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A multicriteria ranking of organotin(IV) compounds with fungicidal properties

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The application of multicriteria decision-making methods to the results of *in vitro* antifungal properties of organotin compounds of the type Ph_xSnX_z ($x = 2$ or 3; $X = O_2CC_6H_4OH$, $O_2CC_6H_4OCOCH_3$, Cl or O_2CCH_3; $z = 1$ or 2) and of free 2-hydroxybenzoic and 2-acetoxybenzoic acids against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Penicillium citrinum*, *Trichophyton rubrum* and *Trichophyton violaceum* have been described. Ranking information necessary to select one toxicant in preference to others and to assess the properties influencing the preference has been obtained. Patterns in the multivariate analyses suggest that cationic and anionic moieties of the toxicant play some roles in their fungicidal activities. The triphenyltin compounds were generally more active than their diphenyltin analogues, but the acetoxybenzoates were more active than the corresponding hydroxybenzoates, acetates or chlorides. Thus, triphenyltin acetoxybenzoate is up to 7.5 times as active as the corresponding acetate, which is commercially marketed as a fungicide. The results of the analyses have been discussed in the light of the mechanism of antifungal activity of organotin compounds and the potential of multivariate data analysis techniques to facilitate the screening and ranking of antifungal agents. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: multicriteria ranking; organotin compounds; fungicidal properties

INTRODUCTION

Organotin compounds continue to attract interest, not only from the inorganic point of view but also from their potential as agricultural biocides[1–5] and pharmaceutical agents.[6–13] For example, in addition to the rich structural possibilities (four-coordinate, tetrahedral, monomeric cis, trans and mer five-coordinate, or five-coordinate bridged polymers) open to triorganotin compounds, they exhibit significant fungicidal activities.[14–17] Thus, triphenyltin acetate has been marketed commercially as a fungicide for a long time[18,19] and the antifungal activities of organotin(IV) compounds containing various other anionic groups have been reported.[14–17,20–23] Nevertheless, the mechanism of antifungal activity of many organotin compounds is not fully understood. A wide range of mechanisms has, therefore, been explored. Some of these have attempted to rationalize the antifungal properties of organotin compounds in terms of differences in (a) the molecular sizes of the substrates,[24] (b) the coordination numbers about the tin atoms,[25] (c) the hydrophobicities of the molecules,[26] (d) the nucleophilicities of the anionic groups,[22,23,27] (e) the penetration of the substrates,[28,29] (f) the delivery of the reactive cationic tin moieties to the fungi[30,31] (g) the derangement of the mitochondria of the fungi[32,33] and (h) the inhibition of the oxidative phosphorylation function of the fungi[34,35].

In this paper, firstly a comparison of the antifungal properties of some tri- and di-phenyltin compounds containing different anionic moieties, as well as free 2-hydroxybenzoic and 2-acetoxybenzoic acids, is reported. Such a direct comparison is important in order to ascertain whether the anionic groups play a part in the fungitoxicity of organotin compounds.[30,31] Secondly, since 2-hydroxybenzoic acid and its derivatives[36–38] are themselves fungitoxic, there is an interest in the antifungal activities of organotin(IV) benzoates, both in the context of finding metal-based antifungal agents

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that are more effective than triphenyltin acetate, and in elucidating structure–activity relationships. Thirdly, in order to conduct ranking analysis and pattern recognition on the cytotoxicities of the compounds, we have employed the multicriteria decision-making (MCDM) methods PROMETHEE (Preference Ranking Organization METHod for Enrichment Evaluation) and GAIA (Geometrical Analysis for Interactive Aid).[39–41] PROMETHEE quantifies the degree of preference of one object compared with another for each variable, and GAIA displays PROMETHEE results as biplots and facilitates the interpretation of the significance of the variables under investigation. The methods were originally developed to aid the selection of locations for the siting of factories, warehouses, etc.,[42] but they have been successfully employed to rank (i) digestion and chemometrics methods,[43–45] (ii) assess rice quality and land-mine detection strategies,[46,47] and (iii) criteria influencing air quality in residential indoor environments (Ayoko *et al*., unpublished results, 2002).

EXPERIMENTAL

Materials and methods

Reagent-grade triphenyltin chloride, diphenyltin dichloride, silver oxide and the carboxylic acids were obtained from the Aldrich Chemical Company. With the exception of the organotin chlorides, which were recrystallized from CH_2Cl_2–hexane before use, the chemicals were used as received. Organotin carboxylates are generally prepared by the reactions of organotin hydroxides or oxides with carboxylic acids, reactions of organotin halides with metal carboxylates or cleavage of tin–carbon bonds with carboxylic acid.[1–4,20,48–50]. In the present study, however, they have been prepared from the corresponding organotin chlorides and silver salts of the carboxylic acid using methods analogous to those reported earlier.[22,23,27] In a typical reaction, the organotin chloride (4 mmol) and silver benzoate (10 mmol) in CH_2Cl_2 (200 ml) were stirred for 48 h under an aluminium foil cover. The mixture was then filtered and the solvent evaporated. Each solid product was recrystallized from dry acetone. The yields for $Ph_3Sn(O_2CC_6H_4OH\text{-}2)$ (abbreviated to Ph_3SnSA), $Ph_3Sn(O_2CC_6H_4OCOCH_3\text{-}2)$ (Ph_3SnASA), $Ph_2Sn(O_2CC_6H_4OH\text{-}2)_2$ ($Ph_2Sn(SA)_2$), $Ph_2Sn(O_2CC_6H_4OCOCH_3\text{-}2)_2$ ($Ph_2Sn(ASA)_2$), $Ph_3SnO_2CCH_3$ (Ph_3SnOAc), and $Ph_2Sn(O_2CCH_3)_2$ ($Ph_2Sn(OAc)_2$) were respectively 86%, 91%, 85%, 78%, 90% and 80%. The strong benzoate bands at *ca* 1390 cm^{-1} and *ca* 1600 cm^{-1}, Sn–Ph deformation bands at 1080 cm^{-1}, and Sn–O–C bands at *ca* 1000 cm^{-1} and ^1H NMR data were in agreement with literature data for the compounds or similar compounds.[6–9,20,22,23,27,48–50] Elemental analysis data (%) were as follows. Found (calc.) Ph_3SnSA: C, 61.58 (61.64); H, 4.11 (4.14); Sn, 24.37 (24.37); Ph_3SnASA: C, 61.22 (61.29); H, 4.16 (4.19); Sn, 22.43 (22.68); $Ph_2Sn(SA)_2$: C, 57.02 (57.08); H, 3.66 (3.89); Sn, 21.69 (21.69); $Ph_2Sn(ASA)_2$: C, 58.14 (58.47); H, 4.61 (4.21); Sn, 20.64 (20.64); Ph_3SnOAc: C, 58.53 (58.67); H, 4.39 (4.40); Sn, 29.00 (29.09); $Ph_2Sn(OAc)_2$: C, 49.13 (49.10); H, 4.16 (4.09); Sn, 29.90 (30.30).

The compounds were dissolved in 25% aqueous acetone and their antifungal activities against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, *Candida albicans*, *Trichophyton rubrum* and *Trichophyton violaceum* evaluated by three different methods. Firstly, the minimum inhibitory concentrations (MICs) were determined in Sabouraud dextrose broth by a method essentially similar to that described previously.[27] Different concentrations of each of the compounds were mixed with double-strength Sabouraud dextrose agar in sterile Petri dishes. Each dish was inoculated with 20 µl of 10^8 cfu/ml (cfu: colony forming units) culture of a fungus and then left undisturbed on the bench for 1 h. The dish was subsequently incubated at 30 °C for 3 days. A control dish without the test compound was similarly treated. Test and control dishes were examined for fungi growth and the lowest concentration of the test compound that completely inhibited fungi growth was taken as the MIC. The MICs were initially obtained in micrograms per millilitre and then recalculated on a molar basis to facilitate comparison.

Secondly, the minimum fungicidal concentrations (MFCs) were determined. The membrane filter discs, which showed no visible growths from the results of the MIC experiments, were removed and placed in toxicant-free Czapek dox containing inactivators (3% Tween 80) and incubated for a further 5 days. The lowest concentration of the toxicant that killed the organisms (shown by lack of viable subculture) was taken as the MFC.

Thirdly, the zone of inhibition (ZOI) of the compounds against the fungi was measured by the disc plate (5 mm diameter) method at 400, 200, 40, 20, 4, 2, 0.4 and 0.2 ppm concentrations of the toxicants. The discs were soaked with organisms in Petri dishes and stored in an incubator at 30 ± 1 °C. The inhibition zone (mm) around each disc was measured after 36 h. Replicated measurements agreed within 5%.

All antifungal activity data were subjected to PROMCALC software[51–53] for MCDM analysis by the PROMETHEE and GAIA procedures, detailed mathematical treatment and application tutorials of which are available in the literature.[39–41] Essentially, PROMETHEE provides a choice of six preference functions, which supply a mathematical basis for selecting one object in preference to another. If the difference between the values of a variable c (say MIC in this study) for different objects a and b (e.g. organotin compounds) is $d = c(a) - c(b)$, then a preference function, $P(a, b)$ translates the difference into a degree of preference ranging from zero to unity, such that when $P(a, b) = 1$ the object a is strongly preferred to object b and when $P(a, b) = 0$ the object a is not preferred to b. Regardless of the function selected (for each variable), all entries in the data matrix were compared pairwise in all possible combinations by subtraction, leading to a difference d for each comparison. It was also necessary to specify whether higher or lower variable values are preferred by choosing to 'minimize' or to 'maximize' each variable.

In this work, unless indicated otherwise, each fungus was considered as a variable. The MIC or MFC of the organotin compound against the fungus was 'minimized', since the lower the value of the MIC and MFC of a compound, then the higher is its potency as an antifungal agent. The data for the ZOI, on the other hand, were 'maximized', since the higher the ZOI of a compound, then the higher is its fungitoxicity. The preference function P selected for the 'minimized' variables was:

$$P = 1 \qquad \text{for } d < 0 \tag{1}$$

$$P = d/z \qquad \text{for } 0 \leq d < z \tag{2}$$

$$P = 0 \qquad \text{for } d > z \tag{3}$$

where d is the difference for each pairwise comparison and z is the threshold, which was set at -1. The negative signs indicate that in a comparison it is the smaller of the two residual values that is preferred in accordance with the 'minimize' condition. A preference function selected for each variable was used to allocate a preference value for each difference, resulting in a preference table. The sum of preference values for each object gives a value called a 'global preference index' π, which indicates the preference of one object over another.

To refine the preference selection process, positive and negative outranking flows ϕ^+ and ϕ^- respectively were computed. The former expresses how each object outranks all others and the latter indicates how each object is outranked by all the other objects. By applying a set of simple rules,[39–41] a partial ranking order was obtained by PROMETHEE I. This order highlighted one of the following three possible outcomes. (A) one object is preferred to another; (B) there is no difference between the two objects; (C) the objects cannot be compared. The result of PROMETHEE I partial ranking is presented in the form of a flow chart (e.g. see Fig. 1). As a rule, comparable objects are joined by one or more arrows, incomparable objects are unconnected by arrows and comparable objects to the left of any object are preferred to that object. To establish a complete ranking order, the net out ranking flow, $\phi = \phi^+ - \phi^-$, was calculated. This procedure is known as PROMETHEE II. The main advantage of PROMETHEE is that it is a non-parametric multivariate ranking procedure and, therefore, it is applicable to a matrix consisting of just a few samples.

GAIA, on the other hand, displays PROMETHEE results visually and facilitates the interpretation of the significance of the different variables. The GAIA principal component biplots obtained in this study were interpreted according to the guidelines reported earlier.[39–41]

RESULTS AND DISCUSSION

Tables 1–3 present the antifungal activities of the organotin carboxylates, along with those of the organotin chlorides and the free acids from which the organotin carboxylates were prepared. The ZOI studies were carried out at different concentrations of the organotin compounds in the range 0.2–400 ppm. However, only results at 0.4, 40 and 400 ppm are presented in Table 3, since the trends in the results obtained at other concentrations are broadly similar to those listed. It is evident from Tables 1–3 that the starting materials (viz. Ph_3SnCl, Ph_2SnCl_2, 2-hydroxybenzoic and 2-acetoxybenzoic acids) and the organotin benzoates show significant fungicidal activities against the fungi used. Tables 1–3 reveal the following details:

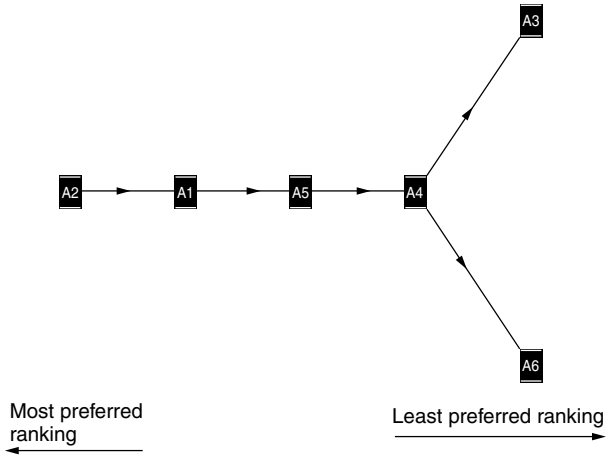

Figure 1. PROMETHEE I ranking for the MIC for the first six compounds in Table 1 (A1: Ph_3SnSA; A2: Ph_3SnASA; A3: Ph_2SnSA_2; A4: Ph_2SnASA_2; A5: Ph_3SnCl; A6: Ph_2SnCl_2).

1. The organotin carboxylates/chlorides and organic acids exhibited varying degrees of inhibition on the growth of the fungi tested.
2. The benzoates are effective inhibitors of the fungi, since their observed MICs, MFCs and ZOIs are equal to or better than those observed for Ph_3SnOAc and Ph_3SnCl, both of which are known commercial fungicides.[18,19,54] Interestingly, triphenyltin acetate is itself more active *in vitro* against *T. rubrum*[5] than common fungicides like clotrimazole, tioconazole and chlormidazole. Thus, *in vitro* activities of the triphenyltin compounds reported in the present study are encouraging in terms of their potential as antifungal agents.
3. Apart from compound A3, the compounds do not generally exhibit appreciable differential cytotoxicity, although compound A2 appears to be somewhat more active towards *A. niger* and *P. citrinum*, and compound A4 is less active towards *A. flavus* and *T. violcaeum* than they are towards the other fungi. This suggests that they can be used as broad-spectrum rather than selective antifungal agents.
4. The results of the ZOI studies at different concentrations of the organotin compounds (i.e. 0.2, 0.4, 2, 4, 20, 40, 400 ppm) showed that inhibition by the organotin compounds was

Table 1. MIC (mmol l^{-1}) of the compounds against fungi species

Compound	*A. flavus*	*A. niger*	*P. citrinum*	*T. violcaeum*	*C. albicans*	*T. rubrum*
Ph_3SnSA	0.10	0.10	0.10	0.10	0.10	0.10
Ph_3SnASA	0.10	0.04	0.04	0.10	0.10	0.10
Ph_2SnSA_2	0.40	0.10	0.20	0.40	0.17	0.30
Ph_2SnASA_2	0.40	0.10	0.10	0.40	0.13	0.10
Ph_3SnOAc	ND	0.20	0.30	ND	0.20	0.30
$Ph_2Sn(OAc)_2$	ND	0.30	0.70	ND	0.30	0.70
Ph_3SnCl	0.10	0.10	0.10	0.10	ND	ND
Ph_2SnCl_2	0.70	0.70	0.80	0.60	ND	ND
Bu_2SnSA_2[a]	4.90	5.0	5.20	ND	ND	ND
Bu_2SnASA_2[a]	0.90	0.90	0.90	ND	ND	ND
$Bu_2Sn(OAc)_2$[a]	7.10	7.50	7.40	ND	ND	ND
Bu_2SnCl_2[a]	16.50	8.20	16.50	ND	ND	ND
Bu_2SnPht_2[a]	12.60	12.60	12.60	ND	ND	ND
Phthalic acid[a]	15.00	15.05	15.05	ND	ND	ND
Acetic acid[a]	16.40	16.40	16.40	ND	ND	ND
2-Hydroxybenzoic acid[a]	18.0	18.10	18.10	18.1	ND	ND
2-Acetoxybenzoic acid[a]	13.9	15.1	15.0	15.2	ND	ND

ND: not determined.
[a] Taken from Ref. 27.

Table 2. MFC (mmol l^{-1}) of the compounds against fungi species

Compound	*A. flavus*	*A. niger*	*P. citrinum*	*T. violcaeum*
Ph_3SnSA	0.50	0.10	0.10	0.10
Ph_3SnASA	0.10	0.05	0.10	0.10
Ph_2SnSA_2	0.90	0.90	0.90	0.90
Ph_2SnASA_2	0.90	0.90	0.90	0.90
Ph_3SnCl	>1.3	0.40	0.20	0.10
Ph_2SnCl_2	>1.5	>1.5	>1.5	0.90

dose dependent; the higher the dose, the better the fungitoxicity.

5. In line with observations from similar studies,[10–13,27] the triphenyltin compounds are usually more effective against the fungi than their diphenyltin analogues. This is probably because the biologically active moiety, Ph_3Sn^+, separates faster than Ph_2Sn^{2+}.

Triphenyltin acetoxybenzoate has already been shown to have a structure[28,29], in which the benzoate group is asymmetrically bidentate to give a compound with a distorted tetrahedral tin centre. Although the structure of a compound can only be confirmed by crystallographic studies, it is noteworthy that the $\Delta\nu(COO^-)$ (where $\Delta\nu(COO^-) = \nu_{as}(COO^-) - \nu_s(COO^-)$ of *ca* 210 cm^{-1} generally observed for the benzoates investigated in this study has been associated[10–13] with the presence of a bidentate ligand in other organotin carboxylates. Thus, it appears that the differences in the antifungal activities of this suite of compounds are unlikely to be due to differences in the coordination number about the tin atoms.

PROMETHEE and GAIA analysis

Any meaningful ranking analysis and recognition of patterns in the data presented in Tables 1–3 must consider all objects and variables simultaneously and systematically. Therefore, the data were subjected to the PROMETHEE and GAIA MCDM procedures. Only some of the compounds in Tables 1–3 were tested against all of the fungi. Yet, the analysis produced meaningful outcomes because PROMETHEE is a non-parametric method. The PROMETHEE and GAIA results obtained from the study are presented and discussed below.

For the MFC data in Table 2 analysed by PROMETHEE and GAIA, the PROMETHEE I (partial) ranking presented in Fig. 1 shows that the best-performing compound is Ph_3SnASA, followed by Ph_3SnSA. In turn, Ph_3SnSA is better performing than Ph_2SnASA_2 and Ph_3SnCl. Ph_3SnCl is better than Ph_2SnASA_2, Ph_2SnSA_2 and Ph_2SnCl_2. Ph_2SnASA_2 is better than Ph_2SnSA_2 and Ph_2SnCl_2, but Ph_2SnSA_2 and Ph_2SnCl_2 cannot be compared (i.e. these two compounds have comparable antifungal efficiencies, but on different fungi). However, the PROMETHEE II complete ranking (Table 4) of the compounds (from the most preferred to least preferred) is:

$$Ph_3SnASA \geq Ph_3SnSA \geq Ph_2SnASA_2 > Ph_3SnCl > Ph_2SnSA_2 > Ph_2SnCl_2$$

This order shows that triphenyltin compounds with a particular anionic moiety are more active than their diphenyl

Table 3. Diameter of inhibition zone (mm) at $a = 400$, $b = 40$ and $c = 0.4$ ppm concentrations of the organotin compounds

	A. flavus			*A. niger*			*P. citrinum*			*T. violaceum*		
Compound	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Ph_3SnSA	26	21	13	29	25	18	36	31	20	26	19	25
Ph_3SnASA	27	22	17	30	27	19	41	35	8	42	37	11
Ph_2SnSA_2	14	14	10	24	19	11	25	22	12	12	11	9
Ph_2SnASA_2	21	15	10	24	21	11	25	24	12	12	11	9
Ph_3SnCl	25	20	12	26	21	13	28	23	14	20	15	11
Ph_2SnCl_2	14	15	9	19	17	9	22	15	10	15	11	12

Table 4. PROMETHEE II complete ranking results for the compounds according to their fungicidal activities

	Fungicidal activity					
	MIC		MFC		ZOI	
Ranking[a]	Compound	ϕ	Compound	ϕ	Compound	ϕ
1	Ph_3SnASA	0.05	Ph_3SnASA	0.46	Ph_3SnASA	0.34
2	Ph_3SnSA	0.04	Ph_3SnSA	0.37	Ph_3SnSA	0.17
3	Ph_2SnASA_2	0.033	Ph_2SnASA_2	−0.03	Ph_3SnCl	−0.03
4	Ph_3SnOAc	−0.002	Ph_3SnCl	−0.14	Ph_2SnASA_2	−0.11
5	Ph_2SnSA_2	−0.015	Ph_2SnSA_2	−0.30	Ph_2SnSA_2	−0.19
6	$Ph_2Sn(OAc)_2$	−0.112	Ph_2SnCl_2	−0.36	Ph_2SnCl_2	−0.24

[a] From most preferred to least preferred.

counterparts. Likewise, the acetoxybenzoates are more active than the hydroxybenzoates, which are in turn more active than the chlorides.

When the MIC data for the first six compounds in Table 1 were considered (in order to rank the fungitoxicities of the compounds against *A. niger*, *P. citrinum*, *C. albicans* and *T. rubrum*), the best-performing toxicant was Ph_3SnASA and the worst performing was $Ph_2Sn(OAc)_2$; the PROMETHEE II complete ranking (Table 4) of their fungicidal activities was

$$Ph_3SnASA > Ph_3SnSA > Ph_2SnASA_2 > Ph_2SnSA_2 > Ph_3SnOAc > Ph_2Sn(OAc)_2$$

(Since the MICs for the interactions of Ph_3SnOAc and $Ph_2Sn(OAc)$ against *A. flavus* and *T. violcaeum* were not determined under the experimental conditions used for the other compounds, these fungi were not included in this multivariate analysis.) The results suggest that the benzoates have better antifungal activities than their acetate counterparts. On the other hand, GAIA showed that the first two principal components (PCs) accounted for almost 100% of the variance (Fig. 2). The loadings vectors for *A. niger* and *P. citrinum* almost overlap. (A loading vector denotes a projected vector that describes the ability of a particular variable to differentiate the objects; the longer the vector, the more variance it contains and the more important it is in differentiating the objects.) Similarly, the loadings vectors for *C. albicans* and *T. rubrum* almost overlap but are roughly orthogonal to those for *A. niger* and *P. citrinum*. The rules[39–41] employed for the interpretation of GAIA plots stipulates that if the vectors of two variables overlap then the variables are correlated, and if they are orthogonal then the variables are independent of each other. Therefore, the susceptibilities of *A. niger* and *P. citrinum* to the toxicants are similar. In the same way, the susceptibilities of *C. albicans* and *T. rubrum* to the toxicants are similar but are independent of the susceptibilities of *A. niger* and *P. citrinum*.

If the fungi are made the objects and the organotin compounds the variables (in order to rank the susceptibilities of the fungi to the compounds), then analysis of their MIC data by PROMCALC showed the PROMETHEE II complete ranking (from the most to least preferred) and outranking net flow (in parentheses) to be fungi:

$$A.\ niger\ (0.25) > C.\ albicans\ (0.04) > P.\ citrinum\ (-0.02) > T.\ rubrum\ (-0.27)$$

GAIA showed that approximately 99.5% of the variance is explained by the first two PCs (Fig. 3). The loadings vectors for the acetates and for the benzoates correlated with one another, but these sets of vectors (with the exception of that for Ph_2SnSA_2) are almost at 90° to each other. Thus, the activities of the acetates and benzoates are mostly independent of one another. In the context of arguments in the literature[27,30,31]

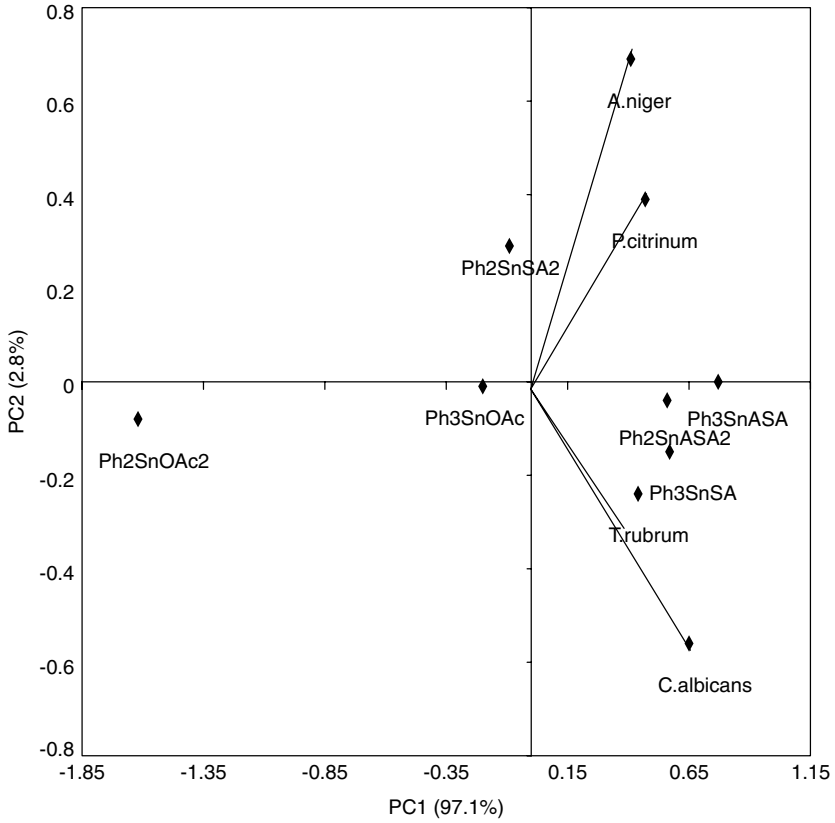

Figure 2. GAIA biplot of the first six compounds in Table 1 against MIC results for *C. albicans*, *A. niger*, *T. rubrum* and *P. citrinum*.

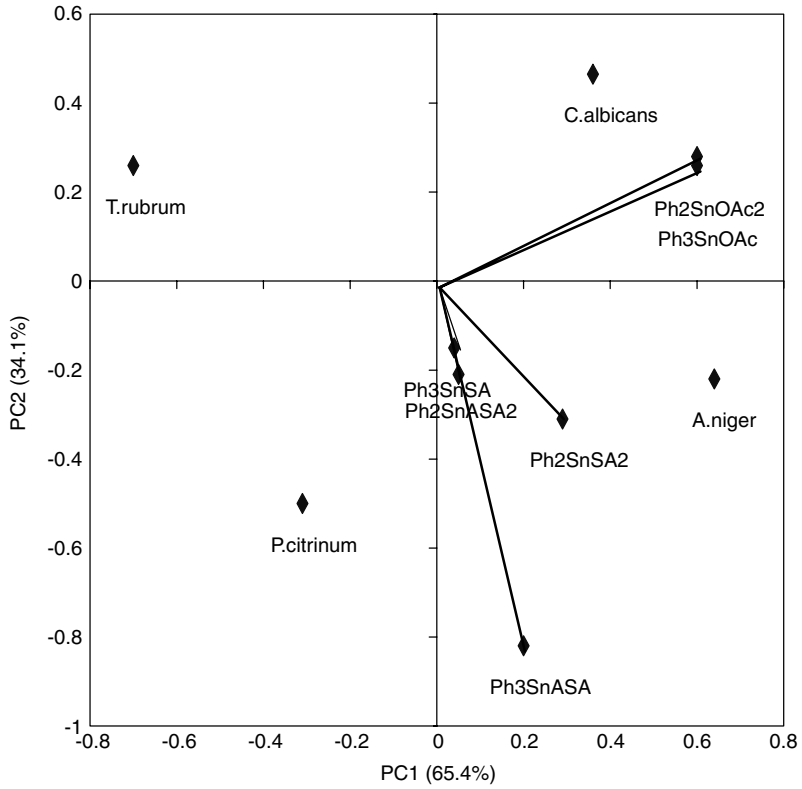

Figure 3. GAIA biplot of the first six compounds in Table 1 against MFC results for *C. albicans*, *A. niger*, *T. rubrum* and *P. citrinum*.

regarding the role of the anionic group in the fungicidal activity of an organotin compound, it appears that the anionic groups in these compounds play some part in their activities. If only the benzoates are examined against the six fungi then their order of susceptibility and outranking net flow (in parentheses) is

A. niger (0.34) > *P. citrinum* (0.27) > *C. albicans* (0.07)
> *T. rubrum* (−0.05) > *A. flavus* (−0.31)
≈ *T. violaceum* (−0.31)

All compounds studied in this work, as well as those reported in Ref. 27, have been tested against *A. niger* and *P. citrinum* under similar conditions. Therefore, using the MIC data in Table 1, a matrix consisting of the two fungi as variables and the compounds as objects was submitted to PROMETHEE in order to rank the antifungal activity of all of the compounds. The resulting PROMETHEE II ranking showed the order of antifungal efficacy of the compounds to be

$Ph_3SnASA > Ph_2SnASA_2 > Ph_3SnSA = Ph_3SnCl$
$> Ph_2SnSA_2 > Ph_3SnOAc > Ph_2Sn(OAc)_2 > Ph_2SnCl_2$
$> Bu_2SnASA_2 > Bu_2SnSA_2 > Bu_2Sn(OAc)_2 > Bu_2SnCl_2$
$> Bu_2SnPth_2 >$ 2-acetoxybenzoic acid = phthalic acid
> acetic acid > 2-hydroxybenzoic acid

This order confirmed an earlier suggestion[27,30,31] that phenyl-containing organotin compounds are usually more active than their butyl analogues. However, it is noteworthy that Gielen and co-workers[6–9] found butyl-containing compounds to be more active antitumour agents than the corresponding phenyl compounds. Several interesting observations are also evident from the PROMETHEE and GAIA results. Firstly, although 2-hydroxybenzoic acid and its derivatives have long been known[36,37] to show antifungal activity, relative to the organotin compounds used in the current work, the free acids showed weak antifungal activities against the fungi tested. This agreed with well-established observations (e.g. see Refs 10–13) that the activities of many biologically active ligands are enhanced when they form complexes with metal ion complexes. Secondly, the GAIA PC biplots (Fig. 4) showed that almost 100% of the variance were accounted for by the first two PCs and that there are three main clusters along PC1. One of the clusters consisted of objects with negative PC1

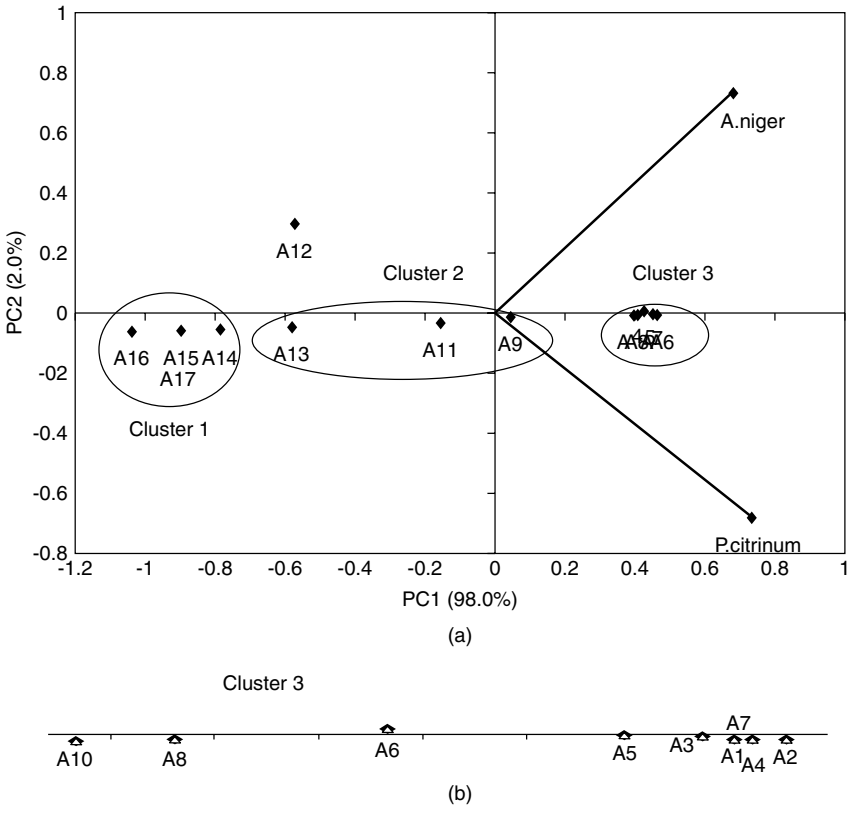

Figure 4. (a) GAIA biplot for the MICs of the compounds in Table 1 against *A. niger* and *P. citrinum* (A1: Ph_3SnSA; A2: Ph_3SnASA; A3: Ph_2SnSA_2; A4: Ph_2SnASA_2; A5: Ph_3SnOAc; A6: Ph_2SnOAc_2; A7: Ph_3SnCl; A8: Ph_2SnCl_2; A9: Bu_2SnCl_2; A9: Bu_2SnSA_2; A10: Bu_2SnASA_2; A11: Bu_2SnOAc_2; A12: Bu_2SnCl_2; A13: Bu_2SnPth_2; A14: phthalic acid; A15: acetic acid; A16: 2-hydroxybenzoic acid; A17: 2-acetoxybenzoic acid). (b) A detailed representation of cluster 3, which consisted of A10, A1, A2, A3, A4, A5, A6, A7 and A8.

scores (SA, acetic, phthalic and ASA), another contained the butyl compounds (Bu_2SnPht, Bu_2SnOAc_2 and Bu_2SnSA_2) and the third, with positive PC1 scores, consisted of the best-performing organotin compounds (Ph_3SnASA, Ph_3SnSA, Ph_2SnASA_2, Ph_3SnCl, Ph_2SnSA_2, Ph_3SnOAc, $Ph_2Sn(OAc)_2$, Ph_2SnCl_2 and Bu_2SnASA_2). Thirdly, the only dibutyl in the last group is the Bu_2SnASA_2, as Bu_2SnCl_2 appears to be an outlier. These results corroborated the observation that phenyl-containing organotin compounds are better-performing antifugal agents against these fungi than their butyl counterparts.[27,30,31] Fourthly, the loadings vectors for *A. niger* and *P. citrinum* are orthogonal, suggesting that the susceptibility of the fungi to these compounds is independent of each other.

The PROMETHEE II result obtained when the MFC data in Table 2 were used showed that the order of antifungal efficiency of the compounds against *A. flavus*, *A. niger*, *P. citrinum*, and *T. violaceum* is (Table 4)

$$Ph_3SnASA > Ph_3SnSA > Ph_2SnASA_2 > Ph_3SnCl > Ph_2SnSA_2 > Ph_2SnCl_2$$

The compounds were separated on the PC1 of the GAIA biplot (Fig. 5) according to their cationic moieties. Thus, compounds with the Ph_3Sn moiety have positive PC1 scores and those with the Ph_2Sn moiety have negative PC1 scores. This suggests that the cationic species are important contributors to the antifungal activities of the compounds. The loadings vectors for *A. niger* and *P. citrinum* almost overlap, showing that the fungi have similar susceptibilities towards the same compounds.

When the ZOI (at 400 ppm) data in Table 3 were analysed by PROMETHEE and GAIA, the PROMTHEE II results indicated that the most preferred compound is Ph_3SnASA and the least preferred is Ph_2SnCl_2. The complete ranking (Table 4) was

$$Ph_3SnASA > Ph_3SnSA > Ph_3SnCl > Ph_2SnASA_2 > Ph_2SnSA_2 > Ph_2SnCl_2$$

This order not only confirmed that compounds with the Ph_3Sn moiety[10–13,27] are more active than their diphenyl analogues, but it also suggests that the benzoates are more active *in vitro* than the chlorides, although the latter are well-known fungicides.[54] The first two GAIA PCs account for 97% of the variance, and the objects were arranged in two distinct lines: one consisting of the compounds with the Ph_2Sn moiety and the other consisting of compounds with the Ph_3Sn

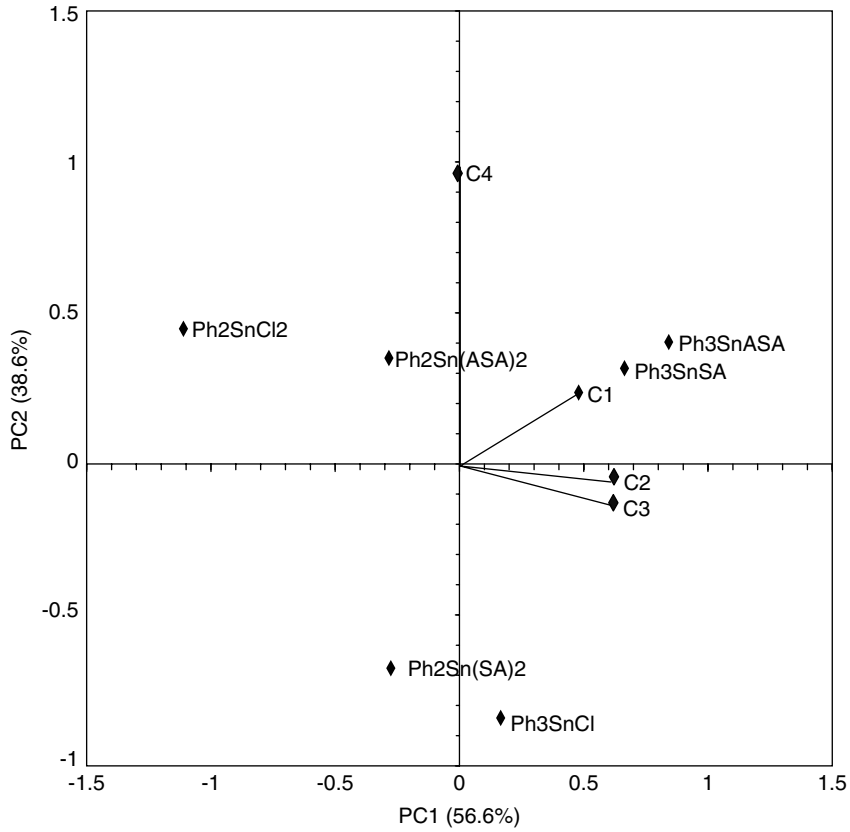

Figure 5. GAIA biplot for the MFCs of the compounds in Table 2 against *A. flavus* (C1), *A. niger* (C2), *P. citrinum* (C3) and *T. violaceum* (C4).

moiety (Fig. 6). (Since the data for 40 ppm and 0.4 ppm of the toxicants followed a similar trend to that for 400 ppm, only the data for the 400 ppm were used for this multivariate analysis.)

The organotin carboxylates and their parent organotin chlorides showed varying degrees of inhibitory effects on the fungi, and this has implications on the mechanism of their fungitoxicities. Although our present results do not allow us to propose unequivocal modes of action for the antifungal activities of these organotin compounds against the fungi investigated, it was suggested previously[30,31] that differences in the fungicidal activities of a series of closely related compounds depends on their varied abilities to produce the organotin cations thought to be the sole species responsible for their biological activities. If this were the case, then, the only role played by the anionic groups in the fungicidal activities of organotin compounds is to assist the delivery of the active cationic moiety to the target. The PROMETHEE results generally indicate that Ph_3SnSA and Ph_3SnASA have better fungicidal activities than Ph_3SnOAc and Ph_3SnCl, even though they are all expected to give the same triphenyltin cation, Ph_3Sn^+. This might imply that, in addition to the cationic species, the anionic groups also play a role in the fungitoxicities of these compounds. Since 2-hydroxybenzoic acid and 2-acetoxybenzoic acid are themselves fungitoxic,[27,36–38] some synergistic or additive effects of the cationic moieties and anionic groups may be operating in the present systems.

However, the possibility that the compounds manifest their antifungal activities by means of nucleophilic reactions against the target, as suggested in a previous study,[22,23,55] or by inhibition of oxidative phosphorylation in the fungi[35] cannot be excluded. If the compounds exhibit their fungicidal properties by nucleophilic reactions against the target fungi, then the electron-donating and -withdrawing ability of anionic groups would play a role in determining the antifungal efficacy of the compounds. Thus, it would be reasonable to expect that weakly ionizing groups such as the benzoates would possess the appropriate electronic environments around tin to facilitate the formation of weak bonds between the metal and donor sites of the fungi. If, on the other hand, the compounds exert their activities by interfering with the function of mitochondria, then the biologically active anionic group would promote anion–hydroxide exchange reactions across the inner membrane of the mitochondria.[56]

To summarize, our results clearly demonstrate the inhibitory effects of the organotin(IV) benzoates against all of the fungi tested and the ability of the MCDM methods, PROMETHEE and GAIA, to produce ranking information and pattern recognition from antifungal activity data. Although *in vitro* potency has not always been translated into *in vivo* activities, the fact that the *in vitro* activities of the organotin(IV) benzoates reported in this study are always ranked higher than those for Ph_3SnOAc and Ph_3SnCl suggests that the potential of the benzoates as fungicides is encouraging. Regardless of the mode of action adopted by

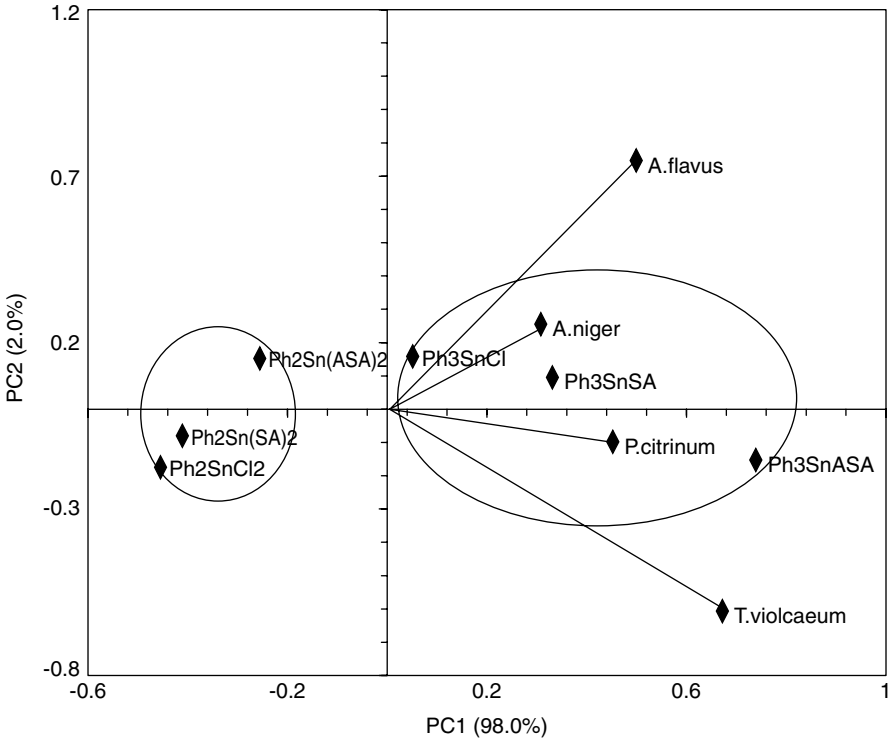

Figure 6. GAIA biplot of the ZOI results in Table 4.

these organotin compounds, it appears that the biologically active anionic ligand plays some role in their fungicidal activity. Organotin compounds containing such anionic moieties may well hold the key to the development of future metal-based antimicrobial agents, and the use of multivariate data analysis techniques could facilitate the screening and ranking of potentially useful drugs. Further investigations of the biological activities on related compounds are in progress.

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