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Environmental Contamination and Toxicology

Acute Toxicity of Tributyltin (TBT) to Early Life History Stages of the Hard Shell Clam, *Mercenaria mercenaria*

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Tributyltin compounds (TBT) are effective biocides in marine antifouling coatings, wood preservatives and disinfectants due to their high, non-specific toxicity. When used in antifouling paints, TBT is released into water, causing sublethal and acute toxicity to non-target organisms (Hall and Pinkney 1985). Significant reductions in spawning success, larval recruitment and postsettlement growth of the Pacific oyster, Crassostrea gigas, mainstay of French and English shellfisheries, occurs in the presence of TBT (Alzieu et al. 1980; Waldock and Thain 1983). Declines of the the dog whelk, Nucella lapillus, have also been attributed to exposure to TBT from antifouling paints in England. Interestingly, in the case of the dog whelk, mortality occurs secondarily as a result of a condition called 'imposex', the imposition of male sexual morphological characteristics on female snails. Generalizations from these and other studies suggest that molluscs are the most sensitive taxon to chronic, low-level exposure to TBT. Examples cited above have motivated examination of indigenous North American bivalve mollusc species. In experiments reported here, we compare the sensitivity of veligers and post-larvae of the clam, Mercenaria mercenaria to determine acute and sublethal responses of a commercially-important bivalve species during 8-day exposures. This interval spans the duration of larval development under the temperature regime used.

MATERIALS AND METHODS

Adult clams, Mercenaria mercenaria, collected from the Indian River Lagoon, Florida (USA), were induced to spawn in the laboratory by cyclic temperature exposures, first 20° C, followed by abrupt transfer to 30-32° C. Eggs from several females were pooled, then fertilized with sperm from 1 male.

Veligers were 24-48 h old at initiation of exposure to TBT. Approximately 100 veligers were placed into beakers containing 1 L 30 °/oo seawater. Isochrysis galbana Tahiti strain (40-160 x 10^5 cells/mL) was added as food. At each sampling period indicated in the figures, the entire contents of a beaker were collected by

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straining through a fine mesh (Nitex, 51 µm) and preserved in buffered seawater-formalin until counting. Every other day, veligers in remaining beakers were similarly strained, reintroduced to freshly prepared exposure solutions in clean glassware, and fresh microalgae added as food.

Clam postlarvae, used in a second set of experiments, were obtained from an experimental aquaculture project conducted by the Division of Applied Biology, Harbor Branch Oceanographic Institution. They were approximately 4-wk old and 1 mm in valve length at initiation of the experiment. Approximately 40 post larvae were placed into a 4-cm finger bowl covered with nylon mesh. There were three of these bowls per exposure. All were placed into a larger (20 cm) finger bowl containing 500 mL of 30 °/oo seawater to which TBT had been added. Water in the larger bowl was renewed by siphoning TBT-seawater solutions from a larger reservoir so that flow rates were ~2-L/day. Once each day, Isochrysis galbana were added as food for the post larvae. The 2 L/day flow rate was selected to renew toxicant solution while minimizing dilution of microalgal cells before they could be consumed by the postlarvae. Every third day, each small bowl containing clam postlarvae was examined to count living post-larvae and to remove dead ones. Bowls and mesh coverings were also wiped as necessary to remove slime.

Bis tri-n-butyltin oxide (Alpha Products, Danvers, Massachusetts) was the test toxicant in all experiments. It was first dissolved in acetone to make a series of stock solutions of sufficient strength so that addition of 10 µL/L seawater yielded desired concentrations. Exposure solutions in bioassays were analyzed for TBT by derivatization of butyltins with borohydride, purge and trap of volatile butyltin hydrides followed by detection and quantification by atomic absorption spectroscopy (Hodge et al. 1979; Valkirs et al. 1985; 1987).

RESULTS AND DISCUSSION

All clam post larvae in 10 µg/L were dead at the end of 25 days exposure to TBT (Fig. 1). Survival of remaining exposure groups was between 40 and 50%, typical of laboratory and hatchery survival rates of this species. Within this narrow range, it was not consistently exposure dependent. These data do not lend themselves to rigorous estimation of an LC50 value, but it falls within the range of 7.5 to 10 µg/L for exposure durations of 25 days. Experience with TBT suggests that a dose-dependent pattern would likely be exhibited during longer exposure periods (Laughlin et al. 1982). The one used here, 25 days, is long enough, however, to support the conclusion that postlarvae are not the most sensitive stage.

Compared to postlarvae, mortality of clam veligers, the planktonic larval stages, occurred at lower concentrations and after shorter duration exposure to TBT (Fig. 2). All veligers were dead by day 2 in 2.5, 5.0 and 7.5 µg/L. Those in 1 µg/L displayed very little mortality relative to controls until day 4. By day 7, all were

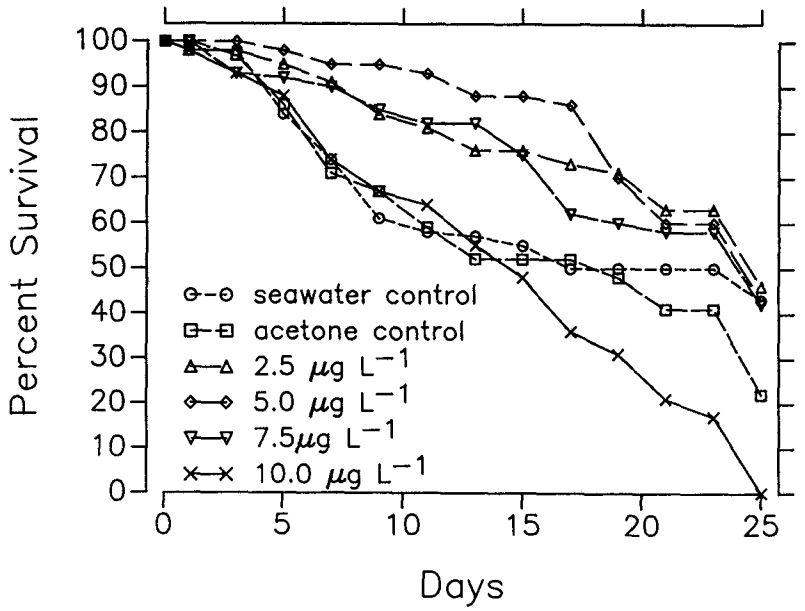

Figure 1. Survival of clam postlarvae, Mercenaria mercenaria, exposed to tributyltin.

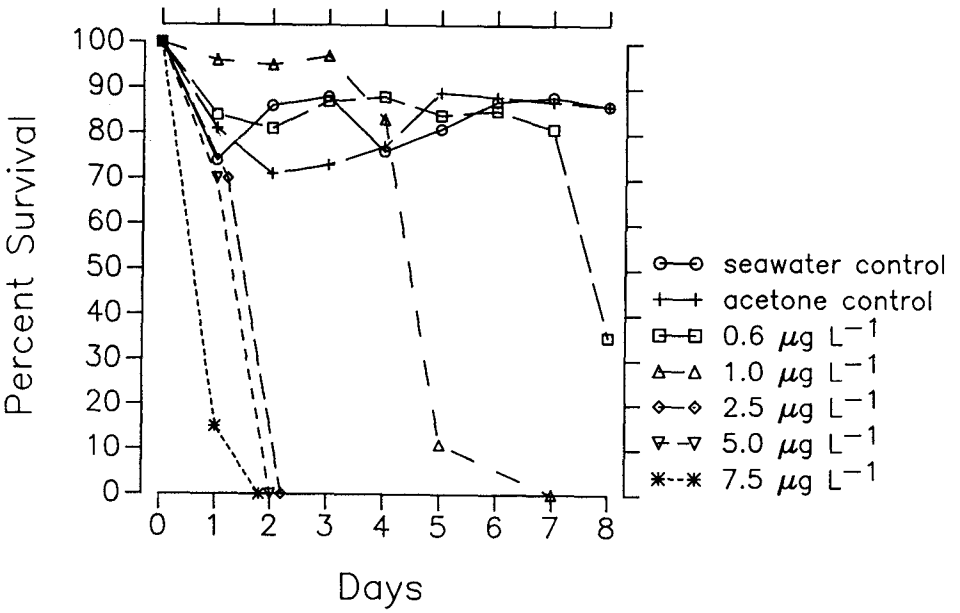

Figure 2. Survival of clam veligers, Mercenaria mercenaria exposed to tributyltin for 8 days.

dead. Veligers exposed to 0.6 µg/L had survival similar to controls until day 8, then it fell to 36%. Like data for post-larvae, those for veligers do not lend themselves to typical LC50 calculations because of lack of partial kills necessary for rigorous determinations of statistics. It is, nevertheless clear that the 48-hr LC50 value is between 1 and 2.5 µg/L. Roberts (1987) recently published a 48-hr LC50 estimate of 1.65 µg/L for M. mercenaria veligers from Virginia. Our value and his are consistent. We conclude from our data that an LC50 for the duration of larval development is less than 0.6 µg/L.

Highly variable and occasionally low survival of acetone controls was a characteristic of both postlarval and veliger bioassays. This situation has been repeatedly observed in a number of bioassays we have conducted. Addition of acetone or ethanol in excess of 100 µL per L seawater leads to microbial growth which fouls test containers and kills larvae outright. Reduction of carrier volume to 10 µL per L was not, apparently, sufficient to completely stop microbial induction by added carbon sources. That its effects were not observed in TBT solutions may be due to toxicity of the organotin compound to some bacteria. Other explanations of carrier control mortality are also plausible. Subsequent experience has suggested that use of an aqueous stock, if possible, is preferable to one with acetone, ethanol or acetate.

Growth of veligers was markedly reduced by exposure to TBT (Fig. 3). Valve length of controls increased from 101.2 ± 1.6 to 232 ± 8.8 µm (mean ± 1 standard deviation) in 8 days. Veligers exposed to 0.6 µg/L TBT, the only concentration with any survival for the duration of the experiment, increased their mean valve length to only 119 ± 4.7 µm (measurement on day 8). Given the standard deviation of mean valve length during the week of rearing, it is difficult to assume any significant growth occurred after the first day in veligers exposed to TBT.

Analysis of TBT in exposure solutions from experiments showed that measured concentrations were within 87% of nominal. After 24 hr, exposure solutions for the postlarval experiments were ~50% of nominal and those in the veliger experiments (static renewal) were 28% of nominal. Analysis of 24-hr solutions was performed without filtration or any other treatment to remove microalgae. In their presence, recovery of added TBT (0.120 µg/L) was only half that for oceanic seawater with virtually no suspended particulates. Thus our analyses report dissolved TBT and are likely to underestimate TBT bound to microalgae and potentially available by ingestion.

These experiments were motivated, in part, by a reported 96-hr LC50 value of 0.015 µg/L for M. mercenaria veligers (Becerra-Huencho, 1984). Our acute mortality data do not support such a low estimate. They are in excellent agreement, as noted above, with those of Roberts (1987).

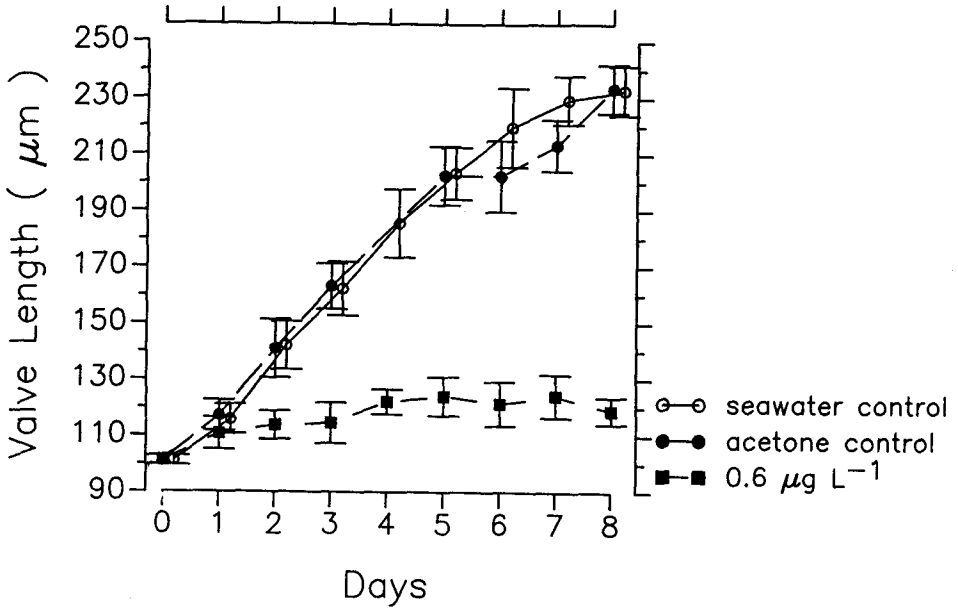

Figure 3. Cumulative growth of clam veligers, Mercenaria mercenaria exposed for 8 days to tributyltin.

These results clearly indicate that planktonic larval stages of Mercenaria mercenaria are the ones of greatest concern for effects of environmental exposure to TBT. Compared to other mollusc species such as Crassostrea gigas (Alzieu et al. 1980), Mytilus edulis (Beaumont and Budd 1984) or the dog whelk, Nucella lapillus (Bryan et al. 1986), M. mercenaria is relatively resistant to acute effects of TBT exposure. The complete interruption of growth, however, implies that death of veligers would likely result as a failure to metamorphose and recruit into benthic populations. This particular effect is beyond the scope of laboratory experiments, but should be critically examined in field studies.

Results of these studies are useful for comparisons of sensitivity of different life history stages of M. mercenaria and also for interspecific comparisons. It should be noted, however, that exposure to concentrations in habitat areas of clams, away from marinas and heavy boating traffic are approximately an order of magnitude lower than those tested here (Valkirs et. al. 1986; Grovhoug et al. 1986; Seligman et al. 1987; Unger et al. 1986; Hall et al. 1987). Experiments in progress will test sublethal responses during chronic exposures to 10-500 ng/L TBT, those more typical in estuarine habitats.

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