Bull. Environ. Contam. Toxicol. (1998) 60:318-322
© 1998 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

Toxicity of Tributyltin to *Lemna minor* L. and *Azolla filiculoides Lamk*

Song Zhihui, Chen Tianyi

Department of Environmental Sciences, Nankai University, Tianjin 300071, People's Republic of China

Received: 25 March 1997/Accepted: 10 December 1997

Tributyltin (TBT), which is used as a marine antifouling biocide and in several industrial process, is one of the most toxic compounds to aquatic organisms ever introduced deliberately to water (Krull, 1989). Common duckweed *Lemna minor* is a widespread vascular plant. Duckweed is small. fast growing and easy to cultivate. *Azolla* is a floating water fern common in many places of the world, and is used as a fertilizer in rice fields. The purpose of this study was to determine the toxicity of tributyltin chloride to *Lemna minor* and *Azolla filiculoides.*

MATERIALS AND METHODS

Lemna minor was collected from the Shuishang Lake in Tianjin City. The stock was cultured in the laboratory using Hunter solution (Hunter 1953). Toxicity tests were conducted in a series of 100 mL beakers. Each beaker contained 80 mL of test water and 12 fronds (or 6 plants). Five concentrations of TBT (0,5.0, 10.0, 25.0, 50.0 µg/L) were used as experimental treatments, each replicated 3 times. The number of fronds in each beaker was counted every day. After 4 days the chlorophyll content of each beaker was determined. A photoperiod of 16hr light 8hr dark was used at 25 ± 1 °C .

Azolla filiculoides was collected from the Weijin River in Tianjin City. The stock was cultured using Hogland solution (Mordechai, 1989). Tests were conducted in 250 mL beakers. 200 mL of test water containing seven concentrations of TBT (0, 1.0, 2.5, 5.0, 10.0, 25.0,

Correspondence to: Song Zhihui

50.0 µg/L) and 0.2g (fresh wt) plant were placed in beakers, each replicated 3 times. After 7 days, the plant wet weight and the chlorophyll content of each beaker were determined. A photoperiod of 16hr light/ 8hr dark was used at 25 ± 1 °C

After each test. the whole plant was homogenized with 10 mL acetone to extract chlorophyll. The extraction solution was put into refrigerator for 96 hours at 4 %. Then the solution was centrifuged at 3000 x g for 15 minutes. The absorbance of clear solution was determined at 663 and 64.5 nm (Zhu, 1990).

The chlorophyll content was calculated as follows:

$$C_{a+b} = 20.2A_{645} + 8.02A_{663}$$

Growth inhibition was calculated as follows:

$$V = \ln(N_t/N_0)/t$$

$$I = (V_0 - V_t)/V_0 \times 100\%$$

where V was the growth rate, N was the number of fronds in *L. minor* test, or weight in *A. ,filiculoides* test, or the chlorophyll content (C_{a+b}) in both tests. N_t and N_0 were N at t time and initial time. V_0 was the growth rate of control, V_t was the growth rate of treatment at t time, t was the time and I was growth inhibition. I was regressed with the values of logarithm of TBT concentrations so as to calculate the value of IC_{50} (median inhibition concentration). The tests data were analyzed for statistical differences by an analysis of variance (ANOVA), studying the effect of each treatment of TBT.

RESULTS AND DISCUSSION

The initial chlorophyll content was 0.44 mg/L of each beaker in *L. minor* tests. The data (table 1) show the toxic effect of TBT on the growth and chlorophyll content in beakers of *L. minor*. According to the fronds data, the 96hr IC_{50} of TBT to *L. minor* was 30.83 µg/L. The correlation coefficient was 0.877. The 96hr IC_{50} of TBT for chlorophyll content in *L. minor* test was 12.4 µg/L. The correlation coefficient of the data was 0.916.

Table 1. Toxic effect of TBT on *L. minor*

	TBT concentration (μg/L)				
	0.0	5.0	10.0	25.0	50.0
number of fronds (at the end of test)	75[a]	61	60	53	33
chlorophyll content (mg/L)	1.38 ± 0.04[b]	1.23 ± 0.09	1.06 ± 0.02	0.68 ± 0.03	0.19 ± 0.06

a. total fronds number of three replicates

b. mean value of three replicates

Table 2. Toxic effect of TBT on *A. filiculoides*[c]

TBT concentration (μg/L)	wet weight /g (at the end of test)	chlorophyll content(mg/L) (at the end of test)
0.0	0.288 ± 0.013	20.77 ± 0.21
1.0	0.268 ± 0.020	17.36 ± 0.35
2.5	0.260 ± 0.019	15.77 ± 0.48
5.0	0.249 ± 0.051	14.57 ± 0.69
10.0	0.241 ± 0.043	13.52 ± 0.72
25.0	0.235 ± 0.031	12.94 ± 0.54
50.0	0.221 ± 0.024	11.39 ± 0.46

c. mean value of three replicates

The initial chlorophyll content was 14.44 mg/L in *A. filiculoides* tests.The data (table 2) show the toxic effect of TBT on the growth and chlorophyll content in beakers of *A. filiculoides*. The IC_{50} of TBT (7 days) for growth of *A. filiculoides* was 8.17 μg/L. The IC_{50} of TBT (7 days) for chlorophyll content of *A. filiculoides* was 1.84 μg/L. All correlation coefficients were over 0.9.

The results showed that concentration levels of TBT being at μg/L. the toxicity of TBT to *L. minor* and *A. filiculoides* was very high. The data suggested that chlorophyll content in beakers was more susceptible than growth.

The 48hr LC_{50} of TBT to *Solea solea* was 88 μg/L. The 7 days LC_{50} of TBT to *Cyprinodon variegutus* was 5 μg/L, the 14 days LC_{50} was 3μg/L, the 48hr LC_{50} of TBT to *Daphnia magna* was 2 μg/L (Mamie,

1991). In comparison with the IC_{50} for growth, *L. minor* and *A. filiculoides* were same sensitive to TBT as fish, and less sensitive than *D. magna*. In comparison with the IC_{50} for chlorophyll content, the sensitivity of *L. minor* and *A. filiculoides* were proximate to that of *D. magna*.

In studies of the toxicity of organotins compounds the test organisms were often aquatic animals; plants were limited to algae (Reader, 1992, Marsot, 1995). Higher plants provide habitats for aquatic animals and also serve as food organisms of the animals. Though the target organisms of TBT are mollusks, crustaceans and worms. it is also toxic to many other aquatic organisms. So it is necessary to use higher plants as test organisms. In this study, we found that duckweed and water fern were very sensitive to TBT, which suggested that the toxicity of TBT to aquatic plants can be critical.

Acknowledgment: This research was supported by the National Nature Sciences Foundation of China through a grant to CT.

REFERENCES

Hunter. S. H. (1953) Growth and differentiation in plants, Iowa State College Press. Ames. Iowa.

Krull IS (1989) The determination of organotins (TBT) in fish and shellfish via gas chromatography-flame photometric detection and direct currant plasma emission spectroscopy (GC-FPD/DCP). Organomet Chem 3: 295-308

Mamie May-ming Lau (Wong) (1991) Tributyltin antifoulings: A threat to the Hong Kong environment. Arch Environ Contam Toxicol 20: 299-304

P. Marso, E. Pelletier, R. St-Louis (1995) Effects of triphenyltin chloride on growth of the marine micoalga *Pavlova lutheri* in continuous culture. Bull Environ Contam Toxicol 54: 389-395

S. Reader E. Pelletier (1992) Biosorption and degradation of butyltin compounds by the marine diatom *Skeletonema costatum* and the

associated bacterial community at low temperature. Bull Environ Contam Toxicol 48: 599-607

Zhu guanglian (1990) Botanical physiological test. Peiking University Press.