EFFECTS OF ORGANOTIN ANTIFOULING PAINT LEACHATES ON PEARL HARBOR ORGANISMS:
A SITE SPECIFIC FLOWTHROUGH BIOASSAY

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ABSTRACT

Tributyltin (TBT) concentrations in the range of 0.04 to 2.5 $\mu\text{g/l}$ were used for treatments on marine communities at Pearl Harbor, Hawaii. Over an exposure phase of three months and a recovery phase of two months, significant effects were noted on many species of fouling organisms. Four of the six most abundant fouling taxa sustained 100% mortality at 0.5 $\mu\text{g/l}$ TBT. Overall species abundances of fouling organisms were reduced by 50, 60 and 80% by TBT treatments of 0.5, 1.8 and 2.5 $\mu\text{g/l}$, respectively. TBT concentrations of 0.1 $\mu\text{g/l}$ and higher caused significant reductions in numbers of species and species diversity of organisms that settled on virgin panel surfaces. Significant mortality of oysters (*Crassostrea virginica*) was observed only in 2.5 $\mu\text{g/l}$ treatments. However, condition indices showed that general health of oysters is markedly reduced by TBT concentrations of 0.1 $\mu\text{g/l}$ and higher.

INTRODUCTION

Long-term flow-through experiments were conducted from 1980 to 1983 at the flow-through marine microcosm facility of Naval Ocean Systems Center (NOSC) at Mokapu, Oahu, Hawaii to evaluate the effects of organotin antifouling leachates on inshore bottom communities. In those experiments, exposure to 0.5 to 1.8 $\mu\text{g/l}$ tributyltin (TBT) resulted in decreased density for several species and groups of biota¹. Seawater and organisms used in the microcosm experiments were obtained from an unpolluted, open-coast shoreline site. There is a possibility that leachate effects might be considerably less in typical harbor waters where organisms dwell in relatively high levels of pollutants, organics, and suspended particulates. Therefore, we decided to perform flowthrough bioassay experiments at a harbor locale using water and organisms recruited from that site. This report describes those experiments accomplished in 1984 at Ford Island, Pearl Harbor, Hawaii.

METHODS

Experimental System

This bioassay experiment was performed at the southwest end of Ford Island approximately 1 km downcurrent of Southeast Loch, the portion of Pearl Harbor most heavily polluted by industrial wastes and most extensively used for ship moorage.

The bioassay tank and plumbing array used is a prototype of a Portable Environmental Test System (PETS) being developed under U.S. Navy funding to develop the capability to accomplish flow-through bioassays at specific harbor locales.

The operational core of the PETS consists of 18 unshaded 155-l polyethylene tanks. Twin fiber-glass pumps each provide 230 l/minute of seawater flow to a polyethylene 380-l receiving tank which supplies water to 5.1-cm (2-in) diameter feed pipes laying across the tops of the bioassay tanks. Pressure head of water in the feed pipes is reduced to a constant low level by by open, upward-facing overflow elbows at the ends of each pipe. An open elbow of 2-cm (0.75-in) diameter pipe taps off the feed pipe over each bioassay tank. Each tank-feed elbow is pivoted to adjust its overflow height and flow of water. A flow rate of 4 l/min was used to produce an average bioassay tank water residence time of about 40 minutes. Pumped seawater is drawn from about 1-m depth and is filtered only through a coarse screen with 1-cm holes. All seawater plumbing is made of polyvinyl chloride (PVC) plastic.

Surface water temperatures in Pearl Harbor vary between 25° to 28°C (76° to 82°F) during summer months, and salinities in the vicinity of south Ford Island range from 33 to 36 ppt. Harbor waters are generally very turbid and green due to rich plankton populations consisting largely of diatoms (algae), copepods (microcrustaceans), and chaetognaths (arrow worms). Abundance of plankton in turn supports dense populations of fouling and bottom-living organisms².

Experimental Design and Analytical Techniques

International Paint Co. formulation BFA 956 Pink SPC-9 HiSol paint was used as a source of treatment leachate for this experiment. Composition of SPC-9 is listed as 9.4% tributyltin methacrylate (as part of a polymer), 0.5% bis(tri-n-butyltin) oxide, 44.7% cuprous oxide, and 45.4% inert ingredients. The paint was applied by a roller in two moderately thick coats onto plexiglass panels which were then leached for 4 months in flowthrough tanks. When in the experimental tanks, painted panels were tilted against the west and east tank walls with painted sides facing toward the tank centers.

All organisms tested in this bioassay experiment were obtained from Pearl Harbor waters. About 30 species of larger epifauna typical of shallow-water harbor fouling communities were obtained through natural settlement and growth on 72 roughened 20-cm by 25-cm plexiglass panels which were suspended 0.5 m deep in water near the bioassay facility water intake. Panels were allowed 14 weeks of field colonization and then were transferred to the bioassay tanks and allowed an additional 5 weeks of colonization and tank adaptation. Four pre-fouled panels were tilted at a gentle angle against the inside walls of each bioassay tank and panel backsides were used for monitoring changes in the fouling communities.

Changes in areal coverage of fouling panel organisms were quantified by taking 35-mm color photo slides of an 8.5-cm by 12.5-cm area in the center back side of each panel at weekly or biweekly intervals during the experiment. Panels were photographed with an underwater camera equipped with a 3:1 closeup lens, framer, and strobe flash. Slides were later projected onto a table-top dot grid to determine percent coverage of recognizable taxa.

Data for the most abundant taxa from the fouling panels in the various treatments were plotted as mean percent cover versus days of exposure. Also plotted were time series for total number of faunal species and species diversity per treatment. The diversity index used is the Shannon-Weiner diversity index³ which is a combined measure of number of species and "evenness" of number of individuals per species. High index values indicate more uniform distribution between the component species. The index equation used is a version that has been modified by the Botany Department of the University of Hawaii for use with smaller sample sizes.

Arcsine transformation⁴ was applied to percent coverage data for individual species on pre-fouled panels. One-way analysis of variance (ANOVA)⁴ was then performed on those data. Significant results for all statistical tests were determined by the critical values ($\alpha = 0.01$) of the approximate distributions. Number of species data for pre-fouled and settlement panels were first tested for between-treatment differences at the $\alpha = 0.01$ level. If overall ANOVA demonstrated significant difference, Duncan's New Multiple Range Test⁵ was used to define significant differences

between the various treatments.

American oysters (*Crassostrea virginica*) of 5- to 8-cm length were collected from West Loch, Pearl Harbor, and were placed on clay panels on the tank bottoms for 5 weeks of adaptation prior to the experiment. At the beginning of treatment, there were 18 oysters in each tank. In the course of the experiment, oysters were shaken free of accumulated sediment every few days, and the presence of dead individuals was logged. Several oysters were collected from each treatment at the ends of treatment and recovery phases for later analysis of condition indices and organotin body burdens. Collected individuals were frozen in plastic bags.

Condition indices were measured on all sampled oysters to determine the relative health levels or "conditions" of individuals in the various treatments. The index used is a modification of that described in Galtsoff⁶ which defined condition index as the dry weight (in grams) of the oyster meat divided by the oyster shell cavity volume (in milliliters) multiplied by 100. For the present experiment, wet weight of meat was used instead of dry weight to avoid possible alteration of organotin compounds in the samples. For comparison to work of others, oyster wet weights were converted to approximate dry weights by multiplying them by 0.12, a dry-weight to wet-weight ratio cited in Galtsoff⁶ for oysters from southern United States coastal waters.

The treatment (exposure) phase of the experiment began on 25 July 1984 when painted panels of various areas were put into the bioassay tanks to produce target nominal organotin concentrations of 0.05, 0.13, 0.31, 0.78 and 1.95 $\mu\text{g/l}$. Three tanks were used for each control and treatment level. One week after beginning the treatment phase, a single 18-cm by 20-cm panel of grey PVC plastic was put into each tank to monitor the recruitment and settlement of fouling organisms. Those panels were tilted against the drain standpipes in the tank centers. Photos were taken of the panel back sides using the same methods and the schedule described for the pre-fouled panels.

Water samples were collected on seven occasions during the 2-month treatment phase for analysis of organotin and/or copper content. Samples for organotin analysis were stored frozen in 500-ml polycarbonate bottles and were analyzed using a volatile hydride derivatization and atomic adsorption spectrophotometry technique⁷ which determines quantities of tri-, di-, and monobutyltins. Because of rapid hydrolysis, the anion is not known; therefore, the speciated compound is defined as the cation (e.g., tributyltin or TBT).

Copper water samples were stored frozen in 750-ml polyethylene bottles and were analyzed using a spectrophotometric technique of Strickland and Parsons⁸.

After 2 months of treatment, the paint panels were removed from the tanks, and the bioassay communities were allowed 2 months of recovery (depura-

tion) under flowthrough conditions. All remaining oysters were samples at the end of recovery for condition index and organotin body burden analysis. Control tanks contained no antifouling paint panels during the experiment and had community compositions and sampling protocols similar to those of treatment tanks.

RESULTS AND DISCUSSION

Chemistry

Mean measured concentrations of TBT were close to predicted nominal concentrations for the control and two lower level treatments, but were substantially higher than nominal for the three higher level treatments (table 1). Overall mean TBT leach rate calculated for panels in the five leachate treatments was $3.3 \text{ ug/cm}^2/\text{day}$.

Mean measured copper concentrations were significantly higher than control levels in the three high-level leachate treatments (table 1). Calculated mean copper leach rate for those panels was $13.2 \text{ ug/cm}^2/\text{day}$.

Table 1. Mean TBT and copper concentrations measured in nominal TBT bioassay treatments.

Nominal TBT	Measured TBT	Measured Copper
Control	0.01 + 0.007 (5)	1.0 + 0.16 (4)
0.05	0.04 + 0.007 (5)	1.2 + 0.53 (4)
0.13	0.10 + 0.052 (10)	1.2 + 0.20 (4)
0.31	0.54 + 0.234 (10)	3.1 + 0.34 (8)
0.78	1.77 + 0.735 (6)	4.4 + 0.31 (4)
1.95	2.52 + 1.253 (6)	5.0 + 0.26 (4)

Mean, + standard deviation (no. of values).
Units = ug/l .

Pre-fouled Panels and Walls

Precipitous declines in total numbers of faunal species and species diversity occurred during leachate exposure on the 0.5, 1.8, and 2.5 ug/l treatment pre-fouled panels (figures 1 and 2). At the beginning of exposure, mean numbers of faunal species in exposure treatments were statistically similar to mean control level (table 2). By the end of the exposure phase, mean species abundances in the three higher level treatments were significantly lower than the two lower level treatment and control means. The mean number of species in the 0.1 ug/l treatment was significantly higher than in the control and 0.04 ug/l treatment. At the end of the recovery, species abundance in all treatments was similar to controls.

Overall effects on fouling organisms are typified by the end-of-exposure mortality status of the six most common fouling species on the pre-fouled panels (table 3). *Botrylloides* spp. (orange colo-

nial tunicates) exhibited highest TBT sensitivity with 100-percent mortality at TBT concentrations of 0.1 ug/l and higher. Treatment mortality for *Schizoporella errata* (encrusting bryozoan) was 49-, 100-, 84-, and 100-percent at TBT concentrations of 0.1 -, 0.5 -, 1.8 -, and 2.5 ug/l , respectively. Each of these were significantly different than the controls at the 99-percent confidence level. Total mortality occurred in populations of *Didemnum candidum* (white colonial tunicate) exposed to TBT concentrations of 0.5 ug/l and higher.

Figure 1. Total number of faunal species on pre-fouled panels plotted versus time for bioassay control and leachate treatments.

Figure 2. Species diversity (modified Shannon-Wiener index) of fauna on pre-fouled panels plotted versus time for bioassay control and leachate treatments.

Table 2. Significant differences between organotin bioassay treatments for mean total number of faunal species on pre-fouled panels at ends of pre-treatment, treatment, and recovery phases.

	TBT in $\mu\text{g/L}$					
	Control	0.04	0.1	0.5	1.8	2.5
End Pre-Treatment						
End Treatment						
End Recovery						

Analysis with Duncan's New Multiple Range Test. Any two treatments not underscored by the same bar are significantly different at 99% level. Any two treatments underscored by the same bar are not significantly different.

At the end of the exposure phase, all *Anomia nobilis* (saddle oysters) in the 0.5, 1.8, and 2.5 $\mu\text{g/l}$ treatment panel photo areas were dead, and those in the 0.1 $\mu\text{g/l}$ treatment were severely reduced in numbers. Unfortunately, *A. nobilis* percent coverage on the control and 0.04 $\mu\text{g/l}$ treatment panels was very low to nil throughout the experiment, precluding the use of those data as a meaningful base for statistical comparisons with other treatment data. Those low abundances were apparently artifacts of senescence and die-off of the mature control oyster populations. However, at the beginning of the experiment, saddle oysters were observed among the early succession stage fouling populations on the tank walls in a typical "normal" coverage of 2 to 3 percent.

Succeeding observations showed that after 60 days of leachate exposure, the number of juvenile saddle oysters was unchanged on 0.04 $\mu\text{g/l}$ treatment tank walls, a few individuals in the 0.1 $\mu\text{g/l}$ tanks had died, and all individuals on tank walls of the other three treatments had died. Saddle oysters on control walls remained at normal abundance through the exposure phase. Therefore, observations of substrates outside of the photo areas indicated there was no significant mortality of saddle oysters under 0.04- $\mu\text{g/l}$ TBT exposure and control survival of that organism was normal.

Early senescence of organisms on panels also affected the statistical utility of photo data for *Hydroides elegans* (tube worms) and *Ascidia* spp. (solitary tunicates). Again, observations of younger populations on tank walls confirmed that reductions in percent coverage in the higher level treatments had occurred to the same degree as seen in the mean photo data. Also control coverage of organisms throughout treatment was nearly identical to mean percent cover seen in all treatments before exposure. The percent mortality values given in table 3 for tube worms and solitary tunicates, therefore were derived primarily through examination of large areas of wall.

Table 3. Significant mortality summary for most common species on pre-fouled panels.

		Mean Measured TBT Concentration ($\mu\text{g/L}$)					
		Control	0.04	0.1	0.5	1.8	2.5
<i>Botrylloides</i> spp. (Orange colonial tunicate)	**				100% Mortality		
<i>Schizoporella errata</i> (Encrusting bryozoan)	**			49%	100% Mortality	84%	100%
<i>Didemnum candidum</i> (White colonial tunicate)	*				100% Mortality		
<i>Anomia nobilis</i> (Saddle oyster)	(0)				100% Mortality		
<i>Hydroides elegans</i> (Tube worm)	(0)					77%	68%
<i>Ascidia</i> spp. (Solitary tunicates)	(0)						91%

Any two means not underscored by the same bar are significantly different. Any two means underscored by the same bar are not significantly different. ** = significant difference at 99% level. * = 95% level. (0) = significant differences not evident in ANOVA analysis of panel data because of sparse control panel populations, but listed mortalities obtained by observations of populations on other substrates.

Twenty taxa of epifauna were found to be ubiquitous in all treatments and controls on pre-fouled panels and other tank substrates prior to leachate exposure (table 4). Presence/absence observations at the end of exposure phase revealed that only one of those ubiquitous species was absent in the 0.04 $\mu\text{g/l}$ treatment and two species were absent in the 0.10 $\mu\text{g/l}$ treatment. Major reductions in numbers of ubiquitous species occurred during exposure in the three high-level TBT exposures. Those reductions were 55, 60 and 80 percent in the 0.5, 1.8, and 2.5 $\mu\text{g/l}$ treatments, respectively. Only five taxa, the anemone *Haliplanella luciae*, some *Ascidia* species solitary tunicates, the tube worms *Hydroides elegans* and *Pileolaria militaris/pseudo-militaris*, and a single individual of tube-dwelling gastropod, *Vermetus alii* were observed in 2.5 $\mu\text{g/l}$ TBT treatments at the end of exposure. Fouling populations at the end of recovery showed no major differences in overall presence/absence in the various treatments.

Settlement Panels

On settlement panels, the total number of faunal species per treatment and species diversity per treatment were relatively high at the end of treatment in the control and 0.04 $\mu\text{g/l}$ TBT tanks but

Table 4. Presence or absence of fouling organisms in organotin bioassay treatments at ends of pre-treatment, treatment, and recovery phases.

	PRE-TREATMENT					TREATMENT					RECOVERY				
	µg/L					µg/L					µg/L				
	2.5	1.8	0.5	0.1	CTRL	2.5	1.8	0.5	0.1	CTRL	2.5	1.8	0.5	0.1	CTRL
PORIFERA (Sponges)															
Demospongiae															
Erecting sponges	+	+	+	+	X						+	+	+	X	X
Calcarea															
<i>Leuconia halana</i>	+	+	+	+							X	X	X	X	X
Cnidaria (Coelenterates)															
Anthozoa (Anemones)															
<i>Alipazia pulchella</i>	+	+	+	X	X						X	+	+	X	+
<i>Haliplanella luciae</i>	+	+	+	+							+	+	+	+	
BRYOZOA															
<i>Bugula neritina</i>	+	X	X	X	+						X	X	X	+	X
<i>Schizoporella errata</i>	X	X	X	X	X						X	X	X	X	X
<i>Holoporella</i> sp.					X										X
<i>Waterporia</i> sp.		X					X								
MOLLUSCA															
Pelecypoda (Bivalves)															
<i>Anomia nobilis</i>	X	X	X	X	+						X	+	+	+	X
<i>Hiatella arctica</i>	X	X	X	X	+						+	+	+	+	+
Gastropoda															
<i>Crepidula aculeata</i>	X	X	X	X	+						X	X	X	X	+
<i>Vermatux alii</i>															X
ANNELIDA (Polychaeta)															
Sedentaria															
<i>Branchionia cingulata</i>	+	X	X	X	+						X	+	+	+	X
<i>Hydroides elegans</i>	X	X	X	X	X						X	X	X	X	X
<i>Ptilolaria militaris/pseudomilitaris</i> (undif.)	X	X	X	X	X						X	X	X	X	X
ARTHROPODA															
Cirripedia (Barnacles)															
<i>Balanus</i> spp.	X	+	X	+	+						+	+	+	+	+
TUNICATA (Sea squirts)															
<i>Ciona intestinalis</i>				X	X										
<i>Polyclinum</i> sp. ¹	+	+	+	+	+						+	+	+	+	+
<i>Didemnum candidum</i>	X	X	X	X	X						X	X	X	X	X
<i>Didemnum edmonsoni</i>															X
<i>Diplosoma macdonaldi</i>	X	X	X	X	X						X	X	X		
<i>Didemna</i> (undif.)				X											
<i>Perophora</i> sp.	+	+	+	+	+						X	X	X	X	X
<i>Ascidia</i> spp. ²	X	X	X	X	X						X	X	X	X	X
<i>Pyrosoma</i> ³	X	X	X	X	X						X	X	X	X	+
<i>Botryllodes</i> sp. A (brown/tan)	X	X	X	X	X						X	+	+	+	+
<i>Botryllodes</i> sp. B (red/orange)	X	X	X	X	X						X	+	+	+	+
<i>Symploca viridis</i>											X				

X = organism(s) inventoried by analysis of pre-fouled panel photo, + = organism(s) noted in overall tank observations, blank = no organisms observed by either method.

1 *Polyclinum vasculosum* (?)

2 Includes *Ascidia nigra* and *A. interrupta*

3 Includes *Herdmania momus* and *Microcosmus exasperatus*

were substantially lower in the four higher level treatments (figures 3 and 4). End-of-treatment differences were statistically significant between those groups, but differences between treatments within the two groups were not significant (table 5). Numbers of species in TBT-exposed populations were not significantly different from control populations at the end of recovery.

Seventeen taxa were readily identified from settlement panels in the course of the experiment. Presence/absence data for taxa identified after TBT exposure indicate that phyla of lower evolutionary status (e.g., Porifera, Cnidaria, Bryozoa, and Mollusca) were most sensitive to TBT (table 6). Organisms least affected were those capable of complete enclosure in hard, tubular shells such as *Hydroides* and *Pileolaria* spp. or in thick body walls such as *Ascidia* spp. solitary tunicates. The tube worms *Hydroides elegans*, *Pileolaria militaris*, and *P. pseudomilitaris* were the only organisms that colonized panels in the 2.5-µg/l TBT exposures.

Figure 3. Total number of faunal species on settlement panels plotted versus time for bioassay control and leachate treatments.

Figure 4. Species diversity (modified Shannon-Wiener index) of fauna on settlement panels plotted versus time for bioassay control and leachate treatments.

Table 5. Significant differences between bioassay treatments for mean total number of faunal species on settlement panels at ends of treatment and recovery phases.

	Control	TBT in $\mu\text{g/L}$				
		0.04	0.1	0.5	1.8	2.5
End Treatment						
End Recovery						

Analysis with Duncan's New Multiple Range Test. Any two treatments not underscored by the same bar are significantly different at 99% level. Any two treatments underscored by the same bar are not significantly different.

Table 6. Presence or absence of fouling organisms noted on settlement panels in bioassay treatments at ends of treatment and recovery phases.

	END OF TREATMENT					END OF RECOVERY				
	TBT in $\mu\text{g/L}$					TBT in $\mu\text{g/L}$				
	2.5	1.8	0.5	0.1	CTRL	2.5	1.8	0.5	0.1	CTRL
PORIFERA (Sponges)										
Calcarea (Calcarea sponges)										
Leucosia kalae						X	X	X	X	X
OXIDARIA (Coelenterates)										
Anthozoa (Anemones)										
Aiptasia pulchella									X	X
BRYOZOA										
Bugula neratilis									X	X
MOLLUSCA										
Palaeopoda (Bivalves)										
Anomia aculeata						X	X			
Gastropoda										
Caprellidae aculeata						X				
ANNELIDA										
Polychaeta										
Sabellidae (unid.)										X
Hydroids alagans						X	X	X	X	X
Pileolaria militaris/ P. pseudomilitaris (unidi.)						X	X	X	X	X
TUNICATA (Sea squirts)										
Ciona intestinalis						X	X			
Polysiphonia cf. vasculosum									X	X
Didemnum candidum						X	X	X	X	X
Diplosoma macdonaldi						X	X			X
Paraphora sp.										X
Ascidia spp. ¹						X	X	X	X	X
Pyrosoma ²										X
Botryllodes sp. A (brown/brown)						X	X	X	X	X

X = organism(s) inventoried by analysis of panel photo, blank = no organisms inventoried.

¹ Includes Ascidia nigra and A. interrupta

² Includes Herdmania momus and Microcosmus exasperatus

Algae

Dominant algae that colonized the tank surfaces consisted essentially of blue-green algae films, diatom mats, the green algae Cladophora socialis and Ulva sp., the brown alga Dictyota acutiloba, and calcareous red algae. Throughout both the exposure and recovery phases of the experiment,

no significant between-treatment differences were observed in the abundances of algae. Coralline algae, which were quantified on pre-fouled panels, actually increased in coverage during the exposure phase in all treatments.

Oysters

Survival of oysters exposed to TBT concentrations of 1.8 or less was similar to the controls (figure 5). Slightly enhanced survival of oysters in the three low-TBT treatments was probably due to reduced predation of oysters by flatworms. This was attributed to direct toxic effects on the worms and to oyster valves being closed for longer periods, thus allowing less opportunity for worms to invade and prey on oysters. The mortality rate for oysters exposed to 2.5- $\mu\text{g/L}$ TBT was 50 percent after 30 days of exposure (figure 5).

Figure 5. Total number of live oysters (Crassostrea virginica) plotted versus time for bioassay control and leachate treatments.

Figure 6. Mean condition indices of oysters (Crassostrea virginica) after 57 days exposure to control or leachate treatment and 59 days of recovery.

Vertical bars are 95% confidence limits.

Condition indices for oysters sampled after 57 days of exposure were significantly lower in oysters exposed to TBT concentrations of 0.1 µg/l and greater (figure 6). Mean condition indices for TBT-exposed oysters correlate well with exposure concentrations and clearly indicate that even though oysters exhibit normal mortality rates under moderate TBT concentrations for periods of several weeks, their ability to maintain tissue mass and possibly their long-term survivability are affected by TBT concentrations of about 0.1 µg/l and higher.

The mean condition index for control oysters is very similar to the mean condition index of 5.3 obtained for oysters collected from West Loch, Pearl Harbor by Sakuda⁹. Note that all condition indices of the present study and of Sakuda's study are substantially lower than a minimum level of about eight for marketable quality oysters. Additionally, the pattern of variability in condition indices seen in the present study matches trends documented by Westly¹⁰ for a closely related species of oyster. Specifically he noted that "standard deviation of *Crassostrea gigas* samples indicate consistency of results when oyster condition is poor with increased variation in better oysters". For future oyster condition index studies, variability could probably be reduced by using dry weight tissue weights instead of wet weights.

After 2 months of recovery, condition indices of surviving treatment oysters had returned to near-control levels (figure 6). This relatively rapid recovery indicates that oysters had resumed normal feeding and toxic effects from TBT were apparently short-lived. As of this writing, analyses of oyster tissue organotin content had not been completed, so it is not known to what degree the oysters had accumulated and depurated leachates. Those results will be reported later.

SUMMARY

The experiment described is unique in that no other studies are known that have examined the effects of chronic exposure to organotin leachates on complex harbor communities under flowthrough of unaltered typical harbor water. Leachate effects on harbor organisms in "estuarine" water were shown to be of essentially the same character and magnitude as were observed under equivalent leachate exposure experiments with complex soft-bottom communities collected from and maintained in clean open-coast water¹.

The hierarchy of chronic TBT sensitivities of the various phyla tested in this experiment corresponds to the pattern revealed by previous short-term bioassays. That pattern generally relates increased tolerance to TBT with increasing level of evolutionary development. Exceptions to this pattern exist for some species that apparently received lessened contact with dissolved toxins because they live within impervious shells or integuments or are capable of high-volume output of protective mucus.

Effects of higher TBT concentrations on both colonizing and mature fouling communities indicate that chronically high organotin leachate levels, such as might be encountered near concentrations of large ships in low-circulation harbors, could cause significant shifts in abundances of fouling species. In some cases, those shifts could lead to the dominance of pollution-tolerant "nuisance" foulers such as tube-worms, tunicates, and vermetid mollusks. The potential for adverse effects from such changes would be greatest in relatively pristine areas and slight in harbors where nuisance foulers are already abundant.

Of primary concern from a fisheries management aspect is the demonstrated sensitivity of adult oysters to sub-µg/l TBT concentrations. Recent studies on oysters and mussels indicate exposure to TBT concentrations in the 0.1 to 0.5-µg/l range resulted in varying degrees of malformation, reduced growth, and mortality^{11, 12 & 13}. Combined effects on bivalve mollusks seen in the present and previous studies substantiate the need to ensure that chronic TBT levels in and around shellfish beds do not reach 0.1 µg/l.

In nearly all aspects of this bioassay, 0.5-µg/l was found to be the critical concentration at which major adverse effects such as high mortality, reduced settlement, and obvious stress occurred in fouling organisms. Effects generated by that exposure level included significant reductions in species diversity and number of species of fouling organisms, nearly 50-percent reduction in adult oyster condition index, and 100-percent mortality of colonial tunicates, an encrusting bryozoan (*Schizoporella errata*), and a bivalve mollusk (*Anomia nobilis*). Collectively, these experimental results reinforce the Department of Navy's determination¹⁴ of 0.5 µg/l as a maximal TBT concentration that should not be exceeded in marine waters to avoid significant acute effects on harbor ecosystems.

By application of a 10-fold "safety factor" to the 0.5 µg/l value, an average TBT concentration of 0.05 µg/l should not be exceeded to ensure protection of more sensitive species and larvae. This value has also been recommended by the Navy as an average target TBT limit in harbor waters. Results of the present study reinforce the selection of 0.05-µg/l TBT as a "no-effect" level because no significant deleterious effects were evident on either the settlement or survival of fouling organisms, or the condition index of oysters in long-term 0.04-µg/l TBT exposures.

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