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Preliminary evidence for *in vitro* methylation of tributyltin in a marine sediment

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Recent reports from our laboratory on the occurrence of methylbutyltins in marine sediments and seawater suggest that these compounds are formed in the environment by the methylation of both tributyltin (TBT) and that of its degradation products, *i.e.* dibutyltin and monobutyltin, to give $Me_nBu_{(4-n)}Sn$ for which $n = 1$, 2 and 3 respectively. We investigated the possibility of inducing methylation of TBT in seawater–sediment mixtures in experiments carried out *in vitro* using environmental materials collected from a yacht marina in Msida, Malta. Three water–sediment mixtures, which were shown to contain TBT, dibutyltin and monobutyltin but no other organotins, were spiked with tributyltin chloride (90 mg in 100 ml sea-water/100 ml sediment); to one mixture was added sodium acetate and to another methanol, to act as possible additional carbon sources, and all mixtures were allowed to stand at 25 °C in stoppered clear-glass bottles in diffused light for a maximum of 315 days. Speciation and quantification of organotins was performed using aqueous phase boroethylation with simultaneous solvent extraction followed by gas chromatography with flame photometric detection. The atmosphere inside the bottles quickly became reducing with abundant presence of H_2S, and after an induction period of about 112 days, and only in the reaction mixture containing methanol, methyltributyltin ($MeBu_3Sn$) was observed in both sediment (maximum concentration 0.87 $\mu g_{Sn}\ g^{-1}$) and overlying water (maximum concentration 6.0 $\mu g_{Sn}\ l^{-1}$). The minimum conversion yield of TBT into $MeBu_3Sn$ was estimated to be 0.3%. $MeBu_3Sn$ has a significantly lower affinity for sediment than TBT and, therefore, is more mobile in the marine environment, possibly also migrating into the atmosphere to generate a hitherto unsuspected flux of organotin into that phase.

Keywords: tributyltin (TBT); methylation; environment; marine pollution; methylbutyltin

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INTRODUCTION

The widespread use of tributyltin (TBT) compounds as antifouling agents in boat paints had led to global concern about their effects as marine pollutants. The literature on the occurrence of TBT in the marine environment is vast and constantly increasing. The fate of dissolved TBT is believed to be dominated by: *(a)* dispersion in the water column; *(b)* accumulation in sediments, in the surface microlayer and in marine organisms; and *(c)* degradation by successive debutylation to dibutyltin (DBT), monobutyltin (MBT) and inorganic tin(IV) species. Although the presence of organotin compounds in sediments is well documented, little is known about the bioavailability of sorbed organotins, and hence the effect they exert on organisms that inhabit this environmental phase. Factors that are known to determine the tendency for the bioconcentration of organotins include pH and the presence of dissolved humic acid, which has recently been shown to inhibit strongly the bioavailability of triorganotins.[1]

We have previously reported the occurrence in the marine environment of tetrasubstituted organotins of the type $Me_nBu_{(4-n)}Sn$ (Me = methyl; Bu = butyl), where $n = 1$–3.[2] Previously, these compounds were only reported in lacustrine environments, and then very infrequently.[3,4] In the Malta coastal zone, these compounds were found occasionally in the water column and frequently in sediments, where, for example, the concentration of Me_3BuSn was found at a maximum value of 9 $\mu g_{Sn}\ g^{-1}$. The presence of these tetraorganotins is significant, because their lipophilicity and their Lewis acidity

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Table 1 Sediment–seawater mixtures spiked with TBT chloride and added carbon sources

Experiment	Mass of TBT chloride spike (mg)	Carbon source and mass (g)
X	90	None
Y	90	$CH_3CO_2Na{\cdot}3H_2O$ 1.25
Z	90	CH_3OH 0.3

towards ligands, such as marine humic acids, is expected to be quite different from that of ionogenic tri-, di- and mono-organotins.

In this paper we report our preliminary findings based on *in vitro* experiments that demonstrate that a mixed methylbutyltin can form from tributyltin in the marine sedimentary environment under largely anoxic conditions. Biomethylation of mercury is, of course, a well-known phenomenon, and there is evidence for environmental methylation of inorganic tin(IV) and tin(II) in sediments,[5] but, to our knowledge, this is the first reported observation of the direct methylation of tributyltin under environmental conditions.

Materials and Methods

TBT chloride and tripentyltin chloride were purchased from Aldrich Chemicals Co. Sodium tetraethylborate ($NaBEt_4$) was from Strem Chemicals and hexane and *iso*-octane were from Rathburn and BDH Ltd respectively. Butylmethyltins were prepared and purified according to the method of Maguire and Huneault.[6]

Samples of seawater and marine sediments were obtained from Msida Creek, Malta. This is the site of one of the major yacht marinas on this central Mediterranean island; the creek also receives considerable runoff from one of the valley systems in Malta. This site was chosen as it provides an organic-matter-rich calcitic sediment that is known to be moderately contaminated with organotins; TBT and methylbutyltins had been identified in various sediments from this marina[2] and, therefore, biota resistant to TBT and possibly capable of transforming organotins biochemically to methylated forms were considered to be present in the sediment. Around 10 l[3] of seawater was collected from a depth of 1 m, and around 2 kg of a greyish-black sediment were removed from the sea bed at 2.5 m water depth using a grab sampler. The samples were taken to the laboratory and analysed for the presence of organotins (see below) within 6 h of collection.

Mixtures of seawater (100 ml) and sediment (100 ml) were placed in clear-glass 250 ml reagent bottles equipped with polypropylene stoppers, and to each of these bottles was added 90 mg TBT chloride; in addition, the organic compounds shown in Table 1 were also added to two of the mixtures to act as carbon sources for possible biological transformation. The amount of TBT chloride added was about two orders of magnitude higher than that originally found to be present in the sample. The amount of organic compound added as carbon source was an excess (30-fold) intended to stimulate the rate of any possible biomethylation. The reaction mixtures were kept in diffused light, as available on the bench in the laboratory. All three stoppered bottles were kept in a thermostatic bath set at 25 °C and the content of these reaction bottles (both water and sediment) were analysed regularly, approximately once every 4 weeks as follows. About 5 ml of the supernatant water layer was pipetted out of the bottle and analysed for organotins. Each water sample was analysed in triplicate. About 3 g of sediment was scooped out of the bottle and 2 g of wet sediment was analysed for organotins; a further smaller sample was used for moisture determination. Sediment analysis was performed in duplicate.

Analysis for organotins

The method adopted was that described by Carlier-Pinasseau *et al.*[7] using boroethylation (with $NaBEt_4$) to convert the ionogenic organotins into ethylated tetraorganotins with simultaneous solvent extraction followed by gas chromatography–flame photometric detection (GC–FPD) of the solvent extract. Sediments were analysed as follows: 2 g was treated with 20.0 ml acetic acid for 4 h and the mixture was then separated by centrifugation. A volume of 2.0 ml supernatant was diluted with 50 ml deionized water and 12 ml of 20% sodium acetate trihydrate (to pH 4.6) and filtered through a 0.45 μm cellulose acetate filter (Whatman). Organotins in the filtrate were derivatized and analysed by gas chromatography as described below. Seawater was prepared for derivatization and gas chromatography by filtering a 5.0 ml sample through a 0.45 μm cellulose acetate

filter and adding to the filtrate 5.0 ml acetic acid–sodium acetate buffer of pH 4.6.

Derivatization was performed as follows: a volume of tripentyltin chloride in hexane (100–200 µl; 7 mg l^{-1}) was added as internal standard to 5 ml of sample (seawater or sediment extract) followed by 200 µl $NaBEt_4$ solution (0.02 g ml^{-1}) followed by 800 µl *iso*-octane in a specially designed reactor[7] and the mixture was stoppered and stirred magnetically for 20 min. Enough deionized water was then added to force the *iso*-octane layer into the narrow part of the reaction vessel, thus permitting easy retrieval by Pasteur pipette and transfer to a small vial. This volume was blown down under nitrogen to about 50 µl and analysed by GC–FPD. A Perkin Elmer Model 8000 gas chromatograph equipped with a flame photometric detector and a 610 nm filter was employed, and the analytical column was a 25 m fused silica narrow bore capillary column having a nonpolar bonded phase (BP1, SGE Australia). Peak identities were confirmed by co-chromatography with authentic standards. The instrumental tin detection limit was 0.36 ng (signal/noise ratio equal to three), which limited detection of organotins in seawater to 12 ng_{Sn} l^{-1} and in sediments to 3.6 ng_{Sn} g^{-1}.

Using this protocol, we established that tetra-organotins are extracted without change into the *iso*-octane layer; this is an important (albeit anticipated) finding, since the main substance targeted for analysis was a compound of this type.

RESULTS AND DISCUSSION

The mean concentrations ($N = 3$) of TBT, DBT and MBT in the seawater samples from Msida Creek were found to be respectively 83 ng_{Sn} l^{-1}, 75 ng_{Sn} l^{-1} and 55 ng_{Sn} l^{-1}. The mean concentrations ($N = 3$) of the same analytes in the sediment were respectively 300 ng_{Sn} g^{-1}, 330 ng_{Sn} g^{-1} and 160 ng_{Sn} g^{-1}. No methylbutyltins were found in either phase.

As described earlier, both seawater and sediment from each reaction bottle were analysed about once every 4 weeks. Each time the bottles were opened, a strong odour of hydrogen sulfide was observed, especially from reaction mixture Y. The presence of H_2S in the headspace of the bottles was confirmed by reaction with lead acetate paper. The concentrations of organotins found in the water samples as a function of time are shown in Fig. 1. Except for experiment X, where the decay of TBT was found to be monotonic, it was observed that TBT concentra-

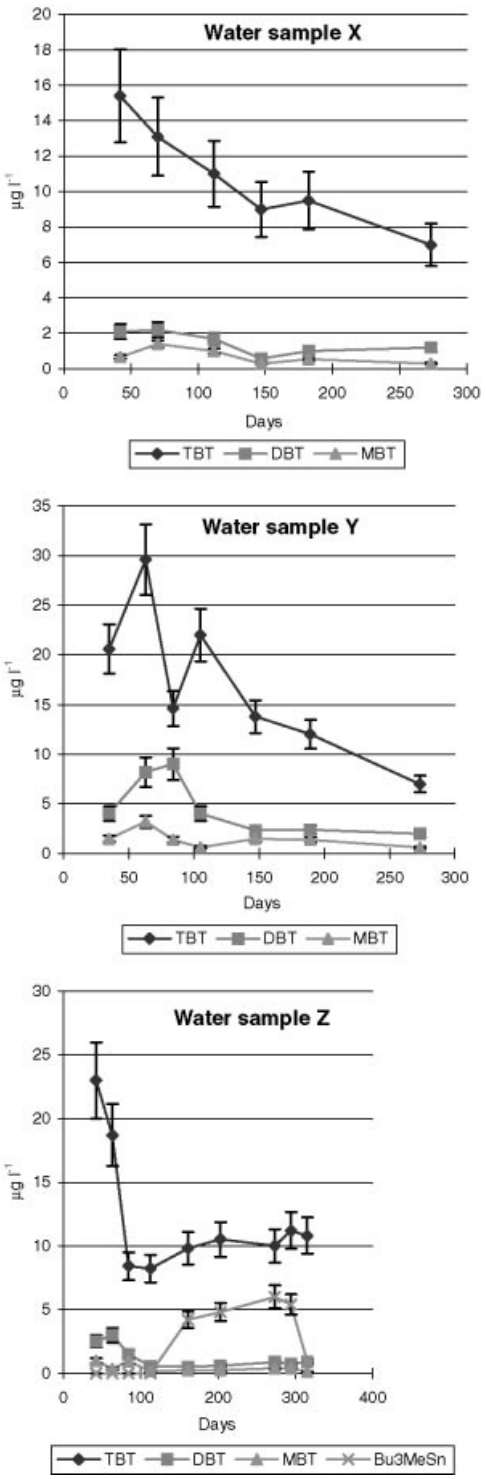

Figure 1 Variation with time of concentration of organotins (µg_{Sn} l^{-1}) measured in water samples in experiments X, Y and Z.

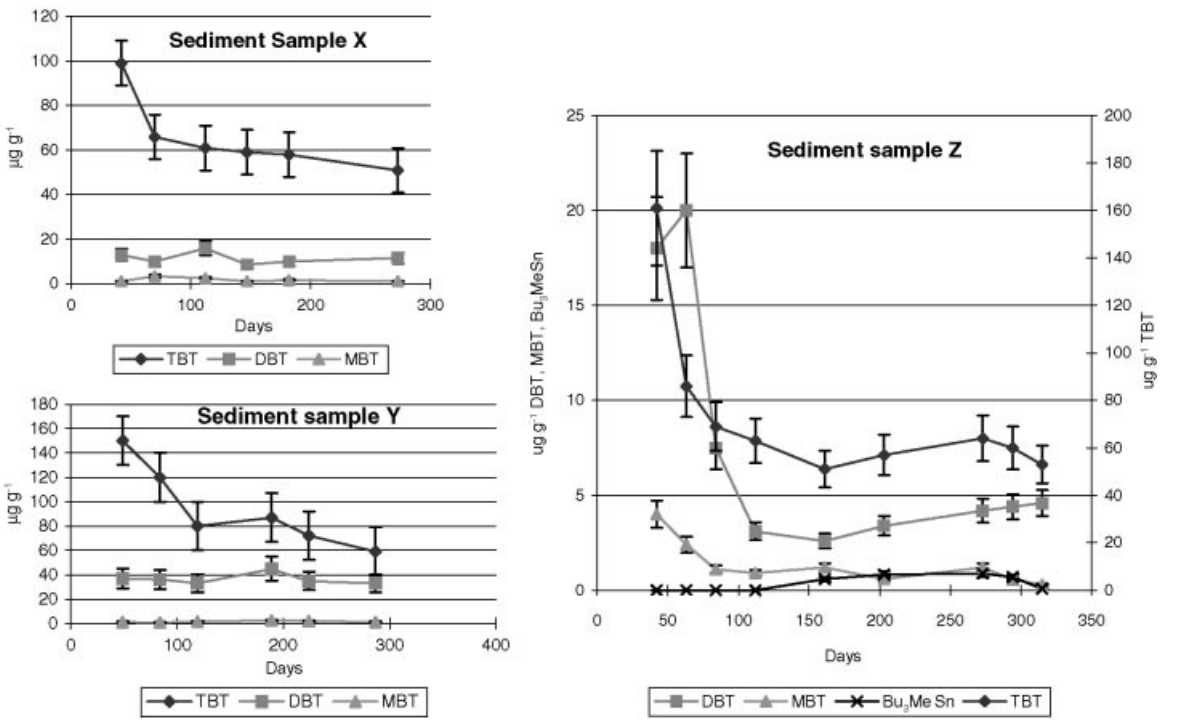

Figure 2 Variation with time of concentration of organotins ($\mu g_{Sn}\ g^{-1}$) measured in sediment samples in experiments X, Y and Z.

tions varied irregularly with time; this behaviour could be due to effects caused by repartitioning of the organotin between the liquid phase and the sediment, possibly in response to changing conditions with time or even due to disturbance caused during sampling.

In the case of experiment Z only, among the products of degradation of TBT, there was observed a significant presence of methyltributyltin. This organotin appeared in the water column after an induction period of at least 112 days, and its concentration continued to increase over the next 161 days; it then decreased slowly and eventually more rapidly over the next 42 days of the experiment. For over 150 days, $MeBu_3Sn$ was the second most abundant organotin in solution, reaching peak concentrations equal to 60% that of residual TBT. It was significant that $MeBu_3Sn$ did not form in the water column in experiments X and Y, and we conclude that its formation in experiment Z was a direct result of the presence of methanol added as carbon source. Presumably, mixture X did not contain a sufficient amount of an appropriate carbon source to allow for methylation of TBT; also, results from experiment Y suggest that acetate is not an appropriate carbon source for this conversion.

The results from the sediment samples are shown in Fig. 2. Here, in all cases, TBT in the sediment decreases regularly with time. These results cannot readily be used to deduce the kinetics of decomposition of sedimentary TBT, since the added dose of organotin alters profoundly the microbiological composition of the material.

In sediment Z only, there appeared the presence of methyltributyltin among the products of degradation of TBT. This coincided with the appearance of the substance in the water column. No other methylbutyltins were observed in either the sediment or the water in the reactor. It is noted that whereas methyltributyltin was the second most abundant organotin (after TBT) in the dissolved state, it was the least abundant sedimentary organotin compound until day 161 and remained comparable to MBT in abundance and always subsidiary to DBT in this phase. This suggests that, once formed in the sediment, $MeBu_3Sn$ tends to migrate and to concentrate preferentially in the water column. The much more polar ionogenic organotins are preferentially retained by the sediment. This conclusion is supported by the distribution constant (K_d; sediment/water) values reported by Tabone Adami[8] for sediment from Msida Creek, Malta: for Me-

Bu_3Sn this is 0.13 ± 0.04 l g^{-1}, and the corresponding value for TBT is 6.2 ± 1.1 l g^{-1}. It is not clear why a hydrophobic compound like methyltributyltin should partition preferably in the aqueous phase, but there is a strong affinity of these compounds for water. We have shown in our laboratory[9] that all three methylbutyltins $Me_nBu_{(4-n)}Sn$ ($n = 1$, 2 or 3), when dissolved in water, are very difficult to evaporate off even when the solution is vigorously agitated and air is bubbled through.

Comparison of the corresponding concentrations of methyltributyltin in the two phases (Table 2) reveals that, in all cases except for the 315 days sample, the organotin is partitioned between the phases at the equilibrium point. At 315 days, the sediment/water concentration quotient is well below the equilibrium value; this suggests that the generation of $MeBu_3Sn$ had ceased and the compound was apparently surviving longer in the water column than in the sediment. This is a peculiar result (and one which would have to be tested against much more data than presently available) since, for TBT, the opposite behaviour would be expected.[5]

The methylation of TBT under the chosen experimental conditions, though clearly possible, is apparently only a minor route of degradation for TBT. Using the maximum concentration found for $MeBu_3Sn$ sorbed in the sediment, and comparing it with the total mass of spiked TBT, one calculates an approximate methylation yield of 0.3%. However, the measured concentration of $MeBu_3Sn$ represents the balance between its rate of formation and that of its degradation and, therefore, should be regarded as a minimum value. Also, conditions may have been far from optimal for methylation, and this aspect of the phenomenon requires additional investigation. The tetraorganotin concentration decreases rapidly beyond about day 300 in both phases when the residual TBT concentration is still high. This disappearance could result from alteration of the chemical or microbiological conditions in reactor Z, possibly in response to the complete consumption of the added carbon source, not necessarily by the process of methylation but also by other competing and concurrent pathways.

Environmental methylation of TBT can be due to both biotic and abiotic mechanisms, and the findings in this work do not allow differentiation between these two mechanisms. Indeed, both mechanisms could possibly be operative in the marine environment. This was the conclusion reached by Guard *et al.*[10] with regards to methylation of trimethyltin to Me_4Sn in estuarine sediments. The time for appearance of a methylated product from TBT observed in this work was comparable to, if somewhat longer than, that for trimethyltin, where peak Me_4Sn concentrations were detected after 80 to 90 days. Presumably, biomethylation of Bu_3Sn^+ would require a nucleophilic species (*e.g.* methylcobalamine), as for the methylation of Hg(II).[11] The efficiency of methylation of TBT is likely to be dependent on the sediment type, and if, as is likely, a biotic mechanism is active, also on the microbial community in the sediment. As suggested earlier, it is highly probable that inoculating the sediment with TBT will alter considerably the microbial ecosystem, so that the observed rate of methylation would also depend on the tolerance to high TBT levels of any microorganism(s) responsible for biomethylation. Methanol appears to have a determining role in the conversion of TBT to $MeBu_3Sn$, although this aspect is the subject of further study. We are actively investigating the effects on the methylation of TBT of using isotopically labelled methanol and other substrates in order to shed more light on this matter.

Table 2 Concentration quotients $[MeBu_3Sn]_{sed}/[MeBu_3Sn]_{water}$ for sediment–seawater mixture Z as measured on different days of the experiment. The distribution constant (sediment/water) for $MeBu_3Sn$ is 0.13 ± 0.04 l g^{-1}

Day	$[MeBu_3Sn]_{sed}/[MeBu_3Sn]_{water}$ (l g^{-1})
161	0.14
203	0.17
273	0.14
294	0.13
315	0.073

CONCLUSIONS

We have presented preliminary data showing that *in vitro* methylation of TBT in a marine sediment–seawater mixture can occur to form $MeBu_3Sn$ under anoxic conditions if an appropriate carbon source is available. The transformation can presumably occur by both biotic and abiotic mechanisms, and this aspect of the phenomenon is the subject of further study in our laboratory.

The presence in the environment of methylated TBT, and also of methylated DBT (as Me_2Bu_2Sn) and MBT (as Me_3BuSn), has been reported only infrequently in the literature.[2–4] It appears likely that these compounds might be more ubiquitous than is presently apparent, and they could have remained

unidentified due to the fact that attention may have been reserved solely for specifically targeted organotin forms. It is not uncommon to see reports in the literature where FPD-generated gas chromatograms are presented that contain more peaks than are identified and discussed by the authors, and methylbutyltins could well be among such unidentified peaks.[12]

Although sediments are fairly good sinks for TBT and its debutylated analogues, methyltributyltin has a significantly lower affinity for this phase. Once formed, it has a tendency to migrate preferentially into the water column and possibly also to ex-solve into the atmosphere to generate a hitherto unsuspected flux of organotin compounds into marine air. Not much is known about the ecotoxicity of $MeBu_3Sn$ and that of similar tetraorganotins, although they might be expected to be less toxic than TBT.[13] In view of their greater environmental mobility, it would appear desirable to study more closely the impact of these compounds as part of the ecotoxicology of TBT.

REFERENCES

1. Looser PW. Bioaccumulation of triorganotin compounds by a sediment-dwelling organism (Chironomus riparius): assessment of bioavailability, uptake and elimination. Doctor of Natural Sciences dissertation (ETH No. 13′661), Swiss Federal Institute of Technology, Zurich, 2000.
2. Vella AJ, Mintoff B, Axiak V, Agius D and Cassone R. *Toxicol. Environ. Chem.* 1998; **67**: 491.
3. Maguire RJ. *Environ. Sci. Technol.* 1984; **18**: 291.
4. Maguire RJ, Tkacz RJ, Chau YK, Bengert GA and Wong PTS. *Chemosphere* 1986; **15**: 253.
5. Batley G. The distribution and fate of tributyltin in the marine environment. In *Tributyltin: Case Study of an Environmental Contaminant*, de Mora SJ (ed.). Cambridge University Press: Cambridge, 1996; 139–166.
6. Maguire RJ and Huneault H. *J. Chromatogr.* 1981; **209**: 458.
7. Carlier-Pinasseau C, Lespes G and Astruc M. *Appl. Organomet. Chem.* 1996; **10**: 505.
8. Tabone Adami JP. Methylation of tributyltin species in marine sediments. M.Sc. dissertation, Department of Chemistry, University of Malta, 2000.
9. Vassallo R. Organotin compounds in the atmosphere. M.Sc. dissertation, Department of Chemistry, University of Malta, 1998.
10. Guard HE, Cobet AB and Coleman WM. *Science* 1981; **213**: 770.
11. Wood JM. *Science* 1974; **183**: 1049.
12. Vella AJ, Mintoff B and Axiak V. *Sci. Total Environ.* 2000; **258**: 81.
13. Cooney JJ and Wuertz S. *J. Ind. Microbiol.* 1989; **4**: 375.

