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Comparison of Effects of Tributyl-, Triphenyl-, and Tribenzyltin Compounds on Freshwater Benthos and Alga *Scenedesmus quadricauda*

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The purpose of this study was to evaluate the sensitivity of tributyltin (TBT), tribenzyltin (TBeT) and triphenyltin (TPT) compounds to three aquatic organisms belonging to freshwater benthos (Tubifex tubifex and Chironomus plumosus) and algae (Scenedesmus quadricauda).

The documented presence of organotins, especially tributyltin compounds (TBT) in surface waters and sediments has prompted a large number of studies on its potential adverse effects on nontarget organisms to which belongs also freshwater algae (Blaise 1993) and benthos (Chen 1994). Recently pollution with organotin compounds (OTCs) used as an antifouling paint has been reported in several aquatic areas (Champ and Pugh 1987). Organotin compounds such as TBT and TPT have been widely used in diverse industries such as biocides, heat stabilizers for polyvinyl chloride and catalysts in a variety of chemical reactions (Blunden and Chapman 1986). In particular, the use of tributyltin and triphenyltin as antifouling agents in boat paints has been widespread because of their superior effectiveness compared to previously used copper oxide paints. Tributyltin (TBT) was often found in water at concentrations which could case chronic toxicity in a sensitive species. Its concentration was occasionally found in the surface microlayer of fresh water much higher than in subsurface water (Maguire 1992; Tolosa et al. 1992). Triphenyltin (TPT) was also identified from the water and sediments exhibiting concentrations similar to TBT (Stab et al. 1993).

At the present time, the impact of xenobiotics on aquatic environment is estimated using bioassays, such as algal growth inhibition, daphnia immobilization, fish toxicity tests and benthic organisms mortality tests. Most of these tests are carried out in laboratories under static conditions on environmental samples (Pandard et al. 1993). Microalgae are the most important primary producers in aquatic habitats and are potential indicators of water quality. Algal tests are recognized by regulating authorities (U.S. EPA 1985) as being both relevant and sensitive. Three approaches to determine algal response to organotin compounds (OTCs) were investigated in this study. First, the growth inhibition was based on monitoring with a

hemocytometer. In the second approach, photosynthetic oxygen evolution was measured by the oxygen electrode connected with a computer (Drtil et al. 1993) and in the third approach chlorophyll a content was determined spectrophotometrically (Harris 1989). Tests with benthic organisms provide information on the bioavailability and adverse effects of contaminants associated with whole sediment. Tubifex tubifex and Chironomus sp. have been used successfully in freshwater toxicity tests because these species are fairly large with a short generation time, and are in direct contact with the sediment. They have been shown to be sensitive to many contaminants associated with sediments. Methods for culture and testing are summarized in the ASTM (1992). The endpoints of concern are survival, as well as monitoring in our tests, and growth.

MATERIALS AND METHODS

Scenedesmus quadricauda (TURP.) BRÉB., strain Greifswald 15 was obtained on agar slants from the Institute of Botany AS CR, Tøeboò, Czech Republic, and was grown in a liquid culture of modified Knapp solution without calcium (Fargašová 1994). The culture was maintained under constant temperature and permanent light conditions (25±l °C; 2,000 lux). The algae were statically cultivated, during the tests for growth and chlorophyll a content in 100-mL Erlenmeyer flasks with 50 mL cultivation medium, and for photosynthesis measurement 500-mL Erlenmeyer flasks with 200 mL cultivation medium were used. Each followed concentration was tested in triplicate. For inoculation, algae in the exponential phase of the growth were used and the total inoculum in test and control media was 25,000 coenobia (four cells connected in one unit). Algal counts were conducted every two days during a 12 day cultivation period (Fargašová and Kizlink 1996). For photosynthesis and chlorophyll a content after 7 days of cultivation in an unsupplemented medium 1 mL of OTCs, at an appropriate concentration, (20 various concentrations from 0.01 to 100 µg/L) was added to the cultivation medium. The cultivation lasted 2 days longer under the same conditions. Then, photosynthesis was measured with the oxygen electrode connected with a computer (Drtil et al. 1993) and chlorophyll a content was determined using a spectrophotometric method (Fargašová 1996).

Tubificid worms Tubifex tubifex and Chironomus plumosus larvae were acquired from natural water sediments. The temperature of used overboiled tap water was 20 °C and 20 mm long worms and larvae were used. Specimens were kept out of direct sunlight, solutions were not aerated, and organisms were not fed during the tests. The tests were carried out in glass Petri dishes, diameter 80 mm. The volume of solution per dish used was 20 mL. Control and each concentration was done in triplicate and for each parallel 10 organisms were used. The survival in 10 various concentrations of OTCs (from 0.01 to 10 µg/L) was observed and compared with control after 96 h. The number of dead organisms in all particular Petri dishes of all triplicates holding a total of 30 organisms (3x10) did not differ by more than ± 2 organisms. This split of reading constituted an experimental error of 10 % at the utmost.

The tests were initiated by adding tributyltin-, triphenyltin- and tribenzyltin diethyldithiocarbamate compounds at different concentrations to the test medium. The testing compounds were dissolved in 1 mL of warm ethanol and tilled in 10 mL with distilled water. For the control, an equivalent amount of ethanol solvent was added. The tested compounds were:

TBTC - tributyltin N,N-diethyldithiocarbamate [5847-53-0]
TBeTC - tribenzyltin bis-N,N-diethyldithiocarbamate [55349-54-7]
TPTC - triphenyltin N,N-diethyldithiocarbamate [17523-08-09]

The probit analysis (Gelber et al. 1985) was used to derive the 96-h LC_{50} values for benthos and all EC_{50} values (EC_{50}^{G} - growth, EC_{so}^{Ph} - photosynthesis, EC_{50}^{Ch} - chlorophyll a content) for algae. The statistically significant effects of triorganotins on mortality, growth, photosynthesis and chlorophyll a content were determined using analysis of variance (ANOVA) in conjunction with Duncan's multiple range test (SAS 1989) with 95 % confidence intervals ($P<0.05$).

RESULTS AND DISCUSSION

The inhibitory effects of triorganotins on algal growth, measured by hemocytometer, photosynthesis, determined by oxygen amount measurement, and chlorophyll a content of green algae S quadricauda as well as lethal effects on benthic organisms are presented in Figure 1. as LC_{50} and EC_{50} values and their 95 % confidence limits.From LC_{50} and EC_{50} values introduced in this figure the following rank orders of toxicity and inhibition for the tested triorganotin compounds were established:

Tubifex tubifex:	TBTC>TPTC>TBeTC
Chironomus plumosus:	TBTC>TPTC>TBeTC
Scenedesmus quadricauda:	
growth inhibition:	TBeTC=TPTC>TBTC
photosynthesis inhibition:	TPTC>TBeTC>TBTC
chlorophyll a content:	TBTC>TPTC>TBeTC

From these rank orders, it is evident that for benthic organisms, the tributyltin compound was the most toxic, and the least toxicological effect for acute toxicity determined as mortality was estimated for the tribenzyltin compound. This fully confirmed the high toxicity of tributyltin compounds introduced by many authors (McDonald and Trevors 1988; Saint-Louis at al. 1994; Bruschweiler et al. 1995). Avery et al. (1993) reported that the biosorption of triorganotin compounds was increased with a molecular mass of the organotins. He introduced the order in which triphenyltin is more toxic than the tributyl- and tripropyltin compound. This statement was confirmed when the growth and photosynthesis inhibition of alga S. quadricauda were determined. For chlorophyll a content the sequence of a triorganotin inhibitory effect was the same as for benthos, i.e. tributyltin was the most toxic compound. The benzyl group, as introduced by Davies and Smith (1980) and

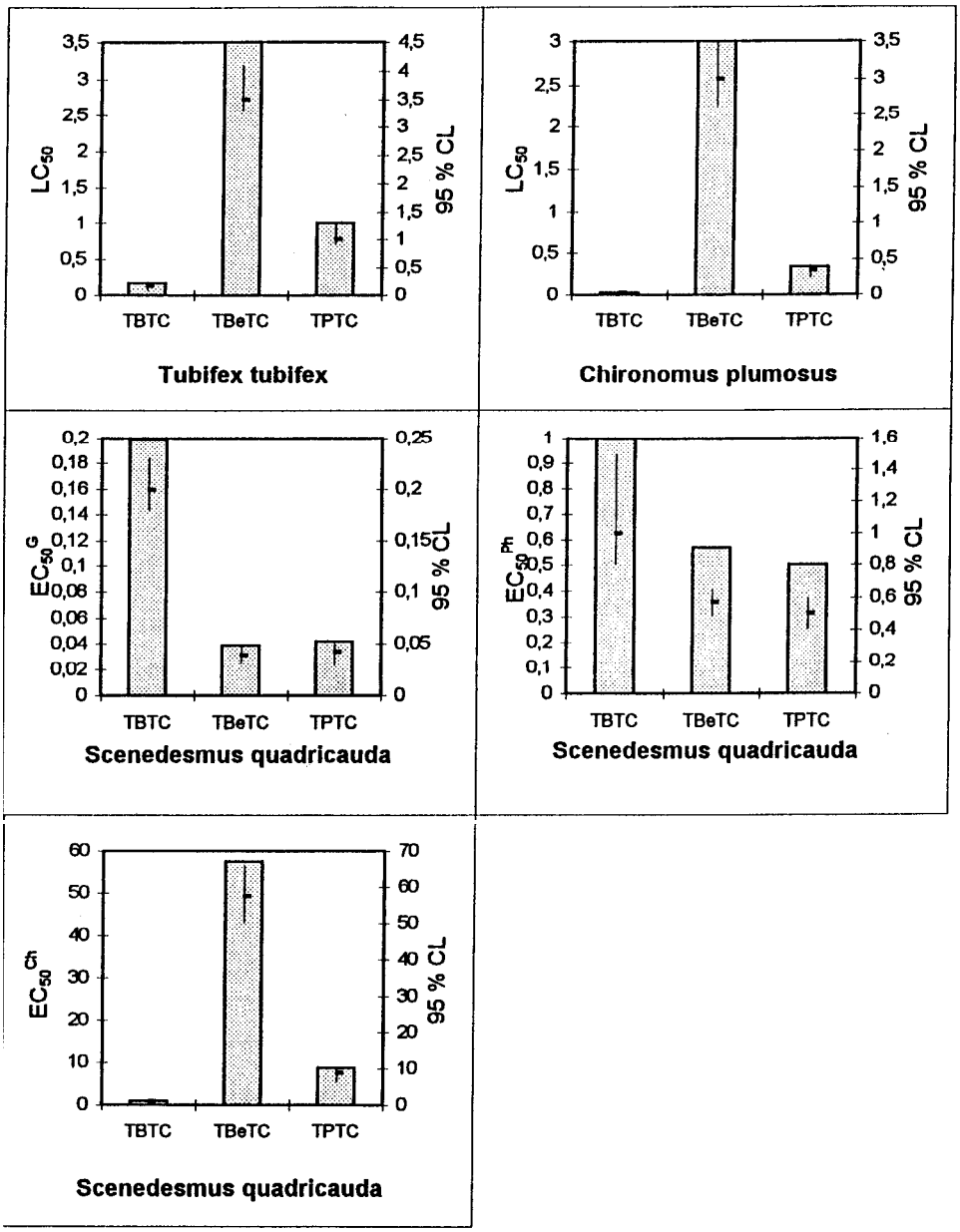

Figure 1. The LC_{50} values (µg/L) for Tubifex tubifex and Chironomus plumosum and EC_{50} values (µg/L) for Scenedesmus quadricauda and their 95% confidence limits (CL) for tested triorganotin compounds. LC_{50} - concentration for 50 % mortality; EC_{50}^{G} - concentration for 50 % growth inhibition; EC_{50}^{Ph} - concentration for 50 % photosynthesis inhibition; EC_{50}^{Ch} - concentration for 50 % chlorophyll a content

Fargašová and Kizlink (1995), caused a sharp decrease in biological activity, thiswas fully confirmed especially for growth rate and photosynthesis activity of alga S. quadricauda. The effective concentration of TBeTC was in these cases many times lower than those for TBTC and TPTC (see Figure 1.). For S. quadricauda chlorophyll a content this statement was not confirmed. The differences between EC_{50}^{G} and EC_{50}^{Ph} values for TBeTC and TPTC were not significant but for growth the efficiency of these two triorganotins was almost-equal-to 5 times and for photosynthesis 2 times higher than TBTC activity. The effect of TBeTC and TPTC on chlorophyll a content in alga S. quadricauda was very week especially for TBeTC. In comparison with TBTC, the activity of TBeTC was about 66 times lower than that for TBTC. The effect of TPTC was about 7 times higher than that for TBeTC and 10 times lower than for TBTC.

In alga, triorganotins had the strongest influence on algal growth and the slightest on the chlorophyll a content. Triorganotins with butyl and phenyl radical inhibited chlorophyll a production more than organotins with benzyl radical. Thayer (1983) noted that the toxicity of organotins is influenced by the length of the side chain. Organotin compounds having a butyl group have maximum efficiency and phenyl has approximately the same biocidal effectiveness. This state was not fully confirmed in our tests.

When the LC_{50} values for individual triorganotin compounds were compared, the values for Ch. plumosus were in all cases lower (for TBTC 10^{-6}, for TBeTC 10^{-1} and for TPTC $l0^{-3}$) than those for T. tubifex. That means that Ch. plumosus was more sensitive to tested triorganotin compounds than T. tubifex. The high sensitivity of chironomid larvae, especially those of Ch. plumosus, was also reported by Chen (1994). When in our tests the obtained LC^{50} values for T. tubifex and Ch. plumosus are compared with OTCs, and especially with TBT, levels in water sediments (Dowson et al. 1992; Stab et al. 1993), we can claim that all LC^{50} values are lower than the given OTCs (especially TBT) concentrations in water sediments. This means that triorganotins leaching into the water and accumulating in sediments are very harmful to benthic organisms. They decrease the vitality of benthos (Chen 1994; Fent and Looser 1995) and increase bioaccumulation of triorganotins in all water organisms. Since benthic organisms as well as algae are at the beginning of the food chain, the penetration of toxic compounds through them to higher trophic levels is very intensive and dangerous.

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