Structure-Affected Bioactivity of Triorganotin Compounds

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The biocidal activity of 8 triorganotins R_3SnX (R = butyl, phenyl, benzyl; X = oxide, chloride, acetate, naphthenate, *N*,*N*-diethyldithiocarbamate) on brown rot fungi, moulds mixture, benthic organisms, plankton alga *Scenedesmus quadricauda*, and crop plant has been investigated. The results suggest dependence between lipophilicity of bounded R groups and intensity of unfavourable effect. The most effective triorganotins were compounds with butyl group in n-alkyl chain, the least active were the tribenzyltins. In general, independently on the investigated biological object, the strongest biocidal activity was shown by bis(tributyltin) oxide whereas the lowest inhibitory efficiency was exhibited by tributyltin naphthenate. The effect of X group on biocidal activity was not significantly manifested.

Organotin compounds have a wide range of industrial applications. It has been estimated that some 35 000 t are currently being produced per year and from that figure 28 000 t are stabilizers, 2 000 t are catalysts, and 5 000 t are biocides [1]. Nowadays, the industrial applications of tin chemicals are so widespread and in many cases specialized that it is difficult to grasp the full potential of these compounds [2]. Tin is capable of forming stannous (Sn^{2+}) and stannic (Sn^{4+}) compounds as well as organometallic compounds, termed "organotins", in which from 1 to 4 carbon atoms may be bonded directly to the tin atom. The number and nature of the organic groups attached to the tin atom dramatically affect the properties of the compound (particularly its bioactivity), and this has resulted in organotins finding application in a wide range of areas, accompanied by considerable growth in their consumption over the last forty years [1].

For the purpose of biological effects, bis(tributyltin) oxide has a very wide application [1, 3]. For this aim it is possible to use also other obvious trialkyltin compounds as acetates, benzoates, and sulfonates [4] but also alkanesulfonates [5], alkanedisulfonates [6], and dithiocarbamates [7]. The study of the effects of organotin compounds on the synthesis of nucleic acids and ATP in the growth process of *Bacillus subtilis* showed that these compounds inhibited energy transformation of the cell and thereby induced the prolongation of DNA and RNA synthesis [8]. The organotin compounds were found to inhibit oxidative phosphorylation in the eukaryotic cells by damage of the mitochondria membranes [9] and tributyltin chloride is also known to inhibit ATP formation and coupled electron transport in isolated chloroplasts [10].

Triorganotin compounds (R_3SnX) are probably the most widely studied and most biologically active tin compounds. The R group strongly influences the nature of the biocidal properties, while the X group mainly affects their volatility and solubility [11]. The two most important classes of triorganotins are the tributyl and triphenyl derivatives, since these combine high biological activity [12] with low mammalian toxicity [11, 13]. The toxicity of organotins is influenced by the length of the side chain [14]. Organotin compounds having methyl groups are considered to be the least toxic and toxicity is increasing up to the butyl group (maximum efficiency) [13]. Triorganotins are very often used as biocides for water treatment and antifouling paints; preservatives for wood, textiles, paper, and paints; insecticides; and household and industrial germicides [1, 4]. The biological activity of organotin compounds shows an increase with increasing lipophilicity of the alkyl substituents, however, the increase in n-alkyl chain length to octyl resp. dodecyl causes a sharp decrease in biological activity. This so-called "cut-off" effect, *i.e.* the quasi-parabolic course of the dependence of biological activity on the lipophilicity of the alkyl substituent has been confirmed for many homologous series of organic compounds [15, 16]. It was reported that butyl and phenyl substituents having comparable lipophilicity have approximately the same biocidal activity [17].

In the presented paper we would like to turn attention to relations between structure and biocidal activity of triorganotin compounds. The biological efficiency of tested compounds was examined on freshwater benthos and plankton algae, wood-destroying fungi and moulds, and agricultural crop plant.

EXPERIMENTAL

In the tests the following organotin compounds were investigated: some commercially available liquid (bis(tributyltin) oxide (*I*) and tributyltin naphthenate (*II*); Schering AG, Germany) and solid products (triphenyltin chloride – Brestanol (*III*) and triphenyltin acetate – Brestan (*IV*); Bayer AG, Germany) were used as standards as well as tribenzyltin chloride (*V*), tributyltin *N*,*N*-diethyldithiocarbamate (*VI*), triphenyltin *N*,*N*-diethyldithiocarbamate (*VII*), and tribenzyltin *N*,*N*-diethyldithiocarbamate (*VIII*). Tributyltin *N*,*N*-dialkyldithiocarbamates of the type $Bu_3Sn—SSC—NR_2$ were prepared by the reaction of *V* with alkaline salts of *N*,*N*-dialkyldithiocarbamic acids or by condensation of *I* with dialkylamine and carbon disulfide [18, 19]. The analytical evaluation of prepared compounds was carried out by the GLC method elaborated for various *N*,*N*-dialkyldithiocarbamates [20].

Separate stock solutions (V(ethanol) : $V(H_2O)$ = 0.1) were made for each final concentration of organotin required. The final ethanol concentration in experimental solutions (V(ethanol) : $V(H_2O)$ = 0.0033) did not affect vitality and activity of observed subjects. All of the triorganotins studied were soluble in final ethanol solution at the concentrations specified for introduced tests.

The testing of prepared compounds as biocides was carried out by the methods introduced in References [2, 21—24] and is more detailedly described below.

The testing of standard and prepared compounds as fungicides for wood preservation against rot (brown rot fungi *Coniophora puteana* (Schum. ex Fr.) P. Karst. and *Serpula lacrymans* (Wulf. ex Fr.) Schoet., and mould suspension prepared as a mixture of five strains, *Aspergillus amstelodami, Aspergillus niger* (Van Theghem), *Paecilomyces variotii* (Bainier), *Penicillium cyclopium* (Westling) and *Penicillium brevicompactum* (Dierolax)) was in more details described by the method of "poisoned soil" in 3 % malt agar at 20 °C according to the gradual content (or mass fraction) of the organotin compounds in the range 0.01—0.0001 % [18, 19, 21, 23]. The tests lasted 17 d.

For alga tests freshwater alga *Scenedesmus quadricauda* (Turp.) Bréb., strain Greifswald 15 was used. Algae grew statically in a calcium-depleted modified Knop solution (pH = 7.2) for 12 d under constant temperature ((25 ± 1) °C) and permanent light conditions (24 μmol s^{-1} m^{-2} PAR (PAR = photosynthetically active radiation)). Chlorophyll *a* content was determined by using spectrophotometric method [2]. The photosynthetic oxygen evolution was measured with an oxygen electrode connected with a computer after 12 d cultivation [24]. For these measurements the same cultures as for chlorophyll *a* content determination were used. During experiments triorganotins were tested in 10 various concentrations falling into the interval 0.01—200.0 μg dm^{-3}. For all determined parameters the EC_{50} values (50 % inhibition) and their 95 % confidence intervals (CI) were estimated by probit analysis [25] and expressed chronic effect of tested compounds.

As freshwater benthic organisms *Tubifex tubifex* worms and *Chironomus plumosus* larvae were used. The mortality of animals in 10 various concentrations of tested organotins (from the range 0.01—10.0 μg dm^{-3}) was observed and compared with control after 96 h treatment. Results were expressed as LC_{50} values and their 95 % confidence intervals (CI) and were estimated using probit analysis and expressed acute toxic effect of tested compounds [12].

In agricultural crop plant tests seeds and seedlings of *Sinapis alba* were used as the test subject. The seeds were placed in Petri dishes (50 seeds per dish) with a 14-cm diameter and filter paper on the bottom. The organotins were used in the concentration range 1.0—500.0 mg dm^{-3}. The exposure lasted (in the dark at the temperature (25 ± 1) °C) 72 h and then germination (%) and root length (cm) were evaluated. The IC_{50} values for root growth inhibition by using probit analysis and 95 % confidence intervals (CI) were calculated by moving average methods [26].

RESULTS AND DISCUSSION

By using modification of the "poisoned soil" method the slow down or completely stopped growth of mycelium from wood-destroying fungi *Coniophora puteana* (Schum. ex Fr.) P. Karst., *Serpula lacrymans* (Wulf. ex Fr.) Schoet. and mould suspension was determined and introduced in Table 1. For the majority of tested triorganotins *S. lacrymans* was the most sensitive and the moulds the least sensitive fungi. The most effective triorganotins against fungi were those containing butyl group in n-alkyl chain, such as *I* and *VI*. The most lipophilic compound from the investigated set, *i.e.* compound *I* with two tin atoms and six butyl chains in its molecule, exhibited the highest biological activity. Comparable high inhibitory activity was shown also by compound *VI* which would be suitable for the extension of the present assortment of commercially available products [18]. The fungicidal activity of triphenyltins (*III, VII*) was lower than that of tributyltin compounds (*VI*), but higher than that of tribenzyltins (*V, VIII*) indicating that the biological activity shows a decrease with increasing lipophilicity of R substituent and simultaneous increase of the molecular volume of R_3Sn group. It is probable that the R_3Sn group with smaller molecular volume can better approach the site of inhibitory action than that with higher molecular volume. Consequently, tribenzyltins *V* and *VIII* were found to exhibit the lowest biological activity. The obtained results indicate that biocidal activity of triorganotins is closely connected with the bounded R group and

Table 1. The Growth Inhibition of Brown Rot Fungi *Coniophora puteana* and *Serpula lacrymans* and Mould Suspension Expressed as Mean Daily Values after 17 d Cultivation on "Poisoned Soil" with Triorganotins

Compound	w_i/%	*I*/%		TI/%
		C. puteana	*S. lacrymans*	Moulds
I	0.01	100	100	100
	0.001	93	100	100
	0.0001	63	79	8
II	0.01	97	100	92
	0.001	92	100	83
	0.0001	49	73	6
III	0.01	84	93	47
	0.001	72	87	16
	0.0001	51	59	7
IV	0.01	91	100	100
	0.001	83	96	22
	0.0001	57	35	8
V	0.01	48	74	15
	0.001	14	15	5
	0.0001	3	0	3
VI	0.01	100	100	100
	0.001	90	100	100
	0.0001	75	93	25
VII	0.01	80	100	99
	0.001	73	90	37
	0.0001	55	44	6
VIII	0.01	57	62	5
	0.001	40	23	4
	0.0001	8	5	3

I – growth inhibition (%); TI – total inhibition index calculated by the equation TI = 100 − MS, where MS is the mould surface (in %) on the vital soil with the addition of the tested compounds.

fully confirm findings of other authors [11, 13, 14, 17].

The inhibition of photosynthetic oxygen evolution and chlorophyll *a* production in freshwater alga *S. quadricauda* reflected the long-time effects of the studied compounds on this model alga (Table 2). The strongest biological activity on both observed parameters was shown by the most lipophilic compound *I*. Decrease in chlorophyll *a* production was also connected with the changes in the appearance of the alga suspension. Some cultures treated with triorganotins (*VIII*, *V*, *VI*, *I*) were pallid and exhibited a colour change from green to yellow-green. These cultures indicated under microscopic observation deformations and disintegration of coenobia (four cells connected in one unit). The higher EC_{50} values were calculated for chlorophyll *a* content (EC_{50}^{Chla}) than for oxygen evolution (EC_{50}^{Ox}). The inhibition of photosynthetic electron transport (PET) by the studied compounds resulted in inhibition of oxygen evolution whereas inhibition of alga chlorophyll production was connected probably with changes in its biosynthesis. Consequently

Table 2. EC_{50} Values and their 95 % Confidence Intervals (CI) for *Scenedesmus quadricauda* Chlorophyll *a* Content (Chla) and Oxygen Evolution (Ox) after 12 d Cultivation in Media Supplemented with Triorganotins

Compound	EC_{50}^{Chla}/(μmol dm^{-3}) (CI/(μmol dm^{-3}))	EC_{50}^{Ox}/(μmol dm^{-3}) (CI/(μmol dm^{-3}))
I	0.21 (0.19—0.24)	0.32 (0.28—0.34)
II	158.50 (149.62—164.06)	18.62 (18.42—19.05)
III	2.98 (2.87—3.10)	3.43 (3.29—3.45)
IV	1.00 (0.92—1.10)	4.51 (4.32—4.73)
V	5.50 (5.12—5.60)	1.00 (0.91—1.05)
VI	0.87 (0.83—0.90)	0.93 (0.89—1.10)
VII	8.71 (8.58—9.12)	0.50 (0.48—0.52)
VIII	57.54 (56.70—61.10)	0.57 (0.54—0.58)

Table 3. LC_{50} Values for *Tubifex tubifex* and *Chironomus plumosus* and IC_{50} Values for *Sinapis alba* Root Growth Inhibition and their 95 % Confidence Intervals (CI) for Tested Triorganotins

Compound	$LC_{50}/(\mu g\ dm^{-3})$ (CI/($\mu g\ dm^{-3}$))[a]		$IC_{50}/(mg\ dm^{-3})$ (CI/($mg\ dm^{-3}$))[b]
	T. tubifex	*C. plumosus*	*S. alba*
I	0.1 (0.09—0.13)	0.05 (0.04—0.06)	5.00 (4.46—5.70)
II	0.25 (0.22—0.26)	0.09 (0.08—0.10)	446.68 (426.58—467.73)
III	2.40 (1.50—3.10)	0.09 (0.08—0.09)	12.02 (11.36—15.74)
IV	1.90 (1.60—2.70)	0.33 (0.29—0.38)	25.12 (22.59—33.67)
V	0.95 (0.82—1.10)	1.90 (1.70—2.30)	15.12 (12.90—17.70)
VI	0.17 (0.15—0.18)	0.03 (0.02—0.03)	31.62 (29.17—34.28)
VII	1.00 (0.90—1.30)	0.34 (0.27—0.40)	33.34 (31.62—39.44)
VIII	3.50 (3.30—4.10)	3.00 (2.60—3.50)	37.51 (35.89—40.35)

a) After 96 h treatment; *b*) after 72 h treatment.

it can be concluded that some of studied compounds (mainly compounds *II, VII,* and *VIII*) are more effective inhibitors of PET than of biosynthesis of alga chlorophyll. This was reflected in dependences of biological activities on the lipophilicity of the compounds. Whereas the inhibition of oxygen evolution rate showed an increase with increasing lipophilicity of the compounds, for inhibitory activity concerning alga chlorophyll production the dependence of biological activity on the lipophilicity of the compounds showed a quasi-parabolic course (with the exception of compound *I*).

Compound *II* was found to be the less effective inhibitor from the studied set. Low biocidal activity of this compound was also introduced in the literature [2, 24, 26]. Naphthenic acid is a natural product of different isomers and naphthenes correspond to certain saturated hydrocarbons, specifically to five- and six-carbon cycloparaffins and their alkyl derivatives, found in crude petroleum. Compound *II* was found to be the less effective inhibitor in the investigated set, probably due to its lower aqueous solubility causing limited passage of this inhibitor through the intact outer alga membrane. On the other hand, *II* inhibited PET in the suspension of partially broken spinach chloroplasts [27]. Using EPR spectroscopy it was confirmed that *II* interacts with tyrosine radicals Tyr_Z and Tyr_D which are located in 161st position in D_1 and D_2 proteins on the donor side of photosystem 2 and in the presence of *II* the release of Mn^{2+} ions from the oxygen-evolving complex occurs [27].

The investigated triorganotin compounds are amphiphilic compounds showing characteristics of both metal ions and hydrophobic materials and it has been found that the uptake of these compounds by phytoplankton cells has usually been considered as a simple partitioning mechanism driven mostly by their hydrophobic character [28, 29]. Positively charged tin atom in R_3Sn^+ cations interacts with anionic sites on the cell surface such as sulfate and carboxylate groups [30, 31]. Tributyltins are lipid-soluble and possibly penetrate the cell by a direct interaction with membrane lipids [32] and an excess of tributyltin crossing the membrane might affect its fluidity by modifying the arrangement and interactions of the different lipids and protein-composing membrane. The principal effect of organotin compounds on the function of the eukaryotic cell is ascribed to the inhibition of oxidative phosphorylation caused by the damage of the mitochondria membranes [9] and compound *V* is also known to inhibit ATP formation and coupled electron transport in isolated chloroplasts [10].

The results concerning inhibition of root growth of *S. alba* by the studied organotin compounds are presented in Table 3. With the exception of the least active compound *II*, relatively small differences in the inhibitory activity of investigated compounds were

found. Compound *I* was repeatedly the most active inhibitor.

The LC_{50} values for the studied organotin compounds concerning mortality of *Tubifex tubifex* worms and *Chironomus plumosus* larvae are summarized in Table 3. Similarly to the above described results high biological activity was exhibited by *I*. In general it can be concluded that the replacement of butyl substituent (*VI*) by phenyl (*VII*) and benzyl (*VIII*) led to the decrease of biological activity, supporting the role of lipophilicity of R substituent as well as that of molar volume of R_3Sn group for the biological activity of investigated triorganotin compounds (the molar volumes calculated for $(C_4H_9)_3$—C, $(C_6H_5)_3$—C, and $(C_6H_5—CH_2)_3$—C groups were 2.251×10^{-28} m^3, 2.431×10^{-28} m^3, and 2.796×10^{-28} m^3, and it can be assumed that also the differences between molar volumes of $(C_4H_9)_3$—Sn, $(C_6H_5)_3$—Sn and $(C_6H_5—CH_2)_3$—Sn groups will be similar) [33].

As for X group it can be concluded that its effect is not expressive enough in comparison with the effect of R group [11]. For brown rot fungi, moulds, and benthic organisms the dominant effect of R group was unambiguously confirmed [12, 21, 34]. Summarizing it can be concluded that the biocidal activity of the studied triorganotin (R_3Sn—X) compounds is closely connected with the lipophilicity of the alkyl substituent R as well as with the molar volume of R_3Sn group and the effect of X group on biocidal activity is not significantly manifested.

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