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Review

Microbial transformations of tin and tin compounds

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SUMMARY

The use of organotins for agricultural and industrial purposes and in the marine environment has been increasing steadily for more than 20 years. Recently, reliable methodologies have been developed to permit quantification of individual molecular species of organotins in cultures and in the environment. Particular attention has been given to methyltins which can be formed abiotically and by microorganisms, and to tributyltins which are toxic components of effective antifouling paints. In the aquatic environment tin, tributyltins, and other organotins accumulate in the surface microlayer, in sediments, and on suspended particulates. Tin compounds are toxic to a variety of organisms and some aquatic organisms can bioaccumulate them. When tin compounds, particularly di- or tri-substituted tins, enter an ecosystem, a portion of the microbial population is killed. Among the survivors are organisms which can methylate inorganic or organic tins, but the relative contribution of biotic and abiotic mechanisms is not clear. While many details of methylations and demethylations need to be worked out, it is clear that transformations of tins can influence the toxicity, volatility and mobility of tin in natural ecosystems. Tributyltins can be debutylated by microorganisms, and hydroxybutyl tins may be intermediates, as they are in mammalian systems. Little is known of the potential and probable microbial transformations of other economically important organotins, but the transformations should be studied for they may have industrial and environmental importance.

About 2×10^5 tons (1.8×10^8 kg) of tin are mined each year [52]. Most of it is used for plating of tin cans, but $2.5–3.0 \times 10^4$ tons (2.5×10^7 kg, or 14%) per year are used as organotins [102]. Davies and Smith [32] estimated that the use of organotins in industry had risen to $3.0–3.5 \times 10^7$ kg in 1980 from 5×10^6 kg in 1965.

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The principal uses of organotins include serving as polymerizing and stabilizing agents for chlorinated hydrocarbons such as polyvinyl chlorides and for oils, paints and other plastics; as chemical catalysts; as antioxidants, poultry feed additives, wood preservatives, slimicides in cooling towers, in sprays for fruit trees; and as antifouling agents in marine paints [72,91]. Some organotins have potential as agents for cancer chemotherapy [19].

One of the measures of pollution by an element

is the degree to which the element is enriched in atmospheric particulates in comparison to its abundance in the earth's crust, presumably having been moved from the crust to the atmosphere by natural forces and by human activities. By this criterion tin is the third most important elemental pollutant, after lead and tellurium [18]. Estimates of anthropogenic fluxes of tin in the environment may be as high as ten times the natural fluxes [18]. Concentrations of tin in recent marine and terrestrial sediments are ten times higher than in sediments deposited before the industrial revolution [77]. Thus, it is clear that tin is a pollutant of human origin.

The aims of this review are to summarize briefly the main points in our current knowledge of tin in the environment and of tin's toxicity, and then to review microbial transformations of tin and tin compounds.

TIN IN THE ENVIRONMENT

It was not until the 1980's that two key advances were made which allowed us to begin to understand tin in the environment. First, it was realized that individual molecular species of tin must be measured rather than total tin. Second, reliable methodologies were developed to quantify important molecular species, particularly methyl- and butyltins.

In the last several years methyl- and/or butyltins have been measured in a number of bodies of water, including Chesapeake Bay [45,51,68], a New Hampshire estuary [36,73], San Diego Bay [93], a number of Canadian fresh waters [62,64], at several sites in Canada, France and southern England [23,37], a Portuguese estuary [3], a Florida estuary, the Gulf Stream and the Sargasso Sea [18], in a variety of waters and sediments, and in rain water [92]. Particular attention has been given to methyl- and butyltins because methyltins can be produced by microbial action and because tributyltin (TBT) has received attention as a component of antifouling paints. In seawater, butyltins have been reported at levels as high as tens of ppb of Sn. 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 [36,62,67]. The surface microlayer may be involved in movement of organotins to areas of biological productivity and ecological sensitivity. Tin may be associated with dissolved organic matter [42]. Byrd and Andreae [18] calculated that most of the pollutant tin in rivers becomes attached to suspended particulates which are eventually deposited on the continental shelves. Tugrul et al [92] reported that monomethyltin predominated in anoxic polluted marine sediments, dimethyltin in oxic-polluted sediments, and trimethyltin in oxic-nonpolluted sediments. Although 95% of the tributyltin in the water column is associated with particles, environmental concentrations are about three orders of magnitude greater in sediments than in the water column [93]. Values for tin in sediments are harder to obtain because of analytic difficulties, but in contaminated areas butyltins can reach ppb–ppm levels. Binding of tin compounds to sediments varies greatly with the sediment and the tin species, and binding is influenced by salinity, pH and amounts of particulates present [74]. TBT binds strongly to sterile sediments from Toronto Harbor. The $t_{1/2}$ for desorption (the time required for half the TBT to desorb) was at least 10 months at 20°C [63]. Thus, sediments may be a trap for TBT.

Concentrations of butyltins are higher in harbors and at marinas than in open waters, making a strong connection between tributyltin-based antifouling paints and organotin pollution [64,68,79]. Furthermore, butyltins are highest in marina areas in late spring and early summer after freshly painted recreational boats are launched [45].

Photolysis may play a role in organotin degradation [20,61,63] although in some cases it is not clear whether light degrades tin directly or stimulates photosynthetic organisms which accomplish the degradation [68,79].

Levels of total tin can reach hundreds of ppm in sewage sludge [87]. At 100 ppm TBT can adversely affect the settling ability of sludge [4].

TOXICITY

Tin and organotins are toxic to a wide variety of organisms at levels reported to be present in polluted environments. Recent reviews are available [20,88,91]. In general, organotins are toxic in the 1–50 ppb range. Eggs, larvae and juvenile forms of aquatic animals are more sensitive than are adult forms.

It is equally important that some aquatic organisms can bioaccumulate tin compounds. These include shellfish [33,94] and some finfish [80,95]. Trophic level does not appear to be very important since both predators and filter feeders can accumulate TBT from their food [57].

Microorganisms, at the base of the food web, can also bioaccumulate tin. The alga *Isochrysis galbana* had a bioconcentration factor (BCF) of 5500 for TBT [58] while the alga *Ankistrodesmus falcatus* had a BCF of 30 000 [65]. Pure cultures of eight bacteria isolated from Chesapeake Bay had BCF values of 350–850 when exposed to TBT [12], and six methyltin-resistant bacteria from Chesapeake Bay had a BCF of 220 when exposed to TBT (Jonas, R.B. and J.J. Cooney, Abstr. Annu. Meet. Am. Soc. Microbiol. 83: 227, 1983). This area needs additional work.

Tin and organotins are toxic to some microorganisms. When tin or organotins enter an ecosystem, a portion of the indigenous microorganisms is killed. Thus, 45–88% of the total plate counts from sediments from nine sites in Chesapeake Bay were inhibited or killed in medium containing 15 mg Sn per liter added as dimethyltin [46]. The toxicity of tin to microorganisms was reviewed by Thompson et al. [91]. Briefly, tins and organotins are toxic to a variety of algae, fungi and bacteria, including indicator bacteria [46,47,70,71]. Di- and tri-substituted compounds are more toxic than mono- or tetra-substituted compounds, and tri-substituted are usually the most toxic. Among tri-substituted tins, those with 9–12 total carbons show maximum activity against fungi and those with 9–15 total carbons are most effective against bacteria. Gram-positive bacteria are generally more sensitive than Gram-negative bacteria. At the cellular level, organotins can inhibit mitochondrial and chloroplast functions in eucaryotes. Less information is available for their action in bacteria, but inhibition of reactions related to energy transduction and cell permeability point to the cell membrane as a prime site of activity, as reviewed by Thompson et al. [91].

A wide variety of physical and chemical factors can affect the apparent toxicity of metals to microorganisms as measured in the laboratory. Likewise, the physicochemical characteristics of an ecosystem determine the chemical speciation of a metal and the bioavailability of the metal to microorganisms in the ecosystem. Reviews are available [5,6,27,67]. Of relevance here are the findings that the toxicity of inorganic tin to populations of estuarine microorganisms is influenced by organic components of the medium, by inorganic components and even by the solidifying agent used to prepare plates [46,49]. Therefore, a thorough study of toxic effects of metals on microorganisms should use more than one measure of toxicity, toxicity should be estimated under conditions as close to in situ as possible, and one should extrapolate from the laboratory to the field with caution.

MICROBIAL TRANSFORMATIONS

All microbe-mediated reactions shown to date involve the formation or breakage of tin–carbon bonds, and possibly transformations of organic moieties bonded to tin. There is no evidence that stannous ions can be oxidized or that stannic ions can be reduced by direct microbial action.

Biomethylation/demethylation reactions

Among the microorganisms which survive stress by tin compounds are some which can methylate inorganic or organic tins. In a review published in 1982 [89] Thayer and Brinckman stated, 'There is presently no firm evidence available for the biosynthesis of organometals having organic groups larger than methyl sigma bonded to a metal...'.

Methyltin species have been found in rain, in sediments, and in a variety of natural waters, as reviewed by Thompson et al. [91]. They may be de-

rived from anthropogenic sources or as a result of abiotic methylation reactions as well as by biotic methylation. The relative contributions of these sources are poorly understood. Byrd and Andreae [18] found that mono-, di- and trimethyltin compounds were almost ubiquitous in the rivers surveyed. They concluded that biomethylation was the major source. Brinckman and co-workers [17] suggested that adsorption and concentration of microorganisms and tin on suspended particles and in surface slicks could provide sites for biomethylation in aquatic systems. Tugrul et al. [92] presented evidence which suggested that the net methylation rate in marine coastal sediments is independent of the inorganic tin content of a sediment.

Methyltin compounds can also be demethylated sequentially to inorganic tin by photolysis [13].

Tin can be methylated in nutrient medium or in mixtures of natural water and sediment by populations of aquatic microorganisms. Guard et al. [44] incubated oxygen-deficient sediments from San Francisco Bay with trimethyltin under aerobic conditions. Tetramethyltin was detected in autoclaved and in non-autoclaved flasks, but 2.7 times more was present in the non-autoclaved flasks. Sediments from Canadian lakes incubated in a microbiological medium methylated inorganic Sn(II) and Sn(IV), but methyltin(IV) compounds were methylated more readily than the inorganic compounds [22]. Dimethyltin and trimethyltin were detected by GC and GC/MS when sediments from Chesapeake Bay were incubated with $SnCl_4$. Methylated tins were not detected in poisoned controls or in sterile controls [26,48].

Pure cultures can also effect methylation. Brinckman and colleagues observed methylation of inorganic Sn(II) or Sn(IV) by a *Pseudomonas* sp. [16,17,50]. They provided GC/MS data to confirm formation of trimethyltin species from Sn(IV) and, to a lesser extent, from Sn(II). Dimethyl- and trimethyl stannanes and probably tetramethyltin were also found [11,15,53]. Seidel et al. [77] reported that unidentified bacteria isolated from seaweed produced tetramethyltin, which was identified by its GC retention time. Thus, methyltins which they found in the seaweed could be due to methylation by epibiotic bacteria present on the algae.

A number of reactions in the 'tin cycle' proposed by Ridley et al. [75] occur in laboratory experiments. Methylation reactions are environmentally significant because methylation increases the volatility and the toxicity of tin, as well as potentially altering its mobility in the environment.

Recently, Maguire [60] reported butylmethyltins in the sediments of several harbors in Canada. Since tributyltins from antifouling paints on ship hulls are the principle source of tin in those harbors, this observation provides strong presumptive evidence that butyltins can be partially degraded and then methylated in situ. It is not known whether the process is biotic, abiotic or both.

Gilmour et al. [43] used sediments from Chesapeake Bay as inoculum for laboratory media containing $SnCl_4$ and detected mono- and dimethyltins as products. Cultures incubated anaerobically produced methyltins more rapidly than aerobic cultures. Production of monomethyltin was correlated positively with numbers of sulfate reducers and of sulfate oxidizers. *Desulfovibrio* spp. isolated from the sediments were able to carry out the methylation. Thus, methylation of tin can occur in anaerobic sediments, and sulfate reducers may be major contributors as they are for mercury methylation [24,25].

The question of geochemical tin cycling is made more complex by the knowledge that organotin compounds can give rise to one another by disproportionation reactions (Eqn. 1 and reviewed by Thompson et al. [91]).

$$3\,R_4Sn + 3\,SnCl_4 \rightleftarrows 2\,R_3SnCl + 2\,R_2SnCl_2 + 2\,RSnCl_3 \quad (1)$$

Disproportionation reactions can complicate attempts to distinguish biological and chemical components of methylation and of geochemical cycling of tin. Transmethylation reactions are also possible between inorganic and methylated forms of different elements, e.g. between methyltin and mercury [89]. Moreover, the mobility of methyltin compounds in estuarine waters can be influenced by organic ligands such as fulvic acid and by particulate matter such as iron oxide [36]. In addition,

macroalgae can contain inorganic tin, and methyl- and butyltins. Both healthy and decaying algae can release inorganic tin and methyltins [35]. These observations suggest that algae may serve as a reservoir for tin compounds.

Biochemical mechanisms for methylation are not completely clear. A thorough treatment is beyond the scope of this review. Briefly, methylcobalamin (methyl-B_{12}) has been proposed as the methylating agent [34,75]. Other methylating species, *S*-adenosylmethionine and *N*-5-methyltetrahydrofolate, are not regarded as able to transfer methyl to positively charged metals [75,96]. $SnCl_2$ reacted aerobically with methyl-B_{12} to yield methyltin trichloride. The reaction did not occur under anaerobic conditions [38]. Craig [28] and Craig and Rapsomanikis [30] have pointed out that conditions for the methylcobalamin-mediated reactions are not likely to be met in the natural abiotic or enzymatic environment. Other methylating agents which can function under conditions found in the environment include CH_3I, $(CH_3)_3S^+I^-$, and $(CH_3)_3N^+CH_2COO^-$ [1,29,90]. Abiotic formation of methyltin triiodide by the reaction of tin sulfide and methyl iodide in water has been observed [66]. All of these potential methylating agents and methyl-B_{12} occur or are produced in natural aquatic ecosystems. Potential mechanisms for methylation were reviewed by Thayer [88], Thayer and Brinckman [89], and Thompson et al. [91].

It is possible that some methylating agent(s) can be produced by cells and released to the environment by those cells or released when cells die. It is also possible that cells and/or reactants can be concentrated on soil particles, on suspended particulates or on sediments, increasing reaction rates in situ.

Transformations of butyltins

Tributyltins (TBTs), as the oxide (TBTO) or the fluoride (TBTF), are used as the active agent in antifouling paints for wood, fiberglass and metal boat hulls, where they are more effective than copper-based coatings. In addition, they do not promote corrosion of metal hulls [85]. The U.S. Navy intends to replace copper-based paints with tributyltin copolymer paints on its steel-hulled vessels by 1991. This will result in a demand for an additional 41 000 kg of TBT per year. However, concern over the toxicity of TBT led to a decision to ban their use on U.S. naval vessels until more is known of their fates and effects [82]. TBTO is also used as a wood preservative and as a disinfectant.

In seawater, TBT reaches an equilibrium distribution which includes tributyltin chloride, tributyltin hydroxide, the aquo complex ($TBTOH_2^+$) and a carbanato species ($TBTOHCO_2^-$). The equilibrium is affected by the chloride ion concentration, dissolved CO_2 and pH. The K_{ow} value for TBT varies with salinity, with a low value of 5500 in water containing 25‰ salt and increasing values at higher or lower salinity to a maximum of 7000 in deionized water [58]. In water TBTO does not debutylate in the dark and does not volatilize over a period of at least 2 months. TBTO in water photolyzes slowly ($t_{1/2}$ > 89 days) in sunlight [61]. Slesinger and Dressler [83] reported a photolytic $t_{1/2}$ of 18 days.

Relatively little is known about metabolism of TBT by microorganisms. Barug [8] showed that a bacterium and two fungi could debutylate TBT. Fungal cultures isolated from wood degraded TBTO, apparently to dibutyltin and monobutyltin [69]. Barug [8] was not able to isolate a pure culture which could use TBTO as sole carbon source, but he obtained cultures of *Pseudomonas aeruginosa, Alcaligines faecalis* and the fungi *Coniophora puteana, Trametes versicolor* and *Chaetomium globosum* which could debutylate TBTO. Barug and Vonk [9] showed that TBTO was metabolized to CO_2 in fertile soils but not in sterilized soil samples. A $t_{1/2}$ of 15–20 weeks was estimated for TBTO.

Blair et al. [12] did not find evidence of TBT metabolism by tin-resistant bacteria isolated from Chesapeake Bay, although the organisms accumulated tin. In a later work from the same laboratory [68] no biodegradation of TBT was observed in samples taken in the winter and incubated at winter temperature, but samples taken in the summer degraded TBT to di- and monobutyltin. Exposure to incandescent light during incubation stimulated biodegradation, suggesting that photosynthetic microorganisms may be involved. Tetrabutyltin

was identified in some light-incubated cultures, but not in dark controls, suggesting the involvement of photosynthetic organisms and/or a disproportionation reaction.

The green alga, *Ankistrodesmus falcatus,* debutylated TBT completely, yielding inorganic tin as well as di- and monobutyltins [65].

Maquire [60] reported tributylmethyltin and dibutylmethyltin in sediments from harbors in Canada, suggesting that some butyltin species can be methylated in aquatic environments.

Maguire and Tkacz [63] reported a half-life of 5 months for TBT in Toronto Harbor water at 20°C, and a half-life of 4 months in sediment-water mixtures. They concluded that the main factors limiting degradation in aquatic ecosystems are photolysis in water and biological degradation in water and sediment. Seligman and co-workers [78,79] estimated degradation rates in San Diego Bay waters. Half-lives calculated from a clean-water site were 9 and 19 days for light and dark treatments, respectively. Water from a yacht harbor yielded half-lives of 6 and 7 days for light and dark treatments. Photolysis was not detected. Complete mineralization to CO_2 was slow, with a half-life of 50–75 days. The principal products of TBT degradation were dibutyl- and monobutyltins. In water samples 30% of the butyltin species was dibutyltin and 10% or less was monobutyltin.

Rat liver microsomes can both hydroxylate and debutylate TBT [21]. Rat liver can form several mono-hydroxylated compounds from TBT without debutylating it [40]. Microsomal preparations from livers of rats, mice and from the abdomen of house flies formed α-, β-, γ- and δ-hydroxybutyl dibutyltins from tributyltin acetate. A monooxygenase was involved [55]. Cell-free preparations of fish tissues incubated with TBT yielded β-hydroxybutyl dibutyltin as well as dibutyltin [59].

There is some evidence for formation of hydroxybutyl intermediates by microorganisms. Culture filtrates from three fungi which degraded TBTO contained an unidentified material which was suspected of being a hydroxylated dibutyltin [69]. Seligman et al. [79] found a compound 'having the properties of' a hydroxybutyl dibutyltin after water samples from San Diego Harbor were incubated with TBT in the light. Olson and Brinckman [68] reported two unidentified compounds in cultures of organisms from Chesapeake Bay which had been incubated with TBT.

Other organotins

Little is known of microbial transformations of ethyl-, propyl-, pentyl-, hexyl-, octyl- or mixed cyclohexylphenyltin compounds.

Di- and triethyltins are toxic in mammalian systems [2,56,76,80] and to bacteria [46,47,70,71]. Triethyltin chloride inhibited growth of *Escherichia coli* [97] and, at concentrations below the minimum inhibitory concentration for growth, oxidation of several substrates was inhibited [54]. Rat liver microsomes convert tetraethyltin to triethyltin [31]. Rats can deethylate diethyltin but do not appear to metabolize monoethyltin [14]. Kimmel et al. [55] noted that ethyl-, propyl-, pentyl-, hexyl-, and cyclohexyl trialkyltins appear to undergo microsomal monooxygenase attack. Thus, ethyltins are probably metabolized by mammals. Their metabolism should be examined in microbial systems.

Ethyl iodide reacted with lead diacetate in aqueous solution at pH 5–6 to produce tetraethyllead [1], suggesting that ethylation of metals can occur in natural ecosystems.

Tripropyltin chloride inhibits membrane functions [99–101] and other cell functions [100] in *E. coli.* Cells of *E. coli* take up tripropyltin chloride rapidly [98], but virtually nothing is known of metabolism of propyltin compounds.

Tricyclohexyltin is used as a miticide in sprays for fruit trees. It can be photolyzed by sunlight on surfaces and in water, apparently first to di- and then to monocyclohexyltin. Photolysis in water produces cyclohexane and cyclohexanol [84]. The mixed compound cyclohexyltriphenyltin was converted to 4-hydroxycyclohexyltriphenyltin by a rat microsomal monooxygenase system [39]. There is some evidence for binding to soil and subsequent microbial dealkylation [10].

Triphenyltins are used as fungicides. They can be degraded abiotically by sunlight photolysis in water or on surfaces. Water-soluble polymeric

mono- and diphenyltin species may also be formed [86]. In the dark, triphenyltin hydroxide is stable in water for at least a month between pH 5 and 10 at 32°C. Kimmel et al. [55] reported that triphenyltin acetate is resistant to monooxygenase attack in rat liver microsome preparations. Soil microorganisms degrade it slowly ($t_{1/2}$ = 140 days) to inorganic tin via di- and monophenyltins [7]. Similar degradation pathways have been found in rats and on the leaves of sugar beets [41], although photolysis was not excluded on leaves.

UNANSWERED QUESTIONS

We have only begun to understand interactions between microorganisms and tin compounds. For almost all tin compounds there are tremendous gaps in our knowledge of whether they can be metabolized, what the key intermediates are, and the enzymes involved. Can organo-moieties larger than methyl be transferred to tin? Are hydroxylated intermediates involved in microbial metabolism of butyltins or of other organotins?

The genetics of these microbial systems is unknown. It is not even known whether resistance to tin or organotins is plasmid-mediated.

Questions related to the physiology of microbial transformations should be asked and answered in the areas of uptake, regulation, and mechanisms of toxicity and resistance.

What are the relative contributions of biotic and abiotic mechanisms in the degradation and geochemical cycling of tin and tin compounds? What are the effects of key environmental variables, including seasonal variations? What are the rates of biotic and abiotic processes in nature and what are the relative rates of aerobic and anaerobic transformations? To what extent can natural systems be manipulated in order to enhance selected reactions, including degradative reactions in natural waters and sediments or in industrial waste streams? Answering these and other questions should lead to environmentally and industrially useful knowledge.

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