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Model reactions for abiotic mercury(II) methylation: kinetics of methylation of mercury(II) by mono-, di-, and tri-methyltin in seawater

Gabriella Cerrati, Michael Bernhard and James H. Weber*
ENEA, Centro Ricerche Ambiente Marino S. Teresa, PO Box 316, I-19100 La Spezia, Italy

The usual presence of mercury(II) with mono-, di-, and tri-methyltin in water, sediments, and plants in estuarine environments suggests possible abiotic formation of methylmercury via methyl transfer from methyltin compounds. Kinetics studies of reactions between mercury(II) and methyltin compounds under pseudo-first-order conditions in seawater show that relative rates of methylmercury formation under the same conditions are: monomethyltin > trimethyltin > dimethyltin. This order is explainable mainly by the speciation and charge of methyltin compounds in seawater and by the existence of mercury(II) as a tetrachloro anion. A factorial experiment with the variables pH and salinity (seawater diluted with deionized water) showed that pH, but not salinity, is significant at the 95% confidence level; and that reaction rates increase as pH increases. These results suggest the possibility of abiotic methylation of mercury(II) in seawater. Additional experiments in seawater demonstrated an absence of methylation of mercury(II) (14 days) and mercury(0) (35 days) by methyl iodide.

Keywords: methylmercury, methyltin, kinetics, abiotic, methylation

INTRODUCTION

Since the confirmation that Minamata disease results from methylmercury (MeHg) poisoning, researchers have recognized that MeHg contamination of food chains leading to man is a potential health hazard. Scientists have identified both point sources of mercury contamination and natural mercury sources, especially in the mercuriferous Mediterranean–Himalaya and circum-Pacific belts.[1] Regulations have greatly reduced mercury contamination from point sources. Recently, new concern has resulted from the large number of lakes in Scandinavia and North America, uncontaminated by point sources, that contain fish with high MeHg concentrations.[2,3] In these lakes 50–90 % of total mercury in fish is MeHg.

Numerous experiments suggest that sediments are major sites of methylation of inorganic mercury [Hg(II)] and that microbiological processes contribute to the methylation.[2,4] Model experiments under anaerobic conditions with specific inhibitors indicate that sulfate-reducing bacteria are the main Hg(II)-methylators[5] and MeHg-demethylators[6] in estuarine environments. However, the specific environment needed for sulfate-reducing bacteria (sufficient but low sulfate concentration) to produce MeHg makes it difficult to explain the ubiquitous occurrence of MeHg in aquatic biota, especially in pelagic organisms far from the coast, and in deep-sea sediments. In addition, the high concentrations of Hg(II) added to samples[2] allows only mercury-resistant species to survive and it is doubtful that these laboratory experiments demonstrate *environmental* methylation of Hg(II) in sediments and water by sulfate-reducing bacteria. For these reasons there is a strong possibility that abiotic methylation of Hg(II) contributes to the formation of MeHg.

The ubiquitous presence of Hg(II),[1,2] and $MeSn^{3+}$, Me_2Sn^{2+} and Me_3Sn^{+} (collectively called MeSn),[7] in marine environments is one indication that MeSn might transfer methyl from MeSn to Hg(II). The concentration of total mercury in seawater is typically 0.001 ng cm^{-3} and in marine and estuarine sediments it is <50–5000 ng g^{-1} dry weight.[1,2] Similarly, MeSn occur in the water, sediment and biota of estuaries at widely varying

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* Author to whom correspondence should be addressed at his permanent address: Chemistry Department, University of New Hampshire, Durham, NH 03824-3598, USA.

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concentrations. For example, our group has determined MeSn concentrations in water,[8] sediment,[9] oysters,[10] macroalgae,[11] eelgrass[12,13] and *Spartina alterniflora*[14] of the Great Bay Estuary (NH, USA). Sometimes MeSn concentrations (as Sn) are below the *ca* 1 ng detection limit of the atomic absorption spectrometry–hydride generation method,[15] but typical concentrations of MeSn[8] are 0.01–0.2 ng cm^{-3} in water, 10–130 ng g^{-1} in sediment, and 1–50 ng g^{-1} in biota.

The presence of Hg(II)[1,2] and MeSn[7] in estuarine and marine waters and other compartments of estuaries and oceans suggests the possibility that MeSn can methylate Hg(II) to MeHg in estuaries. MeSn compounds are unlikely to be anthropogenic pollutants and are probably formed within estuaries by methylation of inorganic tin compounds. Several model studies by our own group suggest the feasibility of methylation of inorganic tin to MeSn in estuaries. For example, methyl iodide (MeI) methylates Sn(II) in 0.1 mol dm^{-3} potassium chloride solution (KCl),[16] simulated seawater[17] and estuarine porewater.[18] In addition a dimethylcobalt compound, which is a synthetic model compound for methylcobalamin, methylates Sn(II) in sediments.[19]

Model methylation studies of Sn(II) are an excellent conceptual basis for the present studies of methylation of Hg(II) by MeSn in seawater. Very little is known about this potentially important methylation process. Howell *et al.*[20] studied methylation of Hg(II) by MeSn (including Me_4Sn) in 0.59 mol dm^{-3} NaCl using ^{199}Hg and ^{119}Sn NMR and polarography to detect reactants and products. NMR confirmed methyl transfer from Me_3Sn^+ toHg(II) by identifying the Me_2Sn^{2+} and MeHgCl products. In addition, polarography demonstrated a *ca* 85 % methyl transfer from Me_3Sn^+ to Hg(II) in two days, but the researchers reported no kinetics data. Bellama and co-workers[21] measured second-order rate constants of *ca* 13–37 dm^3 mol^{-1} h^{-1} for methylation of Hg(II) by Me_3Sn^+ in different media containing sodium chloride (NaCl) and sodium perchlorate ($NaClO_4$), but they did no reactions in seawater and their highest NaCl concentration was 0.076 mol dm^{-3}.

Our paper differs from the two mentioned above by emphasizing a careful study of the kinetics of methylation of Hg(II) by $MeSn^{3+}$, Me_2Sn^{2+} and Me_3Sn^+ at low concentrations in 100 % seawater and 50 % seawater at pH values ranging from 4.5 to 8.0. This work includes experiments of factorial design, which distinguish the effects on the reaction rates of pH (significant at the 99 % confidence level) and salinity (insignificant). Our major finding is that, contrary to typical organometallic reactivity patterns, $MeSn^{3+}$ is the fastest methyl donor, and that reaction rates increase as pH increases. These results are encouraging for future studies of abiotic methylation of mercury(II).

EXPERIMENTAL

Materials and reagents

All chemicals used were analytical grade, were obtained from commercial suppliers, and required no further purification. All water used was deionized. Mono-, di-, and tri-methyltin chlorides were purchased from Alfa, and their stock solutions (*ca* 25 μmol cm^{-3}) were made in 0.05 mol dm^{-3} nitric acid. Tetramethyltin was purchased from Aldrich. A solution of 3.04 mg cm^{-3} $HgCl_2$ (as Hg) containing $^{203}HgCl_2$ (specific activity 9.25 MBq per mg Hg) at pH 1.24 in hydrochloric acid (HCl), which was purchased from Amersham (UK), had an activity of 28.1 MBq cm^{-3} on 8 August 1991. The stock solution was diluted to 0.304 mg Hg cm^{-3} with water. Seawater, collected on 15 January 1990 near to the shore at ENEA (S. Teresa, Italy), had pH 8.25, a salinity of 37.2 and was filtered through a bed of 0.3–0.8 mm silica sand. For some experiments the seawater was diluted 1:1 (v/v) with deionized water to make 50 % seawater.

MeHgCl containing $Me^{203}HgCl$ was synthesized[22] by adding 0.5 cm^3 of aqueous $^{203}HgCl_2$ (9.3×10^3 Bq) and 0.25 cm^3 of 0.040 mol dm^{-3} Me_4Sn in methanol to 5 cm^3 of 1.2 mol dm^{-3} HCl. The mixture was heated at 100 °C for 10 min, cooled, extracted five times with 1 cm^3 toluene and stored in toluene.

Extractions

Radioactive $^{203}HgCl_2$ and $Me^{203}HgCl$ were separated by the following extraction scheme and counted for 1 min by a LBK Wallac 1282 Compu Gamma Universal Gamma Counter. Although both radioactive and non-radioactive forms of mercury occur in all solutions, only the radioactive form will be mentioned for simplicity. In the first step, 1 cm^3 of saturated aqueous KCl that was 1.2 mol dm^{-3} in HCl, and 1 cm^3 toluene were added to 1 cm^3 of seawater sample; the two phases were agitated for 1 min on a Lab-Line Instruments Super Mixer to achieve equilibrium, and the upper toluene phase removed by a pipetter. In the second step, 1 cm^3 of the KCl/HCl solution and 0.1 cm^3 of aqueous $CuCl_2$ (1 : 1, w/w) were added to the toluene solution, the two phases were agitated for 1 min, and the toluene layer removed by the pipetter. The $CuCl_2$ in the second step ensures that complexed Hg(II) will not extract into the toluene phase. Thus, the activity in this second toluene layer is due to $Me^{203}HgCl$.

Percentages recovery experiments were performed separately, beginning with *ca* 3 × 10^6 counts min^{-1} (cpm) $^{203}HgCl_2$ or 3 × 10^3 cpm $^{203}MeHgCl$ to simulate a 0.1 % yield for methylation reactions. The first extraction of $^{203}HgCl_2$ brought 1.3 % of it into the toluene phase and the second step left 0.61 % in toluene. After two steps $^{203}HgCl_2$ in the toluene phase was at background levels (*ca* 200 cpm). The first extraction of $Me^{203}HgCl$ recovered 81 % into the toluene phase and the second step left 82 % of the $Me^{203}HgCl$ in toluene. The overall result was that the second toluene extract achieves 66 % recovery of the original $Me^{203}HgCl$. All reaction data for kinetics calculations were corrected for the 0.66 extraction efficiency.

Methylation of Hg(II) by MeSn

Kinetics experiments with MeSn

In a typical triplicate experiment, 1.75 μmol of a $MeSn^{3+}$, Me_2Sn^{2+} or Me_3Sn^+ chloride solution in 0.05 mol dm^{-3} HNO_3 is put into a plastic test-tube (10 cm^3 total volume, 100 mm × 15 mm) and enough 0.5 mol dm^{-3} aqueous Na_2CO_3 was added to neutralize the acid approximately. Then 6 cm^3 of seawater was added, the pH was measured by a Metrohm 654 pH-meter with a combined pH–glass electrode, and the pH was adjusted to 8.0 ± 0.1. (At the end of the reaction the pH was within 0.1 unit of the initial value.) Then 0.070 μmol of $HgCl_2$ was added. (Since all solutions of $HgCl_2$ and MeHgCl contain radioactive and non-radioactive forms, all concentrations cited below are total mercury concentrations.) Typical initial concentrations were 284 μmol dm^{-3} methyltin chloride (MeSn) and 11.4 μmol dm^{-3} $HgCl_2$. Three controls excluded only addition of MeSn. The solutions were kept in the dark at 17 °C, and 1 cm^3 samples were removed periodically for extraction and measurement of fraction of reaction. A minimum of a 25-fold molar excess of MeSn over $HgCl_2$ ensured pseudo-first-order conditions.

Experiment to confirm methylation

A methylation experiment at higher concentrations was done to demonstrate the presence of non-radioactive MeHg by AA[22] and Fourier transform infrared (FTIR) spectrometry.[23] A sample of 50 μmol Me_3Sn^+ was put into a plastic test-tube and 216 μl of 0.5 mol dm^{-3} aqueous Na_2CO_3 was added to approximately neutralize the HNO_3 of the Me_3Sn^+ solution. Then 6 cm^3 seawater and 0.49 μmol $HgCl_2$ were added from a 4.90 μmol cm^{-3} (1.2 mol dm^{-3} in HCl) stock solution, and the pH was adjusted to 7.8. After 45 min at room temperature, the sample was extracted as usual into 1 cm^3 toluene and immediately frozen for determination of MeHg by FTIR and AA spectrometry.

Attempted methylation of Hg(II) and Hg(0) by MeI

Reaction of Hg(II)

To triplicate 12 cm^3 seawater samples were added 0.114 μmol $HgCl_2$ and 90.2 μmol MeI (5.6 μl, 790-fold excess). The solutions were kept in the dark at 17 °C, and 1 cm^3 samples were removed over a period of 14 days. Three controls were treated similarly without addition of MeI.

Reaction of Hg(0)

Triplicate samples of 0.15 μmol $SnCl_2$ from a 0.0103 mmol cm^{-3} stock solution in 1.2 mol dm^{-3} HCl and 0.18 μmol $HgCl_2$ were added to 2 cm^3 seawater and mixed for 30 s. After 30 min, 10 cm^3 seawater, 0.020 cm^3 of 0.5 mol dm^{-3} aqueous Na_2CO_3, and 148 μmol methyl iodide (MeI) were added. The pH was 8.0 ± 0.1. The reactions were run in the dark at 17 °C. If all reagents were in solution, initial concentrations were: 2.46 μmol dm^{-3} $HgCl_2$, 12.3 μmol dm^{-3} Hg(0), and 12 100 μmol dm^{-3} MeI (1000-fold excess). $SnCl_2$ reduces $HgCl_2$ to Hg(0), but excess $HgCl_2$ ensures that all $SnCl_2$ is oxidized and some $HgCl_2$ remains. Therefore, Sn^{2+} will not be methylated by MeI and will not therefore subsequently transfer a methyl group to any mercury species. Three controls containing $HgCl_2$ and MeI were run. The samples were periodically extracted as usual for 35 days.

Thin-layer chromatography for confirmation of $Me^{203}HgCl$

To a toluene solution potentially containing $Me^{203}HgCl$, 10 μg of non-radioactive MeHgCl was added as a marker. The sample was applied to thin-layer chromatography plates (silica gel 60, F_{254}, Merck) by adding 5 μl aliquots with time for toluene evaporation between each aliquot. The plate was then counted to determine the total radioactivity before development. Then the plate was placed vertically in a glass vial for development with an acetone–hexane (1:9, v/v) mixture. After the solvent reached 75 % of the length of the plate, it was removed for spraying with a solution of 0.05 % (w/v) dithizone in chloroform. The gel at the colored spot, the starting area, and the rest of the plate were separately scraped off with a scalpel and counted. In a typical experiment 25 μl of a toluene extract containing 3.7×10^3 cpm was placed at the starting point of the plate. After development the colored MeHg spot contained 3.0×10^3 cpm, which is 81 % of the total activity on the plate.

Calculations of pseudo-first-order and second-order rate constants, and second-order rate constants corrected for $[H^+]$

The corrected fraction of reaction (f; Eqn [1] below), which is the ratio of MeHg to total mercury, was calculated each measurement day on 1 cm^3 aliquots of exprimental solution that were treated in two steps as described above. The total activity (cpm) of the initial aliquot was corrected for background and counting geometry. The activity of $Me^{203}HgCl$ in the toluene phase of the second step was corrected by subtracting the activity of a control and correcting for counting geometry. The calculated f is the ratio of corrected toluene phase (second step) activity to total activity corrected for the 66 % extraction efficiency. Because f is a ratio of two activities on the same day, no correction for decay was needed.

All methylation reactions were run under conditions of pseudo-first-order kinetics with a minimum of a 25-fold molar excess of MeSn over $HgCl_2$. Pseudo-first-order conditions mean that one reactant is in sufficient excess to have approximately the same concentration after 100 % reaction. Thus, the rate law given by Eqn [1] applies:

$$-\ln(1-f) = kt \qquad [1]$$

where $1-f$ is the corrected fraction of Hg^{2+} remaining, k is the pseudo-first-order rate constant, and t is the time (h). The constant k yields the second-order-rate constant (k_2) at one pH value (Eqn [2]):

$$k = k_2[\mathrm{MeSn}]_0 \qquad [2]$$

where $[\mathrm{MeSn}]_0$ is the initial concentration (mol dm^{-3}) of $MeSn^{3+}$, Me_2Sn^{2+} or Me_3Sn^+.

Pseudo-first-order rate constants were determined by linear regression. The intercept was not significantly different from zero for all but three of the rate constants reported in this paper. The error in rate constants from regression data is typically 5–10 %.

The rate constant k_2 is the following empirical function of $[H^+]^n$, where k_{2H} is the second-order-rate constant corrected for $[H^+]^n$ (Eqn [3]) and in logarithmic form (Eqn [4]):

$$k_2 = k_{2H}[H^+]^n \qquad [3]$$

$$-\log(k_2) = +n\mathrm{pH} - \log(k_{2H}) \qquad [4]$$

A plot of $-\log(k_2)$ vs pH will give the pH dependence as the slope n and $-\log(k_{2H})$ as the constant at pH 0. However, pH must be constant during each reaction.

Comparison of observed and calculated activity

For each sampling time in all experiments, activity corrected for background and counting geometry was divided by corrected activity at time zero to calculate the observed activity loss. The resulting value was divided by the calculated activity loss due to decay (Eqn [5]):

$$1-f = \exp(-0.000619t) \qquad [5]$$

where $1-f$ is the fraction of activity remaining, t is the time (h), and 0.000619 is the disintegration constant of ^{203}Hg. With one exception of a very slow reaction (measured over 21 days), the observed/calculated ratios are 0.95 and higher. Therefore, no significant amount of mercury was lost during the experiments.

Table 1 Methylation of mercury(II) by methyltin compounds (Expts 1–8) and attempted methylation of Hg(II) (Expt 9) and Hg(0) (Expt 10) by methyl iodide in seawater

Expt no.	Methyl donor	Conditions	$k \pm$ SD (h^{-1})	k_2 ($dm^3\,mol^{-1}\,h^{-1}$)	Comments
1	2580 $\mu mol\,dm^{-3}$ $MeSn^{3+}$	25.8 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.0	2.9 ± 0.2	1100	Very fast reaction, approximate data
2	284 $\mu mol\,dm^{-3}$ $MeSn^{3+}$	11.4 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.0	0.273 ± 0.035	960	Good data
3	905 $\mu mol\,dm^{-3}$ Me_2Sn^{2+}	9.05 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 4.5	0.0011 ± 0.0001	1.2	Very slow reaction, good data
4	60.5 $\mu mol\,dm^{-3}$ Me_2Sn^{2+}	1.94 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.1	0.0033 ± 0.0001	53	Very slow reaction
5	2600 $\mu mol\,dm^{-3}$ Me_2Sn^{2+}	26.0 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.0	0.042 ± 0.004	16	Very fast reaction
6	284 $\mu mol\,dm^{-3}$ Me_2Sn^{2+}	11.4 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.1	0.0084 ± 0.0004	30	Good data
7	2580 $\mu mol\,dm^{-3}$ Me_3Sn^{+}	25.8 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.1	0.28 ± 0.01	110	Very fast reaction
8	253 $\mu mol\,dm^{-3}$ Me_3Sn^{+}	11.4 $\mu mol\,dm^{-3}$ $HgCl_2$, pH 8.0	0.076 ± 0.005	300	Good data
9	8150 $\mu mol\,dm^{-3}$ MeI	8.44 $\mu mol\,dm^{-3}$ $HgCl_2$, pH *ca* 8.0	—	—	No reaction in 14 days
10	14 600 $\mu mol\,dm^{-3}$ MeI	12.0 $\mu mol\,dm^{-3}$ Hg^0, pH 8.0	—	—	No reaction in 35 days

RESULTS

Confirmation of extraction of MeHgCl into toluene

First, in spot checks during reactions of Hg(II) and MeSn, we confirmed the presence of MeHgCl by thin-layer chromatography. Typically, *ca* 80 % of the active mercury in the second toluene phase moved on the plate with authentic MeHgCl. Second, after a 45 min reaction between Hg(II) and Me_3Sn^+ at higher concentrations, MeHg was confirmed in two ways: indirectly by AA after separation by extraction,[22] and directly by FTIR.[23]

Rate constants for methyl transfer from methyltin compounds (MeSn) to mercury(II) chloride

A series of experiments (Table 1) with methyl donors $MeSn^{3+}$, Me_2Sn^{2+} or Me_3Sn^+ was run at pH 8 with high concentrations and a series was run at low concentrations. In high-concentration experiments (Expts 1, 5, 7) approximately 26 $\mu mol\,dm^{-3}$ $HgCl_2$ reacted with a 100-fold molar excess of MeSn. In experiments at lower concentrations (Expts 2, 6, 8) about 9 or 11 $\mu mol\,dm^{-3}$ $HgCl_2$ reacted with a 25-fold molar excess of MeSn. One experiment (Expt 3) at pH 4.5 used 9.05 $\mu mol\,dm^{-3}$ $HgCl_2$ and a 100-fold excess of Me_2Sn^{2+}. In one very low-concentration reaction (Expt 4), 1.94 $\mu mol\,dm^{-3}$ $HgCl_2$ and a 31-fold molar excess of Me_2Sn^{2+} reacted.

Even the lowest MeSn excess is sufficient for pseudo-first order kinetics and we always observe the expected first-order kinetics (Eqn [1]), and can calculate the second-order rate constant (k_2) from Eqn [2]. In both the high- and low-concentration series of reactions at pH 8 the relative k_2 values were in the order: $MeSn^{3+} > Me_3Sn^+ > Me_2Sn^{2+}$. However, rate constants are less reliable in the fast series of reactions (Expts 1, 5, 7) and we shall not discuss them further. The slower reactions at pH 8 (Expts 2, 6, 8), which allow quantification, have the following order of k_2 ($dm^3\,mol^{-1}\,h^{-1}$): $MeSn^{3+}$ (960) > Me_3Sn^+ (300) > Me_2Sn^{2+} (30). The k_2 ratio of $MeSn^{3+}/Me_2Sn^{2+}$ is therefore 32/1.

Significance of percentage seawater and pH on second-order rate constants in the factorial experiment

A duplicate 2^2 factorial experiment[24] was run with the two variables 100 and 50 % seawater (S) and pH values (P) of 6.6 and 7.5 (Table 2). The 50 % seawater was obtained by dilution of seawater with deionized water. The errors in rate constants from linear regression were less than 10 % in all cases. At the 95 % confidence level t-tests show that the lowest k_2 value occurs for $S = 100\ \%$ seawater and pH = 6.6 (Expt 2), and that the results of Expts 1, 3 and 4 are indistinguishable from each other.

Analysis of variance (ANOVA) calculations (Table 3) demonstrate that the pH is significant at the 99 % confidence level. Percentage seawater is not significant at the customary 95 % level and we

Table 2 Rate constants for a duplicate 2^2 factorial experiment for methylation of dimethyltin chloride in 100 % and 50 % seawater at pH 6.6 and 7.5[a]

Variable	Levels + (High)	Levels − (Low)
S, % seawater	100	50[b]
P, pH	7.5	6.6

Expt no.	Variable levels *S*	Variable levels *P*	Second-order rate constants ($dm^3 mol^{-1} h^{-1}$) Repl. A	Second-order rate constants ($dm^3 mol^{-1} h^{-1}$) Repl. B
1	+	+	18.1	13.3
2	+	−	3.65	4.72
3	−	+	11.6	15.5
4	−	−	7.96	9.54

[a] In + + and + − [$HgCl_2$] is 10.9 μmol dm^{-3} and [Me_2SnCl_2] is 273 μmol dm^{-3}. In − + and − − [$HgCl_2$] is 11.4 μmol dm^{-3} and [Me_2SnCl_2] is 284 μmol dm^{-3}. The concentration difference is due to the volume of acid and base added to achieve the desired pH. There is a 25-fold molar excess of Me_2Sn^{2+} in all reactions.

[b] The 50 % seawater was prepared by diluting seawater 1:1 (v/v) with deionized water.

shall not discuss any effects due to ionic strength or [Cl^-]. Figure 1 shows that k_2 increases faster in 100 % than in 50 % seawater as the pH increases from 6.6 to 7.5.

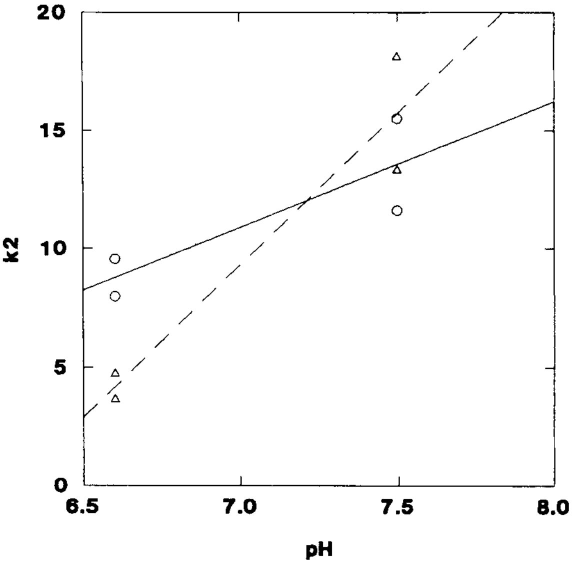

Figure 1 Variation in second-order rate constants ($dm^3 mol^{-1} h^{-1}$) with pH in 100 % (△) and 50 % (○) seawater.

Effect of pH on second-order rate constants for reactions of Me_2Sn^{2+} and Hg(II) in seawater

The value of k_2 for reactions of Me_2Sn^{2+} with Hg(II) in seawater varies from 1.2 at pH 4.5 (Table 1, Expt 3) to 53 h $dm^3 mol^{-1} h^{-1}$ (Table 1, Expt 4) at pH 8.1. Regression analysis of $-\log(k_2)$ vs pH (Eqn [4]) for k_2 values in 100 % seawater at pH 4.4, 6.6, 7.5 and 8.1 gives a value of $n = -0.43$ (± 0.06) in $[H^+]^n$. Therefore, $k_2 = k_{2H}[H^+]^{-0.43}$ (Eqn [3]).

Table 3 Statistical significance of percentage seawater (*S*) and pH (*P*) on pseudo-first-order rate constants for methyl transfer reactions from dimethyltin chloride to Hg(II)[a]

Effect	*F*-value	Probability (%)[b]
S	0.557	50
P	25.4	99
S × *P*	4.31	89

[a] See experimental details in Table 2. [b] Probability that effect is significant.

We cannot explain the observation that the exponent is not an integer and is close to 0.5, but it is clear that $\log(k_2)$ increases linearly with pH. It is not likely that $HgCl_4^{2-}$, the predominant form of Hg(II) in seawater at pH 8.0 (see below), will change as the pH decreases to 4.5; and the increase of k_2 with increasing pH must be due to changes in the nature of Me_2Sn^{2+} in solution. The most likely changes are increases in hydroxide ion and/or carbonate ion coordinated to Me_2Sn^{2+} as pH increases. It is difficult to explain this trend.

Attempted methylation of Hg(II) and Hg^0 by methyl iodide in seawater

An approximate 1000-fold molar excess of MeI in seawater methylated neither Hg(II) in 14 days nor Hg^0 in 35 days (Table 1, Expts 9, 10) in agreement with similar experiments of Craig and Moreton.[25] We did not expect methylation of Hg(II) by MeI because it typically methylates by oxidative addition and the Hg(IV) that would result is unknown. In contrast, reduction potential (E^0) data[26] leave it unclear whether or not MeI could methylate Hg(0) to MeHg(II). MeI methylates Sn(0) ($E^0 = -0.14$ V) while oxidizing it to Sn(II), Pb(0) (−0.13 V) oxidizing it to Pb(II), and Sn(II)

(+0.15 V) oxidizing it to Sn(IV); but not Pd(0) (0.99 V). The reduction potential for Hg(II)/Hg(0) of 0.85 V falls between potentials for known oxidative addition reactions of Sn(0), Pb(0) and Sn(II) by MeI and the absence of the reaction of Pd(0). Our results show that MeI does not methylate Hg(0) in seawater.

DISCUSSION

Comparison of second-order rate constants with literature values

Howell *et al.*[20] did no kinetics studies, but reported that stoichiometric Me_3Sn^+ and Hg(II) in 0.59 mol dm^{-3} NaCl (pH not stated) underwent an 85 % reaction in two days. From these approximate data we calculated a second-order rate constant of 590 $dm^3 mol^{-1} h^{-1}$. Our 300 $dm^3 mol^{-1} h^{-1}$ k_2 value in seawater for Me_3Sn^+ at pH 8.0 (Table 1, Expt 8) is in reasonable agreement, considering the approximate nature of the percentage reaction and time data from Howell and co-workers. Bellama and co-workers[21] reacted Me_3Sn^+ and Hg(II) in solutions of about 0.1 mol dm^{-3} $NaClO_4$ and of the highest NaCl concentration of 0.076 mol dm^{-3}, and found k_2 of 13–37 $dm^3 mol^{-1} h^{-1}$. Our k_2 value for the reaction of Me_3Sn^+ and Hg(II) in seawater is 300 $dm^3 mol^{-1} h^{-1}$ (Table 1, Expt 8). Our data and those from Bellama and co-workers are in acceptable agreement, considering the very different reaction media.

Nature of mercury(II) and methyltin compounds in seawater

In order to rationalize changes in second-order rate constants with changes in pH, it is necessary to speculate on the nature of the reactants in seawater. Detailed speciation calculations on seawater by Turner *et al.*[27] show that Hg(II) exists 100 % as $HgCl_4^{2-}$ even in the presence of 1 μmol dm^{-3} fulvic acid complexing agent. Therefore, we will assume that Hg(II) exists solely as $HgCl_4^{2-}$ in all our experiments.

The speciation of $MeSn^{3+}$, Me_2Sn^{2+} and Me_3Sn^+ (MeSn) in seawater is unknown due to a lack of stability constant data in that medium. Our conclusions about the speciation of MeSn must be speculative. First, Donard and Weber,[28] using all available stability constant data for MeSn, concluded that in simulated seawater MeSn species are close to neutral, and in them OH^- neutralizes the positive ions. Second, Sn(II) is a reasonable model for MeSn, especially Me_2Sn^{2+}, because both have the same ionic charge. Speciation calculations[17] for Sn(II) show that in simulated seawater Sn(II) is nearly 100 % $Sn(OH)_2$ in agreement with conclusions about MeSn.

Experiments of Donard and Weber[28] offer further clues about the nature of MeSn in seawater. In a 2^3+1 factorial experiment they studied adsorption of MeSn on hydrated iron(III) oxide under simulated seawater and estuarine conditions. One experimental condition of interest to this paper measured adsorption of MeSn (0.042 μmol dm^{-3}) at pH 8 in simulated seawater containing 35 g dm^{-3} NaCl as salinity, 5 mg dm^{-3} fulvic acid, and 10 mg dm^{-3} hydrous iron(III) oxide as particulate matter. The order of percentage adsorption of MeSn on hydrated iron(III) oxide for these conditions is: $MeSn^{3+}$ (*ca* 90 %) $\gg$ Me_2Sn^{2+} (35 %) > Me_3Sn^+ (20 %). The same order of percentage adsorption of MeSn also occurred under all other experimental conditions. Colloidal hydrated iron(III) oxide had a negative surface charge due to adsorption of fulvic acid in these experiments. Thus, the observed adsorption order observed by Donard and Weber[28] probably parallels the order of positive charges on MeSn in their simulated seawater media and in the seawater media of this paper.

Rationalization of relative rates of methyl transfer from MeSn to $HgCl_4^{2-}$

As described above, we believe that one major factor affecting the relative reaction rates of MeSn with $HcCl_4^{2-}$ is the relative solution positive charges on MeSn. On this basis alone we expected the relative reaction rates for methyl transfer from MeSn to $HgCl_4^{2-}$ to be $MeSn^{3+}$ > Me_2Sn^{2+} > Me_3Sn^+, i.e. in the same order as percentage adsorption of MeSn on hydrated iron(III) oxide, but this is not the experimental order.

Considerable experimental evidence demonstrates that rates of methyl transfer to metal species in aqueous solution increase as the positive charge of the methyl donor in the solid state decreases, and becomes neutral and then negative. For example, methyl transfer from methylcobalt complexes to Hg(II) strongly follows this trend.[29] These relative reaction rates are readily explainable on the bases of electronegativity and C–Co bond polarity arguments. The relative positive charges of the compounds in the solid state and solution apparently have the same order, because predictions based on solid-state structures of methyl donors agree with experiment. According to this fundamental concept, the rate order for methyl donation from MeSn to Hg(II) should be from least positive to most positive: $Me_3Sn^+ > Me_2Sn^{2+} > MeSn^{3+}$.

Our experimental second-order rate constant order, $MeSn^{3+} > Me_3Sn^+ > Me_2Sn^{2+}$, agrees much better with the order of positive charge in solution from adsorption experiments[28] than with the idea of solid-state charges.[29] Our data show a reversal in the order of rate constants for Me_2Sn^{2+} and Me_3Sn^+ compared with the solution positive charges suggested by adsorption experiments. Such reversals in reactivity are common when two effects favor the opposite order of reactivity in a series. Our experimental reversal probably reflects two opposing trends. First, the prediction, based on fundamental concepts, is that the least positive Me_3Sn^+ among MeSn should transfer methyl groups via carbanions most readily to metal species. Second, the most positive MeSn, $MeSn^{3+}$, should react fastest with $HgCl_4^{2-}$. The mixed reaction order that we observed is due to these two opposing effects: the relative solution charges of MeSn[28] favor Me_2Sn^{2+} over Me_3Sn^+ and the fundamental reactivity effect on the basis of charge[29] favors Me_3Sn^+ over Me_2Sn^{2+}.

CONCLUSIONS

Considerable indirect evidence,[2] based mainly on experiments with sediment samples in the laboratory, suggests that environmental methylation of Hg(II) occurs by a biological process. Typically researchers add Hg(II) to active and sterilized sediment, and observe that MeHg occurs only in active sediments. In addition, Compeau and Bartha[5] found little or no methylation of Hg(II) in the presence of MoO_4^{2-}, which inhibits sulfate-reducing bacteria, and concluded that those bacteria are the main methylating agents of Hg(II) in anoxic sediments.

However, in our opinion evidence for exclusive biological methylation of Hg(II) in the environment is weak, and abiotic methylation may play an important role in MeHg production. We have several reasons for this atypical opinion.

(1) Concentrations of Hg(II) used in laboratory experiments are much higher than trace levels.[2] This results in a large increase in bioavailable Hg(II) and selects mercury-resistant bacteria that do not predominate in the environment.
(2) Sterilization methods change chemistry as well as biology, and conclusions based on experiments comparing active and sterile sediments are not definitive.
(3) Experiments using MoO_4^{2-}, which inhibits sulfate-reducing bacteria, also may often change the chemistry despite the observation in one medium that methylcobalamin methylates Hg(II) in its presence.[5]
(4) Madsen,[30] in a critical review on *in situ* biodegradation, emphasizes the difficulty in relating laboratory experiments to the environment. He points out that a key problem is distinguishing biotic from abiotic processes, and gives criteria for the distinction. These criteria have not been achieved to prove environmental biotic methylation of Hg(II).[2,30]

References in the Introduction show that sufficient amounts of methyltin compounds and Hg(II) are available in estuarine waters, sediments and biota for abiotic formation of MeHg by methyl transfer from one or more of $MeSn^{3+}$, Me_2Sn^{2+}, and Me_3Sn^+ to Hg(II). Our kinetics data demonstrate that in seawater such methyl transfer reactions are quite fast, with k_2 values as high as 1000 $dm^3\,mol^{-1}\,h^{-1}$ for $MeSn^{3+}$. It is particularly significant that $MeSn^{3+}$ often has the highest concentration among methyltin compounds in the estuarine environment,[7,8,14] and is the only one formed by oxidative addition in model experiments.[17,18] Therefore, we believe that in marine environments the abiotic methylation reactions discussed in this paper are viable contributors to environmental methylation.

There are many questions that arise when trying to prove that abiotic processes contribute to environmental methylation of Hg(II) in marine waters, sediments and biota.

(1) Why is inorganic Hg(II) present when MeSn is in molar excess?
(2) What is the speciation of Hg(II) (e.g., $HgCl_4^{2-}$) and MeSn (e.g., $MeSn(OH)_3$) and how does their structure in solution affect reactivity? The reactivity of Hg(II) depends greatly on its structure in reaction media.[25]
(3) What chemicals cause Hg(II) to be soluble

or insoluble in sediments? For example, sulfate-reducing bacteria produce S^{2-} that precipitates HgS, which is probably unavailable for methylation; and ligands, like humic matter, might solubilize it.

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