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Fiespirometric Toxicity Test: Freshwater Alga *Scenedesmus quadricauda* Sensitivity to Organotin Compounds

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Organotin compounds (OTC) are extensively used in a variety of industrial processes and their increasing discharge into the environment is a topic of current concern (Thayer 1988). While inorganic forms of tin are of relatively low toxicity, the more lipid-soluble organotins can be highly toxic to microorganisms and other life forms (Cooney and Wuertz 1989). Generally, trisubstituted (R_3SnX) organotins are more toxic than di- (R_2SnX_2) and monosubstituted ($RSnX_3$) compounds, the anion (X) being considered to have little influence on toxicity (Blunden and Chapman 1986; Cooney 1988). The high biocidal activity of trisubstituted OTC has led to their extensive biocidal use as wood and fabric preservatives and as components of anti-fouling paints (Thayer 1988; Cooney and Wuertz 1989). However, as with other potentially toxic metal compounds (Gadd 1988), many microorganisms may exhibit organotin resistance (Cooney and Wuertz 1989). OTC, the emerging chemicals of the remainder of this century, are without counterparts in natural substances. Thus, both replacement of traditional chemicals and application of novel chemicals are sure to occur (Laughlin et al. 1985). While economic factors are usually easy to assess, environmental issues have generally received less consideration at the beginning, and may tend to be ignored if they contain "bad news". The need for research on environmental effects will grow concomitant with the increasing use of OTC (Laughlin et al. 1985). There are many works about the toxicological effect of OTC (Luijten 1972) but very little about their environmental effects *per se* because laboratory bioassays have several limitations (Laughlin et al. 1985). Additionally, OTC are slowacting toxins.

The respiration rate of a microbial population and short-term biochemical oxygen demand (BOD) are valuable variables for the control of some processes, e.g. intermediate metabolism reflected by ammonia excretion,

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photosynthesis intensity, activity of biomass, the determination of kinetic and stoichiometric constants of microorganisms, the determination of inhibition and stimulation of biomass, etc. In contrast with traditional variables like BOD_5 and TOC (total oxygen capacity) respiration is not only related to the biological process itself but is also one of the few variables which is directly measurable. Additionally, it can also indicate toxic effects (Pérez-García et al. 1993). The respiration rate can be used as a quantity from which otherwise poorly accessible variables, such as the biomass concentration, can be estimated (Takamatsu et al. 1981).

Recently, works about the accumulation, transformation and resistance to OTC have appeared more frequently (McDonald and Trevors 1988; Gadd 1988), but there is still sparse literature about the effect of OTC on some metabolic processes (enzyme production,chlorophyll content, respiration, etc.).Also, some biological subjects are not widely used for these tests (freshwater phytoplankton and zooplankton, higher plants, etc.). In this article we describe the problem of respiration after OTC application to the freshwater alga Scenedesmus quadricauda. We have found only a single report by Wong and Chang (1988) about using of the respirometric method for toxicity tests of algae. Respirometry is widely used for the description of the activated sludge (Suschka and Ferreira 1986; Roš et al. 1988; Drtil et al. 1993). Recently a report about using rapid respirometric toxicity tests for baker's yeast has also appeared (Perez-Garcia et al. 1993).

MATERIALS AND METHODS

Scenedesmus quadricauda (TURP.)BRÉB., strain Greifswald 15, was kindly supplied by the Institute of Botany AS CR, Trebon, Czech Republic. During the tests, the culture was incubated under continuous light at 25±1 °C and a light intensity produced by three 40-W white fluorescent lamps. The culture and control were maintained in a liquid medium containing distilled water and the following chemical ingrediens (g/L): KNO_3 0.1; $K_2HPO_4.7H_2O$ 0.01; $MgSO_4.7H_2O$ 0.001; $FeCl_3.6H_2O$ 0.001; a soil extract of 50 mL; pH=7.18. During the tests, the alga grew in 500-mL Erlenmeyer flasks with a 200 mL cultivation medium supplemented with OTC. Each OTC was tested in four concentrations (mg/L): 0.001; 0.01; 0.1 and 1.0. For the control, only a liquid medium was used. The control and each concentration were duplicated three times. Approximately 25, 000 coenobia (four cells connected into one unit) were inoculated in the test and control media. After 7 d cultivation, 1 mL of OTC, in the appropriate concentration, was added to the cultivation media. The cultivation lasted 2 d longer under

the same conditions and then the respiration was determined by a respirometer. The respirometric measurement consisted of two parts:
1.) the determination of algal dry weight for which 25 mL of algal suspension were filtered through a membrane filter and dried at a temperature of 100-115 °C;
2.) the determination of the respiration rate. In this case the respirometer (Figure 1) was used. The rate of oxygen consumption was determined by using a computer program for the angular coefficient of the respiration rate, which depended on the time chosen for the concentration of OTC.The defined respiration rate was adjusted for the dry weight by using the equation:

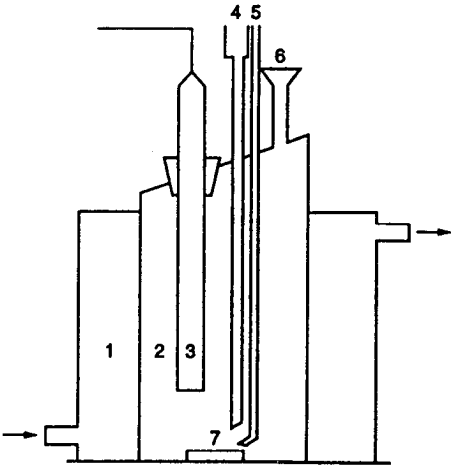

Fig. 1. Respirometer. 1, Water jacket; 2, respirometric cell 3, oxygen electrode; 4, hypodermic syringe; 5, aeration frit; 6, expansion funnel; and 7, magnetic mixing bar.

$$R_{ox,x} = \frac{R_{ox,v}}{x}$$

$R_{ox,v}$ - the volumic respiration rate mg O_2/L/hr
x - dry weight of algal suspension in g/L
$R_{ox,x}$ - the specific respiration rate mg O_2/g/hr

The results are summarized in Table 1 and the efficiency (U) of the tested OTC on the respiration of alga S. quadricauda, in percentages were calculated by the equation:

$$U = \frac{c - Co}{c} .100 \ (\%)$$

c - the value of respiration rate in flask with tested concentration of OTC (mg O_2/g/hr)
Co - value of respiration rate in control flask (mg O_2/g/hr)

The kinetics of respirometry,for the respirometer which we used for our tests (Figure l), was described in more detail by Drtil et al. (1993).

Twelve OTC, synthesized at the Department of Organic Technology, Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovak Republic were tested. The diorganotin compounds (type R_2SnX_2) that were tested were:

A - dibutyl-tin-bis-N,N-diethyl-dithiocarbamate
B - dimethyl-tin-bis-N,N-diethyl-dithiocarbamate

The other tested compounds were triorganotins (type R_3SnX):

C - triphenyl-tin-chloride
D - triphenyl-tin-acetate
E - triphenyl-tin-N,N-diethyl-dithiocarbamate
F - tribenzyl-tin-bis-N,N-diethyl-dithiocarbamate
G - tribenzyl-tin-chloride
H - bis-tributyl-tin-3,4,5,6-tetrachlor-phthalate
I - tributyl-tin-sulfaminate
J - tributyl-tin-N,N-diethyl-dithiocarbamate
K - tributyl-tin-naphthenate
L - tributyl-tin-oxide (TBTO)

RESULTS AND DISCUSSION

Photosynthesis is a process during which green organisms obtaining chlorophyll absorb CO_2 from the surrounding environment and produce oxygen. When light is absent, or the surrounding conditions are not favorable, the respiratory process begins. During respiration, organisms receive oxygen and produce CO_2. In our tests, we observed how the respiration rate increased when OTC were added in the culture media. An increase in respiration means a decrease in photosynthesis and indicates a toxic effect on the metabolic processes of the tested freshwater alga S. quadricauda.

When the effect of OTC, used at four different concentrations on the respiration activity of S. quadricauda, was tested, a significant correlation was observed between the toxicant concentration and the respiration activity stimulation for all OTC, except for compounds D, **J** and **L** at a concentration 0.001 mg/L and compound L at a concentration of 0.01 mg/L. Because our tests used photosynthetic organisms (green alga), the higher respiratory activity response means a higher toxic effect and the more intensive inhibition of photosynthesis. This explains why, in our tests, the highest respiratory rates were observed when the highest concentrations of OTC were tested. As can be seen from the measured values (Table l), the inhibitive effect on photosynthesis was also very strong when the lowest concentration (0.001 mg/L) of OTC was used. For each concentration the rank order of respiration activity under the efficiency value (U) can be arranged:

0.001 mg/L: **F>H>K=I>C>G>B=E>D≥J≥L**
0.01 mg/L: **F ≥I=C≥H>K>A ≥G≥E=J>D>B>L**
0.1 mg/L: **F=C≥I>B=D≥G=H≥E≥L>A>J>K**
1.0 mg/L: **F=C≥G≥I≥H=B≥D>L=A>E>J>K**

From these ranking orders we can see that, in all the above concentrations, the highest respiratory activity had compound **F** which belonged to the triorganotins with benzyl group. Compound **F** inhibited photosynthesis very intensively and had the strongest toxic effect. When compounds **C** and **I** were tested, the respiration activity

Table 1. Evaluation of the influence of organotin compounds on respiration activity of Scenedesmus quadricauda

OTC	c mg/L	$R_{ox,x}$±SD mg O_2/g/hr	U %	OTC	c mg/L	$R_{ox,x}$±SD mg O_2/g/hr	U %
Co		4.57±0.11					
A	1.0	29.59±1.11	84.6	G	1.0	143.28±9.05	96.8
	0.1	23.88±1.77	80.1		0.1	38.89±1.79	88.3
	0.01	11.86±0.55	61.5		0.01	11.08±0.47	58.8
	0.001	-			0.001	9.17±0.08	50.2
B	1.0	67.45±1.63	93.2	H	1.0	73.80±2.50	93.8
	0.1	62.49±1.69	92.7		0.1	39.20±1.65	88.3
	0.01	7.51±0.13	39.2		0.01	27.11±1.28	83.2
	0.001	6.83±0.29	33.1		0.001	17.49±0.80	73.9
C	1.0	301.11±14.02	98.5	I	1.0	93.31±3.78	95.1
	0.1	213.84±1.08	97.9		0.1	105.42±1.83	95.7
	0.01	33.46±1.70	86.4		0.01	34.28±1.68	86.7
	0.001	5.25±0.63	13.0		0.001	14.49±0.88	68.5
D	1.0	57.61±2.78	92.1	J	1.0	13.00±0.76	64.9
	0.1	55.27±1.37	91.7		0.1	18.71±0.76	75.6
	0.01	8.39±0.19	45.6		0.01	10.49±0.52	56.5
	0.001	5.61±0.04	18.6		0.001	5.43±0.44	15.9
E	1.0	22.62±0.24	79.8	K	1.0	10.84±0.34	57.9
	0.1	30.73±1.35	85.1		0.1	14.69±0.24	68.9
	0.01	10.57±0.31	56.8		0.01	15.55±0.29	70.6
	0.001	6.81±0.17	32.9		0.001	14.60±0.27	68.9
F	1.0	360.77±4.85	98.7	L	1.0	30.11±0.81	84.8
	0.1	258.89±2.76	98.2		0.1	29.22±0.63	84.4
	0.01	42.77±2.52	89.3		0.01	5.34±0.12	14.4
	0.001	31.22±0.63	85.4		0.001	5.29±0.16	13.7

Co - control; c - concentration; $R_{ox,x}$ - respiration rate; SD - standard deviation; U - efficiency of the tested OTC on the respiration

was also very high. Very low respiration activity was observed for the 0.001 and 0.01 mg/L concentrations for compound **L** and for higher concentrations 0.1 and 1.0 mg/L for compounds **K** and **J**. When the highest concentration (1.0 mg/L) of OTC was tested, the differences between respiration activity of compounds **B,C,D,F,G,H** and **I** were very small. Because the respiration activity in these cases was very high, we can conclude that photosynthesis was almost completely inhibited,which was also demonstrated by the appearance of the algal suspensions. The intensity of the color of the suspensions had decreased significantly, most of

which ranged from pallid to completely white. Since these compounds contained all kinds of tested R groups (butyl, phenyl and benzyl) it is not possible to conclude which of the R radicals was more toxic. Thayer (1983) and Davies and Smith (1980) report that the butyl group is the most toxic, but we can not confirm this statement. In our tests, when the respiration activity was measured by respirometer for all concentrations used, the most toxic was the compound with the benzyl group **(F).** Low toxicity in these tests came from compounds with the butyl group . For concentrations 0.001 and 0.01 mg/L it was compound **L,** and for concentrations 0.1 and 1.0 mg/L it were compounds **J** and **K.** Variation of the radicals X (usually chloride, fluoride, oxide, hydroxide, carboxylate or thiolate) appeared to have little effect on the biological activity (Davies and Smith 1980), completely agreeing with the results that we obtained.It is not possible to determine whether the respiration rate is demonstrably influenced by triorganotin (R_3SnX) or diorganotin (R_2SnX_2) compounds. Triorganotins are described in literature as more toxic and biologically active than diorganotins (McDonald and Trevors 1988). Usually tributyltin oxide (TBTO - compound L) is used as the representative OTC, as it is generally the most toxic (McDonald and Trevors 1988). In our tests, this effect was not manifested at the concentrations tested. In lower concentrations (0.001 and 0.01 mg/L) TBTO increased respiration from 13.65 to 14.42 %. When higher concentrations (0.1 and 1.0 mg/L) were used, the respiration activities increased about 85 %. That means it had a strong toxic effect and a rapid decrease in the photosynthetic process.

The method described and the results obtained are useful for the evaluation of the influence of OTC on such metabolic pathways as respiration and photosynthetic activity. The respirometric method is very widely used for measuring respiration activity of heterotrophic organisms, especially bacteria and activated sludge. No reports about using this method for toxicity tests of green algae, except for the report by Wong and Chang (1988) about gross photosynthetic rate of Chlamydomonas reinhardtii (mt+) measured with a Gilson Differential Respirometer, were found in the literature.

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