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Effect of Dissolved Humic Material on the Toxicity of Tributyltin Chloride and Triphenyltin Chloride to *Daphnia magna*

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In recent years more works have shown that dissolved organic material (DOM) in natural waters is important in water chemistry and aquatic tosicology of xenobiotics in aquatic systems (Caron and Suffet 1985; Stackhouse and Benson 1988; Kukkonen et al. 1990). The binding or adsorbing of pollutants to DOM alters their bioavailability, and therefore affects the accumulation by and toxicity to aquatic organisms (Servos and Muir 1989).

Tributyltin (TBT) and triphenyltin (TPhT) have been the organotin compounds of most conern because their extremely high toxicity to aquatic biota. TBT and TPhT possess both lipophilic and ionic properties and have capability of binding by both hydrophobic and ionic interactions with macromolecules (i.e. humic material). These interactions could alter the bioavailability of TBT and TPhT to aquatic biota resulting in reduced or increased toxicity.

Dissolved humic materials (DHM) comprise a majority: 60 to 80%, of the total DOM found in aquatic systems and soil (Stackhouse and Benson 1989). Based on acid solubility and molecular weight. DHMs are divided into humic acid (HA) and fulvic acid (FA). Prior to this work. no TBT and TPhT toxicity studies had been reported for measurements in the presence of DHMs. Thus, the purpose of this study was to investigate the toxicity of tributyltin chloride (TBTCl) and triphenyltin chloride (TPhTCl) to *Daphnia magna in* the presence of DHMs from various sources.

MATERIALS AND METHODS

D. magna used in this study were isolated from the natural aquatic environment, and cultured in laboratory at 20°C for more than six months. The *D. magna* were fed daily with a suspension of *Selenastrum capricornutum* and yeast mixture. The rearing medium was DHM-free synthetic water which was prepared by adding 30 mg $MgSO_4$, 30 mg $CaSO_4$, 48 mg $NaHCO_3$, 2 mg KCl and 20 mg $CaCl_2 \cdot 2H_2O$ into 1000 mL distilled water. pH was adjusted to 7.6.

Peat HA was obtained from the Research Center for Eco-Environmental Science, the Academy of Science of China, Beijing. The other DHMs used in this study were isolated from the sediment of Haihe river and the soil of Tianjin Water Park, Tianjin: China. The DHMs stock solutions were prepared by dissolving determined amount of DHM in 250 mL of dilution water prepared as the rearing medium of *D. magna*. These solutions were stirred for 30 min and centrifuged at 5000 rpm for 20 min, and the supernatants were

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filtered through a 0.45-um membrane filter to remove particulates. The dissolved organic carbon content of these stock solutions was measured by a Total Organic Carbon Analyzer (TOC-10B. Shimadzu), and the concentration was calculated as milligram of organic carbon per liter (mgC/L). These DHMs stock solutions were stored in the dark at 4°C before use. Further dilution to the required concentration of DHM was then prepared in dilution water.

Tributyltin chloride (TBTCl) and triphenyltin chloride (TPhTCl) were obtained from commercial sources and used without further purification since their chromatographic purities were greater than 98%. TBTCl and TPhTCl were dissolved in acetone to make solutions (1000 ug/mL). Before conducting the toxicity tests, these solutions were further gradually diluted with DHM solution to the intended concentrations.

Acute toxicity tests were performed on *D. magna* according to the method described by Weber (1991). Young daphnids (15 per each test concentration, 24 h old) were put into 150-mL glass beakers containing 100 mL of test solution. The beakers were then kept in the dark at 20±2°C with a photoperiod of 16 h light and 8 h darkness as in the case of the culture. Percentages of immobilization were recorded after 24 h and 48 h. Each concentration was run in triplicate using humus-free test solutions as controls. Dissolved oxygen, measured at the end of the tests, was always higher than 6.5 mg/L. During the tests, there was no mortality of daphnids in beakers in the presence of DHMs only.

The EC50 (the toxicant concentration that resulted in a 50% immobilization) values (24 h and 48 h) with 95% confidence limits were calculated with a Basic microcomputer program based on probit analysis. The effects of DHMs on the toxicity of TBTCl and TPhTCl were evaluated by comparing of the 95% confidence limits of EC50 values in the presence of each type of DHM with control. If 95% confidence limits did not overlap, the difference between EC50 values was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the measured EC50 values for TBTCl in the presence of 0-40 mg/L of each type of DHM. The results show that TBTCl EC50 values increased in the presence of increasing concentrations of peat HA and that in the presence of 20 and 40 mgC/L the EC50 values were statistically significantly different compared to the control. 24-h EC50 values of TBTCI were approximately 1.4- and 1.7-fold greater at 20 and 40 mgC/L of peat HA, respectively, 48-h EC50 values were increased by 1.7- and 1.9-fold, respectively. In addition, 40 mgC/L of soil HA also decreased the toxicity of TBTCl by 1.4- and 1.7-fold at 24 and 48 h. respectively. The lower concentrations of peat HA (0.5 and 5.0 mgC/L) and soil HA (0.5-20 mgC/L) only slightly decreased the toxicity of TBTCl. For peat HA, there seems to be some direct relationship between the concentration of HA in water and its capability to decrease the toxicity. Over the tested concentration range of river sediment HA and FA, 0.5-40 mgC/L, no significant influence on the toxicity of TBTCl was observed. Conversely, the soil FA appeared to increase the toxicity of TBTCl slightly, as evidenced by smaller EC50 values, however, there were no statistically significant differences between the control and the soil FA treatments.

For TPhTCl, the EC50 values observed with DHMs are reported in Table 2. The EC50 values for TPhTCl to *D. magna* were significantly increased in the presence of 20 and 40

Table 1. Influence of DHMs on the toxicity of TBTCl to *D. magna.*

DHM Type	DHM (mgC/L)	EC50 and 95% confidence limits (μg/L)	
		24-h	48-h
Control	0	13.2 (15.3 - 10.6)	4.38 (5.75 - 3.19)
Peat HA	0.5	15.2 (17.4 - 11.8)	5.03 (7.09 - 3.18)
	5.0	17.3 (19.1 - 14.5)	6.31 (8.19 - 4.22)
	20.0	18.1*(20.6 - 16.3)	7.42*(9.87 - 6.19)
	40.0	22.3*(24.9 - 18.7)	8.37*(11.2 - 7.13)
Sediment HA	0.5	14.3 (16.1 - 11.7)	4.09 (5.74 - 2.92)
	5.0	15.4 (18.3 - 12.2)	4.48 (6.09 - 3.14)
	20.0	14.8 (17.7 - 12.4)	4.70 (6.78 - 3.01)
	40.0	16.9 (19.2 - 14.1)	5.83 (7.76 - 4.29)
Sediment FA	0.5	13.9 (15.1 - 11.2)	4.41 (5.89 - 3.10)
	5.0	14.8 (17.9 - 12.4)	4.28 (6.01 - 3.06)
	20.0	15.9 (19.7 - 13.2)	4.47 (6.48 - 3.31)
	40.0	15.4 (19.0 - 13.8)	4.83 (7.01 - 3.57)
Soil HA	0.5	13.4 (16.0 - 9.36)	4.87 (6.42 - 3.26)
	5.0	15.1 (17.1 - 12.6)	5.65 (7.42 - 3.75)
	20.0	16.6 (19.1 - 13.9)	6.32 (8.37 - 4.45)
	40.0	18.9*(21.7 - 16.1)	7.64*(9.54 - 6.22)
Soil FA	0.5	13.8 (15.9 - 10.2)	4.33 (6.05 - 3.07)
	5.0	12.3 (14.9 - 9.42)	3.97 (5.62 - 2.42)
	20.0	11.2 (14.3 - 9.10)	3.65 (5.11 - 2.67)
	40.0	12.6 (15.1 - 10.3)	3.62 (5.44 - 2.49)

*: Significantly different ($p < 0.05$) from control (0 mgC/L of DHM).

mgC/L of peat HA or soil HA as compared with the control. For peat HA, the 24-h EC50 values were 1.4- and 1.6-fold greater at 20 and 40 mgC/L, respectively, and the 48-h EC50 values were 1.8- and 2.0-fold greater, respectively. Respective concentrations of soil HA caused the TPhTCl 24-h EC50 values and 48-h EC50 values to increase by 1.3-, 1.4-fold and 1.6-, 1.7-fold, respectively, whereas river sediment HA and FA had no significant influence on the toxicity of TPhTCl at any experimental concentrations (0-40 mgC/L). As the results in Table 1 show, the soil FA appeared to slightly increase the toxicity of TPhTCl to *D. magna* (not significantly different from control, $p < 0.05$).

The results shown in Table 1 and 2 indicate that DHM-toxicant interactions can alter the toxicity of organotin compounds and the extent of alteration was dependent on the source of DHM and the concentration of DHM. In the present study, peat HA had a much greater influence on the toxicity of TBTCl and TPhTCl, followed by soil HA. River sediment HA

Table 2. Influence of DHMs on the toxicity of TPhTCl to *D. magna*

DHM type	DHM (mgC/L)	EC50 and 95% confidence limits (μg/L)	
		24-h	48-h
Control	0	30.4 (33.2 - 27.0)	10.2 (12.5 - 8.43)
Peat HA	0.5	32.7 (35.1 - 29.6)	11.1 (13.7 - 8.37)
	5.0	35.5 (39.3 - 32.7)	14.1 (16.6 - 11.7)
	20.0	43.1*(46.5 - 39.7)	18.3*(20.7 - 14.9)
	40.0	48.4*(52.6 - 44.7)	20.6*(23.9 - 17.1)
Sediment HA	0.5	32.1 (35.8 - 29.7)	10.4 (13.1 - 7.95)
	5.0	30.9 (34.4 - 28.3)	10.9 (13.6 - 8.65)
	20.0	33.6 (37.5 - 30.4)	12.0 (15.4 - 9.14)
	40.0	35.3 (39.4 - 32.7)	12.4 (15.9 - 9.39)
Sediment FA	0.5	30.7 (34.8 - 28.1)	10.7 (13.5 - 8.15)
	5.0	31.1 (35.4 - 29.7)	11.9 (14.6 - 8.55)
	20.0	31.8 (36.3 - 30.1)	10.1 (14.4 - 8.14)
	40.0	33.9 (37.8 - 30.7)	9.47 (13.7 - 8.31)
Soil HA	0.5	31.6 (34.2 - 26.4)	9.97 (13.2 - 8.18)
	5.0	34.7 (38.3 - 30.7)	12.3 (15.1 - 9.79)
	20.0	38.3*(41.9 - 35.3)	16.1*(19.1 - 13.8)
	40.0	41.9*(44.2 - 37.1)	17.8*(21.7 - 15.3)
Soil FA	0.5	29.4 (34.1 - 27.2)	10.7 (12.7 - 8.61)
	5.0	29.3 (33.6 - 26.7)	9.06 (11.2 - 7.67)
	20.0	28.1 (31.9 - 26.1)	8.63 (10.9 - 7.21)
	40.0	28.8 (32.3 - 26.4)	8.89 (11.7 - 6.95)

*: Significantly different ($p < 0.05$) from control (0 mgC/L of DHM)

and FA, and soil FA had no significant effect on the toxicity of TBTCl and TPhTCl to *D. magna*. These findings are consistent with the observations of Ortego and Benson (1992) who found that commercial HA had a much greater influence on the toxicity of pyrethroid insecticides than aquatic HA and FA. Garvey et al. (1991) also demonstrated that terrestrial HA affected the copper toxicity to alga to a greater extent than did aquatic HA and FA. It has been reported that commercial HA and soil HA are more hydrophobic with greater binding tendency than aquatic HA and FA (Malcolm and MacCarthy 1986). We determined the organic carbon contents of four types of DHMs used in this study. The results showed that the organic carbon contents of peat HA (60.3%) and soil HA (57.4%) are higher than that of river sediment HA (52.2%) and FA (44.3%) and soil FA (47.4%). It was probable that this difference in organic carbon content is responsible for the observed difference in their ability to interact with toxicants.

Two possible mechanisms may be responsible for the observed decrease in toxicity of TBTCl and TPhTCl in the presence of DHMs. First, there is some direct protection to the cells afforded to the presence of DHMs. Second, the adsorption or binding of toxicants to DHMs decreases the amount of compound available to interact with the biota and thus decreases the toxicity of the compound. It would be difficult to test the first hypothesis by experiment. It has been shown that the effect of DHMs on bioavailability of organic toxicants is a hydrophobic interaction (Versteeg and Shorter 1992), and a relationship between hydrophobicity ($\log K_{ow}$) and the effect of DHM has been demonstrated (Landrum et al. 1985). In general, DHM affects bioavailability of compounds with values of $\log K_{ow} > 3.0$. The $\log K_{ow}$ of TBTCl and TPhTCl are 3.8 and 4.1 (Laughlin et al. 1986; Thompson et al. 1985) respectively, therefore, the effect of peat HA and soil HA on the toxicity of TBTCl and TPhTCl was expected. However, further investigation on the bioavailability of the organotin compounds from the DHM-containing water and on the binding affinity of the organotin compounds to DHM is needed to make sure of the influence mechanisms.

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