sulfate-reducing community in the present sediments may be equally involved in demethylation and methylation, yielding no observable net change. These sulfate-reducing bacteria in sediments are important methylators of mercury(II)[25–27] and demethylators of $MeHg^+$.[23] One could envisage a situation where the addition of growth substrates in bacteriological media in previous experiments[13,14] could inhibit the oxidative demethylation of methyltin species causing them to accumulate. In slurries unamended by nutrients like those used in current experiments, oxidative demethylation by sulfate reducers may remain as a viable energy-yielding pathway since easily decomposed substrates such as glucose or yeast extract were not added. This pathway could demethylate small amounts of methyltin in a process undetectable by us due to experimental error.

Another possible explanation for the lack of methyltin accumulation was that the bulk of the inorganic tin rapidly precipitated as a tin sulfide which was unavailable for methylation. However, it seems logical that in other studies of tin methylation by estuarine sediments in which growth media were added, sulfide production would be at least as prevalent as in our slurries. Nonetheless, it appeared that our salt-marsh sediments, at least when incubated as slurries, do not accumulate MeSn. This result leaves open the question of the source of $MeSn^{3+}$ and occasionally other methyltin species present in the tissues, especially roots, of *S. alterniflora*[5] from the same site as the samples in this study.

Aerobic slurries also failed to accumulate methyltin. The lack of methyltin in aerobic slurries was not surprising since tin methylation seems to be restricted largely to anaerobic environments,[13,14] and aerobic habitats tend to be sites of active demethylation of metals.[23,28] One reason for studying the aerobic slurries was that the salt-marsh rhizosphere contains a complex array of redox gradients due to the growth of roots and rhizomes and the ability of the plants to deliver oxygen below the sediment surface.[29] Slurries which maintain strict anoxia or oxia probably do not mimic the marsh rhizosphere, which undergoes continuous changes in redox conditions due to diurnal and tidal changes. It may be necessary to conduct experiments that periodically change redox conditions in vessels to determine fully the role of the marsh rhizosphere as a source or sink of methyltin.

Loss of inorganic tin in slurries

We were unable to recover inorganic tin present in anaerobic slurries reproducibly. This was evident as a *ca* 75% decrease in the measurable levels of inorganic tin over 10 days (Figs 1a, 1b). The difficulty of recovering inorganic tin occurred only in anaerobic slurries and did not occur in aerobic slurries, where we were able to account for all of the inorganic tin found at Day 0. We did not search for other methods to recover inorganic tin quantitatively since our goal was to study the production and transformation of methyltin. However, it is interesting that the inorganic tin entered a phase that was not extractable by the acid treatment employed. Since the loss of inorganic tin did not occur in aerobic slurries, the formation of a highly insoluble tin phase only in anoxic slurries was responsible for the decrease in extractable inorganic tin. To our knowledge the production and solubility of tin–sulfur compounds in sediments has not been studied in any detail. It would be reasonable to expect oxidized tin(IV), perhaps $Sn(IV)S_2$ or $Sn(IV)O_2$, in aerobic slurries and reduced tin as Sn(II)S in anaerobic ones. Because Sn(II)S is much more soluble than $Sn(IV)S_2$ in HCl,[30] formation of Sn(II)S in anaerobic slurries does not explain the difficulty in extracting inorganic tin. A more reasonable explanation is the presence of a tin(II) polysulfide [$Sn(II)S_n$], particularly $Sn(II)S_2$. Polysulfides, which are involved in sulfur cycling in marine sediments,[31] are exemplified by $Fe(II)S_2$ (pyrite) which is insoluble in HCl. The occurrence of $Fe(II)S_2$ in marine sediments suggests the possible presence of $Sn(II)S_2$, which may be difficult or impossible to extract with non-oxidizing acids.

CONCLUSIONS

A major reason for the use in this study of sediments from a *Spartina alterniflora* salt marsh in the Great Bay Estuary is that these plants are intimately involved in the production of methyltin (MeSn) in the estuary. A seasonal study[5] of *S. alterniflora* consistently found $MeSn^{3+}$ in roots, leaves, rhizomes and surrounding porewater in the concentration order: roots > leaves ≈ rhizomes ≫ porewater. In laboratory model studies, hydroponically grown live *S. alterniflora* plants convert $SnCl_4$ added to the nutrient solution into Me_3S^+ in leaves,[12] and decaying *S. alterniflora* in estuarine water adsorbs added $SnCl_4$

and rearranges MeSn initially present in leaves.[11] The absence of methylation of inorganic tin in the sediment slurries of this study reinforces the idea that *S. alterniflora* are important in the methylation process and suggest that sediments alone cannot effect methylation. Future model systems for methylation of inorganic tin in sediments should include *S. alterniflora*. These model systems of estuarine samples should be unmodified or changed very little in order to relate the results back to the salt marsh better.

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