Journal of Industrial Microbiology, 4 (1989) 375–402
Elsevier

SIM 00204

Review Paper

Toxic effects of tin compounds on microorganisms

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Received 25 July 1988
Revised 7 January 1989
Accepted 27 January 1989

Key words: Toxicity; Organotins; Tin; Methiltins; Butyltins; Tributyltin

SUMMARY

Organotins are used for industrial and agricultural purposes and in antibiologic agents. They are significantly more toxic than inorganic tins, and eventually reach the environment where they can be toxic to a wide variety of organisms. Particular attention has been given to tributyltins which are highly toxic components of antifouling paints. Realization that the molecular species of organotin influences fate and effects of organotins led to development of sensitive methods for quantifying individual molecular species. Even though such methods are now available, little information has been obtained on the ability of microorganisms to bioaccumulate tin compounds. Trisubstituted alkyl and aryltins (R_3Sn's) are more toxic than disubstituted compounds (R_2Sn's) while monosubstituted organotins (RSn's) are still less toxic. R_4Sn's are toxic only if they are metabolized to R_3Sn's. Among trisubstituted compounds propyl-, butyl-, pentyl-, phenyl-, and cyclohexyl Sn's are generally the most toxic to microorganisms. Toxicity in the R_3Sn series is related to total molecular surface area of the tin compound and to the octanol:water partition coefficient, K_{ow}, which is a measure of hydrophobicity; a high K_{ow} indicates greater hydrophobicity and predicts greater toxicity. Care must be taken when testing the toxicity of tin compounds, for a number of biological, physical and chemical factors can influence the apparent toxicity. Although little is known of the effects of tin compounds on microbial processes, a number of bacterial processes can be inhibited by organotins and all relate to membrane functions. They include effects on energy transduction, solute transport and retention and oxidation of substrates. Very little is known of how organotins exert their toxic effects on algae and fungi; Information on effects on chloroplasts and mitochondria stems principally from animal systems and from higher plants. Triorganotins act against chloroplasts and mitochondria by causing swelling, by acting as ionophores and by acting against ATPase, while diorganotins appear to act by binding to dithiol groups on enzymes and cofactors. Nucleic acids do not seem to be affected at environmentally relevant concentrations. Virtually nothing is known of the action of tin compounds on microbial enzymes, but resistant mutants are easy to obtain and should facilitate work to understand modes of microbial interaction with tin compounds and mechanisms of resistance.

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INTRODUCTION

About 5% of the tin mined each year is used for production of organotins, generating some 30 000 tons of organotins. Of these, about 8000 tons are used as biocides and about 22 000 tons for other purposes (Table 1). Tin may have more of its organometallic derivatives in use than any other element [24]. Based on its abundance in atmospheric particulates, tin is the third most important elemental pollutant, after lead and tellurium. Recently, public attention has been drawn to tributyltin, a very effective component of antifouling paints, which has been deemed perhaps the most toxic compound which man has yet introduced to the aquatic environment [40,121].

Tin and its compounds can be metabolized by some microorganisms and are toxic to others [47,48]. Microbial interactions with tin are important because microbes are at the base of the food weg, because microbes are likely to be significant in environmental transformations of tin compounds and because microbes have significant potential for remediation of tin-polluted waste streams and of tin-polluted ecosystems. However, relatively little is known of microbial interactions with tin and its compounds. Each year about 1000 papers are published on the chemistry of organotins. In contrast, the number of papers dealing with microbiological aspects of tin compounds is less than 10. The aims of this review are to call the attention of microbiologists to tin compounds, to summarize current knowledge, and to point out areas which need work if we are to develop the potential of organotins for agricultural and industrial uses and manage those uses in ways which will not damage our environment seriously.

ORGANOTINS AND THEIR USES

Most of the tin compounds of interest involve Sn-C bonds, but some involve Sn-H, Sn-O, Sn-N, Sn-S or Sn-Sn bonds as well as bonds between tin and other metals. The preparation and reactions of organotin compounds have been reviewed [24,55,201].

Tetraorganotins, R_4Sn, do not have a significant industrial application. They are used mainly as intermediates for the synthesis of organotins from $SnCl_4$. Mono- and disubstituted tins, $RSnX_3$ and R_2SnX_2 (where R = alkyl or aryl; X = H, OH, Cl, etc.), are used primarily for non-biological purposes. The largest single use, about 20 000 tons annually, is as stabilizers for polyvinyl chloride. This includes use in PVC beverage bottles, food packaging materials, water-distribution pipes and roofing. Organotins have found application as catalysts in preparation of silicone rubbers, polyurethane foams and phthalates, and for coatings on glass. Dibutyltin dilaurate and dibutyltin maleate are used in poultry feed to control helminthic and pro-

Table 1

Estimated annual world uses of organotin compounds

Compounds	Uses	Tons
$RSnX_3$ and R_2SnX_2	1. PVC stabilizers	
	United states	8 000
	Japan	5 500
	United Kingdom and Western Europe	6 500
	2. Catalysts	1 000
R_3SnX	1. Triphenyltin agrochemicals	1 000
	2. Others (Table 2)	8 000
Total		30 000

Modified from [55], by permission.

tozoal infections (Table 2). Trisubstituted tins, R_3SnX, are employed mainly as antibiological agents (Table 2). In the 1950's the work of Van der Kerk and his colleagues at the Institute for Organic Chemistry in Utrecht, The Netherlands [94,117, 208], revealed the biocidal properties of organotin compounds. Since then, there has been increasing use of organotins as preservatives, fungicides, bactericides and for other purposes.

The uses of organotins have been reviewed [24,53,55,117,148,197,201,232] and the spectrum of uses should increase [65]. As molluscicides, tributyltins have potential for control of schistosomiasis [83,201]. Tributyltins are toxic to some insects [53,160]. Some diethyl- and diphenyltins show activity against *Herpes simplex* virus types 1 and 2 [215]. Organotins are of interest as substitutes for highly toxic Pt compounds as anti-leukemia drugs [169]. Tin-protoporphyrin may be useful in preventing neonatal jaundice [64].

TIN IN THE ENVIRONMENT

It was not until the 1980's that it was fully realized that the toxicity and environmental mobility of tin are dependent on the individual organotin species [26,47,108]. In the aquatic environment attention has focused largely on methyltins because they can be formed and degraded biotically and abiotically, and on butyltins which are toxic components of antifouling paints.

A number of effective methods are now available for butyltins and have been subjected to interlaboratory comparisons [23,121,207]. Advances in measurement technology are available [43,50,67, 128,132] and have been evaluated [16]. Similar methods have been used for phenyltin compounds [190]. Briefly, quantification methods involve solvent extraction, derivative preparation to facilitate separation, and quantification of individual tin species. Quantification is more difficult in sediments

Table 2

Antibiologic uses of organotin compounds[1]

Application	Compounds[2]
Anthelminthics in poultry feeds	Bu_2Sn (dilaurate and maleate)
Agrochemicals	
Fungicides	Ph_3SnOAc
	Ph_3SnOH
Acaricides	$(CyHex)_3SnOH$
	$[(PhCMe_2CH)_3Sn]_2O$
Disinfectants	$(Bu_3Sn)_2O$
	$Bu_3SnOCOPh$
Marine antifouling paints	$(Bu_3Sn)_2O$
	Bu_3SnX (X = F, Cl, OAc)
	Ph_3SnX (X = OH, OAc, F, Cl, S · CS · NMe_2)
	$Ph_3SnO \cdot CO \cdot CH_2CBr_2 \cdot CO \cdot OSnPh_3$
Molluscicides	Bu_3SnF
	$(Bu_3Sn)_{2O}$
Wood preservative fungicides	$(Bu_3Sn)_2O$
	Bu_3Sn (naphthenate)
	$(Bu_3Sn)_3PO_4$
Stone preservative, textile preservative, slimicide in paper industry, biocide in antifouling rubbers, in-can fungicide for paints	$(Bu_3Sn)_2O$

[1] Compiled from Blunden et al. [24], Davies and Smith [55] and Thompson et al. [20].

[2] Notations used throughout the paper include: Me = methyl, Et = ethyl, Pr = *n*-propyl, Bu = *n*-butyl, Pe = *n*-pentyl, Ph = phenyl and CyHex = cyclohexyl. Among anions, Ac = acetate.

than in water samples because of binding to sediment components [88,207] and potential matrix problems, as well as effects of salinity and pH on binding of tin to sediments [87,151,203].

A variety of units is used to report tin concentrations. Wherever possible, we have converted units to molar concentrations. Where the anion was indicated by the authors we have included it, although the anion does not appear to affect toxicity.

In general, the solubility of alkyl tins decreases as the chain length of the alkyl moiety increases. In seawater the presence of chloride inhibits the solubility of Bu_3Sn's and Ph_3Sn's. In aqueous solution at environmental pH (6–8) tin compounds, R_nSnX_{4-n} (n = 0–4), and SnX_2 exist mainly as simple neutral hydroxides [24,121].

Triorganotins adsorb strongly to aquatic sediments [124], and to soil particles, from which they leach slowly [24]. In the aquatic environment tin compounds can be concentrated in the surface microlayer and in oily sediments [45,123,125,127]. In the water column tin may be associated with dissolved organic matter [74] and with organic and inorganic ligands [62], but most is associated with suspended particulates [32]. Concentrations are about three orders of magnitude greater in sediments than in the water column [206]. Extensive surveys have been conducted of butyltins in water and sediments in Canada [121,201], the United Kingdom [45,66,212] and the United States [82,89,163,164, 166,195], and a significant number of determinations has been made on more limited areas. It is unfortunate that some surveys have been limited to the water column when the bulk of tin compounds are in the sediments.

Recently deposited terrestrial and marine sediments contain about ten times as much tin as sediments laid down before the onset of the industrial revolution [162]. Thus, soils and aquatic sediments may serve as traps for toxic tin compounds and one would anticipate that soil organisms and benthic organisms might be strongly impacted in ecosystems which receive organotins.

Organotins can give rise to one another by disproportionation reactions (Eqn. 1, [201]).

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

Transmethylation reactions can also occur, as between methyltin and mercury [198].

Degradation of organotins involves breaking a Sn-C bond by UV irradiation, chemical cleavage, biological cleavage, γ-irradiation or thermal cleavage [24]. The first three are of environmental significance. Organotins degrade in sunlight [19,38,122, 124,189,191]. Reported half-lives ($T_{\frac{1}{2}}$) in water for photolytic decomposition in sunlight were >89 days [117] and 18 days [187]. The Sn-C bond can be polarized in either direction, so it can be attacked by nucleophilic and by electrophilic reagents. Biological degradation involves sequential removal or organic moieties, but may also involve hydroxylation in insects [97], in shellfish and finfish [111], in mammals [73,99], and perhaps in fungi [139]. Microbial degradation of organotins has been reviewed [47,48]. $T_{\frac{1}{2}}$ values may be as short as a week in water exposed to sunlight [122,164,165] and of the order of several months or longer in sediments [124,164]. The main factors limiting persistence of Bu_3Sn in aquatic ecosystems are probably photolysis in water and biological degradation in water and sediment [124], but the relative contributions of these mechanisms remain to be determined and will depend on the ecosystem and on seasonal climatic factors.

Inorganic and organic tins can be methylated via abiotic mechanisms and aerobically and anaerobically by microorganisms [47,48]. Mono-, di- and trimethyltin compounds are found in fresh and marine waters, in rain and in sediments [32,201]; biological methylation is generally regarded as the principal source of methyltins in the environment. Methyltins can be demethylated by photolysis caused by sunlight [38,122,124] and probably by photosynthetic organisms whose growth is stimulated by light [138,166]. Tributylmethyltin and dibutylmethyltin were found in harbor sediments [120]. Presumably, they were derived from tributyltins in antifouling paints, suggesting that butyltins can be methylated in the environment. Tentative identification of methylbutyltins has been made in

oysters [152]. Microbial transformations of tin compounds have been reviewed [47,55,201].

Whether mediated by physical, chemical or biological agents the transformations can influence the toxic effects of tin compounds by altering their volatility, adsorptivity and solubility which in turn influence mobility in the environment. Such transformations can also alter the inherent toxicity of organotins, for example by increasing the toxicity of inorganic tin via methylation or by decreasing the toxicity via debutylation or dephenylation.

TOXICITY: GENERAL CONSIDERATIONS

Although inorganic forms of tin can be toxic to microorganisms [84,86], they do not appear to present a danger to humans or wildlife. Organotins, however, are more soluble in lipids; thus, they can penetrate biological membranes more readily than inorganic tins and they are more likely to accumulate in lipid-rich tissues or organelles. Affinity for lipids can be quantified using a compound's partition coefficient between octanol and water (K_{ow}). These values are used to predict the bioaccumulation of nonpolar organic chemicals [119]. K_{ow} values for organotins are affected by pH, salinity and disproportionation reactions [107], which have not always been taken into consideration. Such factors may be involved in situations where ten times more Bu_3Sn accumulated in mussel (*Mytilus edulis*) tissue than was predicted from the K_{ow} [107]. For compounds in the mono-, di- and trisubstituted organotin series K_{ow} increases within each series as the size of the organic substituent is increased over the range from Me to Bu, phenyl (Ph) or cyclohexyl (CyHex), and range from a low value of about 8×10^{-4} for $MeSnCl_3$ to a maximum of about $1\text{–}2 \times 10^4$ for Ph_3- or $CyHex_3Sn$ chlorides [221,223]. Values for Bu_3Sn derivatives range from 200 to 7000 [107,221,223]. Based on these values Maguire et al. [122] suggested that $(Bu_3Sn)_2O$ has a moderate potential for bioaccumulation and Laughlin et al. [106] found $(Bu_3Sn)_2O$ 'moderately lipophilic'. When the toxicity of organotins to primary productivity of the green alga *Ankistrodesmus falcatus* was examined, K_{ow} values correlated with toxicity within the trisubstituted series but less so in the mono- and disubstituted series [228].

Bioaccumulation of tin compounds and in some cases tissue distribution have been reported for plants growing near a tin smelter [150], mangrove trees [145], mussels [106,211], shrimp [112], oysters [58,104,112,211,220], scallops [58], marine algae [90,162], crabs [71,112] and fin fish [112,171,214]. Both predators and filter feeders can accumulate TBT from their food [104]. Bioaccumulation factors (BCF) for Bu_3Sn have been estimated for a number of organisms. BCF values for fish, molluscs, crustaceans and algae range from hundreds- to thousands-fold above concentrations found in the ambient water [153].

Bioaccumulation by microorganisms is particularly important because they are at the base of many food webs, but little is known in this area. Eight bacterial cultures isolated from Chesapeake Bay had BCF values of 350–850 for Bu_3Sn [22] and six methyltin resistant bacteria had BCF's as high as 220 for Bu_3Sn and $SnCl_4$ [Jonas, R.B. and J.J. Cooney, Abstr. Annu. Mtg. Am. Soc. Microbiol 83: 227, 1983]. Two algae, *Isochrysis galbana* and *Ankistrodesmus falcatus* had BCF's for Bu_3Sn of 5500 [106] and 30 000 [126], respectively.

LC_{50} concentrations of 15 di- and triorganotins were determined for larvae of the mud crab *Rhithropanopeus harrisii* [108]. Correlations between the LC_{50} and the total molecular surface area (TSA) estimated for each compound suggested that hydrophobicity, which is a function of the organic moiety, controls bioconcentration and therefore the lethal dose. Dialkyltins in aqueous media have an additional hydroxyl group, decreasing their hydrophobicity and their toxicity. The work was extended to four other organisms, including the green alga, *A. falcatus* and to *Pseudomonas* sp.; for these molecules TSA is related to chromatographic behavior, K_{ow} values, bacterial uptake and toxicity in aqueous systems [27,70]. Toxicity of these organotins is related to their hydrophobicity rather than to the electronic environment of the tin atom or to steric effects. It was suggested that organic moieties on organotins become associated with the surface of

biological membranes rather than penetrating them.

Organotins are toxic to a wide variety of organisms but mechanisms of toxicity at the organ and organismic level are not well understood. An extensive treatment is beyond the scope of this paper and reviews are available [83,197,201,212]. Briefly, R_4Sn compounds show little direct toxicity, but may show delayed toxicity if they are debutylated metabolically to R_3SnX compounds [54,99]. R_3SnX compounds are the most toxic. R_2SnX_2 compounds have somewhat lesser toxicity and $RSnX_3$ compounds have greatly diminished effects. The inorganic radical, X, generally exerts little effect on toxicity [208]. In general, in the R_3SnX series trimethyl tins are most active against insects; triethyltins against mammals; tripropyltins against Gram-negative bacteria; tributyltins against Gram-positive bacteria, fungi, plants, molluscs and fish; triphenyltins against fungi, molluscs and fish; and tricyclohexyltins against mites and fish. Triorganotins with larger R groups have decreased toxicity [24,55].

The greatest amount of information is available on tributyltin compounds which are used in paints applied to ship hulls as an antifouling agent. An increase of only 10 μm in average hull roughness can increase fuel consumption as much as 1% [41] and barnacles increase both greatly. In tropical waters ships may show significant bottom fouling in less than a year if painted with copper-based paints compared to 5–7 years if painted with Bu_3Sn-based paints. Fuel savings for the U.S. Navy if all vessels were painted with Bu_3Sn-based paints are estimated at $ 110 million per year, based on crude oil at $ 16 per barrel. Savings on commercial, fishing and private vessels in the U.S. are estimated at an additional $ 300–400 million. Perhaps as important is the saving of 2 billion gallons of fossil fuel each year. Additional savings would be expected from decreased wear on machinery and decreased boatyard and drydock time. Similar savings would be expected from other nations [41]. Organotin paints are effective by releasing small amounts of biocide that kills fouling organisms [41]. Organotins are released from boat hulls not only in the water but from hosing [212] and from annual hull-maintenance procedures.

Bu_3Sn's are toxic to a wide variety of organisms. They inhibit the settling of oyster spat (larvae) and cause shell thickening and inhibit growth of the Pacific oyster *Crassostrea gigas* [8,9,211,220]. Effects on spat physiology were noted at concentrations of $(Bu_3Sn)_2O$ as low as 17 pM (10 parts per trillion), concentrations for lower than reported for many sites [110]. In the common dogwhelk *Nucella lapillus* Bu_3Sn's cause imposex (the development of male characteristics in females) which interferes with reproductive capacity [56,75,76]. The incidence of imposex was nearly 100% at sites on the coast of southwest England. The degree of imposex was related to the body concentration of Bu_3- and Bu_2Sn's [28] and can be initiated at concentrations as low as 8.4 pM (1 part per trillion) Bu_3Sn [77]. The dog-whelk's sensitivity may be due to its accumulation of Bu_3Sn with a BCF ranging from 3×10^4 to 10^5. Bu_3Sn's have been implicated in decreased recruitment of scallops and other bivalves [131]. Acute toxicity to the clam, *Mercenaria mercenaria,* would not occur from Bu_3Sn-containing paints in most habitats [109]. Larval silversides, *Menidia beryllina,* and juvenile Atlantic menhaden, *Brevoortia tyrannus,* were susceptible to Bu_3Sn as were two copepods lower in the food chain: *Eurytemora affinis* and *Acartia tonsa* [29]. Bu_3Sn's inhibit limb bud differentiation in mammalian cells in tissue culture [101] and $(Bu_3Sn)_2O$ and $(Ph_3Sn)_2O$ inhibit arm regeneration in the brittle star, *Ophioderma brevispina.* Me_3-, Et_3-, Pr_3- and Bu_3Sn's were toxic to larvae of the shore crab, *Hemigrapsus nudus,* and the lobster, *Homarus americanus* [105]. $(Bu_3Sn)_2O$ caused limb deformities and inhibited limb regeneration in the fiddler crab, *Uca pugilator* [219], impaired egg production in the copepod, *Acartia tonsa* [92], and were lethal to the fathead minnow, *Pimephales promelas* [14] and to embryos of the frog, *Rana temporaria.* Bu_3-, Ph_3- and $CyHex_3Sn$'s are toxic to several fish species [24]. Overviews of Bu_3Sn's and their effects are vailable [24, 40,41,121,212].

Toxic effects are not limited to butyltins. Effects of organotins on biological systems have been reviewed [197,201] and LD_{50} values of organotins for mice, rats and rabbits have been listed [188]. To cite

a few examples inorganic Sn(II) and Sn(IV) produced extensive DNA damage in hamster ovary cells in cultures [129]. $SnCl_2$ caused heme breakdown in rat kidney [98]. Me_3Sn (anion not specified) was toxic to fiddler crab larvae [222]. Me_3SnCl is nephrotoxic in rats [155] and causes neuropathology in mice [42]. Me_2SnCl_2, used as a stabilizer in PVC pipe used for transport of potable water, can cross the placental barrier in rats [136]. Ph_3SnAc is toxic to the isopod, *Asellus aquaticus,* larvae of the ciprinid fly, *Chironomus riparius,* and the goldfish, *Carassins auratus* [52].

Organotins, with a special emphasis on butyltins, have been the subject of two international symposia which focused on measurement of tin compounds in the marine environment and on their toxicity to marine animals [11,12]. Such is the concern over Bu_3Sn's that they are being regulated in spite of the great need for effective antifouling paints. In 1982 France banned the use of Bu_3Sn-containing paints on ships shorter than 25 m. Larger ships are viewed as spending the majority of their time at sea, while smaller vessels spend the majority of their time near shore or in harbor where Bu_3Sn's can accumulate in sediments. In 1986, England limited the amount of tin permitted in antifouling paints. Germany and Switzerland have prohibited the use of organotins in antifouling paints used in fresh waters. Canada has regulated Bu_3Sn's as a preservative for fishing nets and proposes a limitation of use on boats. In the United States, states which have regulated or which are considering regulating Bu_3Sn's include California, New Jersey, Oregon, Maryland, Massachusetts, Virginia and Washington. In 1988 the U.S. instituted regulations on organotins in antifouling paints [205].

Organotins are not carcinogenic [24]. While Bu_3Sn's can cause severe irritation of mucous membranes and the skin in mammals, and inhalation of dust or spray mist should be avoided, Bu_3Sn's 'do not represent a mutagenic, teratogenic or carcinogenic hazard to humans' [161]. Nevertheless, organotins can be toxic to humans. Edible muscle of salmon reared in pens treated with Bu_3Sn-based antifouling paints contained Bu_3Sn, which was not destroyed by common cooking practices [67,170,171]. This may provide a portal for entry of organotins into the food chain [171]. Edible shellfish also accumulate butyltins. Bu_3Sn accumulated in scallop [131], the Pacific oyster had a BCF of 3000 to 10 000 [211,220]. The mussel, *Mytilus edulis* had a BCF from food of <2 but from water or 5000 [107] and samples of the oyster *Crassostrea virginica* collected near a marina contained concentrations of Bu_3Sn as high as 5.2 $\mu mol \cdot kg^{-1}$ wet weight [154]. The subject of organotins in the human food chain will undoubtedly be the subject of future work.

TOXICITY TO MICROORGANISMS

It is important to understand the effects of tin compounds on microorganisms because microbes mediate a number of cyclic processes on the planet and because microbes are at the base of food webs. With microbes as with macroorganisms, toxicity is often noted first as a correlation between presence of a chemical and a deleterious biological effect. Attempts to determine if there is a connection between the chemical and the environmental effect usually begin with studies in the laboratory. Such studies are very useful but they must be undertaken with care and interpreted with caution. A large number of conditions can influence the apparent toxicity of a metal or an organometal to microorganisms [17,18,51]. These include the test organism(s) and strain(s) [115], as well as the physical, chemical and nutritional environment in which the potential toxicant is tested. Even the solidifying agent used in the medium can influence the apparent toxicity [84,86]. Thus, if one hopes to extrapolate from the laboratory to the environment the conditions used in the laboratory should approximate field conditions as closely as is feasible. Moreover, different methods for estimating toxicity of organometals to microorganisms can yield values which differ by as much as 100-fold [93].

We will discuss effects on microorganisms at the level of microbial processes, types of organisms, organelles and molecules.

Microbe-mediated processes

It is often useful to estimate effects of a potential toxicant on microbial processes because they reflect effects on mixed populations rather than a single organism, because they reflect effects on sequences of reactions rather than a single reaction, and because in some cases the process can be selected to represent important ecological events. While such processes have begun to be used for other metals [17,51], they have not yet been used in a systematic way to test the toxicity of tin compounds.

The effect of Ph_3SnAc on nitrification was evaluated using soil columns perfused with glucose and ammonium chloride [19]. When columns had reached a steady state, a single dose of Ph_3SnAc was added at a final concentration of 24 Mmol · kg^{-1}. Nitrification was inhibited initially but over a period of 4 days it recovered and surpassed the rate in control columns. Little degradation of the Ph_3SnAc occurred during the 4-day period. In a second experiment Ph_3SnAc was added when the column was packed with soil. It had little effect on nitrification. Among microorganisms isolated from columns were two *Aspergillus* spp and a Gram-negative rod which were able to mineralize Ph_3SnAc.

Addition of $(Bu_3Sn)_2O$ to activated municipal sewage sludge caused inhibition of microbial respiration at concentrations as low as 0.04 μM $(Bu_3Sn)_2O$, but sludge which had been acclimated for an unspecified period with 0.04 mM or 1.7 μM of $(Bu_3Sn)_2O$ could tolerate doses to 14 μM $(Bu_3Sn)_2O$. A continuous loading of 1.7 μM $(Bu_3Sn)_2O$ had no effect on removal of organics but the settling ability of the sludge was decreased at doses as low as 0.17 μM [14].

Similar approaches should be used to assess possible toxicity to microbial processes in ecosystems impacted by tin compounds.

Effects on algae

Ph_3SnCl was taken up by spores and inhibited respiration and photosynthesis of spores of the seaweeds *Enteromorpha intestinalis* and *Ulothrix flacca* [35]. Macroalgae can accumulate tins and may contain inorganic tin as well as methyl- and butyltins. Such algae might serve as a reservoir for tin compounds [61].

Light stimulates degradation of Bu_3Sn's, and in waters with a higher phytoplankton population and a higher chlorophyll content $(Bu_3Sn)_2O$ decomposed faster than in waters with lower contents [111]. Thus, microalgae appear to participate in biodegradation of organotins. Nevertheless, little is known about the effects of tin compounds on microscopic algae. Wong et al. [221] examined the effect of 14 tin compounds on growth and primary productivity of mixed algal flora from Lake Ontario and on three pure cultures: *Ankistrodesmus falcatus, Scenedesmus quadricauda* and *Anabaena flos-aquae.* Inorganic Sn(II) and Sn(IV) showed the least toxicity. Trisubstituted organotins were more toxic than mono- or disubstituted compounds and Pr_3-, Bu_3- and Ph_3Sn's were most toxic, showing inhibition of primary productivity at about 0.84 nM and inhibition of growth at slightly lower concentrations. Trisubstituted compounds showed a direct relationship between K_{ow} values and toxicity, but other tin compounds did not have a direct relationship. Maguire et al. [126] reported that *A. falcatus* exposed to $(Bu_3Sn)_2O$ accumulated Bu_3Sn^+ and lesser amounts of Bu_2Sn^+ with a BCF of 30 000 for Bu_3Sn^+. This green alga metabolized 50% of the $(Bu_3Sn)_2O$ to Bu_2Sn^{2+} over a 4-week period. Walsh et al. [213] determined that *Skeletonema costatum* and *Thalassiosira pseudonanna* were inhibited by $(Bu_3Sn)_2O$ at 0.55 nM and 1.7 nM, respectively. Correlation was reported between total surface area of organotins and toxicity to *A. falcatus* [70]. Growth of *S. costatum* stopped during an 18-day incubation in cultures which received 0.17 nM $(Bu_3Sn)_2O$ [21]. *Pavlova lutheri, Dunaliella tertiolecta* and *S. costatum* were inhibited by 0.17 nM $(Bu_3Sn)_2O$ and killed by 8.4 nM, death occurring within 2 days.

At 1.7 nM $(Bu_3Sn)_2O$ respiration of *Pavlova lutheri* increased significantly, due perhaps to an increased metabolic energy requirement or to uncoupling or damage to mitochondria. In sandy substrate microcosms inocculated each day with *P. lutheri,* higher chlorophyll *a* levels were found in microcosms which received 0.17 nM or 1.7 nM $(Bu_3Sn)_2O$ than in control tanks. Even though invertebrate species did not survive in the presence of

tin which reduced grazing on algae, chlorophyll *a* levels were higher in controls than in tin-treated tanks indicating that growth of *P. lutheri* was inhibited by organotin [20].

Bu_3SnF inhibited growth of the diatom, *Amphora coffeaeformis*. The growth rate in the presence of the organotin was directly related to initial culture density, suggesting that resistance is not due simply to exclusion of Bu_3Sn from the cell. Exposure to Bu_3SnF increased the organism's need for nitrate and silicate [200].

Effects on algae that are primarily interactions with mitochondria and chloroplasts are summarized below. Considerable work is needed on toxic interactions between tin compounds and microalgae, including uptake of compounds, effects on reproduction, specifics of toxic action, resistance, metabolism and genetics.

Effects on fungi

In 1950 a systematic investigation on the antifungal activity of organotins was begun at the Institute for Organic Chemistry at Utrecht, the Netherlands. The work was summarized by Kaars Sijpesteijn et al. [94] and by Luijten [117]. The initial published work was based on examination of four organisms: *Aspergillus niger, Botrytis allii, Penicillium italicum* and *Rhizopus nigricans*. Trialkyltins were more effective than other alkyltins. Among 11 tri-substituted organotins, Pr_3- and Bu_3Sn's were the most effective inhibitors, being active at 0.28–3.3 μM [117,208]. This is in contrast to reports for insects and mammals in which Me_3Sn's and Et_3Sn's, respectively, showed maximum toxicity. In the series Et_3SnX, where X = OH, CN, benzoate, acetate, phenoxide, *p*-nitrophenoxide, methane-sulfonamide, toluene-*p*-sulphonamide or phthalimide, the anionic moiety had little effect on toxicity [208]. This is surprising since the nature of the anion should influence the polarity of the molecule and its transport into the cell. Perhaps organotins become associated with the surfaces of biological membranes rather than penetrating them [27,70], although they must penetrate the cytoplasmic membrane if they act against chloroplasts and mitochondria as discussed below. Studies of the effects of unsymmetrical trialkyltins led to the conclusion that the total number of carbons in the R groups rather than the nature of the individual groups determined antifungal activity. The most active compounds had 9 to 12 carbons [209]. These observations were extended to members of 14 additional genera. While there were variations in sensitivity among organisms, the relative effectiveness of the tin compounds was the same as in earlier studies [94]. The results have been confirmed in other laboratories [117,226,231]. Triisoalkyltins had activity comparable to normal isomers [95]. $(CyPe)_3SnAc$ and $(CyHex)_3SnAc$ were more effective than the corresponding *n*-alkyl derivatives. Ph_3SnAc had about the same activity as Et_3Ac. Tri-*m*-tolyl- and tri-*p*-tolyl tin acetate had about the same activity as Ph_3SnAc and more activity than tribenzyl tin acetate. Tri-α-naphthyltin acetate did not have antifungal activity, presumably because of its low solubility [118]. Introduction of a functional group, expecially a hydrophilic group, on an R-group reduced antifungal activity [141]. Except for Ph_2SnCl_2, disubstituted compounds were not effective. The only R_4Sn's which were effective could easily lose one R group by hydrolysis to yield an R_3Sn [137]. At the lowest effective concentrations, the tin compounds were fungistatic rather than fungicidal [117].

Assays for the antifungal activity of organotins are somewhat difficult because organotins have limited solubility in water and limited ability to diffuse through agar [49,140]. Thus, comparisons of antifungal activity which compare toxicity of organotins in agar media and which suggest that tributyltins are ten times more toxic than dibutyltins [135] are measuring the combined effects of diffusibility and toxicity. The use of gradient diffusion plates and release of potassium from cells may prove useful for estimating antimicrobial action of organotins [49].

Polyethyleneimine products are used in a variety of industrial applications including fabric treatments, sludge dispersants and as adhesive agents. Formulation of the product with Ph_3SnCl as a component gave a product which inhibited *A. fumagatus* and a *Penicillium* sp. [39]. The growth of *Pen-*

icillium purpurogenum was inhibited by ditributyl stannate ester of fumaric acid at 7 nM. The fungicidal activity was enhanced by addition of *N*-cetylpyridinium chloride. The two compounds together appeared to interfere with protein synthesis [141].

Debaryomyces hansenii isolated from Chesapeake Bay was most sensitive to Et_3- and Pr_3SnCl, slightly less sensitive to Bu_3- or Ph_3SnCl, and was less sensitive to Me_3SnCl and insensitive to $(CyHex)_3SnBr$ [85]. Bu_3Sn's induced morphological changes and increased melanin synthesis in colonies of the common spoilage fungi *Penicillium funiculosum, Phoma glomerata* and *Aureobasidium pullulans* [134]. The effects of nine organotins were examined against 29 yeasts and *A. pullulans* [49]. While sensitivity varied from strain to strain, the most effective compound was Bu_3SnCl. Ph_3-, Bu_2-, Bu-, Me_3-, Et_3- and Et_2Sn's showed intermediate toxicity. Me- and Me_2Sn's were least toxic. Effective compounds caused release of potassium from cells, implicating the cytoplasmic membrane as a site of action. Addition of NaCl decreased the efflux of potassium.

The principal uses of organotins as antifungal agents are to protect growing plants and to prevent fungal growth on surfaces such as metal, wood and stone [197]. Ph_3Sn's have been used against *Cercospora beticola* which causes leaf spot in sugar beets and *Colletotrichum coffeanum* and *Hemelia vasatrix* which attack coffee plants, *Phytophthora infestans* on tomatoes, *Alternaria radicina* and *A. dauci* on carrots and *Nectria galligena* on apples. An undesired effect was observed when Ph_3SnOH was toxic to the fungi *Beauveria bassiana* and *Metarhizium anisopliae* which attack the peanut weavil. Strains of *C. beticola* have been reported which are resistant to Ph_3Sn's [197].

In an early study of materials protection, pine blocks impregnated with Et_3SnOH or Et_3SnAc resisted attack by three wood-rotting fungi: *Lentinus squamosus, Polystictus versicolor* and *Coniophora cerebella* [208]. Trisubstistuted organotins are used as antifungal (and antimicrobial and antibiologic) agents for wood, mortar and cement, and fibers in fishnets [197]. Additional formulations are being developed. For example, seven new complexes of Bu_3- and Ph_3Sn's with amino acids are toxic to *Aspergillus niger* and *Helminthosporium taulosum.* Two of the complexes, Ph_3Sn(PhthGlyO) and Ph_3Sn(AcCysO) were as effective as Bu_3SnCl [130].

Very little is known of how organotins exert their effects on fungi. As indicated, they may act by causing potassium efflux [49] and they can act against mitochondria, as described below. Their mode of action as well as their transport and metabolism and the development, nature and stability of resistant mutants should be examined.

Effects on bacteria

The pioneering work of the group at Utrecht included examining the effects of organotins on bacteria. The initial published works dealt with effects on *Bacillus subtilis, Mycobacterium phlei, Streptococcus lactis, Escherichia coli* and *Pseudomonas fluorescens* [94,117]. The results were similar to those for fungi. Trialkyltins were more effective than dialkyltins. Among trialkyltins maximum activity against the three Gram-positive species was shown by Pr_3- and Bu_3Sn's at concentrations in the range of 0.33 to 16 μM. The dialkyls Pr_2-, Bu_2- and Pe_2SnCl_2, which were not active against fungi, inhibited these three organisms at concentrations of 60 μM to 0.18 mM. Ph_3SnAc was as active as Pr_3- and Bu_3Sn's. In contrast, the two Gram-negative organisms were most sensitive to Et_3- and Pr_3SnAc's, but concentrations of 75 μM to 0.16 mM were required. As with fungi the lowest doses which inhibited growth were bacteriostatic, not bactericidal. These results were confirmed and extended to *Staphylococcus aureus* and *Pseudomonas aeruginosa* [231] and to eight bacteria [226], although not all Gram-negatives were more resistant than Gram-positives and Et_3SnCl was not particularly effective against Gram-negatives [226].

Ten isolates from Chesapeake Bay were screened against $SnCl_4$ and 18 organotins. Trisubstituted compounds were the most effective compound, but Pr_2- and Bu_2Sn's showed considerable activity. In contrast to the results of the Utrecht group, the four Gram-positive organisms were not more sensitive to trisubstituted organotins than the six Gram-negative organisms. Moreover, the Gram-positive organisms were all sensitive to Et_3- Pr_3-, Bu_3-, Ph_3-

and $CyHex_3Sn$'s while the six Gram-negative organisms ranged in sensitivity from one strain which was most sensitive to Et_3SnCl through one which was sensitive to Me_3-, Et_3-, Pr_3-, Bu_3, Ph_3- and $CyHex_3Sn$'s [85]. Thus, the sensitivity of bacteria to triorganotins does not appear to correlate as well with Gram reaction as originally thought.

A significant portion of the antibacterial activity of mouthwashes which contain SnF_2 may be due to the tin ion [10]. SnF_2 inhibited growth of *Streptococcus mutans* and cells took up tin. In contrast, the fluoride salts of sodium, lead, zinc and copper had little effect on the organism [36].

Organotins can affect pollution indicator organisms. Pure cultures of *Streptococcus faecalis* and a fecal strain of *E. coli* were killed by Me_2SnCl_2, Me_3SnCl and Bu_3SnCl. The MeSn's were effective at concentrations of 84 μM to 0.84 mM and Me_2SnCl_2 was more effective than Me_3SnCl against *S. faecalis*. Bu_3SnCl was toxic at 0.84 μM. The Gram-positive and Gram-negative organisms were equally sensitive. Similar effects were noted on natural populations of fecal coliforms and fecal streptococci. These concentrations are greater than those reported in environmental samples but it was pointed out that both microorganisms and organotins can be concentrated on suspended particles and on sediments which would expose organisms to higher concentrations of organometals. In addition, in the aquatic environment other stressors may interact with the stress caused by organotins [146]. $(Bu_3Sn)_2O$ is toxic to *Legionella pneumophila* [192]. Most of the $(Bu_3Sn)_2O$ was cell associated and cellular responses correlated better with the dose per cell than with the concentration of $(Bu_3Sn)_2O$ used; effective doses being between 1×10^4 and 6×10^7 molecules per cell.

Twenty-two tin compounds were examined in an assay system which used decreased emission of light from *Photobacterium phosphoreum* as indicator of toxicity. Mono-, di-, tri- and tetraalkyltins were all toxic with effectiveness in the order $R_3 > R_2 > R_1 > R_4$. In the R_2 series Et_2-, Pr_2 and Bu_2Sn's were most toxic and in the R_3 series Bu_3Sn's were the most toxic followed by $Pr_3 > Et_3 = Pe_3Sn$'s. Bu_2- and Bu_3 compounds with double bonds (butenes) were less toxic than their saturated counterparts. This correlated with the expected variations in K_{ow} values. Tetrabutenyltins were also less toxic than their saturated analogues but toxicity did not correlate with expected K_{ow} values [63].

Organotins are toxic against natural heterotrophic populations. Each of nine sites in Chesapeake Bay contained organisms sensitive to $SnCl_4$ and to Et_2SnCl_2. $SnCl_4$ was not as toxic in a liquid medium as in the same medium solidified with agar [84]. A toxic tin–agar complex was proposed but evidence was not obtained for such a complex [84,86]. Addition of serine or hydroxyflavone to the medium increased the toxicity of $SnCl_4$. Cysteine, another small potential tin-binding molecule, did not affect toxicity. This led to the suggestion that small molecules which bind tin may carry it into cells if they are taken up. Addition of humic acids or the protein gelatin decreased toxicity, probably by binding tin outside the cell. Me-, Me_2- and Me_2SnCl's were toxic to microbial populations in sediments from Boston Harbor as measured by plate counts and by incorporation of ^{3}H-thymidine [147]. LC_{50} values determined by plate counts for Me-, Me_2- and Me_3SnCl's were 93, 61 and 31 μM, respectively; for ^{3}H-thymidine incoporation they were 0.36, 0.19 and 0.14 mM emphasizing that toxicity as estimated in the laboratory is in part a function of the method used to estimate it. All organisms isolated on one of the three MeSn's were resistant to $MeSnCl_3$ but not all organisms isolated on Me_2SnCl_2 or on Me_3SnCl were resistant to the other two compounds, suggesting that all tin-resistant organisms may not share the same mechanism of resistance [147]. Thayer et al. [199] cultured organisms from estuarine sediments and water in media containing successively greater concentrations of Me_2SnCl_2. Organisms resistant to 3 mM Me_2SnCl_2 were isolated form the fourth serial culture.

Eight Bu_3SnCl-resistant bacteria were also resistant to $SnCl_4$. One isolate, a *Pseudomonas* sp., rapidly accumulated Bu_3SnCl without altering it, up to 2% of the cell dry weight, apparently by adsorption since starved cells, starved cells plus glucose and starved cells plus glucose and the metabolic inhibitor sodium azide accumulated similar amounts of

Bu_3SnCl. Bu_3SnCl could be removed from cells by washing, suggesting that the organotin was surface-bound. When cells were disrupted and fractionated by centrifugation, the Bu_3SnCl was associated with the cell envelope fraction [22]. A fluorescence technique was used to demonstrate that this *Pseudomonas* sp. accumulated $SnCl_4$, Bu_3Sn and BuSn. The results suggested that BuSn was accumulated to a greater extent than Bu_3Sn and $SnCl_4$ [27].

Each of four Gram-positive organisms, one Gram-negative and a yeast which had been isolated on medium containing 0.11 mM Me_2SnCl_2 were exposed to Me_2SnCl_2, Bu_3SnCl and to $SnCl_4$. Neither starved nor killed cells nor cells provided with glucose accumulated Me_2SnCl_2 suggesting that resistance to Me_2SnCl_2 is by exclusion of the compound. In contrast, all six orgnaisms accumulated Bu_3SnCl and $SnCl_4$ rapidly, up to 220 times the initial aqueous concentration. Tin made up as much as 2% of the cell dry weight. One of the isolates, a *Pseudomonas,* accumulated the same amount of Bu_3SnCl or $SnCl_4$ whether cells were viable or killed, suggesting that accumulation is not an active cellular process (Jonas, R.B. and J.J. Cooney, Abstr. Annu. Meet. Am. Soc. Microbiol. 83: 227, 1983).

This limited evidence suggests that bacteria may bind tin compounds passively to their surface where cell membrane functions are inhibited. Additional work is needed on this subject. Evidence regarding the mode of action of tin compounds against bacteria is presented below.

MODE OF ACTION ON MICROORGANISMS

Effects of triorganotins on mitochondria and chloroplasts

The action of tin compounds against microalgae and fungi undoubtedly involves interactions with mitochondria and chloroplasts. Although much of the information available has been obtained on higher organisms, it is included here because we assume that effects on mitochondria and chloroplasts in microorganisms will be similar to effects on these organelles in higher organisms; in many cases it is the only information available. The effects of organotin compounds on mitochondria and chloroplasts have been reviewed [1,2,3,166].

Both mitochondrial and protosynthetic oxidative phosphorylation are highly sensitive to triorganotins. The two systems share certain common features which have been studied using, among other compounds, organotins as investigative tools. Briefly, triorganotins interact with mitochondrial membranes in three ways: (1) by causing large-scale swelling; (2) by acting as ionophores and discharging an energy-dependent hydroxyl-anion gradient leading to limited swelling of membranes; (3) by interfering with the energy conservation apparatus (ATP synthase and hydrolase system).

1. Gross swelling. Gross swelling may be due to binding to low affinity sites [223]. Triorganotin-related actions on calcium transport and calcium release [30,31] have been characterized as non-specific attacks on mitochondrial membranes [2]. Swelling is more predominant in the presence of higher molecular weight organotins and more lipophilic trialkyltins, including Bu_3Sn's. This property is energy-independent and does not require the presence of halides and uncouplers in the medium. The possibility has been raised that at high concentrations long-chain trialkyl- and triphenyltin compounds may cause swelling by a detergent-like behavior [194].

2. Action as ionophores. Triorganotins act as antiporters exchanging Cl^-, SCN^-, I^-, Br^-, or F^- ions for OH^- ions across lipid membranes [167,216]. The movement of OH^- through the membrane mediated by Et_3SnCl is dependent on energy supplied either by ATP or by an oxidizable substrate [186]. The results are limited swelling of mitochondria [7], interference with substrate uptake normally accomplished by the hydroxide gradient [185] and inhibition of uncoupler-stimulated respiration below pH 7 because of suboptimal conditions for the intermitochondrial respiratory system [46,59].

In chloroplasts, Bu_3Sn's can act as uncoupler only at higher levels (2 μM) than are required to inhibit photophosphorylation (0.56 μM). In contrast, the same concentration of Et_3Sn inhibited both functions, and it was suggested that the effects of Bu_3Sn's and Et_3Sn's should be distinguished on this

basis. However, at much higher concentrations (1 mM) this effect was obscured by general inhibitory effects [217]. Bu_3SnCl exerted a progressive uncoupling effect in chloroplasts [78] at concentrations which also completely inhibited ATP synthesis (5 μM) [97]. Bu_3SnCl was a more efficient carrier during anion/hydroxyl exchange than Me_3-, Et_3-, Pr_3- or CyHexSn's at pH values ranging from 5 to 9 [168]. The proton-motive force creates both an electrical potential and a pH gradient. It was postulated that triorganotins neutralize the pH difference by driving an anion–hydroxide exchange. The electric potential difference, however, remains unchanged unless the anion can return across the membrane by its own uniport-type permeability [167,194]. The effectiveness of triorganotin-mediated uncoupling depends strongly on the permeability of the available anion in the medium.

3. Action of ATPase. In order to avoid anion exchange effects and still maintain an osmotic balance in vesicular organelles, one can substitute Cl^- with NO_3^-, SO_4^{2-} or isothionate which do not mediate an anion exchange. In chloroplasts only membrane-bound ATPases are inhibited by Ph_3Sn's [185]. It is the F_o component which can be blocked by Bu_3Sn in mitochondria [60] or by Ph_3Sn in chloroplasts [78,218]. In the absence of chloride, Et_3Sn inhibited uncoupler-stimulated ATP hydrolysis but not uncoupler-stimulated respiration [167].

Et_3Sn-resistant mutants of *S. cerevisiae* have been isolated [103]. Class 1 mutants were cross-resistant to oligomycin, uncoupling agents, antimycin A and valinomycin; they showed a limited degree of resistance to the protein synthesis inhibitors erythromycin and cycloheximide. Class 2 mutants were resistant only to one uncoupling agent ['1799'; bis-(hexafluoroacetonyl)-acetone] and showed no cross-resistance to oligomycin, rutamycin, valinomycin, antimycin, erythromycin or cycloheximide.

The ATPase of class 1 submitochondrial particles retained the Et_3Sn sensitivity of the parent strain indicating that resistance could be due to a permeability change in the mitochondrial membrane. In contrast, class 2 mutants had a resistant ATPase. Mutants exhibiting a high degree of drug specificity were regarded as mitochondrial (cytoplasmic) mutants.

All mutants were highly cross-resistant to Me_3Sn and Et_3Sn but not to Et_2SnCl_2. Et_2Sn affects pyruvate and 2-oxyglutarate dehydrogenase but not oxidative phosphorylation. The level of cross-resistance to Pr_3-, Bu_3-, $CyHex_3$- and Ph_3Sn was low.

Et_3Sn inhibited growth of sensitive strains on glycerol at a 40-fold lower concentration than was required on glucose, suggesting that aerobic respiration was more prone to inhibition than were fermentation or general cell metabolism. This was not observed when Pr_3-, Bu_3-, Ph_3- or $CyHex_3Sn$ were the inhibitors since growth on glucose was inhibited at low concentrations [102].

Et_3-mediated inhibition of whole cell respiration and growth was most effective at pH 7–7.4 and least effective at pH 5.0–5.5 [103]. This is in agreement with reports of pH-dependent inhibition of oxidative phosphorylation and electron transport by Et_3Sn in rat liver mitochondria. Inhibition was reversed by uncouplers [46]. At neutral pH Et_3Sn appeared to act on mitochondrial energy conservation reactions rather then by uncoupling [103].

Mutants were resistant in liquid and on solid growth media. Growth on ethanol or during the aerobic phase of growth on glucose was not inhibited by Et_3Sn. Et_3Sn resistance (TET^R) appeared to be constitutive. Oligomycin and Et_3Sn did not induce identical mutations nor were their sites of action identical. However, the $oligomycin^R$ mutation appeared to interact with the TET^R mutation, lowering the maximum resistance level of the latter. In contrast, the $erythromycin^R$ and $chloramphenol^R$ mutations did not modify the TET^R. This was interpreted as a protein conformational interaction between the two sites of action by Et_3Sn and oligomycin, underscored by the knowledge that both sites form part of an integrated mitochondrial enzyme complex. The $oligomycin^R$ and TET^R mutations are located on the mitochondrial genome but probably in different cistrons [103]. The site of action for Et_3Sn is in the F_oATPase subunit. The authors suggested, in view of the ability of both Et_3Sn and oligomycin to mediate Cl^-/OH^- exchange and inhibit ATPase, that resistance to both agents may be founded in an ion-translocating function within the oxidative phosporylation complex.

Bu_3Sn inhibited H^+-ATPases from mitochondrial and plasma membranes as well as vacuolar membranes in the fungus, *Neurospora crassa* [25]. The concentrations required to give 50% inhibition of activity (I_{50}) were 0.01, 0.06, and 0.6 μM, respectively. Several inhibitors of the ATPase complex were tested at different steps during the cell cycle of the fission yeast, *Schizosaccharomyces pombe* 972 h^-. Both triethyltin and dibutylchloromethyltin were inhibitors of the F_o component [116].

Ph_3Sn had negligible ionophoretic effects in chloroplasts and proposed its use for studied specifically designed to test ATPase inhibition. The efficiency of inhibition of ATP synthesis was stoichiometrically dependent on the amount of chloroplast present and was reversible by the addition of dithiol groups [79]. This was interpreted as possible involvement of SH residues in proton translocation by the F_o component of the ATP complex.

It is significant that complete inhibition of ATP hydrolase activity was observed when submitochondrial particles were preincubated with low concentrations of Bu_3SnCl or dibutylchloromethyltin chloride in the presence of chloride. Succinate-driven ATP synthesis, on the other hand, was not affected. ATP-driven transhydrogenase was also inhibited whereas succinate-driven transhydrogenase activity was stimulated. However, at higher concentrations, ATP synthesis and succinate-driven transhydrogenation were also inhibited. These results suggested a concentrations-dependent activity of these compounds which involved differential inhibition of ATP synthesis and hydrolysis. Only the former was reversible by addition of dithiols [69].

It is important to realize that, except in specially adjusted media, subcellular effects may be due to a combination of events; e.g., in the absence of chloride Me_3Sn's cannot inhibit uncoupler-stimulated ATPase (i.e., hydrolysis of ATP) directly. In contrast, low concentrations of Me_3SnCl are required in the presence of Mg^{2+} ions in a KCl medium [167]. It may not be possible to determine clearly the primary action of triorganotins. It seems likely, however, that for Et_3SnCl and Me_3SnCl in an unspecified cellular environment the anion/hydroxyl exchange, which aids in the release of control of electron transfer or ATP hydrolysis, is the mode of action. On the other hand Ph_3SnCl, unlike Et_3SnCl, does not uncouple oxidative phosphorylation at low concentrations in chloroplasts.

Recently, the Cl^-/OH^- antiporter model was discounted as explanation for the uncoupling capacity of triorganotins because, in the absence of Cl^-, triorganotins could dissipate the membrane potential of the proton motive force [80]. Inhibition of ATP synthesis could thus be caused by uncoupling of oxidative phosphorylation. Although the role of the Cl^-/OH^- exchange was questioned, no mechanism was proposed for dissipation of the membrane potential.

Et_3SnCl was less potent than Bu_3SnCl in inhibiting ATP hydrolase and ATP synthase or in decreasing the membrane potential. This is in agreement with the dependence of Et_3SnCl on Cl^- for its uncoupling action [80]. However, binding of triorganotins to the ATPase complex was not discussed. Information regarding specific binding properties of organotins is summarized in a separate section of this review which deals with molecular mechanisms of organotin toxicity.

It follows that triorganotins may differ in their primary action on mitochondria or chloroplasts. Differential binding ability and solubility as well as the type of membrane preparation used may account for some of the observed differences [167]. Triorganotins continue to be valuable tools for studies on oxidative phosphorylation. The in vivo mode of action depends strongly on the cellular environment.

Effects of diorganotins on mitochondria and chloroplasts

As discussed below dialkyltins appear to act by binding to dithiol groups on enzymes and cofactors [1,5]. Bu_2SnCl_2 also uncoupled oxidative phosphorylation at high concentrations (167 nmol/mg protein or 0.334 mM). Aldridge [2] pointed out that this uncoupling could be caused by one of the mechanisms discussed above for triorganotins, viz., by large scale swelling of mitochondria or by ionophoretic action. In one study, Bu_2SnCl_2 inhibited the ATP synthase complex analogous to the action of

triorganotins [34]. This action was reversible by the addition of dithiol compounds. It was suggested that diorganotins may be able to inhibit any enzyme depending on dithiols for its catalytic activity [3].

Effects on other organelles

It is possible that other microbial organelles can be affected by organotins since they are affected in higher organisms. Organotins altered several key aspects of heme metabolism in mammals [157–159]. δ-Aminolevulinic acid synthase, which is involved in heme synthesis, was inhibited. Incubation of hepatic microsomal preparations in the absence of NADPH with $(Bu_3Sn)_2O$ led to concentration-dependent decreases of cytochrome P-450 content and an increase in cytochrome P-420 accompanied by loss of mixed function oxidase activity. These effects were not mediated by lipid peroxidation of microsomal membrane lipids and were not reversed by the addition of sulfhydryl compounds such as cysteine or glutathione [157]. Et_3SnBr inhibited monooxygenase, converted cytochrome P-450 to P-420, and induced microsomal lipid peroxidation in rat liver microsome preparations [153]. Inactivation of cytochrome P-450 would be highly significant for potential detoxification mechanisms in mammals, and presumably in microorganisms. Several hydroxylated metabolites of Bu_3Sn's were less toxic to mice than the parent Bu_3Sn compound [4]. It is therefore possible that inactivation of cytochrome P-450 by organotins may be in competition with a detoxification mechanisms by a cytochrome P-450 dependent monooxygenase system.

Effects of organotins on bacteria

Triorganotins affect the same mechanisms in bacteria as in mitochondria and chloroplasts [167]. Most information on additional actions in bacteria was obtained from studies with *E. coli*. Briefly, triorganotins can inhibit growth, solute transport,

Table 3

Effect of tributyltin on energy-linked reactions of *Escherichia coli*[a]

Energy-linked reactions	Concentrations causing 50% inhibition of maximal activity (nmol/mg protein)	References
(1) Dissipation of ΔpH	0.15	[179,181]
(2) ATPase activities		
(a) ATP hydrolase	2.5(1.2)[b]	[183,69]
(b) ATP synthase	> 2.5?(8.6)[b]	[69]
(3) Oxidation of substrates (NADH, succinate, D-lactate)	>50.0	[179,182]
(4) Glucolysis (intracellular ATP pools)	>50.0	[181]
(5) Solute transport (amino acids)		
(a) At pH 6.6	3.3	[181]
(b) At pH 7.5	>50.0	[181]
(6) Energy-linked transhydrogenase	8.0	[182]
(7) Synthesis of macromolecules		
(a) Proteins	35.3 μM[c]	[230]
(b) DNA and RNA	70.6 μM[c]	[230]
(8) Growth of		
(a) cytochrome-sufficient cells	100.0 μM	[184]
(b) cytochrome-deficient cells	5.0 μM	[184]

[a] From Singh and Singh [189], with permission.

[b] Numbers in parentheses are values for mitochondrial ATPase complex.

[c] These values refer to tripropyltin chloride.

biosynthesis of macromolecules, ATP synthase and energy-dependent transhydrogenase. Effects of Bu_3Sn's on reactions in *E. coli* are summarized in Table 3.

Triethyltins. Et_3Sn stimulated basal respiration of *E. coli* membrane vesicles in medium containing Cl^- but not in medium containing SO_4^{2-}. Succinate oxidation was inhibited more extensively in Cl^- medium than in SO_4^{2-} medium. Thus, an Et_3Sn-mediated Cl^-/OH^- exchange may discharge the pH gradient and prevent entry of succinate into the cell. Since succinate use as an energy source involves oxidative phosphorylation, it was inferred that in the presence of SO_4^{2-}, Et_3Sn had an effect on phosphorylation which was unrelated to uncoupling [114]. Thus, there is limited evidence that organotins can inhibit two key aspects of energy transfuction, ATPase and ΔH^+.

Tripropyltins. Yamada and his colleagues conducted a series of investigations on the effects of trialkyltins on bacteria. Uptake was followed by monitoring Sn uptake by *E. coli* with the assumption that the organomoiety was also taken up. Pr_3SnCl was taken up rapidly, approaching a maximum in 15 min. Rate of uptake was independent of temperature. This suggested that a passive adsorption to cells was involved. Treatment of cells with surface active agents which interact with the cell membrane inhibited uptake. Isolated fractions containing protoplast membranes, a membrane-cell wall complex, or liposomes prepared from phospholipids and cholesterol also bound Pr_3SnCl [227].

Oxygen uptake by intact *E. coli* was inhibited more by Pr_3SnCl than by Bu_3SnCl or Et_3SnCl. In contrast, spheroplasts were equally sensitive to all three alkyltins. Hence, the outer membrane of *E. coli* may be more permeable to Pr_3Sn than to Bu_3Sn or Et_3Sn [224]. Trialkyltins reacted with phosphates and with phospholipids [225]. Cells of *E. coli* or *B. subtilis* exposed to Pr_3SnCl caused leakage of intracellular materials and cells of *B. subtilis* lysed [228]. Pr_3Sn did not inhibit substrate oxidation directly but interfaced with uptake of Na succinate into cells. Pr_3SnCl inhibited uptake by *E. coli* of L-leucine, L-proline, adenine but not methyl-(α-D-gluco)pyranoside [229]. It caused release of previously incorporated L-leucine but apparently not by interfering with leucine-binding protein [230].

L-leucine and L-proline are both taken up by active transport but differ in their energy requirements. ATP supplies energy for L-leucine transport involving ATPase [13] whereas L-proline transport does not depend on the cellular concentration of ATP but on the energized membrane state of the cell [99]. Assuming the Pr_3Sn inhibition of ATPase in *E. coli* is as effective in vivo as it is in vitro [229], this could explain why L-leucine uptake was inhibited 100% at 35 μM Pr_3Sn while L-proline uptake was inhibited only 40% [229]. Pr_3Sn at 70 μM also inhibited uptake of adenine. The accumulation of α-methyl gluycoside, which in *E. coli* is taken up by group translocation, was scarcely inhibited by Pr_3Sn. The authors concluded that Pr_3Sn probably inhibits the energy transduction step in the active transport system since Pr_3Sn action at the recognition step was not apparent as evident from leucine-binding protein (LCB) experiments [229]. These studies strongly implicate ATP synthesis, hydrolysis or utilization in the inhibitory action of Pr_3Sn.

Pr_3SnCl added to intact cells of *E. coli* did not affect respiration but inhibited protein synthesis, RNA synthesis and DNA synthesis (Table 3). RNA polymerase from cells was inhibited only at high concentrations (5–10 mM) of Et_3-, Pr_3-, Et_2 or Pr_2Sn's. Effects on nucleic acid synthesis were regarded as a secondary effect of the organotins. ATPase in membranes was strongly inhibited but ATPase separated from the membrane was only slightly inhibited suggesting action on the F_0component [221].

Tributyltins. Bu_3Sn (anion not specified) inhibited H^+-ATPase [91]. Of 21 mutants of *E. coli* which were resistant to Bu_3Sn, most were also resistant to uncouplers of oxidative phosphorylation, carbonylcyanide *m*-chlorophenyl-hydrazone (CCCP) and/or pentachlorophenol (PCP). It was concluded that the mutants carried mutations in protein sub-units of H^+-ATPase.

Doses of about 10^7 molecules of $(Bu_3Sn)_2O$ per cell were bacteriostatic and cells remained viable for 5 h after exposure. Within 1 min after exposure the

intracellular ATP level and the adenylate energy charge decreased, followed by declines in the rates of CO_2 production, oxygen consumption and macromolecular synthesis. The results suggested that at these doses energy transduction mechanisms were affected. At doses greater than 10^7 molecules per cell all physiological parameters measured decreased immediately. An immediate 90% decrease in viable cells was followed by an additional exponential loss of viability. These great differential effects with a small increase in dose were interpreted as indicating that the modes of bacteriostatic and bactericidal action are different [192].

Bu_3SnCl inhibited uptake of L-proline and L-glutamic acid by *E. coli* and caused efflux of accumulated amino acids (Table 3). A pH gradient in cells and in everted membrane vesicles prepared from the cells was dissipated by Bu_3SnCl, but cells showed no decrease in intracellular ATP levels. Comparison of the wild type with a lipoic acid auxotroph indicated that the effects do not require lipoic acid. The results suggested that Bu_3SnCl inhibits amino acid uptake catalyzing a transmembrane OH^--anion exchange reaction [180]. Bu_3SnCl also inhibited respiration-dependent and ATP-dependent transhydrogenation (Table 3). This was in contrast to *Micrococcus denitrificans* where only ATP-dependent transhydrogenation was inhibited by Bu_3Sn [15]. In *E. coli* the inhibition was reversed by mono- and dithiol compounds and was not dependent on a permeant anion. The organotin appeared to react with a sulfhydryl group on the transhydrogenase enzyme [181].

Bu_3SnCl was fifty times more inhibitory to a cytochrome-deficient strain of *E. coli* than to a cytochrome-sufficient strain. Bu_3Sn-resistant mutants derived from the cytochrome-sufficient strain (class I mutants) were also resistant to Ph_3SnCl, presumably the parent strain was not. Class I mutants had altered cellular and colonial morphology and reduced uptake of Ph_3SnCl [183]. While the authors did not specify, it is possible that class I mutants were resistant to Bu_3SnCl because they excluded it. A second group of mutants (class II) was isolated from the cytochrome-deficient strain. When compared to class I mutants, class II mutants had a Bu_3Sn-resistant membrane-bound ATPase which probably accounted for their resistance to Bu_3SnCl. The cytochrome-deficient parent strain was examined in a later work [184]. This organism forms cytochromes when the medium is supplemented with 5-aminolevulinic acid (ALA). Two explanations for this change in sensitivity were suggested. First, the differential sensitivity to Bu_3Sn (Table 3) may be explained by the different modes of energy generation in the respective strains. Cytochrome-deficient cells generate ATP aerobically during glycolysis and also anaerobically via phosphoroclastic cleavage of pyruvate [178]. The proton motive force is generated in this case from ATP hydrolysis, catalyzed by ATPase. In contrast, ATP formation via substrate oxidation may not be affected by Bu_3Sn in cytochrome-containing cells if *E. coli* displays different sensitivity of ATP synthetic and hydrolytic reactions of the ATPase complex to Bu_3Sn [184]. A differential sensitivity to Bu_3Sn of ATP synthase and ATP hydrolase, as proposed for the mitochondrial ATPase complex [69], has yet to be demonstrated in *E. coli.*

Second, a more likely explanation is that the pH of culture media decreases in the absence of cytochromes. At pH 5.5 the proton motive force in *E. coli* cells was dominated by the pH gradient whereas at pH 7.7 the electrochemical gradient was the major component [142,150]. In the view of Singh and Singh [184] this corroborated their findings that Bu_3Sn affects mainly the pH gradient because of the observed differences in cytochrome-containing and -deficient cells.

To summarize, the work carried out by Singh and coworkers suggests that in *E. coli,* at environmentally significant Bu_3Sn concentrations, the pH component of the proton motive force (PMF) rather than the electric potential difference is affected by Bu_3Sn. In addition, the ready isolation of H^+-ATPase mutants resistant to Bu_3Sn suggests a direct effect on the energy conservation system.

A mutant of *B. subtilis* which had increased resistance to Bu_3SnCl was resistant to the uncouplers CCCP and 1,4-dinitrophenol. The mutant also had increased resistance to neomycin, had elevated levels of membrane ATPase activity, had slightly ele-

vated respiratory rates without elevated cytochrome content, had no change in proton permeability in the presence or absence of CCCP and had an enhanced sensitivity to valinomycin. Starved cells of the mutant synthesized more ATP than the wild type in the presence of agents which lowered the proton motive force. The mutant was the result of a single, pleiotrophic mutation which was suspected of being a mutation in the coupling membrane. The resistant mutant's membrane lipids contained less mono-unsaturated C_{15} fatty acids. The authors hypothesized that the Bu_3Sn-resistant mutant's membrane phospholipid fatty acid composition was related to the ability to synthesize ATP when the transmembrane proton gradient is low [81].

Tri-organotins interfere with such membrane functions as solute transport, retention of solutes and energy transfuction. These may all be results of a common primary interaction, such as alteration of membrane lipids.

Triphenyltins. Ph_3Sn inhibited the light-induced proton uptake in *Halobacterium halobium* [133]. This organism contains both bacteriorhodopsin and a novel retinal protein pigment, halorhodospin. Ph_3Sn inhibited ΔpH and had a slight effect on the electrochemical membrane potential but did not inhibit ATP synthesis in the light or in the dark in a bacteriorhodopsin-deficient strain. This was interpreted as partial evidence for an additional novel mechanism for H^+ incorporation in this organism, one that does not rely on H^+-ATPase. Consequently, Ph_3Sn was assigned a role as $OH^{\pm}/Cl^{\pm}$ antiporter. This is in contradiction to work by Gould [78] who found no ionophoretic effects of Ph_3Sn in chloroplasts.

Diorganotins. The mode of action of diorganotins on bacteria is virtually unexplored, although they probably act on dithiol-containing enzymes as discussed below. In 1967 Kahana and Kaars Sijpesteijn [96] noted that Et_2SnCl_2 inhibited oxygen uptake by resting cells of *E. coli* with glucose, lactate, pyruvate or glutamate as substrates at a concentration which was below the minimum inhibitory concentration for growth. The patterns of accumulation of products indicated that pyruvate metabolism was inhibited as were the oxidation of succinate and malate, as well as α-keto acid production. High concentrations (5–10 mM) of Et_2- or Pr_2Sn's were required to inhibit RNA polymerase in *E. coli.*

Effects on molecules

$SnCl_2$ caused extensive DNA damage to hamster ovary cells in culture, but $SnCl_4$ did not. DNA damage was not accompanied by loss of ability to form colonies [129]. Me_2SnCl_2 reacted with adenosine, guanosine and inosine in aqueous solution at pH 4.5 yielding polymeric species [37]. Thus tin compounds may be genotoxic. This aspect should be pursued.

Inorganic Sn(II) and Sn(IV) were slightly toxic to lipase preparations [44]. A number of proteins bind trialkyltin compounds. Et_3Sn's bind cat and rat hemoglobin, rat-brain myelin, a protein fraction from guinea pig liver, and yeast mitochondrial membrane [55]. Enzymatic components of red blood cells also bind R_3Sn's [173] as do rat liver mitochondrial membranes [72]. $(Bu_3Sn)_2O$ binds a snail tissue protein [55]. Inhibition of enzymes by organotins is summarized in Table 4.

One molecule of rat haemoglobin binds two molecules of Et_3Sn. Each binding site contains two histidine residues and there is cooperativity between the sites such that binding of the first Et_3Sn molecule increases the affinity constant for the second molecule [156]. There are three binding sites per molecule, the third involving a cysteine residue [68]. Reaction of cat haemoglobin with Bu_3SnBr increased its oxygen affinity [178]. Little or no Et_3Sn binds to the deoxygenated protein and binding requires a specific configuration of cysteine and histidine residues. Effects of Et_3Sn are exerted on both the α- and β-heme groups even though Et_3Sn is bound on α-globin at sites far removed from the heme groups [177]. Et_3Sn did not bind to other animal hemoglobins. Trialkyltins also react with sulfhydryl groups in other proteins [196].

Mossbauer spectroscopy studies indicated that in rat liver mitochondria Et_3Sn interacted in three ways [72]. A low affinity binding site appeared to involve thiol groups. High affinity binding to the

Table 4

Inhibition of enzymes by organotins

Enzyme	Source	Tin compound	References
Hemoglobin	Cat	Et_3Sn's	[156]
		triorganotins with	[68]
		5-coordinate bonding	[177]
AMP-dependent protein kinases	Human rbc	Et_3SnBr	[176]
Glutathione-*S*-transferases	Rat liver	Et_3Sn	[202]
Heme oxygenase, Cytochrome P-450	Rat liver microsomes	Bu_3Sn	[159]
			[157]
Hexokinase	Human rbc, yeast	Me_3-, Et_2-, Et_3- Bu_3Sn's and 2-[(dimethylamino) methyl]-diethyltin bromide	[172, 173]
ATPase	Human rbc, membrane	Et_3Sn	[173]
α-Keto acid dehydrogenase	Rat liver mitochondria	Bu_2Sn	[143]
Lipoic acid, lipoyl dehydrogenase	Mammalian mitochondria	R_2S's	[55]

H^+-ATPase coupler was explained as 4-coordinate. A third component involved non-specific partitioning of Et_3Sn into the membrane. In yeast mitochondrial ATPase there are approximately six binding sites for Et_3Sn per mol of enzyme complex [33].

In addition of Et_3Sn's, triorganotins with 5-coordinate internal bonding [210] also bind to cat hemoglobin, but they do not compete for binding with Et_3Sn and so do not bind at the same sites as Et_3Sn's [68]. 5-Coordinate triorganotins inhibited the same mitochondrial functions as trialkyl compounds but at lower concentrations [6].

In contrast to inorganic metals which form heme chelates, organotins may act directly on heme oxygenase [159]. Bu_3Sn added to cytochrome P-450 decreased P-450 while cytochrome P-420 increased. It was suggested that Bu_3Sn's disrupted coordinate covalent bonds in the heme moiety converting P-450 to P-420 [157].

Et_3SnBr interacts with both regulatory and catalytic subunits of AMP-dependent protein kinases from human red blood cell membranes. Et_3SnBr added to partially purified enzyme preparations stimulated enzyme activity by causing release of catalytic subunits from the intact protein kinase [176].

Et_3Sn inhibited glutathione (GSH)-*S*-transferases A, B and C from rat liver. GSH reacted with Et_3Sn, and it was suggested that the inhibited enzymes are in the form of a ternary complex, enzyme-GSH-Et_3Sn, or a quaternary complex, enzyme-$(GSH)_2$-Et_3Sn [202].

Et_3SnBr inhibited hexokinase from human red blood cells and from yeast. Other glycolytic enzymes were not inhibited [172]. The compound also inhibited hexokinase and membrane ATPase from red blood cells [173]. Me_3-, Et_2-, Et_3-, Bu_3Sn's and (2-[(dimethylamino)methyl]phenyl)diethyltin bromide inhibited yeast hexokinase B. Toxicity was associated with hydrophobicity. Bu_3Sn was the most toxic and Me_3Sn was the least toxic of the compounds. When substrates for the enzyme were present they reduced the binding of Et_3Sn to the enzyme. The enzyme monomer had two sites with different affinities for Et_3Sn. Binding of Et_3Sn increased dissociation of the enzyme to its monomers and inactivation involved a slower subsequent event [174]. Binding of Et_3Sn to the enzyme increased reactivity of sulfhydryl groups which preceded loss of activity. Inactivation was reversed by incubation of the inactivated enzyme with glucose and dithiothreitol. Neither cysteine not histidine appeared to be involved in the binding site [175].

Dialkyltins inhibit oxygen uptake in mitochon-

dria but they act differently than R_3Sn's. They appear to act by combining with enzymes or coenzymes which have two thiol groups in the correct positions (vicinal dithiols). Such enzymes include lipoic acid and lipoyl dehydrogenase and their inhibition would interfere with α-keto acid oxidation. Bu_2SnCl_2 inhibited α-keto acid dehydrogenase by binding to dithiol groups of the cofactor lipoic acid [143]. Mechanisms of reactions have been reviewed [55].

UNANSWERED QUESTIONS

Some aspects of tin and organotins do not need further work. For example, reliable, sensitive methods are available to quantify organotin species in most environmental samples and in culture media. We have sufficient evidence to justify stringent limitations on use of Bu_3Sn's in near-shore environs and in certain types of fishing gear. Champ and Pugh [41] listed a number of questions related to monitoring butyltins in the marine environment, fate and effects in the environment, toxicity and regulatory aspects. Some of these, such as the fate of organotins in surface microlayers, involve microorganisms. Little is known of organometals in general and of organotins in particular in fresh water systems, both surface- and ground waters. This is particularly important in view of the use of organotins as fungicides in agriculture. As noted in earlier reviews [47,48] there are major gaps in our understanding of microbial transformations of inorganic tin and organotins. BCF's should be established for microorganisms in the laboratory, in microcosms and in specific environmental situations. In addition, the role of microorganisms in accumulation of tin compounds in food webs should be addressed. Virtually nothing is known of effects of tin compounds on microbe-mediated processes. Toxicity of tin compounds to microorganisms and microbial processes must be assessed under conditions which exist in specific environmental niches, including aquatic and soil ecosystems.

Some algae can accumulate inorganic tin and organotins [63] and microalgae may participate in biodegradation of organotins [113]. Interactions between algae, fungi, bacteria and tin compounds should be explored. Important aspects to be investigated include potential mutagenicity of tin compounds and their effects on viruses.

Tin resistant organisms can be isolated with ease and should be used to address a number of questions. Does resistance to one tin compound confer resistance to others? A common method of microbial resistance to heavy metals is by excluding them from the cell. What are the mechanism(s) whereby microorganisms resist tin compounds? How are tin compounds taken up by cells? Do they enter the cell or do they act at the external surface of the cytoplasmic membrane as suggested [27,70]? If they act at the cell surface how do they exert their toxic effects on mitochondria and chloroplasts in vivo? If R_3Sn's associate with the cell surface can they be metabolized to R_2Sn's which are then taken up? While it is clear that the nature of such anions as OH, acetate and halogens does not have a significant effect on toxicity of R_3Sn's, newer organotins which have non-polar groups or amino acids in those positions should be examined, for such groups can alter the total surface area and the K_{ow} which can reflect changes in associations with the cell surface, in transport into cells and in toxicity.

Details of the interactions of triorganotins with energy-generating and energy-utilizing systems in chloroplasts, mitochondria and bacteria continue to stimulate research. It is still a matter of contention whether organotins act primarily as uncouplers and if so how. Reports on presumptive differential actions by triorganotins on ATP synthase and ATP hydrolase deserve further consideration in order to understand details of the proton motive force.

There is virtually no information on interactions between tin compounds and microbial enzymes. The genetics of tin metabolism and of resistant organisms is unexplored and represents a powerful tool for understanding microbial interactions with tin compounds.

Can we design organotins or formulations of organotins which will be toxic to target organisms but which will be degraded by indigenous microorganisms shortly after release from a biocide? Under-

standing the nature of microbial interactions with tin compounds is essential to the intelligent development, use and regulation of existing and future useful organotins.

ACKNOWLEDGEMENT

Work done in preparing this manuscript was supported in part by grant No. 5-21092 from the MIT Sea Grant College.

REFERENCES

1 Aldridge, W.N. 1976. The influence of organotin compounds on mitochondrial funtions. In: Organotin compounds: new chemistry and applications (J.J. Zuckerman, ed.), pp. 186–196, Adv. Chem. Series 157.

2 Aldridge, W.N. 1986. The toxicology and biological properties of organotin compounds. In: Tin as a vital nutrient (N.F. Cardarelli, ed.) pp. 245–261, CRC Press, Boca Raton, FL.

3 Aldridge, W.N. and A.W. Brown. 1988. The biological properties of methyl and ethyl derivatives of tin and lead. In: The biological alkylation of heavy metals (P.J. Craig and F. Glocking, eds.), pp. 147–163, Royal Soc. Chem., London.

4 Aldridge, W.N., J.E. Casida, R.H. Fish, E.C. Kimmel and B.W. Street. 1977. Action on mitochondria and toxicity of metabolites of tributyltin compounds. Biochem. Pharmacol. 26: 1997–2000.

5 Aldridge, W.N. and J.E. Cremer. 1955. The biochemistry of organotin compounds. Diethyltin dichloride and triethyltin sulphate. Biochem. J. 61: 406–418.

6 Aldridge, W.N., B.W. Street and J.G. Noltes. 1981. The action of 5-coordinate triorganotin compounds on rat liver mitochondria. Chem.-Biol. Interactions 34: 223–232.

7 Aldridge, W.N., B.W. Street and D.N. Skilleter. 1977. Oxidative phosphorylation: halide dependent and halide-independent effects of triorganotin and triorganolead compounds on mitochondrial functions. Biochem. J. 168: 353–364.

8 Alzieu, C. 1986. TBT detrimental effects on oyster culture in France—evolution since antifouling paint regulation. Oceans 86 Proceedings, Vol. 4, pp. 1130–1134, IEEE Service Center, Piscataway, NJ.

9 Alzieu, C., J. Sanjuan, J.P. Deltreil and M. Borel. 1986. Tin Contamination in Arcachon Bay: Effects on Oyster Shell Anomalies. Mar. Poll. Bull. 17: 494–498.

10 Andres, C.F., J.C. Shaffer and A.S. Windeler. 1974. Comparison of the antibacterial properties of stannous fluoride and sodium fluoride mouthwashes. J. Dent. Res. 53: 457–460.

11 Anonymous. 1986. Oceans 86 Proceedings, Vol. 4, IEEE Service Center, Piscataway, NJ.

12 Anonymous. 1987. Oceans 87 Proceedings, Vol. 4, IEEE Service Center, Piscataway, NJ.

13 Anraku, Y., H. Kobayashi, H. Amanuma and A. Yamaguchi. 1973. VII. Characterization of the reaction of restoration of active transport mediated by binding protein. J. Biochem. 74: 1249–1261.

14 Argaman, Y., C.E. Hucks and S.E. Shelby, Jr., 1984. The effects of organotin on the activated sludge process. Water Res. 18: 535–542.

15 Asano, A., K. Imai and R. Sato. 1967. Oxidative phosphorylation in *Micrococcus denitrificans*. II. The properties of pyridine nucleotide transhydrogenase. Biochim. Biophys. Acta 143: 477–486.

16 Ashby, J., S. Clark and P.J. Craig. 1988. The analysis of organotin compounds from environmental matrices. In: The biological alkylation of heavy metals (P.J. Craig and F. Clocking, eds.), pp. 263–290, Royal Soc. Chem., London.

17 Babich, H. and G. Stotzky. 1980. Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. Crit. Rev. Microbiol. 8: 99–145.

18 Babich, H. and G. Stotzky. 1983. Heavy metal toxicity to microbemediated ecologic processes: a review and potential application to regulatory policies. Environ. Res. 36: 111–137.

19 Barnes, R.D., A.T. Bull and R.C. Poller. 1973. Studies on the persistence of the organotin fungicide fentin acetate (triphenyltin acetate) in the soil and on surfaces exposed to light. Pestic. Sci. 4: 305–317.

20 Beaumont, A.R., D.K. Mills and P.B. Newman. 1987. Some effects of tributyl tin (TBT) on marine algae. Oceans 87 Proceedings, Vol. 4, pp. 1488–1493, IEEE Service Center, Piscataway, NJ.

21 Beaumont, A.R. and M.D. Newman. 1986. Low levels of tributyltin reduce growth of marine microalgae. Mar. Poll. Bull. 17: 457–461.

22 Blair, W.R., G.J. Olson, F.E. Brinckman and W.P. Iverson. 1982. Accumulation and fate of tri-*n*-butyltin cation in estuarine bacteria. Microb. Ecol. 8: 241–251.

23 Blair, W.R., G.J. Olson, F.E. Brinckman, R.C. Paule and D.A. Becker. 1986. An international butyltin measurement methods intercomparison: sample preparation and results of analyses. NBSIR 86-3321. National Bureau of Standards, Gaithersburg, MD.

24 Blunden, S.J., L.A. Hobbs and P.J. Smith. 1984. The environmental chemistry of organotin compounds. In: Environmental Chemistry (H.J. Bowen, ed.), pp. 49–77, Royal Soc. Chem., London.

25 Bowman, B.J. and E.J. Bowman. 1986. H^+-ATPase from mitochondria, plasma membranes and vacuoles of fungal cells. J. Membrane Biol. 94: 83–97.

26 Brinckman, F.E. 1981. Environmental organotin chemistry

today: experiences in the field and laboratory. J. Organomet. Chem. Libr. 12: 343–376.
27 Brinckman, F.E., G.J. Olson, W.R. Blair and E.J. Parks. 1988. Implications of molecular speciation and topology of environmental metals: uptake mechanisms and toxicity of organotins. In: Aquatic Toxicology and Hazard Assessment: 10th Volume (W.J. Adams, G.A. Chapman and W.G. Landis, eds.), pp. 219–232, American Society for Testing and Materials, Philadelphia.
28 Bryan, G.W., P.E. Gibbs, G.R. Burt and L.G. Hummerstone. 1987. The effects of tributyltin (TBT) accumulations on adult dog-whelks, *Nucella lapillus:* long-term field and laboratory experiments. J. Mar. Biol. Ass. U.K. 67: 525–544.
29 Bushong, S.J., W.S. Hall, W.E. Johnson and L.W. Hall, Jr. 1987. Toxicity of tributyltin to selected Chesapeake Bay biota. Oceans 87 Proceedings, Vol. 4, pp. 1499–1503, IEEE Service Center, Piscataway, NJ.
30 Bygrave, F.L., C. Ramachandron and R.N. Robertson. 1978. The interaction of tributyltin with the mitochondrial calcium transport system of rat liver. Arch. Biochem. Biophys. 188: 301–307.
31 Bygrave, F.L. and R.L. Smith. 1978. Inability of tributyltin-induced chloride/hydroxyl exchange to stimulate calcium transport in mitochondria isolated from flight muscle of the sheep blowfly *Lucilia cuprina.* Biochem. J. 174: 1075–1077.
32 Byrd, J.T. and M.O. Andreae. 1982. Tin and methyltin species in seawater: concentrations and fluxes. Science 218: 565–569.
33 Cain, K. and D.E. Griffiths. 1977. Studies of energy-linked reactions. Localization of the site of action of trialkyltin in yeast mitochondria. Biochem. J. 162: 575–580.
34 Cain, K., R.L. Hyams and D.E. Griffiths. 1977. Studies on energy-linked reactions: inhibition of oxidative phosphorylation and energy-linked reactions by dibutyltin dichloride. FEBS Lett. 82: 23–28.
35 Calllow, M.E., P.A. Millner and L.V. Evans. 1978. Organotin resistance in green seaweeds. In: Proc. Ninth Int'l. Seaweed Symposium (A. Jensen and J.R. Stein, eds.), pp. 191–197, Science Press, Princeton, NJ.
36 Camosci, D.A. and N. Tinanoff. 1984. Anti-bacterial determinants of stannous fluoride. J. Dent. Res. 63: 1121–1125.
37 Cardin, C.J. and A. Roy. 1986. Anticancer activity of organometallic compounds. 3. The reaction of dimethyltin dichloride with nucleotides under biologically relevant conditions. Inorg. Chim. Acta 125: 63–66.
38 Cardwell, R.D. and A.W. Sheldon. 1986. A risk assessment concerning the fate and effects of tributyltins on the aquatic environment. Ocean 86 Proceedings, Vol. 4, pp. 1117–1124, IEEE Service Center, Piscataway, NJ.
39 Carraher, C.E., D.J. Giron, W.K. Woelk, J.A. Schroeder and M.F. Feddersen. 1979. Characterization of polyethyleneimine modified with organotin halides. Thermal, solubility and fungal properties. J. Appl. Polym. Sci. 23: 1501–1508.
40 Champ, M.A. and F.L. Lowenstein. 1987. TBT: the dilemma of high technology antifouling paints. Oceanus 30: 68–77.
41 Champ, M.A. and W.L. Pugh. 1987. Tributyltin antifouling paints: Introduction & overview. Oceans 87 Proceedings, Vol. 4, pp. 1296–1308, IEEE Service Center, Piscataway, NJ.
42 Chang, L.W., T.M. Tiemeyer, G.R. Wenger and D.E. McMillan. 1982. Neuropathology of trimethyltin intoxication. III. Changes in the brain stem neurons. Environ. Res. 30: 399–411.
43 Chiba. 1987. Determination of tin in biological materials by atomic-absorption spectrometry with a graphite furnace. J. Anal. Toxicol. 11: 125–130.
44 Christensen, G.M. and B. Riedel. 1981. Effect of water pollutants and other chemicals upon the activity of lipase in vitro. Arch. Environ. Contam. Toxicol. 10: 357–363.
45 Cleary, J.J. and A.R.D. Stebbing. 1985. Organotin and total tin in coastal waters of southwest England. Mar. Poll. Bull. 16: 350–355.
46 Coleman, J.O.D. and J.M. Palmer. 1971. The influence of pH on the inhibition of oxidative phosphorylation and electron transport of triethyltin. Biochim. Biophys. Acta 245: 313–320.
47 Cooney, J.J. 1988. Microbial transformations of tin and tin compounds. J. Ind. Micribiol. 3: 195–204.
48 Cooney, J.J. 1988. Interactions between microorganisms and tin compounds. In: The biological alkylation of heavy metals (P.J. Craig and F. Glockling, eds.), pp. 92–104, Royal Soc. Chem., London.
49 Cooney, J.J., L. deRome, O. Laurence and G.M. Gadd. 1989. Effects of organotin and organolead compounds on yeasts. J. Ind. Microbiol. 4: 279–288.
50 Cooney, J.J., A.T. Kronick, G.J. Olson, W.R. Blair and F.E. Brinckman. 1988. A modified method for quantifying methyl- and butyltins in estuarine sediments. Chemosphere 17: 1795–1802.
51 Cooney, J.J. and G.W. Pettibone. 1986. Metals and microbes in toxicity testing. Toxic Assess. 1: 487–499.
52 Cotta-Ramusino, M. and A. Doci. 1987. Acute toxicity of Brestan and fentin acetate on some freshwater organisms. Bull. Environ. Contam. Toxicol. 38: 647–652.
53 Crowe, A.J. 1987. Review: Organotin compounds in agriculture since 1980. Part 2. Acaricidal. antifeedant, chemosterilant and insecticidal properties. Appl. Organomet. Chem. 1: 331–346.
54 Cremer, J.E. The biochemistry of organotin compounds. 1958. The conversion of tetraethyltin into tirethyltin in mammals. Biochem. J. 68: 685–692.
55 Davies, A. and P.J. Smith. 1980. Recent advances in organotin chemistry. Adv. Inorg. Chem. Radiochem. 23: 1–77.
56 Davies, I.M., S.K. Bailey and D.C. Moore. 1987. Tributyltin in Scottish sea lochs, as indicated by degree of imposex in the dogwhelk, *Nucella lapillus* (L.). Mar. Poll. Bull. 18: 400–404.

57 Davies, I.M. and J.C. McKie. 1987. Accumulation of total tin and tributyltin in muscle tissue of farmed Atlantic salmon. Mar. Poll. Bull. 18: 405–407.

58 Davies, I.M., J.C. McKie and J.D. Paul. 1986. Accumulation of tin and tributyltin from antifouling paint by cultivated scallops (*Pectin maximus*) and Pacific oysters (*Crassostrea gigas*). Aquaculture 55: 103–114.

59 Dawson, A.P. and M.J. Selwyn. 1974. The action of trialkyltin compounds on mitochondrial respiration. The effect of pH. Biochem. J. 138: 349–357.

60 Dawson, A.P. and M.J. Selwyn. 1975. The action of tributyltin on energy coupling in coupling factor deficient submitochondrial particles. Biochem. J. 152: 333–339.

61 Donard, O.F.X., F.T. Short and J.H. Weber. 1987. Regulation of tin and methyltin compounds by the green alga *Enteromorpha* under simulated estuarine conditions. Can. J. Fish. Aquat. Sci. 44: 140–145.

62 Donard, O.F.X. and J.H. Weber. 1985. Behavior of methyltin compounds under simulated conditions. Environ. Sci. Technol. 19: 1104–1110.

63 Dooley, C.A. and P. Kenis. 1987. Response of bioluminescent bacteria to alkyltin compounds. Oceans 87 Proceedings, Vol. 4, pp. 1517–1524, IEEE Service Center, Piscataway, NJ.

64 Drummond, G.S. and A. Kappas. 1982. Chemoprevention of neonatal jaundice: Potency of tin-protoporphyrin in an animal model. Science 217: 1250–1252.

65 Duncan, J. 1980. The toxicology of molluscicides. The organotins. Pharmac. Ther. 10: 407–429.

66 Ebdon, L., K. Evans and S. Hill. 1988. The variation of tributyltin levels with time in selected estuaries prior to the introduction of regulations governing the use of tributyltin-based anti-fouling paints. Sci. Total Environ. 68: 207–223.

67 Ebdon, L., S.J. Hill and P. Jones. 1985. Speciation of tin in natural waters using coupled high performance liquid chromatography-flame atomic absorption spectrometry. Analyst 110: 515–517.

68 Elliott, B.M., W.N. Aldridge and J.W. Bridges. 1979. Triethyltin binding to cat haemoglobin. Biochem. J. 177: 461–470.

69 Emanuel, E.L., M.A. Carver, G.C. Solani and D.E. Griffiths. 1984. Differential inhibition of F_0F_1-ATPase-catalyzed reactions in bovineheart submitochondrial particles by organotin compounds. Biochim. Biophys. Acta 766: 209–214.

70 Eng, G., E.J. Tierney, J.M. Bellama and F.E. Brinckman. 1988. Correlation of molecular total surface area with organotin toxicity for biological and physicochemical applications. Appl. Organomet. Chem. 2: 171–175.

71 Evans, D.W. and R.B. Laughlin, Jr. 1984. Accumulation of *bis*(tributyltin) oxide by the mud crab, *Rhithropanopeus harrisii*. Chemosphere 13: 213–219.

72 Farrow, B.G. and A.P. Dawson. 1978. Investigation of the interaction of triethyltin with rat liver mitochondria using binding studies and Mössbauer spectroscopy. Eur. J. Biochem. 86: 85–95.

73 Fish, R.H., E.C. Kimmel and J.E. Casida. 1975. Bioorganotin chemistry: biological oxidation of tributyltin derivatives. J. Organomet. Chem. 93: C1–C4.

74 Froelich, P.N., L.W. Kaul, J.T. Byrd, M.O. Andreae and K.K. Roe. 1985. Arsenic, barium, germanium, tin, dimethylsulfide and nutrient biogeochemistry in Charlotte Harbor, Florida, a phosphorus-enriched estuary. Estuar. Coast. Shelf Sci. 20: 239–264.

75 Gibbs, P.E. and G.W. Bryan. 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. J. Mar. Biol. Ass. U.K. 66: 767–777.

76 Gibbs, P.E. and G.W. Bryan. 1987. TBT paints and the demise of the dogwhelk, *Nucella lapillus* (Gastropoda). Oceans 87 Proceedings, Vol. 4, pp. 1482–1487, IEEE Service Center, Piscataway, NJ.

77 Gibbs, P.E., G.W. Bryan, P.L. Pascoe and G.R. Burt. 1987. The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. J. Mar. Biol. Ass. U.K. 67: 507–523.

78 Gould, J.M. 1976. Inhibition by triphenyltin chloride of a tightly-bound membrane component involved in photophosphorylation. Eur. J. Biochem. 62: 567–575.

79 Gould, J.M. 1978. Dithiol-specific reversal of triphenyltin inhibition of CF_0-catalyzed transmembrane proton transfer in chloroplasts. FEBS Lett. 94: 90–94.

80 Griffiths, D.E. and I. Connerton. 1988. Effects of trialkyllead compounds on mitochondrial energy conservation. Appl. Organomet. Chem. 2: 177–180.

81 Guffanti, A.A., S. Clejan, L.H. Falk, D.B. Hicks and T.A. Krulwich. 1987. Isolation and characterization of uncoupler-resistant mutants of *Bacillus subtilis*. J. Bacteriol. 169: 4469–4478.

82 Hall, L.W., M.J. Lenkevich, W.S. Hall, A.E. Pinkney and S.J. Bushong. 1987. Evaluation of butyltin compounds in Maryland waters of Chesapeake Bay. Mar. Poll. Bull. 18: 78–83.

83 Hall, L.W., Jr., and A.E. Pinkney. 1985. Acute and sublethal effects of organotin compounds on aquatic biota: an interpretative literature evaluation. CRC Crit. Rev. Toxicol. 14: 159–209.

84 Hallas, L.E. and J.J. Cooney. 1981. Tin and tin-resistant microorganisms in Chesapeake Bay. Appl. Environ. Microbiol. 41: 466–471.

85 Halles, L.E. and J.J. Cooney. 1981. Effects of stannic chloride and organotin compounds on estuarine microorganisms. Dev. Ind. Microbiol. 22: 529–535.

86 Hallas, L.E., J.S. Thayer and J.J. Cooney. 1982. Factors affecting the toxic effect of tin on estuarine microorganisms. Appl. Environ. Microbiol. 44: 193–197.

87 Harris, J.R.W. and J.J. Cleary. 1987. Particle-water partitioning and organotin dispersal in an estuary. Oceans 87 Proceedings, Vol. 4, pp. 1370–1374, IEEE Service Center, Piscataway, NJ.

88 Hinga, K.R., D. Adelman and M.E.Q. Pilson. 1987. Radiolabeled butyl tin studies in the MERE enclosed ecosystems.

Oceans 87 Proceedings, Vol. 4, pp. 1416–1420. IEEE Service Center, Piscataway, NJ.

89 Huggett, R.J., M.A.. Unger and D.J. Westbrook. 1986. Organotin concentrations in the southern Chesapeake Bay. Oceans 86 Proceedings, Vol. 4, pp. 1262–1265, IEEE Service Center, Piscataway, NJ.

90 Ishii, T. 1982. Tin in marine algae. Bull. Japanese Soc. Sci. Fish. 48: 1609–1615.

91 Ito, M. and Y. Ohnishi. 1981. Isolation of *E. coli* mutants which are resistant to an inhibitor of H^+-ATPase, tributyltin and also to uncouplers of oxidative phosphorylation. FEBS Lett. 136: 225–230.

92 Johansen, K. and F. Mohlenberg. 1987. Impairment of egg production in *Acartia tonsa* exposed to tributyltin oxide. Ophelia 27: 137–141.

93 Jonas, R.B., C.C. Gilmour, D.L. Stoner, M.M. Weir and J.H. Tuttle. 1984. Comparison of methods to measure acute metal and organometal toxicity to natural aquatic microbial communities. Appl. Environ. Microbiol. 47: 1005–1011.

94 Kaars Sijpesteijn, A., J.G.A. Luijten and G.J.M. Van der Kerk. 1969. Organometallic fungicides. In: Fungicides – An advanced treatise. Vol. II (D.C. Torgeson, ed.), pp. 331–336, Academic Press, NY and London.

95 Kaars Sijpesteijn, A., F. Rijkens, J.G.A. Luijten and L.C. Willemsens. 1962. On the antifungal and antibacterial activity of some trisubstituted organogermanium, organotin and organolead compounds. Ant. van Leeuwenh. J. Microbiol. Serol. 28: 346–356.

96 Kahana L. and A. Kaars Sijpesteijn. 1967. Studies on the mode of action of the antibacterial agent diethyltin dichloride on *E. coli*. Ant. van Leeuwenh. J. Microbio. Serol. 33: 427–438.

97 Kahn, J.S. 1968. Chlorotri-*n*-butyltin. An inhibitor of phosphorylation in isolated chloroplasts. Biochim. Biophys. Acta 153: 203–210.

98 Kappas, A. and M.D. Maines. 1976. Tin: A potent inducer of heme oxygenase in kidney. Science 192: 60–62.

99 Kimmel, E.C., R.H. Fish and J.E. Casida. 1977. Bioorganotin chemistry. Metabolism of organotin compounds in microsomal monooxygenase systems and in mammals. J. Agric. Food Chem. 25: 1–9.

100 Kobayashi, H., E. Kin and Y. Anraku. 1974. X. Sources of energy and energy coupling reactions of the active transport systems for isoleucine and proline in *Escherichia coli*. J. Biochem. 76: 251–261.

101 Krowke, R., U. Bluth and D. Neubert. 1986. In vitro studies on the embryotoxic potential of (bis[tri-*n*-butyltin])oxide in a limb bud organ culture system. Arch. Toxicol. 58: 125–129.

102 Lancashire, W.E. and D.E. Griffiths. 1971. Biocide resistance in yeast: isolation and general properties of trialkyltin resistant mutants. FEBS Lett. 17: 209–214.

103 Lancashire, W.E. and D.E. Griffiths. 1975. Studies on energy-linked reactions: isolation, characterization and genetic analysis of trialkyltin-resistant mutants of *Saccaromyces cereviseae*. Eur. J. Biochem. 51: 377–392.

104 Laughlin, R.B., Jr. 1986. Bioacumulation of tributyltin: the link between environment and organism. Oceans 86 Proceedings, Vol. 4, pp. 1206–1209. IEEE Service Center, Piscataway, NJ.

105 Laughlin, R.B., Jr. and W.J. French. 1980. Comparative study of the acute toxicity of a homologous series of trialkyltins to larval shore crabs, *hemigrapsus nudus*, and lobster, *homarus americanus*. Bull. Environ. Contam. Toxicol. 25: 802–809.

106 Laughlin, R.B., Jr., W. French and H.E. Guard. 1986. Accumulation of bis(tributyltin) oxide by the marine mussel *mytilus edulis*. Environ. Sci. Technol. 20: 884–890.

107 Laughlin, R.B., Jr., H.E. Guard and W.M. Coleman, III. 1986. Tributyltin in seawater: speciation and octanol-water partition coefficient. Environ. Sci. Technol. 20: 201–204.

108 Laughlin, R.B., Jr., R.B. Johannesen, W. French, H. Guard and F.E. Brinckman. 1985. Structure-activity relationships for organotin compounds. Environ. Toxicol. Chem. 4: 343–351.

109 Laughlin, R.B., Jr., P. Pendoley and R.G. Gustafson. 1987. Sublethal effects of tributyltin on the hard shell clam, *Mercenaria mercenaria*. Oceans 87 Proceedings, Vol. 4, pp. 1494–1498. IEEE Service Center, Piscataway, NJ.

110 Lawler, I.F. and J.C. Aldrich. 1987. Sublethal effects of bis (tributyltin) oxide on *Crassostrea gigas* spat. Mar. Poll. Bull. 18: 274–278.

111 Lee, R.F. 1985. Metabolism of tributyltin oxide by crabs, oysters and fish. Mar. Environ. Res. 17: 145–148.

112 Lee, R.F. 1986. Metabolism of BIS 1 (tributyltin) oxide by estuarine animals. Oceans 86 Proceedings, Vol. 4, pp. 1182–1188, IEEE Service Center, Piscataway, NJ.

113 Lee, R.F., A.O. Valkirs and P.F. Seligman. 1987. Fate of tributyltin in estuarine waters. Oceans 87 Proceedings, Vol. 4, pp. 1411–1415. IEEE Service Center, Piscataway, NJ.

114 Leow, A.C.T. and D.D. Leaver. 1977. Effect of triethyltin on *Escherichia coli* K-12. Chem. Biol. Inter. 19: 339–351.

115 Liu, D. 1985. Effect of bacterial cultures on microbial toxicity assessment. Bull. Environ. Contam. Toxicol. 34: 331–339.

116 Lloyd, D. and S.W. Edwards. 1977. Mitochondrial adenosine triphosphatase of the fission yeast, *Schizsaccharomyces pombe* 972h$^-$. Changes in inhibitor sensitivities during the cell cycle indicate similarities and differences in binding sites. Biochem. J. 162: 581–590.

117 Luijten, J.G.A. 1972. Applications and biological effects of organotin compounds. In: Organotin compounds, Vol. 3, (Sawyer, A.K. ed.), pp. 931–974, Marcel Dekker, Inc., New York.

118 Luijten, J.G.A. and G.J.M. Van der Kerk. 1961. Investigations on organo-tin compounds. XIV. Preparation and antifungal properties of some triaryl- and triarylalkyl-tin acetates. J. Appl. Chem. 11: 35–37.

119 Mackay, D. 1982. Correlation of Bioconcentration Factors. Environ Sci. Technol. 16: 274–278.

120 Maguire, R.J. 1984. Butyltin compounds and inorganic tin in sediments in Ontario. Environ. Sci. Technol. 18: 291–294.

121 Maguire, R.J. 1987. Environmental aspects of tributyltin. Appl. Organomet. Chem. 1: 475–498.
122 Maguire, R.J., J.H. Carey and E.J. Hale. 1983. Degradation of the tri-*n*-butyltin species in water. J. Agric. Food Chem. 31: 1060–1065.
123 Maguire, R.J., Y.K. Chau, G.A. Bengert, E.J. Hale, P.T.S. Wong and O. Kramer. 1982. Occurrence of organotin compounds in Ontario lakes and rivers. Environ. Sci. Technol. 16: 698–702.
124 Maguire, R. J. and R.K. Tkacz. 1985. Degradation of tri-*n*-butyltin species in water and sediment from Toronto harbor. J. Agric. Food Chem. 33: 947–953.
125 Maguire, R.J. and R.K. Tkacz. 1987. Concentration of tributyltin in the surface microlayer of natural waters. Water Poll. Res. J. 22: 227–233.
126 Maguire, R.J., P.T.S. Wong and J.S. Rhamey. 1984. Accumulation and metabolism of tri-*n*-butyltin cation by a green alga, *Ankistrodesmus falcatus*. Can. J. Fish. Aquat. Sci. 41: 537–540.
127 Matthias, C.L., J.M. Bellama, G.J. Olson and F.E. Brinckman. 1986. Comprehensive method for determination of aquatic butyltin and butylmethyltin species at ultratrace levels using simultaneous hydridization/extraction with GC-FPD. Environ. Sci. Technol. 20: 609–615.
128 Matthias, C.L., G.J. Olson, F.E. Brinckman and J.M. Bellama. 1988. In press.
129 McLean, J.R.N., D.H. Blakey, G.R. Douglas and J.G. Kaplan. 1983. The effect of stannous and stannic (tin) chloride on DNA in Chinese hamster ovary cells. Mutat. Res. 119: 195–201.
130 Mesubi, M.A., U.B. Eke and T.T. Bamgboye. 1988. Synthesis, structural and biocidal activity studies of triorganotin (IV) compounds of some N-protected amino-acids. Appl. Organomet. Chem. 2: 121–127.
131 Minchin, D., C.B. Duggan and W. King. 1987. Possible effects of organotins on scallop recruitment. Mar. Poll. Bull. 18: 604–608.
132 Mueller, M.D. 1984. Tributyltin detection at trace levels in water and sediments using GC with flame-photometric detection and GC-MS. Fresenius Z Anal. Chem. 317: 32–36.
133 Mukohata, Y. and Y. Kaji. 1981. Light-induced membrane-potential increase, ATP synthesis, and proton uptake in *Halobacterium halobium* R_1mR Catalyzed by Halorhodopsin: Effects of *N,N'*-dicyclohexylcarbodiimide triphenyltin chloride, and 3,5-di-*tert.*-butyl-4-hydroxybenzylidenemalononitrile (SF6847). Arch. Biochem. Biophys. 206: 72–76.
134 Newby, P.J. and G.M. Gadd. 1988. Morphological response of some common spoilage fungi towards triorganotin compounds. In: The biological alkylation of heavy elements. (Craig, P.J. and F. Glocking eds.), pp. 164–167, Royal Soc. Chem. London.
135 Nishimoto, K. and G. Fuse. 1966. Fungicidal and wood preservative properties of organotin compounds. Tin and its uses. 70: 3–5.
136 Noland, E.A., P.T. McCauley, R.J. Bull. 1983. Dimethyltin dichloride: investigations into its gastrointestinal absorption and transplacental transfer. J. Toxicol. Environ. Health. 12: 89–98.
137 Noltes, J.G., J.G.A. Luijten and G.W.M. Van der Kerk. 1961. Investigations on organotin compounds. XV. The antifungal properties of some functionally substituted organotin compounds. J. Appl. Chem. 11: 38–40.
138 Olson, G.J. and F.E. Brinckman. 1986. Biodegradation of tributyltin by Chesapeake Bay microorganisms. Oceans 86 Proceedings, Vol. 4, pp. 1196–1201, IEEE Service Center, Piscataway, NJ.
139 Orsler R.J. and G.E. Holland. 1982. Degradation of tributyltin oxide by fungal culture filtrates. Int. Biodeterior. Bull. 18: 95–98.
140 Orsler, R.J. and G.E. Holland. 1984. The interaction between tri-*n*-butyltin oxide and agar gel as related to a fungus inhibition assay. Int. Biodeterior. 20: 37–41.
141 Oshchepkova, E.P. and D.A. Kochkin. 1981. Inhibition of *Penicillium purpurogenum* growth by organotin compounds. Microbiol. 50: 885–890.
142 Padan, E., D. Zilberstein and H. Rottenberg. 1976. Proton electrochemical gradient in *Escherichia coli* cells. Eur. J. Biochem. 533–541.
143 Penninks, A.H., P.M. Verschuren and W. Sienen. 1983. Dibutyltin dichloride uncouples oxidative phosphorylation in rat liver mitochondria. Toxicol. Appl. Pharmacol. 70: 115–120.
144 Peterson, P.J., M.A.S. Burton, M. Gregson, S.M. Nye and E.K. Porter. 1976. Tin in plants and surface waters in Malaysian ecosystems. Trace Substances in Environ. Health-X. pp. 123–132.
145 Peterson, P.J., M.A.S. Burton, M. Gregson, S.M. Nye and E.K. Porter. 1979. Accumulation of tin by mangrove species in West Malaysia. Sci. Total Environ. 11: 213–221.
146 Pettibone, G.W. and J.J. Cooney. 1986. Effect of organotins on fecal pollution indicator organisms. Appl. Environ. Microbiol. 52: 562–566.
147 Pettibone, G.W. and J.J. Cooney. 1988. Toxicity of methyltins to microbial populations in estuarine sediments. J. Ind. Microbiol. 2: 373–378.
148 Piver, W.T. 1973. Organotin compounds: industrial applications and biological investigation. Environ. Health Perspect. 4: 61–79.
149 Prough, R.A., M.A. Stalmach, P. Wiebkin and J.W. Bridges. 1981. The microsomal metabolism of the organometallic derivatives of the Group-IV elements, germanium, tin and lead. Biochem. J. 196: 763–770.
150 Ramos, S., S. Schuldiner and H.R. Kaback. 1976. Electrochemical gradient of protons and its relationship to active transport in *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. USA 73: 1892–1896.
151 Randall, L. and J.H. Weber. 1986. Adsorptive behavior of butyltin compounds under simulated estuarine conditions. Sci. Total Environ. 57: 191–203.
152 Rapsomanikis, S. and R.M. Harrison. 1988. Speciation of

butyltin compounds in oyster samples. Appl. Organomet. Chem. 2: 151–157.

153 Rexrode, M. 1987. Ecotoxicity of tributyltin. Oceans 87 Proceedings, Vol. 4, pp. 1443–1455, IEEE Service Center, Piscataway, NJ.

154 Rice, C.D., F.A. Espourteille and R.J. Huggett. 1987. Analysis of tributyltin in estuarine sediments and oyster tissue, *Crassostrea virginica*. Appl. Organomet. Chem. 1: 000–000.

155 Robertson, D.G., S-N. Kim, R.H. Gray and F.A. De La Iglesia. 1987. The pathogenesis of trimethyltin chloride-induced nephrotoxicity. Fundamental Appl. Toxicol. 8: 147–158.

156 Rosa, M.S. 1969. Evidence for histidine in the triethyltin-binding site of rat hemoglobin. Biochem. J. 111: 129–137.

157 Rosenberg, D.W. and G.S. Drummond. 1983. Direct in vitro effects of bis(tributyltin)oxide on hepatic cytochrome P-450. Biochem. Pharmacol. 24: 3823–3829.

158 Rosenberg, D.W., G.S. Drummond, H.H. Cornish and A. Kappas. 1980. Prolonged induction of hepatic heme oxygenase and decrease in cygochrome P-450 content by organotin compounds. Biochem. J. 190: 465–468.

159 Rosenberg, D.W., G.S. Drummond and A. Kappas. 1981. The influence of organometals on heme metabolism. In vivo and in vitro studies with organotins. Molec. Pharmacol. 21: 150–158.

160 Saxena, P.N. and A.J. Crowe. 1988. An investigation of the efficacy of organotin compounds for the control of the cotton stainer, *Dysdercus cingulatus*, the mosquito, *Anophelese stephensi*, and the common house fly, *Musca domestica*. Appl. Organomet. Chem. 2: 185–187.

161 Schweinfurth, H.A. and P. Gunzel. 1987. The tributyltins: mammalian toxocity and risk evaluation for humans. Oceans 87 Proceedings, Vol. 4, pp. 1421–1431, IEEE Service Center, Piscataway, NJ.

162 Seidel, S.L., V.F. Hodge and E.D. Goldber. 1980. Tin as an environmental pollutant. Thalassia Jugosl. 16: 209–223.

163 Seligman, P.F., C.M. Adema, P.M. Stang, A.O. Valkirs and J.G. Grovhoug. 1987. Monitoring and prediction of tributyultin in the Elizabeth River and Hampton Roads. Virginia. 1357–1363, IEEE Service Center, Piscataway, NJ.

164 Seligman, P.F., J.G. Brovhoug, A.O. Valkirs, P.M. Stang, R. Fransham, M.O. Stallard, B. Davidson and R.F. Lee. 1989. Distribution and fate of tributyltin in the marine environment. Appl. Organomet. Chem. (in press).

165 Seligman, P.F., A.O. Valkirs, P.M. Stang and R.F. Lee. 1988. Evidence for rapid degradation of tributyltin in a marina. Mar. Poll. Bull. 19: 531–534.

166 Seligman, P.F., A.O. Valkirs and R.F. Lee. 1986. Degradation of tributyltin in San Diego Bay, California, waters. Environ. Sci. Technol. 20: 1229–1235.

167 Selwyn, M.J. 1976. Triorganotin compounds as ionophores and inhibitors of ion translocating ATPases. Adv. Chem. Ser. 157: 204–226.

168 Selwyn, M.J. and A.S. Watling. 1988. Anion transport by alkyl derivatives of heavy metals. In: The Biological Alkylation of Heavy Elements (Craig, P.J. and F. Glockling eds.) pp. 291–295, Royal Soc. Chem. London.

169 Sherman, L.R. and F. Huber. 1988. Relationship of cytotoxic groups in organotin molecules and the effectiveness of the compounds against leukemia. Appl. Organomet. Chem. 2: 65–72.

170 Short, J.W. 1987. Measuring Tri-*n*-butyltin in salmon by atomic absorption: analysis with and without gas chromatography. Bull. Environ. Contam. Toxicol. 39: 412–416.

171 Short, J.W. and F.P. Thrower. 1986. Accumulation of butyltins in muscle tissue of Chinook salmon reared in sea pens treated with tri-*n*-butyltin. Oceans 86 Proceedings, Vol. 4, pp. 1177–1181, IEEE Service Center, Piscataway, NJ.

172 Siebenlist, K.R. and F. Taketa. 1980. Inhibition of red cell and yeast hexokinase by triethyltin bromide $[(C_2H_5)_3SnBr]$. Biochem. Biophys Res. Comm. 95: 758–764.

173 Siebenlist, K.R. and F. Taketa. 1981. Interactions of triethyltin bromide with components of the red cell. Toxicol. Appl. Pharmacol. 58: 67–75.

174 Siebenlist, K.R. and F. Taketa. 1983a. Inactivation of yeast hexokinase B by triethyltin bromide. Biochem. 22: 4229–4234.

175 Siebenlist, K.R. and F. Taketa. 1983b. Inactivation of yeast hexokinase B by triethyltin bromide and reactivation by dithiothreitol and glucose. Biochem. 22: 4642–4646.

176 Siebenlist, K.R. and F. Taketa. 1983c. The effects of triethyltin bromide on red cell and brain cyclic AMP-dependent protein kinases. J. Biol. Chem. 258: 11384–11390.

177 Siebenlist, K.R. and F. Taketa. 1983. The effects of triethyltin bromide on red cell and brain cyclic AMP-dependent protein kinases. J. Biol. Chem. 258: 11384–11390.

178 Singh, A.P. and P.D. Bragg. 1976. Anaerobic transport of amino acids coupled to the glycerol-3-phosphate-fumarate oxidoreductase system in a cytochrome-deficient mutant of *E. coli*. Biochim. Biophys. Acta 423: 450–461.

179 Singh, A.P. and P.D. Gragg. 1978. Lack of involvement of lipoic acid in membrane-associated energy transduction in *Escherichia coli*. Biochem. Biophys. Res. Commun. 81: 161–167.

180 Singh, A.P. and P.D. Bragg. 1979. The action of tributyltin chloride on the uptake of proline and glutamine by intact cells of *E. coli* cells. Can. J. Biochem. 57: 1376–1383.

181 Singh, A.P. and P.D. Bragg. 1979. The action of tributyltin chloride on energy-dependent transhydrogenation of $NADP^+$ by NADH in membranes of *E. coli*. Can. J. Biochem. 57: 1384–1391.

182 Singh, A.P. 1980. II. The role of mutants in the studies of energy-transduction in *E. coli*. Ind. J. Microbiol. 20: 333–361.

183 Singh, A.P. and K. Singh. 1984. Isolation and characterization of TBT resistant mutants of *E. coli*. Z. Naturf. 39c: 293–299.

184 Singh, A.P. and K. Singh. 1985. Differential sensitivity to TBT of cytochrome-containing and cytochrome-deficient

cells of *E. coli* SASX76. Experientia 41: 764–767.

185 Skilleter, D.N. 1975. The decrease of mitochondrial substrate uptake caused by trialkyltin and trialkyllead compounds in chloride media and its relevance to inhibition of oxidative phosphorylation. Biochem. J. 146: 465–471.

186 Skilleter, D.N. 1976. The influence of adenine nucelotides and oxidizable substrates on triethyltin-mediated chloride uptake by rat liver mitochondria in potassium chloride media. Biochem. J. 154: 271–274.

187 Slesinger, A.E. and I. Dressler. 1978. The environmental chemistry of three organotin chemicals. In: Report of the Organotin Workship, Feb. 17-19, 1978 (Good, M., ed.), pp. 115–162, University of New Orleans, New Orleans, LA.

188 Smith, P.J. 1978. Toxicological data on organotin compounds. Int. Tin Res. Inst. Publ. 538. 20 pp.

189 Smith, G.N., F.S. Fischer and R.J. Axelson. 1976. Volatilization and photodecomposition of Plictran miticide. J. Agric. Food Chem. 24: 1225–1229.

190 Soderquist, C.J. and D.G. Crosby. 1978. Determination of triphenyltin hydroxyde and its degradation products in water. Anal. Chem. 50: 1435–1439.

191 Soderquist, C.J. and D.G. Crosby. 1980. Degradation of triphenyltin hydroxide and its degradation products in water. Anal. Chem. 50: 111–117.

192 Soracco, R.J. and D.H. Pope. 1983. Bacteriostatic and bactericidal modes of action of bis(tributyltin)oxide on *Legionella pneumophila*. Appl. Environ. Microbiol. 45: 48–57.

193 Stephenson, M.D., D.R. Smith, L.W. Hall, Jr., W.E. Johnson, P. Michel, J. Short, M. Waldock, R.J. Huggett, P. Seligman and S. Kila. 1987. An international intercomparison of butyltin determinations in mussel tissue and sediments. Oceans 87 Proceedings, Vol. 4, pp. 1334–1338, IEEE Service Center, Piscataway, NJ.

194 Stockdale, M., A.P. Dawson and M.J. Selwyn. 1970. Effects of trialkyltin and triphenyltin compounds on mitochondrial respiration. Eur. J. Biochem. 15: 342–351.

195 Stallard, M., V. Hodge and E.D. Goldberg. 1987. TBT in California coastal waters: monitoring and assessment. Environ. Monitoring Assess. 9: 195–220.

196 Tan, L.P., M.L. Ng and V.G. Kumar Das. 1978. The effect of triethyltin compounds on tubulin polymerization. J. Neurochem. 31: 1035–1041.

197 Thayer, J.S. 1984. Organometallic compounds and living organisms. Academic Press, New York.

198 Thayer, J.S. and F.E. Brinckman. 1982. The biological methylation of metals and metalloids. Adv. Organomet. Chem. 20: 313–356.

199 Thayer, J.S., R.B. Jonas and J.J. Cooney. 1986. The effects of tin compounds on cell growth and development. In: Tin as a vital nutrient (Cardarelli, N.F. ed.), pp. 85–93, CRC Press, Boca Raton, FL.

200 Thomas, T.E. and M.G. Robinson. 1987. Initial characterization of the mechanisms responsible for the tolerance of *Amphora coffeaeformis* to copper and tributyltin. Bot. Mar. 30: 47–53.

201 Thompson, J.A.J., M.G. Sheffer, R.C. Pierce, Y.K. Chau, J.J. Cooney, W.R. Cullen and J.R. Maguire. 1985. Organotin compounds in the aquatic environment. NRCC Assoc. Comm. Scientific Criteria Environ. Qual., Pub. No. NRCC 22494, National Research Council of Canada, Ottawa, Ontario, 284 pp.

202 Tipping, E., B. Ketterer, L. Christodoulides, B.M. Elliott, W.N. Aldridge and J.W. Bridges. 1979. The interactions of triethyltin with rat glutathione-*S*-transferases A B and C. Enzyme-inhibition and equilibrium-dialysis studies. Chem.-Biol. Interact. 24: 317–327.

203 Unger, M.A., W.G. MacIntyre and R.J. Huggett. 1987. Equilibrium sorption of tributyltin chloride by Chesapeake Bay sediments. Oceans 87 Proceedings, Vol. 4, pp. 1381–1385, IEEE Service Center, Piscataway, NJ.

204 Unsel, M. 1984. Accumulation and loss of tin by the mussel. Ocean. Acta 7: 493–498.

205 U.S. Public Law 100-330 102 Stat. 605. Organotin antifouling paint control act of 1988, June 16, 1988.

206 Valkirs, A.O., P.F. Seligman and R.F. Lee. 1986. Butyltin partitioning in marine waters and sediment. Oceans 86 Proceedings, Vol. 4, pp. 1165–1170, IEEE Service Center, Piscataway, NJ.

207 Valkirs, A.O., P.F. Seligman, G.J. Olson, F.E. Brinckman, C.L. Matthias and J.M. Bellama. 1987. Di- and tributyltin species in marine and estuarine waters. Inter-laboratory comparison of two ultratrace analytical methods employing hydride generation and atomic absorption or flame photometric detection. Analyst 112: 17–20.

208 Van der Kerk, G.J.M. and J.G.A. Luijten. 1954. Investigations on organo-tin compounds. III. The biocidal properties of organo-tin compounds. J. Appl. Chem. 4: 314–319.

209 Van der Kerk, G.J.M. and J.G.A. Luijten. 1956. Investigations on organo-tin compounds. V. The preparation and antifungal properties of unsymmetrical tri-*n*-alkyltin acetates. J. Appl. Chem. 6: 56–60.

210 Van Koten, G. and J.G. Noltes. 1976. Chiral pentacoordinate trioorganotin halides. In: Organotin compounds: new chemistry and applications (Zuckerman, J.J. ed.), p. 275, Advanced Chemical Series 147, American Chemical Society, Washington, DC.

211 Waldock, M.J. and J.E. Thain. 1983. Shell thickening in *Crassostrea gigas:* organotin antifouling or sediment induces? Mar. Poll. Bull. 14: 411–415.

212 Waldock, M.J., J.E. Thain and M.E. Waite. 1987. The distribution and potential toxic effects of TBT in UK estuaries during 1986. Appl. Organomet. Chem. Vol. 1/4: 287–301.

213 Walsh, G.E., L.L. McLaughlin, E.M. Lores, M.K. Louie and C.H. Deans. 1985. Effects of organotin on growth and survival of two marine diatoms *Skeletonema costatum* and *Thalassiosira pseudonana*. Chemosphere 14: 383–392.

214 Ward, S.C., G.C. Cramm, P.R. Parrish, H. Trachman and A. Slesinger. 1981. Bioaccumulation and chronic toxicity of bis(tributyltin)oxide (TBTO): tests with a saltwater fish. In: Aquatic Toxicology and Hazard Assessment: Fourth Con-

ference, ASTM STP 737 (Branson, D.R. and K.L. Dickson, eds.), pp. 183–200, American Society for Testing and Materials, Philadelphia, PA.

215 Ward, S.C., R.C. Taylor and A.J. Crowe. 1988. The in vitro antiherpes activity of some selected antitumor organotin compounds. Appl. Organomet. Chem. 2: 47–52.

216 Watling-Payne, A.S. and M.J. Selwyn. 1970. Effects of some organometallic compounds on the permeability of chloroplast membranes. FEBS Lett. 10: 139–142.

217 Watling-Payne, A.S. and M.J. Selwyn. 1974. Inhibition and uncoupling of photophosphorylation in isolated chloroplasts by organotin, organomercury and diphenyleneiodonium compounds. Biochem. J. 142: 65–74.

218 Watling-Payne, A.S. and M.J. Selwyn. 1975. Decrease of proton permeability of CF_1-deficient chloroplast particles by triphenyltin. FEBS Lett. 58: 57–61.

219 Weis, J.S., J. Gottlieb and Jill Kwiatkowski. 1987. Tributyltin retards regeneration and produces deformities of limbs in the fiddler crab, *Uca pugilator*. Arch. Environ. Contam. Toxicol. 16: 321–326.

220 Wolniakowski, K.U., M.D. Stephenson and G.S. Ichikawa. 1987. Tributyltin concentrations and Pacific oyster deformation in Coos Bay, Oregon. Oceans 87 Proceedings, Vol. 4, pp. 1438–1443, IEEE Service Center, Piscataway, NJ.

221 Wong, P.T.S., Y.K. Chau, O. Kramer and G.A. Bengert. 1982. Structure-toxicity relationship of tin compounds on algae. Can. J. Fish Aquat. Sci. 39: 483–488.

222 Wright, D.A. and W.H. Roosenburg. 1982. Trimethyltin toxicity to larval *Uca pugilator:* effects of temperature and salinity. Arch. Environ. Contam. Toxicol 11: 491–495.

223 Wulf, R.G. and K.H. Byington. 1975. On the structure-activity relations and mechanism of organotin-induced, non-energy dependent swelling of liver mitochondria. Arch. Biochem. Biophys. 167: 176–185.

224 Yamada, J. 1981. Effects of trialkyltin chlorides on *E. coli* spheroplasts. Agric. Biol. Chem. 45: 997–999.

225 Yamada, J., K. Oishi, K. Tatsuguchi and T. Watanabe. 1979. Interaction of trialkyltin chloride with inorganic phosphates and phospholipids. Agric. Biol. Chem. 43: 1015–1020.

226 Yamada, J., K. Tatsuguchi and T. Watanabe. 1978. Effects of trialkyltin chlorides on microbial growth. Agric. Biol. Chem. 42: 1167–1172.

227 Yamada, J., K. Tatsuguchi and T. Watanabe. 1978. Uptake of tripropyltin by *Escherichia coli*. Agric. Biol. Chem. 42: 1867–1870.

228 Yamada, J., K. Tatsuguchi and T. Watanabe. 1979. Cell lysis and leakage of cell consituents by tripropyltin chloride. Agric. Biol. Chem. 43: 125–130.

229 Yamada, J. and T. Watanabe. 1979. Effect of tripropyltin chloride on transport system in *E. coli*. Agric. Biol. Chem. 43: 1681–1686.

230 Yamada, J. and T. Watanabe. 1979. Effect of tripropyltin chloride on functional reactions in *E. coli*. Agric. Biol. Chem. 43: 1293–1300.

231 Zedler, R.J. and C.B. Beiter. 1962. Organotins: biological activity, uses. Soap Cosmet. Chem. Spec. 38: 75–78.

232 Zuckerman, J.J., R.P. Reisdorf, H.V. Ellis, III and R.R. Wilkinson. 1978. Organotins in biology and the environment. In: organometals and organometalloids, Occurrence and Fate in the Environment (Brinckman, F.E. and J.M. Bellema, eds.), American Chemical Society Symposium Series Vol. 82, pp. 388–424, American Chemical Society, Washington, DC.