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Structure–activity studies on the fungitoxicity of some triorganotin(IV) compounds

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Seventeen triorganotin(IV) compounds, with the general formula R_3SnX, containing symmetrical and unsymmetrical combinations of alkyl and aryl groups on tin and with a wide variation in the non-carbon-bonded anionic (X) residues, were examined along with three formally pentacoordinated adducts of triaryltin chlorides with triphenylphosphine oxide for their antifungal activity against nine plant pathogenic and saprophytic fungi. The *in vitro* tests included inhibitory studies on radial growth, mycelial growth, spore germination, and germ tube elongation. A significant finding was the dependence of fungitoxicity on the nature of the X group in both the tributyltin and triaryltin series, in contrast to earlier published reports on the negligible influence of the X groups on overall toxicity relative to the R groups. This suggests that the X group is significantly involved in transporting the biocide to the reactive sites, and that the X group which tends to confer increased solubility to the triorganotin compound gives rise to increased activity.

In studies of R group variations, tri-iso-butyltin bromide was found to be much less fungitoxic than tri-n-butyltin compounds, a result which is reconcilable in terms of increased steric encumbrance at the tin site in the former case. The steric factor is also implicated in the reduced activities observed for tris(*p*-tolyl)tin and tris(*p*-chlorophenyl)tin compounds relative to (Ph_3SnX) towards most of the fungi screened in this study. In general, it was also noted that the triaryltins were more selective in their antifungal action than the trialkyltins, which exhibited broad spectral activity when applied at the concentration level of 10 $\mu g\ cm^{-3}$.

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ABBREVIATIONS USED HERE

Bu	n-Butyl
CMA	Corn meal agar
Et	Ethyl
MA	Malt extract agar
Me	Methyl
MIC	Minimum inhibitory concentration
nd	Not determined
OAc	Acetate
Oct	n-Octyl
Ph	Phenyl
$\mu g\ cm^{-3}$	Micrograms per cubic centimetre

INTRODUCTION

In any organotin series, R_nSnX_{4-n} (R = carbon-bonded organic group; X = inorganic substituent; $n = 1-4$), it is now well established that maximum toxicity to all types of living species occurs with the triorganotin compounds, R_3SnX.[1]

Basic studies on the antifungal properties of oganotin compounds were first initiated in 1954 by van der Kerk and Luijten,[2] who found that antifungal activity was maximal with triorganotin compounds in which the total carbon content of the three organic R groups was in the range of 9–12 carbon atoms. Although this has been subsequently confirmed by other workers[3,4] employing a wider range of compounds, it is noteworthy that the tri-n-butyl- and triphenyl-tins originally investigated by van der Kerk and Luijten[2,5] constitute as yet the only classes of organotin fungicides presently in commercial application.

Van der Kerk and Luijten[2] in their initial investigations also observed that for the triethyltin series (Et_3SnX), variations in the X groups led to no significant changes in fungitoxicity. This suggested that for R_3SnX compounds, fungitoxicity was principally governed by the nature of the organic moieties residing on tin. Although this conclusion is generally supported by bioassay studies on other tri-n-alkyltin systems as, for example, n-Bu_3SnX,[6] exceptions have been documented in several instances, especially involving triaryltin systems,[7–9] where presumably the effects of the X group on solubility and consequent activity are more discriminatory. Conceivably, with biocidal X groups, a substantial increase in fungitoxicity may be anticipated for both the trialkyl and triaryltin systems, but such combinations appear to have been little explored. The triaryltin(IV) compounds combine reduced phytotoxicity with a moderate level of fungicidal activity compared with the trialkyltin derivatives.[10,11]

Replacement of a halide by a pseudohalide group such as isocyanate or isothiocyanate in Ph_3SnX has been shown to lead to a significant increase in fungicidal activity.[12] Adducts of those pseudohalides with substituted triazines[13] as well as adducts of triphenyltin isoselenocyanate with nitrogen and oxygen donor ligands are claimed to be efficient fungicides.[13,14] Mono- and bis-(triphenyltin) sulphides which are much less phytotoxic than triphenyltin acetate are, however, only active against a narrow range of fungi.[15] *N*-Triphenylstannyl sulphamates,[16] triphenylstannyl phosphinates[4] and triphenylstannyl benzoates[17] appear to have a favourable ratio of fungitoxic to phytotoxic activity compared with triphenyltin acetate. Adducts of triphenyltin chloride with phosphine oxides,[18] pyridine *N*-oxide[19] and sulphoxides[20] also exhibit a favourable ratio of fungitoxic to phytotoxic activity. There is considerable current interest in the study of triorganotin(IV) compounds containing ligands which are biologically active in their own right.

The aim of this study was to investigate structure–activity relationships encompassing electronic and steric effects, and lipophilic character, for a range of triorganotin(IV) compounds containing symmetrical and unsymmetrical combinations of alkyl and aryl groups on tin and also with a wide variation in the non-carbon-bonded anionic X residue, including formally pentacoordinated adducts of triaryltin chlorides with triphenylphosphine oxide.

MATERIALS AND METHODS

Triorganotin(IV) compounds

Twenty triorganotin(IV) compounds were screened for their antifungal activities. These included trialkyltin compounds, triaryltin compounds including some pentacoordinated complexes of triaryltin chlorides, and unsymmetrical triorganotin compounds containing mixed alkyls, mixed aryls and alkylaryls (including heterocyclics) (Table 1). In compound B3, the sucrose is linked to phthalic acid as the half-ester and not directly attached to tin.

Test fungi

The test fungi included a range of phytopathogenic and saprophytic fungi and these are listed in Table 2.

In vitro evaluations

(a) Inhibition of radial growth

Known volumes of 2% MA were autoclaved and cooled to approximately 50°C. The triorganotin(IV) compound stocks in 95% ethanol or analytical grade acetone (100 $\mu g\ cm^{-3}$ and 10 $\mu g\ cm^{-3}$) were freshly prepared and aseptically added to the cooled agar to give final concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 $\mu g\ cm^{-3}$. The contents of the tubes were vigorously shaken to obtain an even mixture and aliquots were poured into a series of 9-cm sterile plastic Petri dishes. Each dish was inoculated at the centre with a 4-mm disc cut out from the vegetative growing margins of 2–4-day-old cultures maintained on MA or CMA. Fungi seeded on 2% MA amended with 95% ethanol or acetone served as the control. For each fungus, three replicate plates of each concentration of a compound were used. The dishes were incubated at $27 \pm 2°C$ for six days. The colony diameters, taken as the mean of two diameters at right angles to each other, were measured at 24h intervals.

The percentage inhibition of growth compared with maximum growth on control plates was calculated. A probit-log concentration analysis[21] was carried out to determine the ED_{50} value, which is the concentration causing a 50% reduction in growth measured in $\mu g\ cm^{-3}$.[22]

To determine the minimum concentrations of the

Table 1 Triorganotin(IV) compounds used in the antifungal screening

Compound code	Structural formula chemical name [in brackets]	Molecular weight	Tin content (%)
Tributyltin(IV) compounds			
B1	Bu_3SnOAc [Tri(n-butyl)tin acetate]	348.6	34.0
B2	$Bu_3SnOCOC_6H_4OH$-*p* [Tributyltin *p*-hydroxybenzoate]	426.6	27.8
B3	$Bu_3SnOCOC_6H_4CO_2$–sucrose [Phthalic acid (tributylstannyl)(sucrose) ester]	794.6	14.9
B4	Bu_3SnOCO-(2-thienyl) (Tributyltin thienyl 2-carboxylate)	416.6	28.5
B5	$(Bu_3Sn)_2O$ [Bis(tributyltin)oxide]	595.2	39.8
B6	$(Bu_3Sn)_3BO_3$ [Tris(tributyltin) borate]	927.6	38.4
B7	i-Bu_3SnBr [Tri-isobutyltin bromide]	396.6	32.1
Triaryltin(IV) compounds			
P1	Ph_3SnOAc (Triphenyltin acetate)	408.6	29.0
P2	$(p\text{-}ClC_6H_4)_3SnCl$ [Tris(*p*-chlorophenyl)tin chloride]	488.6	24.3
Triaryltin(IV) compounds			
P3	$Ph_3SnCl \cdot Ph_3PO$ [Triphenyltin chloride–triphenylphosphine oxide]	663.2	17.9
P4	$(p\text{-}ClC_6H_4)_3SnCl \cdot Ph_3PO$ [Tris(*p*-chlorophenyl)tin chloride–triphenylphosphine oxide]	766.7	16.8
P5	$(p\text{-}(MeC_6H_4)_3SnCl \cdot Ph_3PO$ [Tris(*p*-tolyl)tin chloride–triphenylphosphine oxide]	705.2	16.8
P6	$(Ph_3Sn)_2S$ [Bis(triphenyltin)sulphide]	731.2	32.4
P7	$(p\text{-}MeC_6H_4)_3Sn]_2S$ [Bis{tri(*p*-tolyl)tin}sulphide]	815.5	29.1
Mixed triorganotin (IV) compounds			
M1	$Ph_2BuSnBr$ (Diphenylbutyltin bromide)	409.6	29.0
M2	(2-thienyl)Bu_2SnCl [2-thienyldibutyltin chloride]	351.1	33.8
M3	(5-Me-2-thienyl)Bu_2SnCl [(5-methyl-2-thienyl)dibutyltin chloride]	365.1	32.5
Mixed triorganotin (IV) compounds			
M4	$(MeOCOCH_2CH_2)_2PhSnCl$ [Bis(β-carbomethoxyethyl)phenyltin chloride]	406.5	29.2
M5	$Et_2OctSnOAc$ (Diethyloctyltin acetate)	348.6	34.0
M6	$(p\text{-}MeC_6H_4)Ph_2SnOAc$ [(*p*-tolyl)diphenyltin acetate]	422.6	28.1

Table 2 Some features of the fungi used in the antifungal screening

Aspergillus niger	Cellulolytic soil fungus; causes post-harvest storage diseases and crown rot in peanuts; black mould in citrus fruits, pineapple and onion bulbs.
Botryodiplodia theobromae	Causes brown pod rot in cocoa; dieback in rubber and oil palm; blue stain in rubberwood; post-harvest diseases in fruits.
Curvularia eragrostidis	Causes leaf spot in maize and oil palm; seedling blight in coconut.
Curvularia lunata	Causes black kernel in rice.
Fusarium moniliforme	Causes damping-off and wilt of vegetables; ear rot and seedling blight in maize; 'bakanae' disease in rice; 'pokkah boeng' disease in sugar cane.
Pestalotiopsis guepini	Causes leaf spots in crops such as cereals and tea; cankerous lesions on tropical fruits.
Phaeotrichoconis crotalariae	Causes cereal disease, e.g. stack burn in rice.
Trichoderma viride	Cellulolytic soil fungus, used as a biological control of plant pathogens, e.g. *Sclerotium rolfsi* and *Armillaria mellea*.
Ulocladium botrytis	Saprophytic wood-destroying fungus.

triorganotin(IV) compounds required to inhibit radial growth completely, concentrations higher than 10 $\mu g\ cm^{-3}$ were often required. Intermediate concentrations between 0.1 $\mu g\ cm^{-3}$ and 15.0 $\mu g\ cm^{-3}$ were tested where necessary. Intermediate concentrations between 15.0 $\mu g\ cm^{-3}$ and 25.0 $\mu g\ cm^{-3}$; 25.0 $\mu g\ cm^{-3}$ and 50.0 $\mu g\ cm^{-3}$; and 50.0 $\mu g\ cm^{-3}$ and 100.0 $\mu g\ cm^{-3}$, however, were not tested. An MIC (minimum inhibitory concentration) value of 50.0 $\mu g\ cm^{-3}$ may, therefore, actually lie between 25.0 $\mu g\ cm^{-3}$ and 50.0 $\mu g\ cm^{-3}$ for example.

(b) Inhibition of mycelial growth

Aliquots of 50 cm^3 of modified Czapek Dox Liquid Medium at pH 6.8 (Oxford CM 95) were dispensed into 100 cm^3 Erlenmeyer flasks and autoclaved at 15 psi (103 kPa) and 120°C for 20 min. Appropriate volumes of triorganotin(IV) compound stocks in analytical-grade acetone were added to the cooled medium to give final concentrations of 0.01, 0.1, 1.0, 5.0, 10.0 and 50.0 $\mu g\ cm^{-3}$. Modified Czapek Dox Liquid Medium devoid of triorganotin(IV) compounds was used in the control flasks. Each flask was inoculated with a 5-mm mycelial disc cut out from the vegetative growing margins of 2–4-day-old cultures of the test fungi maintained on 2% MA or CMA. For each fungus, three replicate flasks were used per concentration per compound. The flasks were incubated in an orbital shaker incubator set at 30°C and operating at 150 rpm. After six days of growth, the flasks were removed and the mycelia were harvested on pre-dried weighed Whatman No. 1 filter papers. The mycelia and filter paper were washed and dried to a constant weight at 60°C. The dry weights of mycelia were recorded. The percentage inhibition of growth was calculated on the basis of mycelial dry weight compared with that in the control medium. The mycelial dry weights were corrected for the weight of the inoculum disc. The ED_{50} value was determined by carrying out a probit-log concentration analysis[21] for each fungus and compound.

(c) Inhibition of spore germination and germ tube elongation

Appropriate amounts of the stock triorganotin(IV) compounds were added to known volumes of 2% MA to give final concentrations of 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 $\mu g\ cm^{-3}$. 2% MA without the triorganotin(IV) compounds served as control. Spore suspensions in 10 cm^3 of sterile distilled water were prepared from 4–7-day-old cultures of the test fungi. The spore density was determined with a Neubaeur haemocytometer and then diluted to obtain a final spore concentration of 50 000 spores cm^{-3}. Discs of triorganotin(IV)-incorporated agar, each 7 mm in diameter, were cut out and three such discs were placed on sterile glass slides in sterile glass Petri dishes lined with sterile moist filter papers. A drop of spore suspension ($\sim 0.02\ cm^3$) was placed on each agar disc. The filter paper in each plate was moistened so that the plates could be maintained at high humidity. The plates were incubated at 27 ± 2°C. The percentage germination, germ tube lengths and morphology were recorded after 12 h. At least 200 spores were counted for each treatment to determine the percentage germination of spores. If the control did not give 100% germination of spores, the Abbott's correction formula:

$$P = \frac{P_o - P_c}{100 - P_c} \times 100$$

was used, where

P = corrected percentage of ungerminated spores;
P_o = observed percentage of ungerminated spores;
P_c = percentage of spores ungerminated in control discs.[21]

At least 20 germ tubes were measured at random for each treatment to determine the mean germ tube length. Using the concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 $\mu g\ cm^{-3}$ for compounds P1, P4, M1, M3 and M6 and 0.1, 1.0, 5.0, 10.0 and 50.0 $\mu g\ cm^{-3}$ for compound P7, a gradual increase in percentage inhibition of spore germination was not obtained. As such the ED_{50} values for spore germination could not be computed. Alternatively, a fungitoxic index was formulated to compare the effects of the selected triorganotin(IV) compounds on spore germination. The ED_{50} values for reduction in germ tube lengths were computed for each fungus and compound.

RESULTS

(a) Inhibition of radial growth

On control plates all the test fungi commenced growth immediately without exhibiting any noticeable lag period within the six days of incubation. With some triorganotin(IV) compounds, however, a lag period ranging from one to five days within the incubation

Table 3 Antifungal activity[a] spectra of triorganotin(IV) compounds at 1 $\mu g\ cm^{-3}$ and 10 $\mu g\ cm^{-3}$

		A. niger	*B. theobromae*	*C. eragrostidis*	*C. lunata*	*F. moniliforme*	*P. crotalariae*	*P. guepini*	*T. viride*	*U. botrytis*
Compound										
B1	Bu_3SnOAc	*	+	+	+	+	+	+	+	+
B2	$Bu_3SnOCOC_6H_4OH$-*p*	+	+	+	+	+	+	+	+	+
B3	$Bu_3SnOCOC_6H_4CO_2$–sucrose	*	+	*	+	+	+	+	+	+
B4	Bu_3SnOCO-(2-thienyl)	+	+	+	+	+	+	+	+	+
B5	$(Bu_3Sn)_2O$	*	*	*	*	+	+	*	*	*
B6	$(Bu_3Sn)_3BO_3$	*	+	+	+	+	+	+	+	+
B7	i-Bu_3SnBr	+	+	+	+	e	e	+	+	e
P1	Ph_3SnOAc	*	+	+	+	e	e	+	e	+
P2	$(p\text{-}ClC_6H_4)_3SnCl$	+	e	+	+	+	e	e	+	e
P3	$Ph_3SnCl \cdot Ph_3PO$	+	e	e	+	e	e	e	e	e
P4	$(p\text{-}ClC_6H_4)_3SnCl \cdot Ph_3PO$	e	e	e	e	e	e	e	e	e
P5	$(p\text{-}MeC_6H_4)_3SnCl \cdot Ph_3PO$	e	e	e	e	e	e	e	e	e
P6	$(Ph_3Sn)_2S$	+	e	e	e	e	e	e	e	e
P7	$[(p\text{-}MeC_6H_4)_3Sn]_2S$	e	e	e	e	e	e	e	e	e
M1	$Ph_2BuSnBr$	*	+	+	+	+	e	+	+	e
M2	(2-thienyl)Bu_2SnCl	e	e	e	e	e	e	e	e	e
M3	(5-Me-2-thienyl)Bu_2SnCl	e	e	+	e	e	e	e	e	e
M4	$(MeOCOCH_2CH_2)_2PhSnCl$	e	e	e	e	e	e	e	e	e
M5	$Et_2OctSnOAc$	*	*	+	+	*	+	*	+	+
M6	$(p\text{-}MeC_6H_4)Ph_2SnOAc$	+	e	+	+	e	e	+	+	e

[a] All results are the means of three replicates from the radial growth experiment. Antifungal activity is expressed as: *, 81–100% inhibition at 1 $\mu g\ cm^{-3}$; +, 81–100% inhibition at 10 $\mu g\ cm^{-3}$ compared with control; e, 81–100% inhibition at concentrations > 10 $\mu g\ cm^{-3}$.

period of six days was often seen. Antifungal activity spectra of the triorganotin(IV) compounds were determined at a low concentration of 1 $\mu g\ cm^{-3}$ and a high concentration of 10 $\mu g\ cm^{-3}$ (Table 3). At the 1 $\mu g\ cm^{-3}$ concentration level, the tributyltin(IV) compounds exhibited selective antifungal activity, whilst the triaryltin(IV) and the mixed triorganotin (IV) compounds were generally inactive save for compounds P1, P3, P6, M1, M5 and M6. At the concentration level of 10 $\mu g\ cm^{-3}$, however, the tributyltin(IV) compounds showed broad spectral activity, whilst both the triaryltin(IV) and the mixed triorganotin(IV) compounds, with the exception of diethyloctyltin acetate (compound M5), displayed a selective antifungal activity (Table 4). Of the fungi investigated, *Phaeotrichoconis crotalariae* proved to be particularly insensitive to the triaryltin(IV) compounds. In the presence of compound M5 and the tributyltin(IV) compounds, *P. crotalariae* manifested increased aerial hyphal growth compared with the control.

The ED_{50} values of the triorganotin(IV) compounds are given in Table 4. The ED_{50} values ranged from as low as 0.06 $\mu g\ cm^{-3}$ [for bis(tributyltin) oxide, B1, with *Aspergillus niger*] to 4781 $\mu g\ cm^{-3}$ [for bis[tri(*p*-tolyl)tin] sulphide, P8, with *Trichoderma viride*]. Generally, the ED_{50} values of the tributyltin(IV) compounds were much lower than that for the triaryltin(IV) and the mixed triorganotin (IV) compounds. An exception, however, was noted for compound M5 which had an activity comparable with that of the tributyltins.

From a comparison of the ED_{50} values of the tributyltin(IV) compounds, discernible differences in toxicity were noted among the tributyltin(IV) esters

Table 4 ED_{50}[a] values of triorganotin(IV) compounds for radial growth of fungi (2% MA; 27 + 2°C)

Compound		*A. niger*	*B. theobromae*	*C. eragrostidis*	*C. lunata*	*F. moniliforme*	*P. crotalariae*	*P. guepini*	*T. viride*	*U. botrytis*
B1	Bu_3SnOAc	0.30	1.25	0.71	0.82	2.07	1.68	1.17	1.55	1.38
B2	$Bu_3SnOCOC_6H_4OH$-*p*	0.14	0.47	0.43	0.53	1.57	0.66	0.45	0.46	1.24
B3	$Bu_3SnOCOC_6H_4CO_2$–sucrose	0.23	0.39	0.24	0.34	0.56	2.14	0.45	0.76	1.04
B4	Bu_3SnOCO-(2-thienyl)	0.36	2.14	0.67	0.97	3.08	1.44	1.45	1.83	1.70
B5	$(Bu_3Sn)_2O$	0.06	0.41	0.20	0.14	0.40	1.45	0.27	0.24	0.28
B6	$(Bu_3Sn)_3BO_3$	0.14	0.88	0.35	0.71	0.71	1.42	1.08	1.32	1.04
B7	$i\text{-}Bu_3SnBr$	0.57	0.98	1.40	1.65	6.65	2.13	1.34	3.71	2.93
P1	Ph_3SnOAc	0.29	1.63	0.67	0.49	3.86	11.8	0.90	0.75	0.81
P2	$(p\text{-}ClC_6H_4)_3SnCl$	2.10	27.8	3.02	2.00	3.17	38.0	4.14	3.09	17.4
P3	$Ph_3SnCl \cdot Ph_3PO$	1.28	15.1	2.14	1.69	15.0	18.8	11.4	6.99	5.07
P4	$(p\text{-}ClC_6H_4)_3SnCl \cdot Ph_3PO$	11.8	74.6	10.8	11.1	7.08	96.2	7.83	23.1	125
P5	$(p\text{-}MeC_6H_4)_3SnCl \cdot Ph_3PO$	36.0	26.4	9.74	5.69	9.73	>25.0	25.3	10.1	123
P6	$(Ph_3Sn)_2S$	0.65	24.0	3.33	2.37	37.3	114	8.66	40.4	33.3
P7	$[(p\text{-}MeC_6H_4)_3Sn]_2S$	130	30.4	113	85.7	233	183	27.9	4781	47.6
M1	$Ph_2BuSnBr$	0.23	2.38	0.63	0.32	1.46	7.82	0.95	0.66	1.53
M2	(2-thienyl)Bu_2SnCl	204	10.0–50.0	4.32	7.71	24.0	40.5	18.9	17.3	10.9
M3	(5-Me-2-thienyl)Bu_2SnCl	3.23	5.0–10.0	3.12	4.40	17.7	60.0	9.00	10.4	24.6
M4	$(MeOCOCH_2CH_2)_2PhSnCl$	82.8	53.2	1.39	15.8	51.4	32.2	47.7	63.9	16.6
M5	$Et_2OctSnOAc$	0.20	0.1–0.5	1.87	0.69	0.01	1.93	0.20	0.38	0.23
M6	$(p\text{-}MeC_6H_4)Ph_2SnOAc$	0.57	4.77	0.87	0.61	4.10	ND	2.59	1.29	2.13

[a] Concentration ($\mu g\ cm^{-3}$) required to inhibit 50% growth compared with control (2% MA with no triorganotin(IV) compound added) obtained from probit-log concentration regression analysis.

(compounds B1, B2, B3 and B4) as well as in the compounds B1, B5 and B6 containing one to three tributylstannyl units in the molecule, and between tri(n-butyl)tin acetate (compound B1) and tri-isobutyltin bromide (compound B7). The toxicity towards *Botryodiplodia theobromae*, *Fusarium moniliforme* and *Pestalotiopsis guepini*, in particular, showed a marked dependence on the nature of the ester grouping. The sucrose–phthalate linkage in compound B3 enhanced the toxicity of the tributyltin system towards all the nine fungi, compared with the thienyl 2-carboxylate moiety in compound B4. The presence of two tributylstannyl units in compound B5 resulted in an appreciable increase in fungitoxicity relative to one tributylstannyl unit in compound B1. The placement of three tributylstannyl moieties in compound B6, however, caused no further increase in fungitoxicity; instead the ED_{50} values were found to be intermediate between the values for compounds B1 and B5. The ED_{50} values obtained for tri-isobutyltin bromide (compound B7) against all the nine fungi except *B. theorbromae* were higher than that obtained for tri(n-butyl)tin acetate (compound B1).

Generally, the ED_{50} values indicate the following order of activities for the tributyltin(IV) compounds: bis(tributyltin) oxide (B5) > phthalic acid (tributylstannyl)(sucrose) ester (B3) > tributyltin *p*-hydroxybenzoate (B2) > tris(tributyltin) borate (B6) > tri(n-butyl)tin acetate (B1) > tributyltin thienyl 2-carboxylate (B4) > tri-isobutyltin bromide (B7).

Among the triaryltin(IV) compounds, triphenyltin acetate (compound P1) showed the highest toxicity. Compound P1 and its *p*-chlorophenyl analogue (compound P2), however, showed similar activity against *F. moniliforme* (Table 4). The introduction of a chloro substituent in each of the three phenyl rings generally decreased the antifungal activity. This also appears to be the case with the methyl substituent.

Some evidence of selectivity was manifested by triphenyltin chloride–triphenylphosphine oxide (compound P3) and bis(triphenyltin) sulphide (compound P6) against *A. niger*, *Curvularia eragrostidis* and *C. lunata*.

The fungitoxicities of the mixed triorganotin(IV) compounds, diphenylbutyltin bromide (compound M1) and *p*-tolyldiphenyltin acetate (compound M6) were comparable with or higher than that of compound P1. The introduction of a methyl group in one of the phenyl rings (compound M6) evidently resulted in a more favourable antifungal activity than for the case where all the phenyl rings carried methyl substituents (e.g. compound P8) or chloro groups (e.g. compound P2). Similarly, the introduction of a methyl group [i.e. (5-methyl-2-thienyl)dibutyltin chloride, compound M3] in the thienyl ring of 2-thienyldibutylin chloride (compound M2) led to an improvement in toxicity. The antifungal activity of diethyloctyltin acetate (compound M5) was comparable with that of bis(tributyltin) oxide (compound B5). Bis(β-carbomethoxyethyl)phenyltin chloride (compound M4) was selectively active against *C. eragrostidis*.

Table 5 illustrates the minimum concentrations of triorganotin(IV) compounds required to inhibit completely radial growth of the nine species of fungi. The minimum inhibitory concentrations required to cause a 100% inhibition of radial growth (MIC_{100}) ranged from 0.4 $\mu g\ cm^{-3}$ (e.g. diethyloctyltin acetate, M5, with *A. niger*) to greater than 100 $\mu g\ cm^{-3}$. A similar trend to that observed for ED_{50} values was observed for MIC_{100} values of the tributyltin(IV), triaryltin(IV) and mixed triorganotin(IV) compounds. The MIC_{100} values of compounds P5, P6, P7 and M4 were generally greater than 100 $\mu g\ cm^{-3}$. *Botryodioplodia theobromae* was generally tolerant to the triaryltin(IV) compounds whereas *P. crotalariae* was generally tolerant to the mixed triorganotin(IV) compounds.

(b) Inhibition of mycelial growth

The compounds P1, P4, P7, M1, M3 and M6 were selected for mycelial growth and germination studies based on their abilities to inhibit strongly radial extension of the colonies of test fungi. The tributyltin(IV) compounds were not selected because of their known high phytotoxicities.[23] The ED_{50} values of the probit-log concentration regression lines for mycelial dry weight are given in Table 6.

Curvularia lunata was tolerant to compounds P1 and P7. *Phaeotrichoconis crotalariae*, which was tolerant to the triaryltin(IV) and the mixed triorganotin(IV) compounds in the radial growth assay, was sensitive to all the six compounds used in the mycelial growth assay. The following order of activity was discernible based on the ED_{50} and slope values for mycelial dry weight: $Ph_2BuSnBr$ (M1) > Ph_3SnOAc (P1) > (p-MeC_6H_4)Ph_2SnOAc (M6) > $Ph_3SnCl.Ph_3PO$ (P4) > (5-Me-2-Thienyl)Bu_2SnCl (M3) > $(Ph_3Sn)_2S$ (P7). Generally, the slope values were gradual, indicating a low order of activity at low or high concentrations of the selected triorganotin(IV) compounds.

Table 5 Minimum concentrations[a] ($\mu g\ cm^{-3}$) of triorganotin(IV) compounds required for 100% inhibition of radial growth (2% MA; 27 ± 2°C; 6 days)

Compound		*A. niger*	*B. theobromae*	*C. eragrostidis*	*C. lunata*	*F. moniliforme*	*P. crotalariae*	*P. guepini*	*T. viride*	*U. botryitis*
B1	Bu_3SnOAc	0.8	15.0	12.0	11.0	25.0	8.0	10.0	12.0	7.0
B2	$Bu_3SnOCOC_6H_4OH$-*p*	1.0	10.0	15.0	10.0	50.0	10.0	10.0	10.0	5.0
B3	$Bu_3SnOCOC_6H_4CO_2$–sucrose	0.5	10.0	5.0	10.0	10.0	5.0	5.0	5.0	5.0
B4	Bu_3SnOCO-(2-thienyl)	3.0	25.0	25.0	25.0	25.0	10.0	15.0	25.0	15.0
B5	$(Bu_3Sn)_2O$	0.5	5.0	5.0	5.0	7.0	5.0	6.0	5.0	6.0
B6	$(Bu_3Sn)_3BO_3$	1.0	15.0	9.0	11.0	25.0	8.0	6.0	10.0	7.0
B7	i-Bu_3SnBr	15.0	25.0	25.0	25.0	50.0	25.0	10.0	50.0	25.0
P1	Ph_3SnOAc	10.0	X	50.0	10.0	15.0	50.0	50.0	15.0	10.0
P2	$(p\text{-}ClC_6H_4)_3SnCl$	50.0	X	X	50.0	50.0	X	X	X	50.0
P3	$Ph_3SnCl \cdot Ph_3PO$	25.0	X	50.0	50.0	X	50.0	50.0	X	X
P4	$(p\text{-}ClC_6H_4)_3SnCl \cdot Ph_3PO$	X	X	X	X	50.0	X	X	X	X
P5	$(p\text{-}MeC_6H_4)_3SnCl \cdot Ph_3PO$	X	X	50.0	X	X	>25.0	X	X	X
P6	$(Ph_3Sn)_2S$	25.0	X	X	X	X	X	X	X	X
P7	$[(p\text{-}MeC_6H_4)_3Sn]_2S$	X	X	X	X	X	X	X	X	X
M1	$Ph_2SnBuBr$	6.0	50.0	25.0	10.0	50.0	X	10.0	50.0	15.0
M2	(2-thienyl)Bu_2SnCl	X	X	X	X	X	X	X	X	X
M3	(5-Me-2-thienyl)Bu_2SnCl	50.0	X	50.0	50.0	X	X	X	X	50.0
M4	$(MeOCOCH_2CH_2)_2PhSnCl$	X	X	X	X	X	X	X	X	50.0
M5	$Et_2OctSnOAc$	0.4	7.0	50.0	50.0	2.0	50.0	0.5	5.0	5.0
M6	$(p\text{-}MeC_6H_4)Ph_2SnOAc$	9.0	X	50.0	50.0	50.0	X	50.0	50.0	50.0

[a] Minimum inhibitory concentrations were obtained by testing at 0.1–100.0 $\mu g\ cm^{-3}$ of the compounds. Intermediate concentrations between 15.0 and 25.0 $\mu g\ cm^{-3}$; 25.0 and 50.0 $\mu g\ cm^{-3}$; 50.0 and 100.0 $\mu g\ cm^{-3}$ were not tested. X = 100 or >100 $\mu g\ cm^{-3}$.

Table 6 ED_{50} values ($\mu g\ cm^{-3}$) of selected triorganotin(IV) compounds for mycelial dry weight (Czapek Dox modified liquid medium, pH 6.81; 30°C; 6 days)

Compound		*A. niger*	*B. theobromae*	*C. eragrostidis*	*C. lunata*	*F. moniliforme*	*P. crotalariae*	*P. guepini*	*U. botryitis*
P1	Ph_3SnOAc	0.16	0.05	<0.01	290	0.02	<0.01	0.01	0.08
P4	$Ph_3SnCl \cdot Ph_3PO$	<0.01	0.61	0.15	<0.01	0.89	<0.01	<0.01	<0.01
P7	$(Ph_3Sn)_2S$	0.16	0.03	0.37	872	21.2	<0.01	0.54	3.02
M1	$Ph_2BuSnBr$	0.01	0.03	<0.01	8.45	0.07	1.05	<0.01	nd
M3	(5-Me-2-thienyl)Bu_2SnCl	0.20	0.55	0.46	0.50	0.79	0.02	<0.1	<0.01
M6	$(p\text{-}MeC_6H_4)Ph_2SnOAc$	0.15	<0.1	6.38	0.60	<0.01	0.09	<0.1	0.02

(c) Inhibition of spore germination and reduction in germ tube lengths

As the ED_{50} values for spore germination could not be computed, the minimum concentration required to completely inhibit spore germination was expressed in four categories and each given a numerical rating (Table 7). Compound M1 was the most active compound in this test, indicating its high sporicidal activity. Compounds P1, P4, M3 and M6 showed similar potencies. Compound P7 was the least active.

The ED_{50} values for germ tube lengths agree with the trend obtained in the radial growth assay (Table 7). M1 had low ED_{50} values and high slope values. Compounds P7 and M3 selectively reduced germ tube lengths of *C. lunata, F. moniliforme* and *U. botrytis* at low concentrations. *Pestalotiopsis guepini* spores were tolerant to compounds P4, P7 and M3. *Ulocladium botrytis* spores were tolerant to compounds P4 and M3. The compounds did not cause much morphological changes of the germ tubes except for the reduction in length.

Table 8 compares ED_{50} values for radial and mycelial growth as well as germ tube extension for six selected compounds. Generally, lower concentrations of the organotins were required to inhibit mycelial growth in liquid medium compared with that required to inhibit radial growth and reduce germ tube lengths. This is probably because the inoculum is constantly bathed in the fungitoxic liquid medium. The ED_{50} values for inhibition of germ tube extension were generally lower than that for radial growth.

The compounds P4, M1 and M6 were good inhibitors of mycelial growth. Compound P1 was a good inhibitor of mycelial growth of all the fungi except *C. lunata*. Compound P7 was a good inhibitor of germ tube extension. A good inhibition of mycelial and germ tube growth was achieved using compound M3.

Table 7 Effect[a] of selected triorganotin(IV) compounds of spore germination (2% MA; 27 ± 2°C; 12 hours

Compound		*C. lunata*		*F. moniliforme*		*P. guepini*		*U. botrytis*	
		a	b	a	b	a	b	a	b
P1	Ph_3SnOAc	2	0.33	2	0.80	2	1.15	2	0.71
P4	$Ph_3SnCl \cdot Ph_3PO$	nd	nd	2	0.40	2	6.52	2	3.92
P7	$(Ph_3Sn)_2S$	1	0.11	2	0.92	1	4.00	1	0.02
M1	$Ph_2BuSnBr$	nd	nd	4	0.14	4	0.87	4	0.54
M3	(5-Me-2-thienyl)Bu_2SnCl	nd	nd	2	0.47	2	3.47	2	4.29
M6	(*p*-MeC_6H_4)Ph_2SnOAc	2	0.70	2	0.89	2	1.52	2	1.91

Abbreviations: a, Minimum concentration required for complete inhibition of spore germination expressed in four categories: 1.0–5.0 $\mu g\ cm^{-3}$ (4); 5.0–10.0 $\mu g\ cm^{-3}$ (3); 10.0–50.0 $\mu g\ cm^{-3}$ (2); >50.0 $\mu g\ cm^{-3}$ (1). b, ED_{50} value for germ tube length.

Table 8 Comparison of ED_{50} values ($\mu g\ cm^{-3}$) of selected triorganotin(IV) compounds for radial growth (a:2% MA; 27 ± 2°C); mycelial dry weight (b:Czapek Dox liquid medium; pH 6.8; 30°C); germ tube length (c:2% MA; 27 ± 2°C)

Compound		*C. lunata*			*F. moniliforme*			*P. guepini*			*U. botrytis*		
		a	b	c	a	b	c	a	b	c	a	b	c
P1	Ph_3SnOAc	0.49	29.0	0.33	3.86	0.02	0.80	0.90	0.01	1.15	0.81	0.08	0.71
P4	$Ph_3SnCl \cdot Ph_3PO$	1.69	>0.01	nd	15.0	0.89	0.40	11.4	<0.01	6.52	5.07	<0.01	3.92
P7	$(Ph_3Sn)_2S$	2.37	872	0.11	37.3	21.2	0.92	8.66	0.54	4.00	33.3	3.02	0.02
M1	$Ph_2BuSnBr$	0.32	8.42	nd	1.46	0.07	0.14	0.95	<0.01	0.87	1.53	nd	0.54
M3	(5-Me-2-thienyl)Bu_2SnCl	4.40	0.50	nd	17.7	0.79	0.47	9.00	<0.01	3.47	24.6	<0.01	4.29
M6	(*p*-MeC_6H_4)Ph_2OAc	0.61	0.60	0.70	4.10	<0.01	0.89	2.59	<0.01	1.52	2.13	0.02	1.91

DISCUSSION

Contrary to earlier findings,[6] the present investigations indicate discernible differences in fungitoxicity exerted by the anionic radial, X, in the triorganotin(IV) compounds. The high antifungal activity of the tributyltin(IV) carboxylates is remarkable considering their lower tin content (14.9–34.0% Sn) compared with the well-established commercial fungicide, bis(tributyl)tin oxide (39.8% Sn). The attachment of hydrophilic X groups to the tributyltin compounds B2 and B3 is seen to increase the fungitoxicity. By way of contrast, compound B4 containing a lipophilic thienyl 2-carboxylate unit manifests a lower order of antifungal activity than tributyltin acetate, B1. The reduced toxicity of compound B7 compared with B1 may be due to the increased steric encumbrance at the tin atom caused by branching in the hydrocarbon chains. The view is indirectly supported by chemical evidence attesting to the ease of formation of the penta-coordinated cationic species $[n\text{-}Bu_3SnL_2]^+$ relative to $[iso\text{-}Bu_3SnL_2]^+$, where L is a neutral oxygen-donor ligand (including water) attached to the primary coordination sphere of tin (V.G. Kumar Das, unpublished results).

Compound P1 showed the highest toxicity among the triaryltin(IV) compounds. This compound has recently been characterized in the solid state as a weakly polymeric structure in which the geometry at the tin atom is a distorted meridional-$[R_3SnX_3]$ octahedron.[24] In the light of this, the significantly reduced activity of the formally five-co-ordinated derivative, $Ph_3SnCl.Ph_3PO$ (compound P4), is surprising. The bis(triaryltin) sulphides (compounds P7 and P8) showed reduced antifungal activity compared with triphenyltin acetate (compound P1), with activity against only a narrow range of fungi including *A. niger*, *C. eragrostidis*, *C. lunata* and *P. guepini* for compound P7 and *B. theobromae* and *P. guepini* for compound P8. Similar observations on the limited antifungal spectrum of bis(triaryltin) sulphides have been previously reported by van der Kerk[15] and Srivastava and Tandon.[25]

Toxicity considerations of triorganotin compounds require cognizance of electronic and steric properties of various substituents on the metal atom.[26,27] The aryltins have proved to be particularly amenable to studying electronic and steric influences of ring substituents.[28] In the present study on the antifungal properties, the electron-withdrawing chloro group and the electron-donating methyl group have been introduced as the ring substituents. Comparison of the ED_{50} values indicate the order $H > CH_3 \simeq Cl$ for the case of triaryltin chloride–triphenylphosphine oxide compexes (compounds P3, P4 and P5) and the bis(triaryltin) sulphides (compounds P6 and P7). In the case of the mixed heterocyclic tin(IV) compounds (compounds M2 and M3), however, the order $CH_3 > H$ was observed.

Addition of a methyl group to one phenyl ring led to more favourable activity than addition of methyl groups to all three phenyl rings (compounds M6 and P7). Inspection of the toxicity data in Table 5 suggests a general masking of the electronic effects of substituent groups in triaryltin compounds by dominant steric effects. Also implicit in the toxicity data trends (Table 5) is the influence of aqueous solubility of the compounds on fungitoxicity. This is suggested by the higher toxicity of compound P1 over compound P7, and compounds B2 and B3 over compounds B1, B4 and B7. It is generally accepted that increased aqueous solubility would enhance the transport of the biocide from extracellular regions to the sites of toxic action within the fungal cells. It would thus appear that fungitoxicity of the triorganotin(IV) compounds could be enhanced by judiciously introducing substituents at the metal centre, by removal of steric congestion, and by altering the partitioning properties between water and lipids of the whole molecule. A quantitative approach using Hansch's Principle[29,30] would be useful in addressing future studies on the structure–activity relationships of triorganotin(IV) compounds.

The triphenylphosphine oxide complexes (compounds P4, P5 and P6), bis(triphenyltin) sulphide (compound P7) and (5-methyl-2-thienyl)dibutyltin chloride (compound M3) are active against fungi known to be pathogenic such as *F. moniliforme*, *C. lunata*, *C. eragrostidis*, *B. theorbromae*, *P. guepini*, *U. botrytis*, *P. crotalariae* and *A. niger* but less active against *T. viride*. The latter is an important soil cellulolytic fungus and has been reported to be antagonistic to several pathogenic fungi.[31–34] The selectivity of the triaryltin(IV) and mixed triorganotin (IV) compounds at 10 $\mu g\ cm^3$ and their low phytotoxicities[35] would make these compounds likely candidates for use in crop protection to control selectively the fungal pathogens used in this study.

The observed variations in response to radial and mycelial growth, spore germination and germ tube elongation (Table 8) may indicate, in part, the multi-

site toxic action of the triorganotin(IV) compounds. Although multi-site toxic action is a characteristic of protectant pesticides, some recent work on triphenyltin acetate, triphenyltin hydroxide and triphenyltin chloride has shown that these compounds possess some systemic activity in plants.[36–38] Further work in this direction is necessary as systemic activity would increase the potential applications of the triorganotin(IV) compounds, especially in the control of root and vascular fungal diseases of plants such as vascular streak dieback of cocoa caused by *Oncobasidium theobromae*[39,40] and foot rot of black pepper caused by *Phytophthora palmivora.*[41,42]

CONCLUSIONS

Fungitoxicity data secured for a range of tributyltin and triaryltin compounds clearly suggest the influence of the anionic X group in determining the overall toxicity of triorganotin(IV) compounds, R_3SnX.

X groups which tend to impart increased water solubility to the triorganotin(IV) compounds lead to increased availability of the biocide at the reactive sites in the test organisms, and hence manifest higher activity.

Increased steric encumbrance at the tin site results in reduced activities in tris-isobutyltin compared with the n-butyltin analogue and in $(p\text{-}ZC_6H_4)_3SnX$, $(p\text{-}ZC_6H_4)_2PhSnX$ and $(p\text{-}ZC_6H_4)Ph_2SnX$ compared with Ph_3SnX where Z = Me or Cl.

Triaryltin compounds are more selective in their antifungal activities compared with the trialkyltin compounds.

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