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Effect of Tributyltin on Mortality and Telson Regeneration of Grass Shrimp, *Palaemonetes pugio*

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Due to its toxic action, tributyltin (TBT) has been used primarily as an antifouling paint in recreational and commercial water craft to prevent the settlement of fouling organisms. These toxic properties make TBT harmful to nontarget organisms (Hall et al. 1988). Alzieu and Heral (1983) first reported a connection between marinas and malformation of oyster shells. Alzieu (1986) conducted a study in Arcachon Bay in France and found that TBT from antifouling paint caused significant damage (poor growth and thickened shells) to the Pacific oyster, *Crassostrea gigas*. This author also reported that a ban on TBT paints drastically improved oyster production in that area over several years. Bryan et al. (1986) found that 20 ng/L TBT induced imposex in the dog-whelk, *Nucellus lapillus*.

The occurrence of TBT has been reported in waters of North America and England. Maguire et al. (1982) reported concentrations of TBT between 0.01 and 2.91 μg/L in unfiltered surface water from Canada. U'Ren (1983) found between 0.01 and 0.55 μg/L TBT in marine waters off southern California, and Beaumont and Budd (1984) found concentrations of TBT greater than 2 μg/L at marine sites in England. Maguire and Tkacz (1985) reported TBT concentrations of 0.01 - 0.20 μg/L in subsurface water and 0.08 - 0.13 mg/Kg in sediments of Toronto Harbor, Canada. Grass shrimp, *Palaemonetes pugio,* are found in shallow estuarine environments and ingest detritus and sediment particles. This study was designed to determine the toxicity of TBT on *P. pugio* larvae and adults and the effects of TBT on telson regeneration and molting of adult shrimp.

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Materials and Methods

Adult grass shrimp, *Palaemonetes pugio,* were collected from Big Sheepshead Creek, Tuckerton, New Jersey, by seine net during the summer of 1987. The shrimp were acclimated 2-3 weeks at room temperature in 20 ‰ (pers per thousand salinity) seawater (Instant Ocean, Carolina Biological Supply Company, Burlington, North Carolina) with a light : dark cycle of 14:10 hrs. (The range of water salinity in the creek is 20-25 ‰). The shrimp were fed Tetramin (Carolina Biological Supply Company, Burlington, North Carolina) twice daily during the acclimation period.

To determine the 96-h LC_{50} value of TBT on adults, shrimp were exposed to 0, 10, 25, 50, 75, and 100 µg/L TBT (Tri-n-butyltin oxide, M & T Chemicals, Woodbridge, New Jersey). TBT was first dissolved in acetone to provide a stock solution of 0.1 mg/ml. All treatments were diluted to appropriate test concentrations by mixing 20 ‰ seawater with a known stock solution of TBT. In each treatment, five shrimp were held in polycarbonate (Nalgene) trays containing 2 L of solution. Each treatment was replicated 5-10 times with control treatments received a comparable amount of acetone. A total of 25-50 shrimp were used for each concentration. Shrimp were not fed during the 96-h exposure period. The shrimp were checked daily, the dead individuals counted and removed, and the solutions changed. Mortality data were analyzed by Probit analysis (Huber 1984).

To determine the 96-h LC_{50} value for larval shrimp, 1-2 day old larvae were exposed to 0, 5, 10, 15, 50 µg/L TBT. In each treatment, ten larvae were held in polycarbonate (Nalgene) trays containing 1L of appropriate solution. Each treatment was replicated 4-5 times. A total of 40-50 larvae used for each concentration. To collect the larvae, gravid females were placed in individual hatching containers constructed of two nested polystyrene containers. Each inner container had the bottom removed and replaced with plastic screen. The container was covered with half of a plastic petri-dish in which holes had been punched to facilitate water circulation. The resulting chamber was held together with a rubber band. This construction allowed newly hatched larvae to fall through the screen and collect at the bottom of the container, preventing the females from cannibalizing them. The larval hatching containers were suspended in aquaria with 20 ‰ seawater. During TBT exposure, larvae were checked daily and dead individuals were removed. The larvae were unfed during the 96-h exposure period. The solutions were changed daily to maintain test concentrations. Data were analyzed by Probit analysis (Huber 1984).

To evaluate the effect of TBT on regeneration and molting of adult shrimp, telsons were amputated with a razor blade. Animals were exposed to 0, 0.1, 0.3, 0.5 and 5.0 µg/L TBT and telson regeneration measured after 7, 14 and 21 days post amputation using a stereo microscope with a calibrated ocular micrometer. Shrimp were fed Tetramin twice daily. Molting of the shrimp was also recorded daily. Test solutions were changed daily to maintain proper concentrations. A total of 25-28 shrimp were used for each concentration. The lengths of telson regeneration and molting were analyzed by one-way ANOVA (Zar 1984). In the ANOVA test a significant F value does not indicate which mean value differs significantly from another mean value. For this reason, Duncan's Multiple Range Test at the 5% probability level was conducted to separate significant means (Zar 1984).

Results and Discussion

Probit analysis gave 96-h LC_{50} values of 4.07 µg/L TBT for larvae (95% confidence limits of 2.58-6.52 µg/L) and 31.41 µg/L TBT for adult shrimp (95% confidence limits of 25.88-38.12 µg/L). These data indicate that the larvae were much more sensitive than the adults to TBT. Clark et al. (1987) reported that the 96-h LC_{50} for TBT for adult grass shrimp, *Palaemonetes pugio,* was 20 µg/L. Hall et al. (1988) reported 48-h and 72-h LC_{50} values for the estuarine zooplankter, *Eurytemora affinis, as* 2.2 and 0.6 µg/L of TBT, respectively. U'Ren (1983) reported 96-h LC_{50} values of 2.0 µg/L and 1.0 µg/L TBT for the harpacticoid copepod, *Nitocea spinipes,* and harpacticoid copepod, *Acartia tonsa,* respectively. The above studies (Clark et al. 1987, U'Ren 1983) were done in flow through systems, while the studies reported here were conducted in static renewal systems and would probably be less sensitive. Nevertheless, it appears that *P. pugio* is more resistant than *E. affinis, A. tonsa,* and *N. spinipes* to the acute toxicity of TBT, especially in the adult stage.

In the regeneration experiment, adult shrimp exposed to 5.0 µg/L TBT had 71 and 100% mortality by days 14 and 21, respectively. By the end of 7 days, the 0.5 µg/L TBT-exposed shrimp showed a

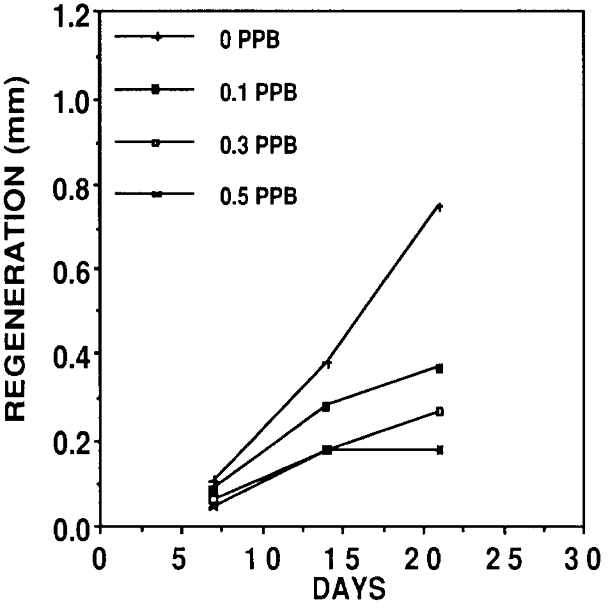

Figure. 1. Effect of TBT on telson regeneration of grass shrimp

significantly slower regeneration rate than the control shrimp (Fig. 1) (F= 4.62); however, neither the 0.1 or 0.3 µg/L TBT-exposed shrimp showed any significant difference from the control shrimp. By the end of 14 days, both the 0.1 and 0.3 µg/L TBT-exposed shrimp showed significant retardation in regeneration compared to control shrimp (F= 24.0). Weis et al. (1987) reported that 0.5 µg/L TBT retarded limb regeneration and caused a variety of deformities in fiddler crabs (*Uca pugilator)* in a static renewal system.

In the molting experiment, after 7 days exposure to 0.1 µg/L TBT, adult shrimp showed no molting retardation compared to control; however, exposure of shrimp to either 0.3 or 0.5 µg/L TBT caused molting retardation (Fig. 2) (F= 12.42). After 14 days, 0.1 µg/L TBT also caused molting retardation (F= 5.41). Likewise, Fingerman and Fingerman (1978) also reported that polychlorinated biphenyls retard molting in fiddler crabs. Grass shrimp, *Palaemonetes pugio* larvae were more sensitive to TBT than the adults.

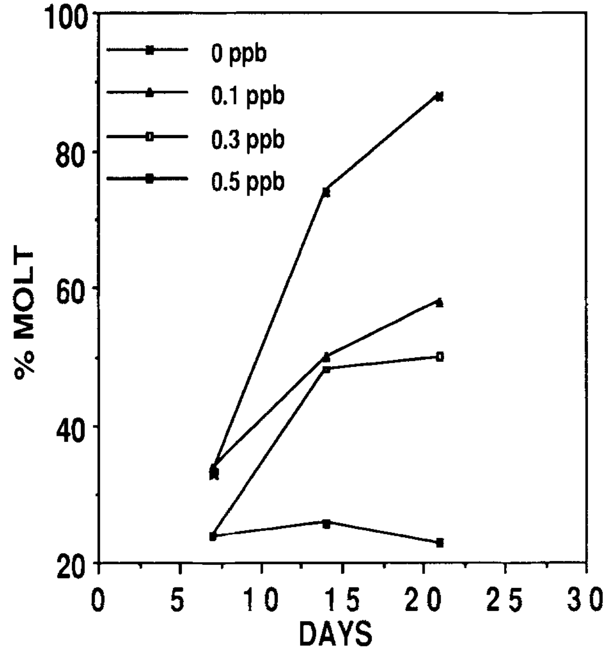

Figure. 2. Effect of TBT on molting of grass shrimp

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