

Organotin compounds and aquatic bacteria: a review*

J. J. Cooney

University of Massachusetts; Boston, MA 02125–3393, USA

ABSTRACT: Organotins are toxic to microorganisms. Trisubstituted organotins (R_3SnX) are considered more toxic than disubstituted (R_2SnX_2) or monosubstituted ($RSnX_3$) compounds, and tetrasubstituted compounds (R_4Sn) are not considered toxic. In the R_3Sn series propyl-, butyl-, pentyl-, phenyl- and cyclohexyltins are the most toxic to microorganisms. Toxicity towards aerobes in the R_3Sn series is related to total molecular surface area and to the octanol:water partition coefficient, K_{ow} , which is a measure of hydrophobicity. Care must be taken when testing the toxicity of tin compounds in the laboratory, for a number of biological, chemical and physical factors can influence the apparent toxicity. Although TBT is generally the most toxic of the butyltins, there are instances where monobutyltin (MBT) is as toxic, or more toxic, than TBT to microorganisms. Thus, debutylation in the sequence $TBT \rightarrow DBT \rightarrow MBT \rightarrow Sn$ does not detoxify TBT for all microorganisms. Some microorganisms can methylate inorganic or organic tins under aerobic or anaerobic conditions. Methylation can also occur by chemical means and the relative contributions of biotic and abiotic mechanisms are not clear. It is difficult to isolate a pure culture which can methylate tin compounds aerobically, and it is difficult to isolate a pure culture which degrades TBT, suggesting that microbial consortiums may be involved in transformations of organotins in the aquatic environment. Methylation and debutylation alter the adsorbitivity and solubility of tin compounds; thus, microorganisms can influence the environmental mobility of tin. TBT-resistant microorganisms can be isolated, and in some of them resistance to TBT can be plasmid-mediated.

INTRODUCTION

The efficiency with which a boat moves through the water depends on the smoothness of its hull. Increasing the average hull roughness by only 10 μm can increase fuel consumption by as much as 1%. If barnacles are present on the hull, they can increase roughness by several centimetres. Therefore, boats which operate in marine or estuarine waters are coated with antifouling paints. The principal types of paints used contain either copper or tributyltin (TBT) as their active antibiologic agent. TBT is the more effective of the two, lasting for up to 7 years, while vessels coated with copper-containing paints are painted yearly. The potential fuel saving on US vessels by using paint with TBT is estimated at US\$ 300–400 million per year, based on crude oil priced at US\$ 16 per barrel. This would represent a saving of 2 billion gallons of fossil fuel (Champ & Pugh, 1987). TBT is also used in coatings or treatments for other surfaces immersed in water, including intake pipes for cooling systems, pilings, buoys, oil rigs, cables and lines, and nets and cages used in aquaculture. Paints containing TBT are the only practical means

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for controlling the zebra mussel, *Dreissena polymorpha*, which is a major freshwater fouling pest (Mackie et al., 1989).

Some 30 000 tons of organotins are produced each year, representing about 5% of the tin mined. Tin may have more of its organometallic derivatives in use than any other element (Blunden et al., 1984; Champ, 1986; Davies & Smith, 1982; Maguire, 1987). Other organotins are widely used in industry and agriculture. Their largest single use is as stabilizers for polyvinyl chlorides, followed by their uses as antibiologic agents and as chemical catalysts (Blunden et al., 1984; Davies & Smith, 1980; Thompson et al., 1985). Much of the organotins used each year will eventually reach the environment. In the aquatic environment, particular attention has been focussed on methyltins, which can be formed there, and are used in industry, and on TBT which is toxic to a number of non-target organisms.

TBT causes morphological aberrations in oysters and mussels (Waldock & Thain, 1983). Considerable attention has been given to effects on the dogwhelk, *Nucella lapillus*, which in TBT-contaminated waters exhibits a condition known as imposex, wherein female animals grow male sex organs and the population cannot reproduce (Gibbs et al., 1991; Spence et al., 1990); exposure to TBT leads to a significant increase in testosterone (Spooner et al., 1991). Imposex can occur at concentrations as low as 1 ng TBT-Sn per litre (1 part per trillion, 8.4 pM) (Gibbs & Bryan, 1986; Oehlmann et al., 1991). Molluscs may be unusually sensitive because they have relatively low activities of cytochrome *p*-450 and mixed function oxidases which lead to TBT accumulation (Lee, 1991). A variety of other macro- and microorganisms is sensitive to TBT (Cooney & Wuertz, 1989; Hall & Pinkney, 1985; Thayer, 1984; Thompson et al., 1985). In general, a high octanol:water partition coefficient, *K_{ow}*, and a high total molecular surface area, predict greater toxicity (Eng et al., 1988). As with many environmental stressors, the early life stages are the most sensitive (Beaumont & Budd, 1984; Fent, 1991; Oehlmann et al., 1991). TBT can accumulate in the flesh of shellfish (Cullen et al., 1990; Davies et al., 1986), and in finfish (Short & Thrower, 1986) held in pens treated with paint containing TBT – thus entering the human food chain.

The most common quantification methods involve solvent extraction, derivative preparation to facilitate separation, and quantification of individual tin species by atomic absorption spectrometry, flame photometry or by mass spectrometry (Ashby et al., 1988; Blair et al., 1986; Han & Weber, 1988; Maguire, 1987; Quevauviller & Donard, 1990; Stallard et al., 1989; Valkirs et al., 1987).

In the aquatic environment, organic and inorganic tin compounds can be concentrated as much as 10 000-fold in the surface microlayer and up to 4000 times in oily sediments (Cleary & Stebbing, 1985; Maguire, 1987; Maguire & Tkacz, 1987). Tin may be associated with dissolved organic matter (Froelich et al., 1985; Donard & Weber, 1985), but most of the pollutant tin in rivers becomes attached to suspended particulates (Byrd & Andraea, 1982; Maguire & Tkacz, 1985) and concentrations are about three orders of magnitude greater in sediments than in the water column (Valkirs et al., 1986), and are a probable source for uptake by benthic deposit feeders (Langston & Burt, 1991). Salinity and pH influence binding to sediments (Harris & Cleary, 1987; Randall & Weber, 1986; Unger et al., 1987). Organotins also occur in freshwaters and their sediments (Fent & Hunn, 1991; Schebek & Andraea, 1991) and in sewage sludge (Fent & Mueller, 1991; Spiegel et al., 1985). Concentrations of TBT and its degradation products dibutyltin (DBT)

and monobutyltin (MBT) are higher in harbours and marinas than in open water (Alzieu et al., 1990; Cullen et al., 1990; Maguire, 1987; Wuertz et al., 1991; Thompson et al., 1985; Valkirs et al., 1986), as expected, since TBT leaches from antifouling paints.

Recently-deposited sediments contain about ten times as much tin as sediments laid down before the industrial revolution (Seidel et al., 1980). Evidence from laboratory microcosms suggests that TBT and its degradation products can accumulate in seagrass beds (Levine et al., 1990). It is to be expected that organotins will interact strongly with benthic organisms.

Organotin compounds also interact with microorganisms, and these interactions have largely been overlooked in considerations of the environmental fate and effects of organotins. It is important to understand these interactions, because microorganisms mediate a number of global processes and because they are at the base of food webs. The purpose of this paper is to summarize what is known of these interactions and to present some experimental results which illustrate the interactions.

ACTION OF TIN COMPOUNDS ON MICROORGANISMS

Pioneering work in this area was begun in the 1950s at the Institute for Organic Chemistry at Utrecht, the Netherlands (Sijpesteijn et al., 1969; Luijten, 1972). Tin compounds are toxic to some algae, fungi and bacteria, and the topic has been reviewed (Cooney & Wuertz, 1989). It is generally considered that toxicity decreases in the order $R_3SnX > R_2SnX_2 > RSnX_3$ (Fig. 1). The anion, X, has little effect on toxicity. Propyl-, butyl-, pentyl-, phenyl- and cyclohexyltins are generally most toxic to microorganisms but, as shown in Table 1, the effect varies from organism to organism, and ethyltins can also be effective. Similarly, the suggestion that Gram-negative bacteria are more sensitive to organotins than are Gram-positive bacteria does not always hold. The general rule that R_4Sn 's and inorganic tin are not toxic does not always apply (Table 1). Organotins and inorganic tin are toxic to some organisms in mixed populations (Hallas & Cooney, 1981b; Pettibone & Cooney, 1988; Wuertz et al., 1991) and to pure cultures (Belay et al., 1990; Boopathy & Daniels, 1991; Hallas & Cooney, 1981a; Hallas et al., 1982b; Soracco & Pope, 1983; Wuertz et al., 1991; and others), including indicator bacteria (Pettibone & Cooney, 1986).

Although the sequence of toxicity $R_3Sn > R_2Sn > RSn$ applies to macroorganisms and to many microorganisms, it does not hold for all microorganisms. For example, MBT was more effective than DBT at causing release of potassium from some marine yeast (Fig. 2, Cooney et al., 1989), and MBT and inorganic tin were more effective than TBT or DBT in inactivating bacteriophage T4 (Fig. 3, Doolittle & Cooney, 1992). Moreover, MBT is more toxic than TBT to methanogenic and sulfate-reducing bacteria (Belay et al., 1990; Boopathy & Daniels, 1991). These results do not agree with the relation verified with a variety of organisms (Eng et al., 1988), that increasing toxicity of organotins correlates with increasing total molecular surface area. Results with methanogens are not due to methanogens being anaerobes because TBT was more effective than MBT against *Escherichia coli*, whether the bacterium was grown aerobically or anaerobically (Boopathy & Daniels, 1991). These toxic effects of DBT and MBT suggest that TBT should not be considered detoxified for microorganisms simply because it has been partially debutylated.

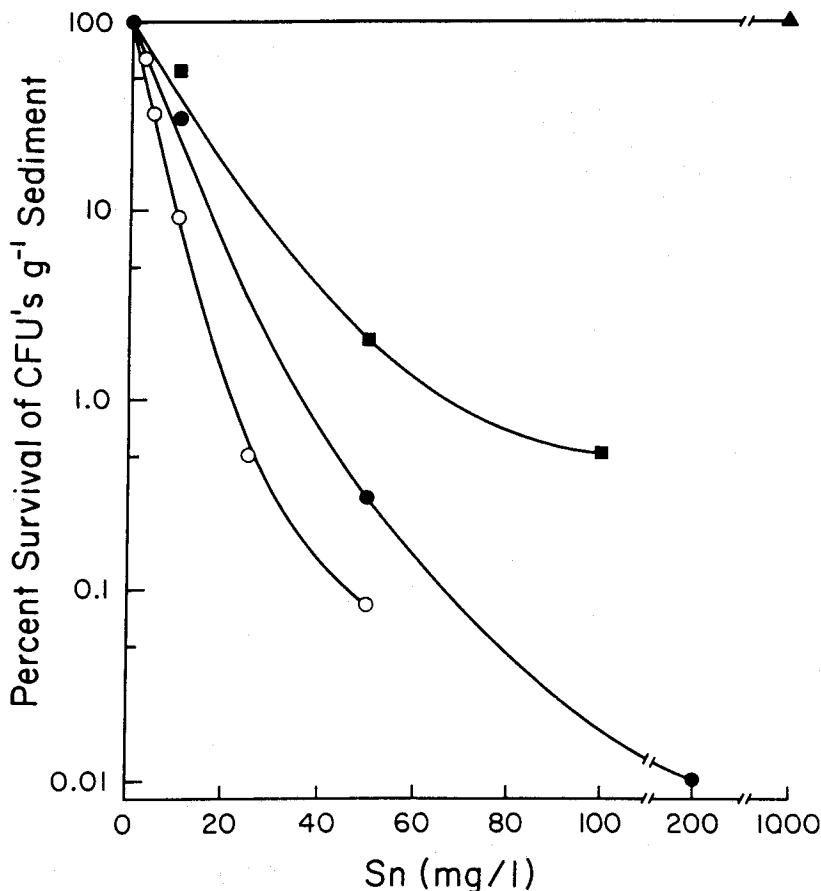


Fig. 1. Effect of methyltin compounds on total viable counts of natural populations from sediments in Boston Harbour. Tin was added as ■, CH_3SnCl_3 ; ●, $(\text{CH}_3)_2\text{SnCl}_2$; ○, $(\text{CH}_3)_3\text{SnCl}$; ▲, $(\text{CH}_3)_4\text{Sn}$. Total viable counts in control suspensions which did not receive any addition of tin ranged from 6.6×10^6 to $4.7 \times 10^7 \cdot \text{g}^{-1}$ of sediment. Reproduced from Pettibone & Cooney (1988), by permission

Mechanisms of toxicity to microorganisms were reviewed by Cooney & Wuertz (1989). Briefly, organotins are likely to accumulate in lipid-rich organelles, and they penetrate biological membranes more readily than inorganic tin. TBT acts as an ionophore, facilitating halide-hydroxyl exchanges, and interferes with energy transduction processes in bacteria, chloroplasts and mitochondria (Aldridge, 1986; Cooney & Wuertz, 1989; Selwyn, 1976). TBT also inhibits energy-linked reactions in *E. coli* (Singh & Singh, 1985) and *Legionella pneumophila* (Soracco & Pope, 1983). Effects include dissipation of ΔpH , and inhibition of: ATPase, oxidation of substrates, glycolysis, solute transport and energy-linked transhydrogenase (reviewed by Cooney & Wuertz, 1989). TBT inhibits microsomal cytochrome *P*-450 in fish (Fent & Stegeman, 1991), and perhaps it can in algae and fungi as well. Effects on mitochondria and chloroplasts include gross swelling, action as ionophores, and inhibition of ATPase (reviewed by Cooney & Wuertz,

Table 1. Minimum inhibitory concentrations (MIC) of stannic chloride and organotin compounds for estuarine isolates. Tin compounds were incorporated into estuarine salts agar (Nelson & Colwell, 1975). Isolates were streaked on the agar, incubated and scored for growth. Reproduced from Hallas & Cooney (1981a), by permission

Isolate	SnCl ₄	MIC ($\mu\text{g} \cdot \text{ml}^{-1}$) for compounds																							
		Methyl				Ethyl				Propyl				Butyl				Phenyl				Cyclohexyl			
		(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
<i>Debaryomyces hansenii</i>	$> 10^3$	$> 10^3$	$> 10^3$	30	$> 10^3$	100	< 1	125	1	175	500	100	5	25	300	15	5	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$
<i>Pseudomonas aeruginosa</i>	225	300	80	15	$> 10^3$	20	< 1	275	25	$> 10^3$	300	175	300	$> 10^3$	500	750	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$
Short gram - rod	250	300	40	5	$> 10^3$	20	< 1	225	20	$> 10^3$	300	50	300	$> 10^3$	200	750	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$
Long gram + rod	225	190	20	15	$> 10^3$	20	< 1	175	< 1	300	175	1	< 1	10	15	< 1	> 1	$> 10^3$	1						
Short gram + rod	125	180	25	15	$> 10^3$	25	< 1	125	1	200	200	10	< 1	< 1	25	15	1	$> 10^3$	< 1						
Short gram - rod	80	160	15	5	$> 10^3$	10	< 1	125	5	$> 10^3$	175	125	5	75	150	100	300	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$
Short gram + rod	80	160	15	15	$> 10^3$	30	< 1	175	< 1	100	175	5	< 1	1	100	1	< 1	$> 10^3$	< 1						
Short gram + rod	80	160	15	15	$> 10^3$	30	< 1	200	< 1	90	175	5	< 1	1	100	1	< 1	$> 10^3$	< 1						
Short gram - rod	125	135	40	15	$> 10^3$	15	< 1	175	< 1	200	100	< 1	< 1	< 1	25	1	< 1	$> 10^3$	< 1						
Short gram - rod	125	130	10	10	$> 10^3$	10	< 1	60	< 1	90	175	< 1	10	50	100	10	75	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$	$> 10^3$
Short gram - rod	12	125	15	2	$> 10^3$	10	< 1	100	< 1	25	100	< 1	< 1	< 1	1	< 1	$> 10^3$	< 1							

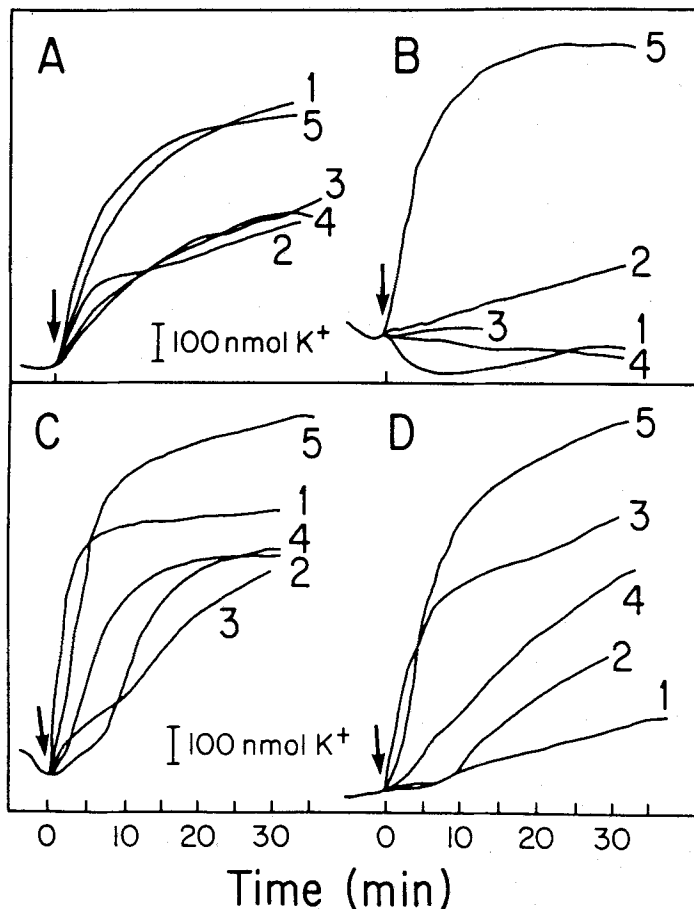


Fig. 2. Organotin-induced release of K^+ from five yeasts. A: monobutyltin trichloride; B: dibutyltin dichloride; C: tributyltin chloride; D: triphenyltin chloride. Final concentrations in the cell suspension were 50 μ molar. 1. *Aureobasidium pullulans*; 2. *Debaryomyces hansenii* NS-75-21; 3. *Pichia pinus*; 4. *Saccharomyces cerevisiae* X2180-1B; 5. *Zygosaccharomyces rouxii*. Organometal solution was added where indicated by the arrow. Reproduced from Cooney et al. (1989), by permission

1989). Disubstituted organotins inhibit enzymes which have dithiol functions, and di- and trisubstituted organotins are active against a number of enzymes (cf. Cooney & Wuertz, 1989; Davies & Smith, 1980).

Considerable caution must be used in extrapolating to the field from results obtained in the laboratory. As with other metals, a large number of conditions can influence the apparent toxicity of a metal or organometal (Babich & Stotzky, 1980, 1983; Cooney & Pettibone, 1986). Different methods for estimating toxicity can yield values which differ by as much as 100-fold (Jonas et al., 1984). As shown in Table 2, methyltins appear to be less toxic to bacteria from estuarine sediment when judged by thymidine uptake, than when judged by viable counts. Changing the organic (Table 3; Wuertz et al., 1991) or

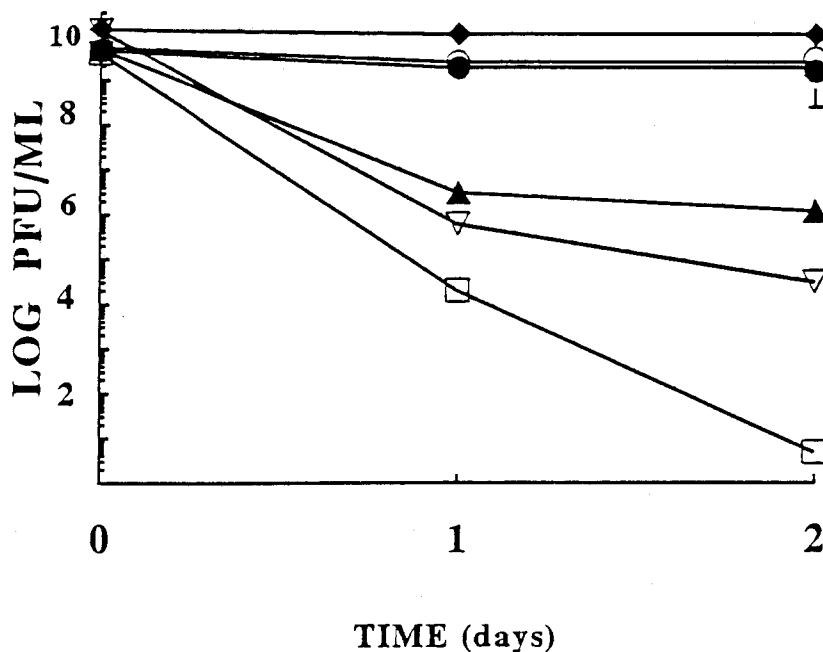


Fig. 3. Effect of tin compounds on infectivity of phage T4. Phage were incubated with 84 μM of the indicated tin compound at pH 6 and at 18 C for the times indicated. Error bars indicate the standard error of the mean (SEM). Where no bar is shown, the SEM was smaller than the symbol. ◆ no tin added; ● Sn²⁺; ○ TBT; ▲ DBT; ▽ MBT; □ Sn⁴⁺. The no-tin control received methanol. Reproduced from Doolittle & Cooney (1992), by permission

inorganic (Hallas et al., 1982b) composition of the assay medium can alter the apparent toxicity. Large molecules – such as humic acid or the protein, gelatin – can bind tin, making it inaccessible to the bacterium and decreasing apparent toxicity of the tin compound. It was suggested that small molecules such as serine or hydroxyflavone, which are known to bind tin and which increase the apparent toxicity, may carry the tin

Table 2. Comparative toxicities of three organotin compounds to natural populations from sediments in Boston Harbour as estimated by viable counts or by thymidine uptake. From Pettibone & Cooney (1988), by permission

Test compound	Sn concentration (mg/l)					
	LC ₂₀ *	Viable count			Thymidine uptake	
		LC ₅₀	LC ₈₀	LC ₂₀	LC ₅₀	LC ₈₀
CH ₃ SnCl ₃	4.3	11	17	26	43	> 50
(CH ₃) ₂ SnCl ₂	2.9	7.2	11.6	8.0	23	> 50
(CH ₃) ₃ SnCl	1.5	3.7	5.9	6.2	17	45

* Values for LC₂₀, LC₅₀, LC₅₀ and LC₈₀ are those concentrations of tin which decreased viable count or thymidine uptake by 20, 50 or 80 %, respectively

Table 3. Effect of organic ligands on the toxicity of stannic chloride to sediment microorganisms. From Hallas et al. (1982b), by permission

Addition to medium*	Concn (mg litre ⁻¹)	% of control**
None		
Single strength		18 ^{d, e}
Double strength		28 ^e
Inorganic nitrogen source***		
Plus cysteine	45	13 ^d
Plus serine	45	16 ^d
		8 ^f
Hydroxyflavone	75	3 ^g
Humic acid	75	51 ^h
	50	125 ⁱ
Gelatin	75	70 ^j

* Medium composition (grams per liter of distilled water): casamino acids, 10; yeast extract, 1; glucose, 2; NaCl, 10; KCl, 0.3; MgSO₄, 2.8

** Colony-forming units on medium containing 75 mg of Sn liter⁻¹ as SnCl₄ · 5H₂O divided by colony-forming units on medium without tin (×100)

*** NH₄NO₃ (1 g/liter) was substituted for yeast extract and casamino acids

^{d-j} Means with different superscripts are significantly different at the 5 % level of confidence

compound with them if they are transported into the cell (Hallas et al., 1982b). Moreover, different results are obtained in solid and liquid media (Hallas et al., 1982b; Wuertz et al., 1991). We have adopted a working definition of a TBT-sensitive bacterium as one which does not grow on trypticase soy agar containing 8.4 µM TBT, a concentration which inhibits the growth of about 90 % of the culturable bacteria from fresh or saltwater sediments (Wuertz et al., 1991). Methods which depend on diffusion of a toxicant through agar cannot be used to compare the toxicity of one tin compound with another, because organotins differ strongly in their solubility, and many of them are essentially water-insoluble. If one hopes to extrapolate from the laboratory to the environment, considerable caution should be used and the conditions used in the laboratory should approximate field conditions as closely as is feasible.

ACTION OF MICROORGANISMS ON ORGANOTINS

Methylation. Among the organisms which survive stress by tin compounds, are some which can methylate inorganic or organic tin compounds. Methyltins are widely distributed. They have been found in rain, in a variety of natural waters and in sediments (Byrd & Andreae, 1982; Maguire, 1987; Schebek & Andreae, 1991; Seligman et al., 1991; Thompson et al., 1985; and others). They may be formed by biotic or abiotic methylation, or be derived from anthropogenic sources, since they are widely used in industry. The relative contributions of these sources is poorly understood

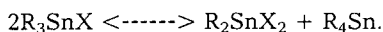
Tin compounds can be methylated by natural populations of microorganisms brought into the laboratory and incubated in natural water and sediment or in nutrient medium

under aerobic conditions. SnCl_2 and SnCl_4 (Chau et al., 1981; Cooney et al., 1981; Hallas et al., 1982a) or methyltins (Chau et al., 1981; Guard et al., 1981) can be methylated. Algae may be important contributors to methylation (Lee et al., 1989). Pure cultures can also effect methylation. A *Pseudomonas* sp. methylated Sn^{2+} and Sn^{4+} , yielding di- and trimethyltin compounds, including the stannanes, and probably tetramethyltin (Blair et al., 1981; Jackson et al., 1982). It is significant that methyltin(s) produced by the *Pseudomonas* sp. can methylate Hg^{2+} abiotically (Huey et al., 1974). Two *Bacillus* spp. isolated from estuarine sediments methylated monomethyltin. Neither organism alone accomplished the methylation (Makkar & Cooney, 1990). This suggests that microbial consortiums may be involved in methylation in situ.

Methylation can also occur anaerobically. Slurries prepared from sulfide-rich sediments incubated anaerobically methylated Sn^{4+} , and did so more rapidly than slurries incubated aerobically. Samples from sediment cores also produced mono-, di- and trimethyltins. *Desulfovibrio* spp. isolated from the sediments methylated at the same rate as the mixed flora in slurries (Gilmour et al., 1985).

Butylmethyltins were found in sediments of several Canadian harbours (Maguire, 1984). Since TBT from antifouling paints is the principal source of tin in those harbours, these observations suggest strongly that butyltins can be partially degraded and then methylated in situ.

Organotin compounds can give rise to one another by disproportionation reactions such as:



The extent of disproportionation reactions in the aquatic environment and their contribution to the geochemical cycling of tin is poorly understood (Blunden et al., 1990; Thompson et al., 1985). They make it more difficult to establish the role of microorganisms in the geochemical cycling of tin.

In summary, a number of the transformations in the "tin cycle" proposed by Ridley et al. (1977) can occur under laboratory conditions. Methylations are environmentally important because methylation increases the volatility, the lipid solubility, the adsorptivity and the toxicity of tin, and probably its mobility in the environment.

Degradation of butyltins. In the marine water column, TBT can be degraded rapidly to DBT and MBT with a half-life of several days (Seligman et al., 1994). Photolytic degradation also occurs but it proceeds slowly, with a half-life of > 89 days (Maguire and Tkacz, 1985). Light stimulates degradation (Seligman et al., 1989) when microalgae are present (Lee et al., 1989) and when nutrients are added (Meyers-Schulte & Dooley, 1990). The green alga *Ankistrodesmus falcatus* degraded TBT to inorganic tin (Maguire et al., 1984). Exposure of water samples from Chesapeake Bay to light, was necessary to obtain TBT degradation (Olson & Brinckman, 1986). These results suggest that photosynthetic organisms may be involved in the degradation.

In sediments, where most of the TBT resides, degradation is slower, with a half-life of several months (Maguire & Tkacz, 1985), years (deMora et al., 1989) or absent (Adelman et al., 1990). Although a strain of *Pseudomonas aeruginosa* and several wood-decaying fungi can degrade TBT, efforts to isolate organisms which can degrade it under aerobic or anaerobic conditions have not been successful in our laboratory or elsewhere (Blair et al., 1982; Olson & Brinckman, 1986). This suggests that microbial consortiums and/or cometabolic events may be involved. Mammals, fish and crustaceans can hydroxylate the

butyl moiety, as well as debutylate TBT (Fish et al., 1975; Lee, 1991), but hydroxylated metabolites have not been demonstrated in microorganisms. Stang et al. (1992) obtained limited biotic degradation of TBT to MBT and inorganic tin in sediments, but sterile, surficial, fine-grained sediments degraded more extensively, to MBT and inorganic tin. The relative contributions of biotic and abiotic degradation remain to be determined in sub-surficial and anaerobic sediments.

Other organotins undoubtedly make their way to the aquatic environment. For example, triphenyltin has been reported in fish tissue (Tas & Opperhuizen, 1991). What little is known of interactions between other organotins and microorganisms is available in reviews (Cooney, 1988; Cooney & Wuertz, 1989).

TBT-resistant microorganisms. We have developed a working definition of a tin-resistant microorganism, as one which grows in the presence of an amount of tin which kills 90%, or more, of the culturable population. For SnCl_4 , this was $0.63 \mu\text{M}$ in an estuarine salts agar (Hallas & Cooney, 1981a) and for TBT, this was $8.4 \mu\text{M}$ in trypticase soy agar (Wuertz et al., 1991). These are pragmatic definitions. Since chemical, biological and physical conditions can influence the apparent toxicity of tin compounds, one should adjust one's working definition to the individual situation.

TBT-resistant bacteria from fresh and estuarine sediments showed a high incidence of resistances to antibiotics and to inorganic forms of heavy metals (Wuertz et al., 1991). Five out of ten estuarine and 12 of 19 freshwater isolates examined contained plasmids. Since some TBT-resistant organisms lack plasmids, TBT-resistance need not be plasmid-mediated. However, when a chromate-resistant and TBT-resistant *Pseudomonas aeruginosa* (which harbours plasmid pCRO1), was cured of the plasmid, it lost TBT-resistance. *Pseudomonas fluorescens* BP4 has four plasmids. In conjugation experiments, it will donate all of them to another strain of *P. fluorescens* and the TBT-resistance of the recipient is increased 10-fold (Miller et al., unpubl.). Thus, TBT-resistance can be plasmid-mediated.

REGULATION OF TRIBUTYL TINS

Because of its effects on non-target organisms at levels as low as 8.4 pM (1 part Sn per trillion), TBT has been called "perhaps the most acutely toxic chemical to aquatic organisms ever deliberately introduced to water", (Maguire, 1987). Thus, TBT has attracted the attention of environmental protection agencies in a number of countries. The first regulation of TBT was in France in 1982, followed by the United Kingdom, Ireland, Germany and Switzerland. The British regulation restricted the amount of tin in antifouling paints, but did not restrict their use. The regulation did not have the desired effect, and in 1987 the use of TBT-paints was banned on small boats and mariculture equipment (Vosser, 1987). In 1988 TBT was regulated in the US. The effective regulations are similar in all countries: paints with TBT may not be used on boats shorter than 25 metres; aluminium-hulled boats are exempt, because copper-based paints will contribute to hull electrolysis. Huggett et al. (1992) provided a useful, brief summary of a number of monitoring studies in the environment and in animals, and of the regulatory history in the US. The restriction on use of paints with TBT has resulted in decreased levels in the environment (Alzieu, 1991; Cleary, 1991; Huggett et al., 1992) and a decrease in effects on shellfish populations (Alzieu, 1986; Bailey & Davies, 1991; Waite et al., 1991). Levels in sediments do not appear to be decreasing as rapidly (Waite et al., 1991), and levels in

water and sediments at many sites exceed acceptable levels (Alzieu et al., 1991). Moreover, paints containing triphenyltin (TPT), which is not covered by the regulations, are now being introduced. TPT is appearing in the environment around marinas and harbours and in fish (Alzieu et al., 1991; Fent & Hunn, 1991; Schebek & Andreae, 1991; Tas & Opperhuizen, 1991). Little is known about the fate and effects of TPT, particularly its interactions with microorganisms.

The TBT story, and stories of other organotins, are not over. The role of microorganisms in their bioaccumulation, transformation, mobilization and degradation is poorly understood – as is the potential use of microorganisms for bioremediating waste streams, and polluted harbours and marinas. We should explore the activities of microbial communities and consortiums in effecting these changes. Similarly, microorganisms can serve as models to expand our understanding of how organotins interact with organisms at the cellular, biochemical and genetic levels.

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