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Structure-Affected Algicidal Activity of Triorganotin Compounds

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Triorganotin compounds (R_3SnX) are probably the most widely studied and most biological active tin compounds. 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 through dodecyl causes a sharp decrease in biological activity (Cooney 1995). 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 (Kráľová *et al.* 1992). It was reported that butyl and phenyl substituents having comparable lipophilicity had approximately the same biocidal activity (Davies and Smith 1980).

The lipophilic nature of most organotins ensures ready penetration of biological membranes and thus access to intracellular sites (Cooney and Wuertz 1989). Briefly, organotins are likely to accumulate in lipid-rich organelles and to penetrate biological membranes more readily than inorganic tin compounds. Tributyltin (TBT) acts as a ionophore, facilitating halide-hydroxyl exchanges, and interfering with energy transduction processes in chloroplasts and mitochondria (Cooney and Wuertz 1989). TBT can inhibit microsomal cytochrome P-450 in algae (Cooney, 1995). Trisubstituted organotins are active against a number of enzymes (Cooney and Wuertz 1989).

Organotins, the emerging chemicals of the end of last century, are without a counterpart in natural substances. They contribute significantly to the pollution of aquatic ecosystems (Fent and Hunn 1991). Because triorganotin compounds of the type R_3SnX, mainly tributyltin (TBT) and triphenyltin (TPT), are used as antifoulants in antifouling paints for the protection of ship hulls and equipment submerged in water, they leach into the aquatic environment and can cause toxic effects to non-target organisms, even at very low concentrations. TBT and TPT are lipid-soluble and have the potential to be concentrated in higher organisms (Maguire 1996). Fent and Hunn (1991) and Avery et al. (1993) reported their toxicity to algae. Microalgae constitute the first trophic level within the aquatic environment and alga tests are recognized by regulating authorities as being relevant and sensitive indicators of pollutants (Tadros et al. 1994). Most infor-

mation about effects of organotins is related to marine water and estuarine organisms (Waite et al. 1991; Alzieu 1996). Relatively few reports have been published on the comparative toxicity of TBTs and other organotins to freshwater environments (Holwerda and Herwig 1986; Fargašová 1996; 1998).

Attention is now being directed to understanding the relationship between structure and antialgal activity of triorganotin compounds. The biological efficiency of tested triorganotin compounds was examined on the freshwater plankton alga, *Scenedesmus quadricauda*, with the observed parameters including growth rate, chlorophyll *a* production, and photosynthetic oxygen evolution.

MATERIALS AND METHODS

In the tests the following organotins were investigated. As standards: commercially available liquid (bis-tributyltin oxide (TBTO) and tributyltin naphthenate (TBTN); Schering AG, Germany) and solid (triphenyltin chloride – Brestanol (TPTCl) and triphenyltin acetate – Brestan (TPTA); Bayer AG, Germany) products were used. Other tested compounds included tribenzyltin chloride (TBeTCl), tributyltin *N,N*-diethyldithiocarbamate (TBTDEDTC), triphenyltin *N,N*-diethyldithiocarbamate (TPTDEDTC), and tribenzyltin *N,N*-diethyldithiocarbamate (TBeTDEDTC) and were prepared at the Department of Organic Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic by known laboratory methods (Kizlink 1991). The analytical evaluation of prepared compounds was carried out by the GC method elaborated for various *N,N*-dialkyldithiocarbamates (Stäb et al. 1993). The purity of all prepared compounds was 97%. Freshly made stock solutions were made in 10% (v/v_{aq}) aqueous ethanol for each final concentration of organotin required prior to the tests. To eliminate or reduce the toxicity of ethanol on the alga cells, a preliminary study established that a concentration of ethanol up to 0.33% (v/v_{aq}) posed no significant effect on the algae studied in terms of their population growth, chlorophyll production and oxygen evolution. All of the organotins tested were soluble in 0.33% (v/v_{aq}) aqueous ethanol at concentrations specified for the tests. During experiments the triorganotins were tested at 10 different concentrations ranging from 0.01-100.0 μM in a logarithmic sequence.

The freshwater alga, *Scenedesmus quadricauda* (TURP.) BRÉB. strain Greifswald 15, was used. Algae were grown statically in a calcium-depleted modified Knop solution (pH = 7.2) (Fargašová 1998) 12 d under constant temperature (25 ± 1 °C) and permanent light conditions 70 μmE/s/m PAR (PAR = photosynthetically active radiation). Algae at the logarithmic phase of growth were used as inocula for experimental studies. Alga cells at initial density of 25,000 ceonobia/mL (four cells connected in one unit) were inoculated aseptically into 50 mL sterilized Knop solution. The concentrated and diluted stock solutions of organotins were spiked to individual cultures directly after inoculation. At two day intervals the growth rate was determined using a spectrophotometer (λ=750 nm) (Bolier and Donze 1989).

Chlorophyll *a* content was determined using a spectrophotometric method (Fargašová 1996) from the same samples used to determine the growth rate. Chlorophyll *a* was determined in 95% (v/v) ethanol extract measuring absorbance (A) at 665 and 649 nm, respectively, and calculated under the equation:

Chlorophyll *a* (Chl *a*) = $13.70(A_{665}) - 5.76(A_{649})$ (in μg/mL culture).

The photosynthetic oxygen evolution after 12 days growth in the presence of tested trioganotins of alga *S. quadricauda* was measured with an oxygen electrode connected to a computer (Fargašová and Drtil 1996). Before each measurement of oxygen evolution, the cultures were flushed with nitrogen gas in order to decrease their oxygen content to 2-3 mg O_2/L. The defined oxygen production was adjusted for the dry weight (DW) of algae.

All experiments were done in triplicates five times (two reference and three elementary tests). Results from repeated tests were incorporated in one file and referred to negative control. The standard deviations between the tests were less than 5%. The EC_{50} values (EC_{50}^{G} – growth; EC_{50}^{Cha} - chlorophyll *a* content; IC_{50}^{PhO} – photosynthetic O_2 evolution) and their 95% confidence intervals (CI) were calculated by the moving average method of Gelber *et al.* (1985) and U.S. EPA (1989).

RESULTS AND DISCUSSION

The EC_{50} values expressing the inhibition of alga growth, photosynthetic oxygen evolution, and chlorophyll *a* production in freshwater alga *S. quadricauda* for the studied triorganotin compounds are given in Table 1. All these values reflect the long-time effects. On the basis of calculated EC_{50} values the following rank orders of inhibition were established:

Growth rate inhibition:

TBTO>TBTDEDTC>TBeTDEDTC=TPTDEDTC>TPTCl>TPTA>TBeTCl>TBTN;

Chlorophyll a content:

TBTO>TBTDEDTC≥TPTA>TPTCl>TBeTCl=TBeTDEDTC>TPTDEDTC>>TBTN;

Photosynthetic oxygen evolution:

TBTO>TPTDEDTC≥TBeTDEDTC>TBTDEDTC=TBeTCl>TPTCl>TPTA>TBTN.

From these ranking it was evident that the triorganotins, which were the most lipophilic, exerted the greatest toxicity, e.g. TBTO, the most lipophilic test agent, was the most toxic one. This compound best inhibited the alga growth and its EC_{50} value was 10-100 times lower than those for other used triorganotins. Triorganotins inhibited growth more intensively than both chlorophyll *a* production and oxygen evolution. A detected decrease in chlorophyll *a* content was also connected with the changes in the appearance of the alga suspension. Some cultures treated with triorganotins (TPTDEDTC, TPTCl, TBTDEDTC, TBTO) were pallid and exhibited a color change from green to yellow-green. Under microscopic observation a number of cultures showed deformations and disintegration of coenobia.

Table 1. EC_{50} values (μM) and their 95% confidence intervals (CI) for *Scenedesmus quadricauda* growth (G), chlorophyll *a* content (Chla) and oxygen evolution (Ox) after 12 days cultivation in media containing triorganotins

Compound	$EC_{50}^{G} \pm 95\%$ CI	$EC_{50}^{Chla} \pm 95\%$ CI	$EC_{50}^{Ox} \pm 95\%$ CI
TBTO	0.02 (0.01-0.02)	0.32 (0.28-0.34)	0.21 (0.19-0.24)
TBTN	4.73 (4.37-5.00)	15.85 (14.96-16.41)	18.62 (18.42-19.05)
TPTCl	0.91 (0.87-0.93)	2.98 (2.87-3.10)	3.43 (3.29-3.45)
TPTA	1.43 (1.38-1.49)	1.00 (0.92-1.10)	4.51 (4.32-4.73)
TBeTCl	2.50 (1.97-2.72)	5.49 (5.12-5.60)	1.00 (0.91-1.38)
TBTDEDTC	0.20 (0.17-0.23)	0.87 (0.83-0.90)	0.93 (0.89-1.10)
TPTDEDTC	0.4 (0.3-0.4)	8.71 (8.58-9.12)	0.50 (0.48-0.52)
TBeTDEDTC	0.4 (0.3-0.4)	5.75 (5.47-6.11)	0.57 (0.54-0.60)

The higher EC_{50} values for the majority of compounds were calculated for chlorophyll *a* content (EC_{50}^{Chla}) rather than for oxygen evolution (EC_{50}^{Ox}), indicating a strongly unfavorable effect of the organotins on alga photosynthesis. The inhibition of photosynthetic electron transport (PET) by the organotin compounds resulted in inhibition of oxygen evolution, whereas inhibition of alga chlorophyll production was probably related to changes in chlorophyll biosynthesis. Consequently, it can be concluded that some compounds (mainly, TBeTCl, TPTDEDTC and TBeTDEDTC) are more effective inhibitors of PET than of biosynthesis of chlorophyll *a*. That was reflected in the dependence of biological activities on lipophilicity of the compounds. Whereas inhibition of the oxygen evolution rate showed an increase with increasing lipophilicity, for inhibitory activity of chlorophyll production the dependence of biological activity on the lipophilicity of the compounds showed a quasi-parabolic plot (with the exception of compound TBTO). TBTN was found to be the least effective inhibitor of the studied set.

The investigated triorganotin compounds are amphiphilic compounds showing affinity for both metal ions and hydrophobic materials. It has been found that the uptake of these compounds by phytoplankton cells proceeded by a simple partitioning mechanism driven mostly by their hydrophobic character. The positively charged tin atom in R_3Sn^+ cations interacts with anionic sites on the cell surface, such as sulfate and carboxylate groups (St-Louis et al. 1997). Tributyltins are lipid-soluble and penetrate the cell by a direct interaction with membrane lipids. An excess of tributyltin crossing the membrane might affect membrane

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 has been ascribed to the inhibition of oxidative phosphorylation caused by the damage of the mitochondria membranes. The compound TBeTCl is also known to inhibit ATP formation and coupled electron transport in isolated chloroplasts (Yatome et al. 1993). However, during our study the inhibitory effect of this compound on alga growth was very low; its inhibitory effect on chlorophyll *a* production and oxygen evolution was moderate. The inhibition of photosynthetic electron transport (PET) was also observed for other tributyl-, triphenyl- and tribenzyltins tested, except the TBeTCl. On the basis of obtained results for oxygen evolution the tested compounds could be arranged into four groups. The first group includes TPTDEDTC and TBeTDEDTC with very strong inhibitory effect on oxygen evolution accompanied by a strong inhibitory effect on PET. The second group includes TBeTCl and TBTDEDTC with moderate inhibitory effect on oxygen evolution. Group three consists of compounds TPTCl and TPTA which have in comparison with compounds from two previous groups significantly lower inhibitory efficiency both to oxygen evolution and to PET. The last group includes only compound TBTN with very low inhibitory effect on the observed parameter. Low biocidal activity of this compound has been known (Fargašová 1996; Fargašová and Drtil 1996). Naphthenic acid is a natural product consisting of different isomers and naphthenes, corresponding to certain saturated hydrocarbons, specifically to five- and six-carbon cycloparaffins and their alkyl derivatives, found in crude petroleum. Compound TBTN was found to be the less effective inhibitor in the investigated set, probably due to its lower water solubility causing limited passage of this inhibitor through the intact outer alga membrane. On the other hand, TBTN inhibited PET in the suspension of partially broken spinach chloroplasts (Šeršeň *et al.* 1997). EPR spectroscopy experiments confirmed that TBTN interacted with tyrosine radicals Tyr_Z and Tyr_D which were located in 161^{st} position in D_1 and D_2 proteins on the donor side of photosystem 2 and that in the presence of TBTN the Mn^{2+} ions are released from the oxygen-evolving complex (Šeršeň et al. 1997). TBTO was excluded from this assessment because of its different structure containing two tin atoms.

In general, the replacement of butyl substituent (TBTDEDTC) by phenyl (TPTDEDTC) and benzyl (TBeTDEDTC) in *N,N*-diethyldithiocarbamates led to decrease of biological activity, supporting the role of lipophilicity of R substituent as well as that of molar volume of R_3Sn group in generating bioactivity of investigated triorganotin compounds (the molar volumes calculated for $(C_4H_9)_3$-C, $(C_6H_5)_3$-C and $(C_6H_5\text{-}CH_2)_3$-C groups were 2.251×10^{-28} m^3, 2.431×10^{-28} m^3 and 2.796×10^{-28} m^3 respectively. It can therefore be assumed that also the differences between molar volumes of $(C_4H_9)_3$-Sn, $(C_6H_5)_3$-Sn and $(C_6H_5\text{-}CH_2)_3$-Sn groups will be similar) (Ertl 1992).

We found that the effect of the X group was not sufficiently expressed in comparison with the effect of an R group (McDonald and Trevors 1988). Therefore we conclude 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. The effect of X group on biocidal activity has not been significantly manifested.

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