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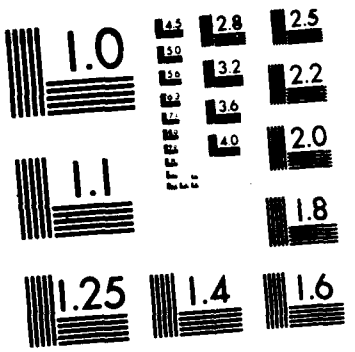

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EFFECTS OF TBT ON MARINE ORGANISMS:

FIELD ASSESSMENT OF A NEW SITE-SPECIFIC BIOASSAY SYSTEM

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ABSTRACT

A Portable Environmental Test System (PETS) was evaluated in San Diego Bay over a 7-month period using tributyltin (TBT) antifouling leachates. Three TBT concentrations ($\bar{x}$ = 0.065, 0.077 and 0.193 ug/l) were tested against seawater controls with three replicates of each using 340-l tanks. Unfiltered seawater was pumped over a TBT-coated panel, creating a TBT-leachate which was diluted with seawater in mixing bins and distributed to test tanks. There were no significant effects attributable to TBT on fouling communities (species abundance and biomass), mussel and clam condition index, mussel gonad index or oyster growth. TBT reduced juvenile mussel growth rate. Mussels and clams accumulated TBT at all test concentrations. Although mussels accumulated more TBT at higher test concentrations, there was an inverse relationship between dose and bioconcentration factor.

INTRODUCTION

Microcosms have been used to study a variety of physical, chemical and biological processes. The shortcomings of microcosms are primarily associated with adequately reproducing the components of whole systems and applying results to natural ecosystems (1). However, they combine some of the advantages of laboratory and field studies in a single experimental unit.

The flow-through microcosm facility at the Naval Ocean Systems Center (NOSC) Hawaii Laboratory described by Evans (2) has been used for several years to study the effects of pollutants on harbor organisms. Anticipating U. S. Navy Fleet implementation of organotin-based antifouling (AF) coatings, NOSC researchers used this facility to study the effects of tributyltin (TBT), the primary toxic component of organotin AF paints, on selected benthic organisms and fouling communities (3). A portable, flow-through version of that system was developed for site-specific bioassays with TBT and endemic species (4). After testing that system in Hawaii, a subsequent Portable Environmental Test System (PETS) was developed and evaluated in San Diego with the TBT experiments described below.

The purpose of the San Diego Bay study was to 1) obtain TBT bioassay data at sub-part-per-billion concentrations on bivalves of economic and recreational value and epifaunal organisms and 2) test the efficacy of the PETS. The results of the TBT study, the system evaluation and suggestions for improving PETS are presented here.

METHODS

The PETS study was conducted at the end of a pier at the Naval Amphibious Base, Coronado, California (Figure 1). The system consisted of the following: 1) a seawater intake, 2) a receiving tank, 3) the leachate tank, 4) two sets of mixing bins, 5) twelve 340-l flow-through polyethylene test tanks with aeration, and 6) a van modified for power and laboratory space (Figure 2). The tanks were shaded with a 70% sunscreen to reduce some adverse effects of direct sunlight. Two intake pumps were situated on a floating dock approximately 30 meters from the test tanks. Unfiltered seawater was pumped from a depth of 2 meters to the elevated receiving tank. The TBT-dosed seawater (leachate) was produced in the leachate tank by circulating aerated ambient seawater around a plexiglas panel, coated with a self-polishing, copolymer organotin AF paint (International Paint Co., BFA 956 Pink SPC-9 HiSol). Flow rate and AF paint surface area were adjusted to yield a leachate concentration of ~0.2 ug/l. Unfiltered seawater and TBT leachates were distributed by gravity flow.

Treatment solutions were generated in the mixing bins by combining different volumes of unfiltered seawater and leachate water. The treatments were 100, 25 and 10% leachate solutions, representing nominal TBT concentrations of 0.200, 0.050 and 0.020 ug/l, respectively. Unfiltered ambient seawater was used for the controls. Flow rate to each tank was ~3 l/min.

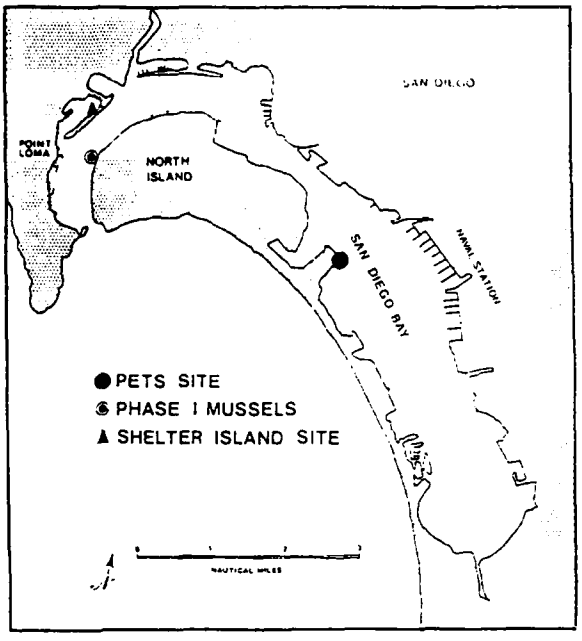

Figure 1. San Diego Bay site locations: PETS, Shelter Island and Phase I mussel collection.

The PETS experiments were performed in two phases. Phase I was conducted for 110 days (May to Sep 1986) and examined TBT effects on fouling communities, adult mussels, scallops, clams, and juvenile mussels. Phase II was conducted for 56 days (Oct to Dec 1986) and examined TBT effects on juvenile mussels and four species of juvenile oysters. System modifications were made between Phases I and II. All tests included a Tank Control and three TBT treatments with three replicates of each. To evaluate tank effects, animals were suspended in the bay immediately adjacent to the seawater intake. This Pier Control included adult mussels in Phase I and juvenile mussels and oysters in Phase II. During Phase I adult mussels were also suspended at a TBT-contaminated site in a Shelter Island marina (Figure 1). This allowed a comparison between TBT effects on mussels under naturally stressful conditions and those artificially treated in PETS. Plastic holding trays for mussels, clams and oysters were leached for at least 2 weeks in clean seawater.

Seawater samples for TBT analysis were collected in 500-ml polycarbonate bottles weekly from test tanks and less frequently from the seawater intake, Shelter Island site and the leachate tank. These samples were analyzed immediately or frozen and stored. TBT measurements were made by hydride derivatization and atomic absorption detection (5).

Temperature, pH, conductivity and dissolved oxygen were measured twice per week in each tank and at the seawater intake. Similar measurements were taken hourly over a 24-hour period to determine daily fluctuations. Salinity was calculated from conductivity and temperature data.

Phase I: Fouling Study

The effects of TBT on newly developing and established fouling communities were assessed by censusing plexiglas panels for attached organisms (4). Unfouled panels (three replicate panels/tank) provided substrate for newly developing communities in PETS. Prefouled panels provided established fouling communities in PETS (three replicate panels/tank). These communities were established by suspending panels under the floating dock at the test site for 126 days before the experiment.

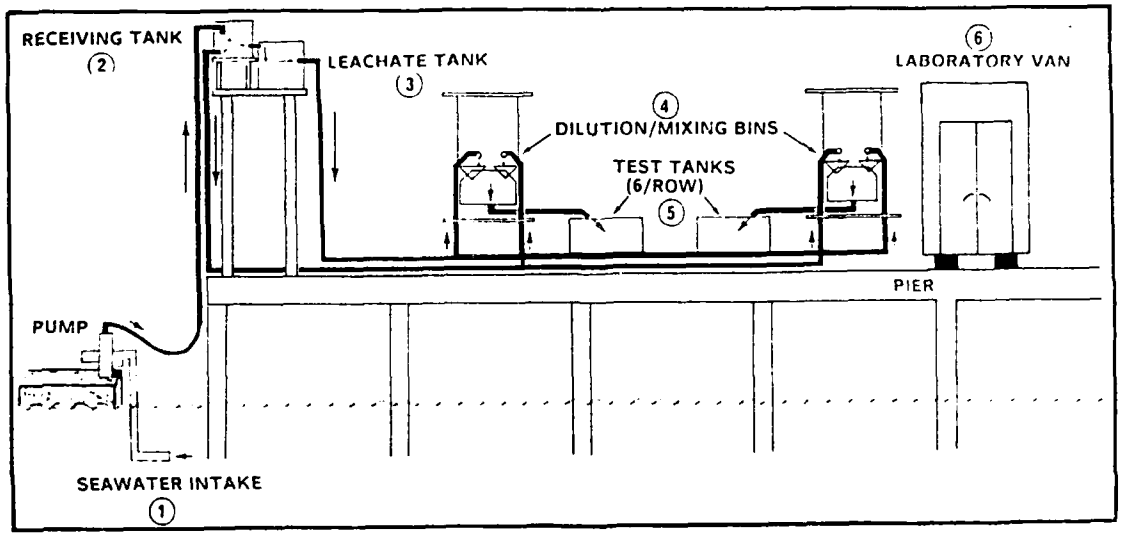

Figure 2. PETS Schematic.

Every 2 weeks total settlement on the unfouled panels was documented using an underwater camera. Species counts were made from the projected slides. Thick tunicate growth on the prefouled panels precluded photographic analysis. Therefore, species counts were made directly from the panels. Biomass of attached organisms was also measured for each panel. Multiple one-way analyses of variance (ANOVA) ($P < 0.05$) were used to test for differences in abundance and biomass among controls and treatments at each time interval.

Phase I: Bivalve Tests

The Phase I bivalve tests examined the effects of TBT on mussels (Mytilus edulis), clams (Macoma nasuta) and scallops (Hinnites multirugosus). Adult mussels and clams were monitored for TBT bioaccumulation and condition index; gonad indices were measured in mussels only. Animals (9 mussels and 10 clams) were collected from each tank, the Pier Control (mussels only) and Shelter Island (mussels only) every two weeks for these measurements. Scallop tissues were collected but were not analyzed for TBT accumulation. Lengths and weights of juvenile mussels (M. edulis) were measured weekly as a growth estimate. These methods are discussed elsewhere (6).

Eighty-three adult mussels (40-60 mm in length) were held in plastic mesh trays suspended in the test tanks. Mussels at the Shelter Island marina were suspended 0.5 m below a floating dock in a plastic mesh bag. Ten clams (20-60 mm in length) were placed in plastic tubs containing 1.5 liters of 1 mm-sieved sediment collected from an area adjacent to the test site. Ten tubs were placed on the bottom of each test tank.

Mussel and clam tissues were frozen immediately after collection for TBT analysis. For each sampling interval tissues of replicates from each tank were pooled to obtain sufficient biomass. Tissue analyses were made on mussels collected from the Shelter Island, Pier Control, Tank Control and 100% leachate exposures and clams collected from the Tank Control and 100% leachate exposures. The tissues were extracted in methylene chloride/HCl and rinsed with NaOH to remove mono- and dialkyltin species. TBT measurements were made with graphite furnace atomic absorption detection using a matrix modification (7).

Condition indices have been used to measure the relative health of experimental bivalves (8, 9, 10, 11). The index described by Baird (9) and Galtsoff (12) was modified and the ratio of total soft tissues (g wet weight) to internal shell volume (ml) was used. A wet weight method was necessary because tissues were used for index measurements and then bioaccumulation. Dry weight methods would have affected the subsequent TBT analyses. Gonad indices provide a measure of developing gametes for experimental individuals (13). The index used was the ratio of mantle (g wet weight) to total soft tissues (g wet weight) (14).

Two null hypotheses were tested by one-way ANOVAs ($P < 0.05$): 1) H_o = Exposure to TBT has no effect on the condition index of mussels or clams. 2) H_o = Exposure to TBT has no effect on the gonad index of mussels. If the H_o was rejected, a Duncan's multiple range test determined at which TBT concentrations the significant differences occurred.

System Modifications

The system was modified between Phases I and II to solve some of the problems encountered in Phase I. After draining the tanks, attached biota and accumulated sediment were removed. Flow rates were increased and diluters were adjusted to bring actual TBT concentrations closer to nominal. The total biomass/tank was markedly reduced at the beginning of Phase II.

Phase II: Juvenile Bivalves

The Phase II study monitored growth in juvenile mussels and oysters. The juvenile mussel portion of this study is reported elsewhere (6). The oyster species used were Crassostrea gigas, Crassostrea virginica, Ostrea edulis, and Ostrea lurida. Oysters were selected because of concern over potential TBT effects on the oyster industry. Although O. lurida is not commercially cultured, it was used because it is the only endemic oyster species found in San Diego Bay.

The initial weights of oysters were: C. gigas - 150 to 300 mg; C. virginica - 96 to 1256 mg; O. edulis - 140 to 280 mg; and O. lurida - 100 to 300 mg. All juvenile bivalves were maintained in each tank and at the Pier Control in partitioned, plastic mesh trays. Each tray contained 18 individuals of a given species, except C. virginica, with 15 individuals per tray. Whole-animal wet weights were measured weekly as an estimate of growth.

Only data for animals surviving at the end of the study were used in the statistical analyses. For each species at each treatment weekly mean and cumulative percent increases in weight were determined to normalize size effects and for graphical representation. ANOVAs ($P < 0.05$) were performed on weight data at each sampling interval to test the null hypothesis, H_o = Exposure to TBT has no effect on growth of test organisms. If the H_o was rejected, a Duncan's multiple-range test determined at which TBT concentrations significant differences occurred.

RESULTS

TBT Concentrations and Water Quality

Overall mean TBT concentrations were 0.193, 0.077 and 0.065 ug/l for the 100%, 25% and 10% leachate treatments, respectively (Figure 3, Table 1). Mean TBT concentrations in Phase I were 0.204, 0.092 and 0.079 ug/l for the respective treatments. Mean TBT concentrations in Phase II were 0.157, 0.051 and 0.038 ug/l TBT for the respective treatments. Overall mean TBT concentration in seawater at the intake and in control tanks was 0.009 ug/l; Phase I and Phase II averaged 0.006 and 0.010 ug/l TBT, respectively (Table 1). The mean TBT concentration measured at the Shelter Island site was 0.452 ug/l (±0.247).

TBT concentrations in the leachate and 100% treatment tanks fluctuated markedly during the experiment. Measured variability in TBT concentrations was high among replicates. There was very little separation between TBT concentrations in the 10 and 25% dilutions. Instead of differing by a factor of 2.5, these dilutions only differed by a factor of 1.2.

All physical parameters measured were reasonably constant except temperature. Mean salinity, dissolved oxygen and pH values for Phases I and II, respectively, were: salinity - 36.4 and 35.7 ppt; Dissolved oxygen - 7.3 and 7.6 ml/l; pH - 7.6 and 7.7. There were large variations in temperature from day to day and diurnally. Temperature ranged from 19.5 to 25.9°C in Phase I and from 15.0 to 21.7°C in Phase II. The 24-hour study showed daily temperature ranging from 13.5 to 16.9°C in the tanks but only 15.0 to 16.0°C in the bay.

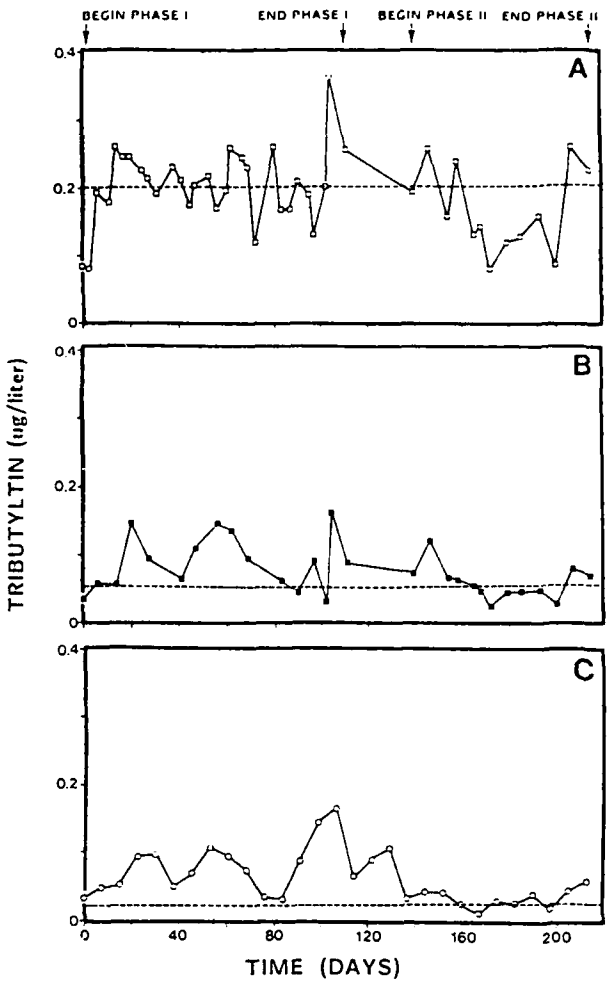

Figure 3. TBT leachate treatments: A - 100%, B - 25%, C - 10%; (———) measured TBT concentration, (- - -) nominal TBT concentration.

Table 1. Mean measured TBT concentrations (ug/l) by treatment, tank and Phase.

			Overall Concentrations (16 May - 16 Dec 1986)		Phase I Concentrations (16 May - 4 Sep 1986)		Phase II Concentrations (21 Oct - 16 Dec 1986)	
		Tank	$\dot{X}$ (±S. D.)	$\bar{\dot{X}}$ (±S. D.)	$\dot{X}$ (±S. D.)	$\bar{\dot{X}}$ (±S. D.)	$\dot{X}$ (±S. D.)	$\bar{\dot{X}}$ (±S. D.)
TREATMENT	100%	1	0.181 (±0.074)	0.193 (±0.071)	0.189 (±0.077)	0.204 (±0.070)	0.161 (±0.068)	0.157 (±0.066)
		11	0.203 (±0.074)		0.212 (±0.074)		0.166 (±0.076)	
		12	0.194 (±0.059)		0.212 (±0.055)		0.143 (±0.049)	
	25%	3	0.073 (±0.040)	0.077 (±0.040)	0.086 (±0.046)	0.092 (±0.044)	0.050 (±0.016)	0.051 (±0.019)
		8	0.074 (±0.040)		0.096 (±0.045)		0.048 (±0.015)	
		10	0.082 (±0.038)		0.098 (±0.041)		0.056 (±0.023)	
	10%	4	0.065 (±0.036)	0.065 (±0.039)	0.076 (±0.036)	0.079 (±0.041)	0.038 (±0.012)	0.038 (±0.015)
		6	0.066 (±0.042)		0.079 (±0.048)		0.041 (±0.017)	
		7	0.063 (±0.038)		0.083 (±0.040)		0.035 (±0.014)	
	CON	2	0.007 (±0.008)	0.009 (±0.007)	*	0.006 (±0.006)	0.011 (±0.008)	0.010 (±0.007)
		5	0.006 (±0.007)		*		0.010 (±0.007)	
		9	0.008 (±0.009)		*		0.010 (±0.005)	

* = Samples pooled from Tanks 2, 5 and 9 to yield one result.

Fouling Study

Prefouled Panels. At the start of the test, prefouled panels were primarily covered with solitary tunicates. Also present were mussels, arthropods and sponges. After placement in the test tanks, the tunicates began to slough off, and by day 60 nearly 75% of the total panel area was unfouled. They were partially recolonized by sponges, anemones, tunicates, worms, mussels and arthropods.

Unfouled Panels. Settlement on the unfouled panels was slow. After 95 days in test tanks, there was relatively little fouling. Approximately 20% of the total panel area was colonized by tube worms, tube-building amphipods, bryzoans, an unidentified turbellarian or flatworm, limpets and gastropod egg masses.

Statistical analyses of the fouling data indicate no significant difference between controls and treatments in abundance of species or biomass of attached organisms. Variability among replicate tanks was high.

Bioaccumulation

Mussels and clams exposed to TBT accumulated increasing amounts of TBT in their tissues for 60 days, after which TBT body burdens appeared to stabilize and approach a threshold (Figure 4). Control mussels and clams maintained nearly constant body burdens at 0.42 and 0.22 ug TBT/g tissue, respectively.

Mussels accumulated more TBT than clams. The amount of TBT measured in mussel tissues was proportional to the exposure concentration. The average maximum body burdens for mussels during the threshold period (day 60 to 110) were 10.38, 5.40 and 2.96 ug TBT/g tissue for the Shelter Island (0.452 ug/l TBT), 0.204 and 0.079 ug/l TBT exposures, respectively. Mean concentration in clam tissues at the 0.204 ug/l TBT exposure was 2.13 ug TBT/g tissue. For the same three treatments, mussel bioconcentration factors (BCF's), were 23000, 26500 and 37500. The BCF for clams was 10400. BCF's for control mussels and clams were 70000 and 36700, respectively.

Bivalve Indices

Condition and gonad indices decreased over time for all control and treatment mussels (Figure 5). Pier Control mussels had consistently higher indices than Tank Control mussels. Condition indices in the 100% treatment were significantly lower than Tank Controls on days 31, 47 and 80. Gonad indices for the same treatments were significantly lower on days 47 and 95.

None of the measurements indicated that TBT affected clams. Clams from all test tanks exhibited high mortalities and highly variable condition indices which decreased with time for all controls and treatments. No significant differences were found.

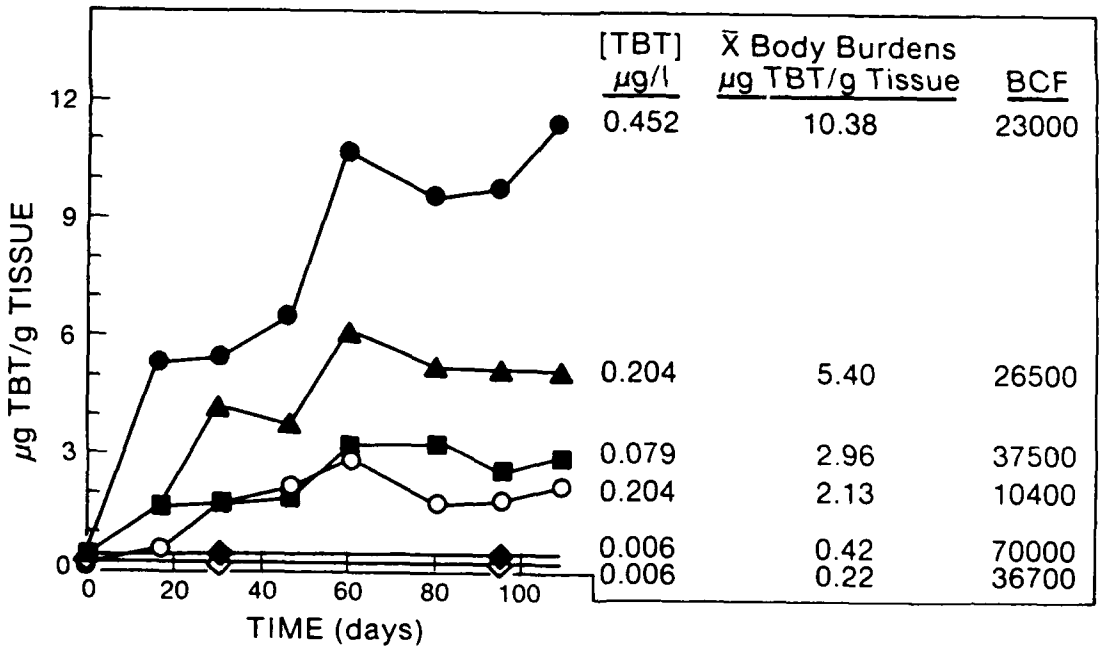

Figure 4. TBT bioaccumulation (ug TBT/g tissue): (●) Shelter Island mussels; (▲) 100% leachate mussels; (■) 10% leachate mussels; (○) 100% leachate clams; (♦) Control mussels; (◊) Control clams.

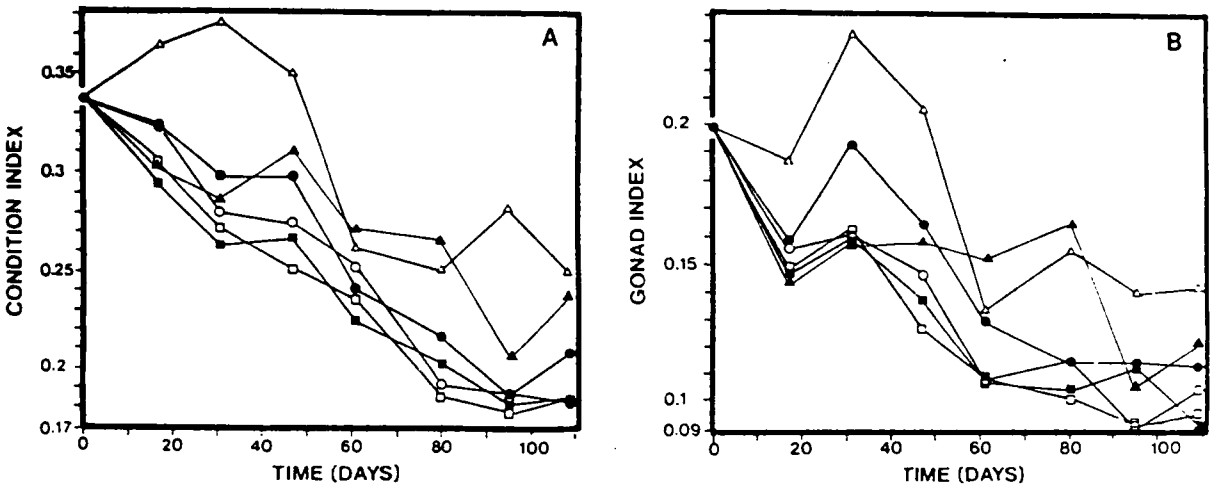

Figure 5. TBT effects on adult mussel condition index (A) and gonad index (B); (△) Pier Control (●) Tank Control; (▲) Shelter Island; (□) 100% leachate; (■) 25% leachate; (○) 10% leachate.

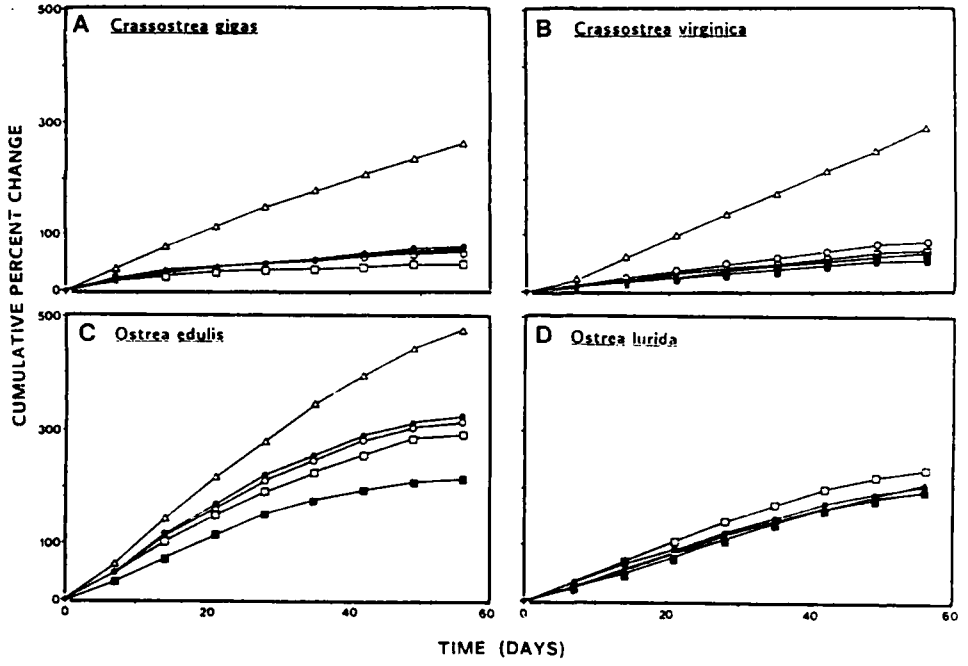

Figure 6. TBT effects on juvenile oyster growth: (△) Pier Control; (●) Tank Control; (□) 100% leachate; (■) 25% leachate; (○) 10% leachate.

Phase II: Juvenile Bivalve Growth Study - Oysters

Cumulative percent increases in weights for all species of oysters are presented in Figure 6. Except for O. lurida, growth of the Pier Controls was significantly greater than growth in any Tank Control or treatment. The difference was most pronounced in C. virginica which grew five times faster than tank-held animals. TBT did not significantly affect oyster growth when treatments were compared to Tank Controls.

DISCUSSION

The prototype site-specific microcosm system evaluated in San Diego Bay is probably more environmentally realistic than most laboratory tests even though conditions within our system did not duplicate the surrounding bay waters. Even at the highest TBT concentrations used in PETS, there were no significant effects attributable to TBT on fouling communities (species abundance and biomass), mussel and clam condition index, mussel gonad index or oyster growth. However, high variability within and among replicates in TBT concentrations, temperature, and available light may have masked TBT effects. In many cases statistical results indicated tank effects were high enough to obscure TBT effects. For these reasons the biological results of the TBT studies must be interpreted with caution. The only portion of this test which showed adverse TBT effects was the juvenile mussel growth study. However, those effects may have been overestimated because the animals were under significant system stress (6).

Biological Effects

The paucity of settlement on all fouling panels suggests that not all larvae passed through the system to the test tanks, or that they were quickly filtered from suspension by the animals in the tanks. Although not quantified as part of the fouling study, the presence of epifauna in the seawater distribution lines, on tank walls and on test containers indicates that some larvae were able to settle. Settlement of O. lurida, M. edulis, and Musculista senhousia, occurred at concentrations shown to be highly toxic in laboratory studies (15). This suggests that wild larvae of these species may not be as sensitive to TBT as laboratory-reared individuals or that laboratory studies are not realistic indicators of TBT toxicity. Further, TBT, the molluscicide developed to control freshwater snails, had no apparent effect on the marine snail Navanax inermiss. It settled, grew, and laid eggs in all tanks. The effects of TBT on the survival and development of these eggs were not monitored.

In general, mussel condition and gonad indices decreased over the entire test period. It is not clear whether this decrease is part of the natural cycle for mussels in San Diego Bay, or if it can be attributed to stress in the test tanks. The decrease in condition index between May and September is in general agreement with that observed by others (16, 17, 18). Condition indices for the Pier Controls declined similarly after an initial 30-day increase. However, Pier Control condition indices were always greater than those for tank-held animals. Although gonad indices also decreased over time, there were always some individuals that appeared to have mature gametes at each sampling period. No apparent differences in gamete development were observed at the TBT concentrations tested.

The Phase II juvenile bivalve growth study should have been more realistic and informative than Phase I studies because flow rate and stability of TBT concentrations were improved and total biomass for Phase II was reduced. Growth results and temperature measurements suggest Phase I animals were under temperature and nutritive stress. Bayne et al. (13) have described the effects of temperature and nutritive stress on reducing growth rate in M. edulis. The reduced biomass and increased flow rates in Phase II experiments should have eliminated these problems. However, bivalve growth indicates that system modifications and improvements were insufficient to provide growth conditions equivalent to surrounding bay waters. For all oyster species except O. lurida, Pier Control animals grew considerably faster than Tank Controls. M. edulis grew four times faster in the bay than in test tanks (6). This strongly suggests that all of our test animals, including Tank Controls, were stressed by the test system.

Growth of O. edulis Tank Controls was similar to that reported in a laboratory study (19). However, in that study growth of 3 mm juvenile O. edulis was markedly reduced at 0.060 ug/l TBT after 20 days. In contrast, no significant reductions in growth of 10 mm (0.200 g) juvenile O. edulis exposed to TBT concentrations as high as 0.157 ug/l for 56 days were found in the PETS study. Thain and Waldock indicated no reductions in growth of 5 g O. edulis exposed to 0.24 ug/l TBT for 45 days while growth of 2.5 g C. gigas under similar conditions was significantly reduced. In the PETS study juvenile (~15 mm, 0.211 g) C. gigas were not affected by 0.157 ug/l TBT after 56 days of exposure.

There are several possible explanations for these differences in results. Thain and Waldock (19) suggest that the sensitivity of juvenile bivalves is size dependent. Since juvenile oysters used in PETS were larger, they might be expected to be more resistant to TBT. Further, if test animals in the laboratory study were under more stress, greater sensitivity might be expected. Differences in TBT effects could be attributed to differences in bioavailability between the laboratory and PETS microcosm (20). Although no statistically significant differences in growth attributable to TBT were found for any oyster species, tank variability may have precluded detecting such differences. Therefore, the PETS study may not have been sensitive enough to detect differences at these low concentrations either.

TBT tissue values show that both mussels and clams accumulated significant amounts of TBT from bay and experimental environments and approached constant TBT tissue burdens after 60 days of exposure. This could be attributed to either approaching an equilibrium condition or metabolic decreases associated with stress from containment and TBT. Although mussels accumulated more TBT at higher concentrations, the BCF decreased with increasing TBT concentration.

Other investigators have found a similar inverse relationship with TBT concentration and BCF for O. edulis and C. gigas in the laboratory (21) and C. virginica in the NOSC Hawaii microcosm (22). Laughlin et al. (23) suggest that a calculated BCF of ~5,000 is about an order of magnitude above what can be predicted from model compounds and octanol-water partitioning coefficients. The BCF calculated for animals held at Shelter Island and in 100% leachate test tanks was about 25,000. This value is nearly 50 times higher than predicted. However, Laughlin et al. indicate that laboratory-determined values are not reliable measures of the environmental bioconcentration process.

Bioavailability may be correlated with suspended particulates, which were higher in Shelter Island. Differences in the BCF were expected between Shelter Island mussels and those in test tanks because of sediment losses in the plumbing. However, bioaccumulation was similar for both groups of mussels. Bioavailability may have been similar between 100% leachate tanks and Shelter Island because only a small portion of TBT was associated with particulates in Shelter Island as suggested by Valkirs et al. (24). The environmental significance of the BCF remains unclear.

System Assessment

In theory, a microcosm system combines the advantages of controlled laboratory dosing with realistic field conditions. This permits meaningful environmental studies to be conducted over extended periods of time. Although not truly portable, the PETS design facilitates deployment in a small area at almost any location. The main improvement over the Hawaii prototype was removing the leachate panels from individual tanks and using a primary leachate tank with a dilution and distribution system. This solved two problems: possible direct contact between test animals and leachate panels, and differential leaching from individual panels. Although there is variability in a leachate-dosing system, the authors believe this is the best available system for long-term, flow-through tests.

Improvements made between Phases I and II resulted in concentrations closer to nominal for all but the highest concentration. At the highest test concentration, TBT values varied by almost a factor of two over the entire test period and during the 24-hour sampling period. This variability was also observed in a 66-day flow-through laboratory test (25) and is characteristic of a TBT leachate dosing system. Even more variability was observed in the field, where TBT concentrations near marinas fluctuated by more than a factor of 20 during a tidal cycle (26). It is not clear how this type of variation affects the biota compared to a stable concentration with a similar mean.

The arrangement of tanks appeared to affect test tank conditions. Tanks were placed in two rows of six tanks each, with the rows approximately 1 meter apart. Even though a 70% sunscreen was provided, some tanks still received more light than others. This resulted in significant differences in temperature among the four end tanks and the inside eight and added to the temperature stress problem. This also influenced the density of algae on the surface and sides of the tanks. Davis et al. (27) have suggested a circular distribution of tanks to help eliminate some of these problems.

Numerous authors have stressed the need for field validation of microcosm experiments (28, 29, 30, 31, 32, 33, 34, 35); fewer have actually done so (28, 34, 36, 37). Considering the marked differences between Pier and Tank controls in PETS, the authors feel field controls are absolutely necessary in the validation of site-specific bioassays.

Suggestions for improving this particular system are as follows: 1) Increase the flow rate to provide seawater containing its complete particulate load to reduce tank effects; 2) Configure the test system and utilize shading to minimize effects of atmospheric conditions; 3) Always include a field control to verify that the measured biological parameters are responding as they would in the field.

The results of this microcosm study are helpful in assessing the fate and effect of TBT from organotin AF coatings. Under site-specific microcosm conditions, juvenile mussel growth rates were shown to be affected by TBT stress (6). The bioaccumulation study confirmed that TBT is accumulated by mussels and clams. TBT accumulation by mussels is directly proportional while the BCF is inversely proportional to TBT concentration. Results from the other portions of this study were less tangible and less useful for assessing TBT effects. The absence of measurable biological effects associated with TBT exposure could be interpreted to mean that there would be no effects in nature or that the measurements were too insensitive given the variability of the test system. Microcosms can be effective assessment tools in environmental research only if the investigator is aware of their limitations and is prudent in the application of results.

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