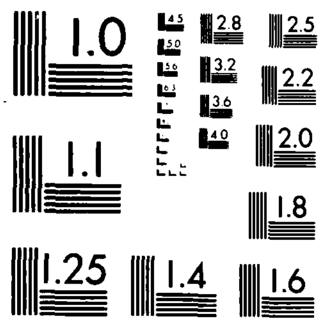
AD-A172 985 EFFECTS OF TRIBUTYLTIN ANTIFOULING PAINT LEACHATES ON PEARL HARBOR ORGANI..(U) NAVAL OCEAN SYSTEMS CENTER SAN DIEGO CA R S HENDERSON DEC 85 NOSC/TR-1079 1/1

UNCLASSIFIED F/G 6/20 NL



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TR 1079

(12)

TR 1079

Technical Report 1079
December 1985

EFFECTS OF TRIBUTYLTIN ANTIFOULING PAINT LEACHATES ON PEARL HARBOR ORGANISMS

Site-Specific Flowthrough Bioassay Tests

R. Scott Henderson

Naval Ocean Systems Center
San Diego, California 92152-5000

NAVAL OCEAN SYSTEMS CENTER

San Diego, California 92152-5000

F. M. PESTORIUS, CAPT, USN
Commander

R. M. HILLYER
Technical Director

ADMINISTRATIVE INFORMATION

This work described was performed by the Marine Environment Branch, Code 522, Naval Ocean Systems Center, for the Energy Research and Development Office of the David W. Taylor Naval Ship Research and Development Center, and the Office of Chief of Naval Research. Additional support for PET development was provided by Naval Facilities Engineering Command.

Released by
P. F. Seligman, Head
Marine Environment Branch

Under authority of
S. Yamamoto, Head
Environmental Services
Division

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of employees who assisted in these experiments by performing maintenance and monitoring tasks, chemical analyses, biological sorting and identification, and field collection. Naval Ocean Systems Center (NAVOCEANSYSCEN)-Hawaii personnel included W. Cooke, R. Fransham, J. Groves, J. Grovhoug, and D. Pawloski. NAVOCEANSYSCEN-San Diego personnel were C. Dooley, V. Homer, S. Lieberman, P. Seligman, P. Stang, G. Vafa, and A. Valkirs. The Research Corporation of the University of Hawaii and Computer Sciences Corporation provided contractual support.

JG

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NOSC TR 1079	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Naval Ocean Systems Center 6b. OFFICE SYMBOL (If applicable) Code 522	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State and ZIP Code) San Diego, CA 92152-5000	7b. ADDRESS (City, State and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION D.W. Taylor Naval Ship R&D Center Office of Chief of Naval Research Naval Facilities Engineering Command 8b. OFFICE SYMBOL DTNSRDC-2759 OCNR 126 NAVFAC 032	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State and ZIP Code) Annapolis, MD 21402 Washington, DC 20360 Washington, DC 20390	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 63724N, 63721 PROJECT NO: Z0838011, Y0817 TASK NO: Agency Accession No.: DN888 749

11. TITLE (Include Security Classification)
EFFECTS OF TRIBUTYLTIN ANTIFOULING PAINT LEACHATES ON PEARL HARBOR ORGANISMS
Site-Specific Flowthrough Bioassay Tests

12. PERSONAL AUTHOR(S)
R. Scott Henderson

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 1983 TO 1985	December 1985	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Organotin paint leachates, antifouling coatings, marine microcosms, benthic organisms, harbor pollutants, tributyltin

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Site-specific bioassay studies were performed to evaluate the effects of tributyltin (TBT) antifouling paint leachates on complex communities of organisms. Test communities were maintained in flowthrough seawater tanks at Ford Island, Pearl Harbor, Hawaii, and were composed of about 30 common fouling invertebrates (attached to panels and tank surfaces), American oysters, swimming crabs, glass shrimp, and feather-duster worms. Organisms were exposed to five treatment levels ranging from 0.04- to 2.5-µg/L TBT. Organisms that exhibited no visible stress responses to all levels of TBT were swimming crabs, glass shrimp, the anemone Haliplanella luciae, and all genera of algae encountered in the tanks.

The American oyster (Crassostrea virginica) experienced sublethal effects (reduced condition index) from organotin leachates at 0.1-µg/L TBT and high mortality at 2.5 µg/L. Substantial declines in numbers of species and lowered species diversity were observed on fouling panels and surfaces exposed to 0.5-µg/L TBT. No significant effects were noted at 0.04 µg/L.

Tolerance of some species of nuisance foulers, such as tube worms and solitary tunicates, to moderate-to-high concentrations of TBT suggests that areas affected by significant amounts of organotins should be closely monitored for possible shifts in dominance of specific organisms.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
[X] UNCLASSIFIED/UNLIMITED [ ] SAME AS RPT [ ] DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL R. Scott Henderson	22b. TELEPHONE (Include Area Code) (808) 254-4465
	22c. OFFICE SYMBOL Code 522

DD FORM 1473, 84 JAN

83 APR EDITION MAY BE USED UNTIL EXHAUSTED
ALL OTHER EDITIONS ARE OBSOLETE

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

EXECUTIVE SUMMARY

Site-specific bioassay tests were performed to determine the effects of organotin paint leachate on complex communities of organisms. Test communities were maintained in flowthrough seawater tanks at Ford Island, Pearl Harbor, Hawaii, and were composed of about 30 common fouling invertebrates (attached to panels), American oysters, swimming crabs, glass shrimp, and feather-duster worms. Panels of varying surface area painted with a commercial ablative co-polymer coating containing tributyltin and cuprous oxide toxicants were "aged" for 4 months in flowing seawater. The aged panels were placed in the bioassay tanks. The established communities were then subjected to a 3-month leachate treatment phase followed by a 2-month recovery phase after removing the panels.

Mean measured tributyltin (TBT) concentrations in leachate treatments were 0.04, 0.1, 0.5, 1.8, and 2.5 μg/L (ppb). The lower concentrations (0.04 to 0.5 μg/L) encompassed TBT levels that could be attained in low-circulation areas of harbors, near docked ships, or discharging drydocks with high use of organotin-based antifouling paints. The higher concentrations were not predicted to be observed in harbors.

Major biological effects observed during the treatment phase included the following:

1. Substantial declines in numbers of species and species diversity of pre-established fouling communities exposed to 0.5-μg/L TBT and greater.

2. Significant reductions in numbers of species and species diversity of larval forms settling on virgin panel surfaces exposed to 0.1-μg/L TBT and greater.

3. Sublethal effects (reduced condition index) on American oysters (*Crassostrea virginica*) exposed to 0.1-μg/L TBT and greater. Mortality was observed only in oysters exposed to 2.5-μg/L TBT.

4. Ninety-five- to 100-percent mortality of feather-duster worms exposed to 0.5-μg/L TBT and higher.

5. One-hundred-percent mortality of common anemones in TBT treatments of 0.5 μg/L and higher.

Swimming crabs, glass shrimp, the anemone *Haliplanella luciae*, and all genera of algae encountered in the tanks exhibited no visible stress responses to all levels of TBT treatment. Tolerance of some species of "nuisance" foulers, such as tube worms and solitary tunicates, to moderate-to-high concentrations of TBT suggests that areas affected by significant amounts of organotins should be closely monitored for possible shifts in dominance of specific organisms.

The American oyster was the only organism of fisheries importance that experienced detrimental effects from organotin leachates. As evidenced by significant decreases in the condition index of oysters exposed to 0.1-μg/L TBT, average field concentrations proximal to oyster beds should be kept substantially below that level. Concentrations of 0.04-μg/L TBT elicited no significant changes in oyster condition index, species diversity, or mortality.

CONTENTS

ILLUSTRATIONS

TABLES

INTRODUCTION

The Navy has been examining the feasibility of fleetwide conversion from copper-based to organotin-based antifouling paints. Such a conversion would generate substantial fuel and dry-dock savings and would increase fleet readiness and reliability. Potential marine environmental effects of organotin leachates are issues that will have a major influence on decisions related to possible implementation of the organotin-based paints.

To evaluate the effects of organotin antifouling leachates on inshore bottom communities, long-term experiments were conducted from 1980 to 1983 at the flow-through marine microcosm facility of Naval Ocean Systems Center (NOSC) at Mokapu, Oahu, Hawaii. In those experiments, exposure to 0.5 to 1.8 μg/L tributyltin (TBT) resulted in decreased abundance of several species and groups of biota (Henderson, 1985). 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 live under relatively high levels of pollutants, organics, and suspended particulates. Therefore, we decided to perform a series of 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 Facility

The site selected for the bioassay experiments is located on the southwest end of Ford Island approximately 1 kilometer downcurrent of Southeast Loch, the portion of Pearl Harbor most heavily polluted by industrial wastes and most extensively used for ship moorage (figure 1). Southeast Loch itself was not considered for location of the experimental facility because of the unavailability of space and potential problems with surface oil pollution. Water quality and biological conditions at the Ford Island site are typical of those found throughout the harbor (Evans, 1974).

The primary bioassay system consists of eighteen 155-L polyethylene tanks situated on an unshaded deck (figure 2). Twin fiberglass swimming pool pumps each provide 230 L/minute of seawater flow to a polyethylene 380-L receiving tank. Gravity flow distributes water from the receiving tank to a 5.1-cm (2-in.) diameter feed pipe, which lies across the tops of the bioassay tanks. Pressure head of water in the feed pipe is reduced to a constant low level by an open, upward-facing overflow elbow at the end of the pipe. Individual open elbows of 2-cm (0.75-in.) diameter pipe tap off the feed pipe over each bioassay tank. Each tank-feed elbow can be pivoted to adjust its overflow height and flow of water. Tank flow rates can be regulated to $\pm$0.1 L/minute from a range of 1 to 10 L/minute and remain nearly constant for periods of several days.

A flow rate of 4 L/minute was selected to produce an average bioassay tank water residence time of about 40 minutes. That flushing rate was deemed sufficient to provide

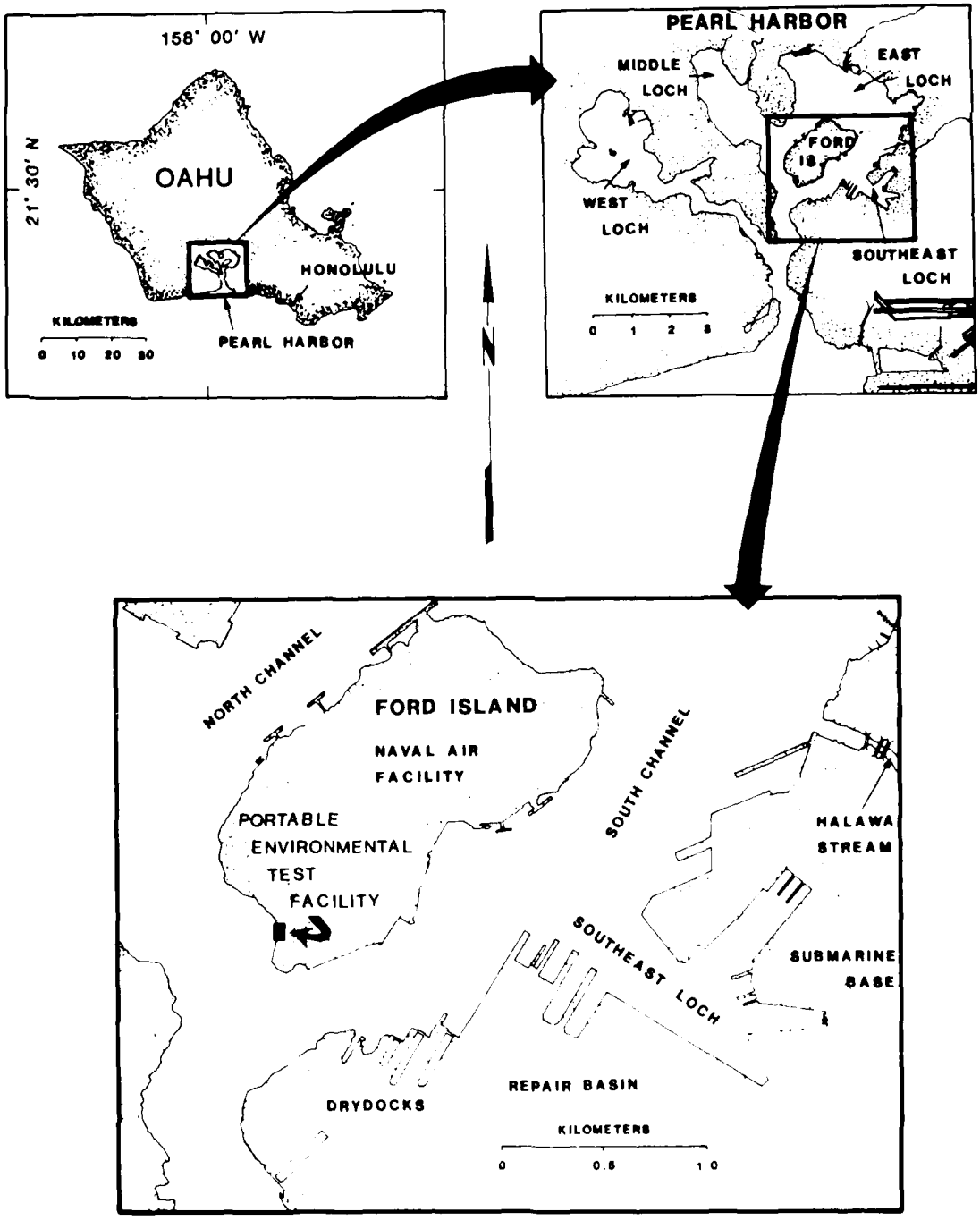


Figure 1 Location of portable environmental test facility for organotin antifouling paint leachates bioassay

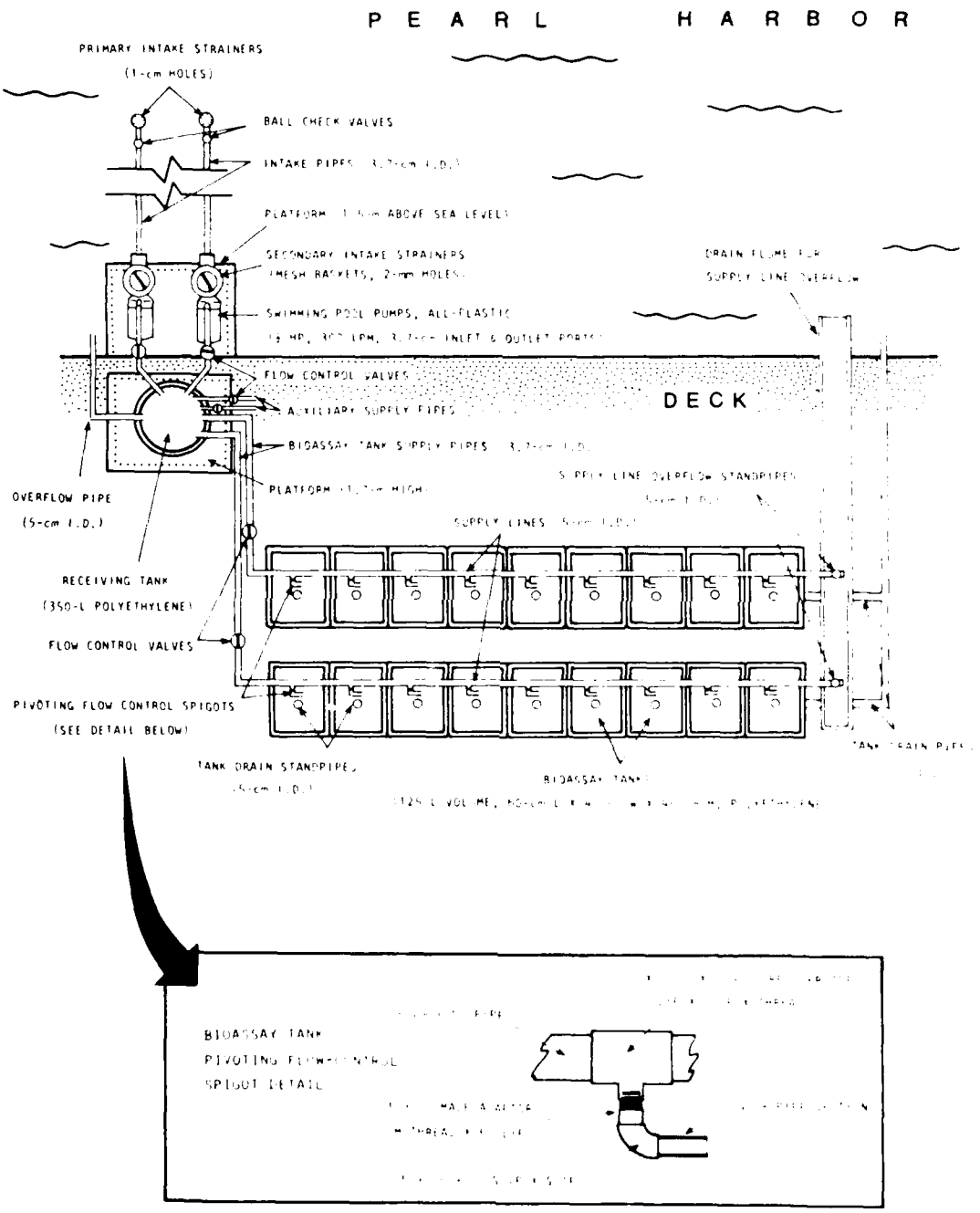


Figure 2. Plan-view schematic of portable environmental test facility and flowthrough control apparatus.

a steady supply of planktonic food for filter feeding and detrivorous organisms that would be maintained for the experiments. Pumped seawater is drawn from about a 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. Influx of dissolved nutrients (N and P compounds) to Pearl Harbor waters is high, largely because of high input of fresh water from streams and springs. However, most nutrients are assimilated by plankton very close to point sources. 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 organisms (Evans, 1974).

Experimental Design and Analytical Techniques

A self polishing copolymer antifouling paint (International Paint Co. formulation BFA 956 Pink SPC 9 HiSol) was selected as a typical dual toxin (organotin and copper) paint that might be used on Navy craft. Composition of this paint is listed by the manufacturer as 9.4 percent tributyltin methacrylate (as part of a polymer), 0.5 percent bis (tri-n-butyltin) oxide (TBTO), 44.7 percent cuprous oxide, and 45.4 percent inert ingredients. The paint was applied by a roller in two moderately thick coats onto sanded (180 grit) plexiglass panels. Painted panels were leached for 4 months in 400 L flowthrough tanks at a flow rate of about 10 L/minute. 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 25.5 cm plexiglass panels. The panels were suspended on fixed arrays 0.5 m deep in unshaded water 5 m deep, about 200 m from the bioassay facility water intake. Panels were allowed 14 weeks of field colonization after which time they 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. The out-facing, sun-exposed sides of the panels accumulated thick mats of diatoms and filamentous algae because fish grazing activities were not present in the tanks as they were in the field. Consequently, invertebrate epifauna were relatively sparse on the sun-exposed panel sides, and only panel back sides 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 on 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. While being photographed, panels were immersed in ambient seawater in a plastic tub. 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 index (Wilhm & Dorris, 1958), 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 transformation (Sokal & Rohlf, 1969) was applied to percent coverage data for individual species on pre-fouled panels. One-way analysis of variance (ANOVA) (Sokal & Rohlf, 1969) 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 $\alpha = 0.01$. If overall ANOVA demonstrated significant difference, Duncan's New Multiple Range Test (Alder & Roessler, 1972) 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 beginning of the experimental treatment phase. 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 Galtsoff (1964), 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 that were immediately frozen for later analysis of organotin content.

For comparison to work of others, oyster wet weights were converted to approximate dry weights by multiplying them by a conversion factor of 0.12. That dry-weight-to-wet-weight ratio is derived from Galtsoff (1964) for oysters from southern United States coastal waters.

Approximately 10 feather-duster worms (*Sabellastarte sanctijosephi*) were introduced to each tank 5 weeks prior to the beginning of the experiment. These polychaete worms are common throughout Pearl Harbor and live in leathery tubes attached to hard

substrate. Average adult tube diameter and length were 1.2 cm and 11 cm, respectively. Upright short segments of 1.3-cm-diameter PVC pipe bound together in clusters with plastic ties were used as attachment substrates for the worm colonies. The relative health of worms in the various treatments was determined periodically by examining the degree of distention, size, rigidity, and speed of alarm withdrawal of individual feeding caps. Heavily stressed individuals would shed feeding caps and, in extreme cases, would completely relax and float free of their tubes.

The glass shrimp (*Palaemon* spp.) was selected for bioassay testing because it is a crustacean harvested as bait and food species in moderate quantities from harbor waters. Thirty individuals of 3- to 5-cm body length were added to each tank 2 weeks before treatment. Abundances and general behavior of the shrimp were monitored by visually scanning the tanks periodically with a transparent "look box."

Swimming crabs (*Thalamita admete*) appeared in most of the bioassay tanks, apparently being introduced by the influx of larvae in the supply seawater or transferred from the field as small juveniles on pre-fouled panels. Most tanks harbored two or three small individuals of 1- to 2-cm carapace width and one or two adults of 3- to 4-cm carapace width. Larger individuals were removed from the tanks and frozen for organotin analysis if their browsing activities were disruptive to oysters and fouling panels.

The treatment (exposure) phase of the experiment began on 25 July 1984 when painted panels of various areas were put into the bioassay tanks (table 1). Target nominal organotin concentrations started at 0.05 and increased successively by a factor of 2.5. These were identical to concentrations selected for use in concurrent bioassay experiments at NOSC, San Diego.

Table 1. Paint area and nominal TBT concentration treatments for bioassay experiment.

Nominal TBT Concentration (μg/L)	Control 0.00	0.05	0.13	0.31	0.78	1.95
SPC-9 HiSol Paint Area (cm^2)	0	95	238	595	1,488	3,720
Number of Tanks	3	3	3	3	3	3

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 side 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 absorption spectrophotometry technique described in Valkirs, et.al. (1985). That technique 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 (Strickland & Parsons, 1972) based on carbon tetrachloride extraction of copper complexed by sodium diethyldithiocarbamate.

Five days before termination of the treatment phase, four feather-duster worms and six oysters were collected from most tanks for organotin analysis. In higher level treatments where mortality had severely reduced amounts of worms and oysters available, all remaining individuals were sampled.

After 2 months of treatment, the paint panels were removed from the tanks, and the bioassay communities were allowed 2 months of recovery (depuration) under flowthrough conditions. All remaining oysters, adult crabs, and feather-duster worms were sampled at the end of recovery for organotin body burden analysis. At the end of the recovery phase, one-half of the volume of fine sediment that had accumulated on the tank bottoms was siphoned and washed through a 0.8-mm screen to recover infauna for inventory. Fouling and recruitment panels with biota were preserved in 10-percent formaldehyde to allow for verification of organisms in panel photos where needed.

Control tanks contained no antifouling paint panels during the experiment and had community/sediment 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 2). Higher apparent TBT leach rates in the higher level treatment may have been caused by reduced development of bacterial/algal slime layers on the panels due to toxic effects of the higher concentrations of TBT. Overall mean TBT leach rate calculated for panels in the five leachate treatments was 3.3 $\mu g/cm^2/day$.

Table 2. 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 (number of values). Units = $\mu g/L$.

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

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 $\mu g/L$ treatment pre-fouled panels (figures 3 and 4). At the beginning of exposure, mean numbers of faunal species in all exposure treatments were statistically similar to mean control level (table 3). 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 $\mu g/L$ treatment was significantly higher than in the control and 0.04 $\mu g/L$ treatment. At the end of the recovery, species abundance in all treatments was similar to controls.

Table 4. Significant mortality summary for most common species on pre-fouled panels

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

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

Early senescence of organisms on panels also affected the statistical utility of photo data for *Hydroides elegans* (tube worms) and *Ascidea* 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 percent coverage of those organisms throughout treatment was nearly identical to mean percent cover seen in all treatments before exposure. The percent mortality values given in table 4 for tube worms and solitary tunicates, therefore, were derived primarily through examination of large areas of wall.

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 5). Presence/absence observations at the end of the exposure phase revealed that only one of those ubiquitous species was absent in the 0.04 μg/L treatment and two species were absent in the 0.1 μg/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 μg/L treatments, respectively. Only five taxa (the anemone *Haliplanella luciae*, some *Ascidia* species solitary tunicates, the tube worms *Hydroides elegans* and *Pileolaria militaris/pseudomilitaris*, and a single individual of tube-dwelling gastropod, *Vermetus alii*) were observed in 2.5 μg/L TBT treatments at the end of exposure. Fouling populations at the end of recovery showed no major differences in overall presence/absence of taxa 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 μg/L TBT tanks but were substantially lower in the four higher level treatments (figures 5 and 6). End-of-treatment differences were statistically significant between those two groups, but differences between treatments within the two groups were not significant (table 6). Numbers of species in TBT-exposed populations were not significantly different from control populations at the end of recovery.

Table 5. 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	1.0	0.5	0.1	0.04	CTRL	2.5	1.0	0.5	0.1	0.04	CTRL	2.5	1.0	0.5	0.1	0.04	CTRL
PORIFERA (Sponges)																		
Demospongiae																		
Encrusting sponges	•	•	•	•	X	•				•	•	•	•	•	•	X	X	X
Calcarea																		
Leucosia kaiana	•	•	•	•	•	•				X	•	•	X	X	X	X	X	X
CNIDARIA (Coelenterates)																		
Anthozoa (Anemones)																		
Aiptasia pulchella	•	•	•	X	X	•				X	•	•	•	•	X	X	•	•
Haliplanella luciae	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
BRYOZOA																		
Bugula turrita	•	X	X	X	•	X			X	X	•	X	•	•	X	X	•	X
Schizoporella errata	X	X	X	X	X	X		X		X	X	X			X		X	X
Holeporella sp.						X												X
Watersiporia sp.			X						X									
MOLLUSCA																		
Pelecypoda (Bivalves)																		
Anomia nobilis	X	X	X	X	•	X				X	•	•	X		•	•	•	X
Hiatella arctica	X	X	X	X	•	•					•	•	•	•	•	•	•	•
Gastropoda																		
Crepidula aculeata	X	X	X	X	•	•		X	X	X	•	•		X	X	X	X	•
Vermetus sp.							•						•		X			
ANNELIDA (Polychaeta)																		
Sedentaria																		
Branchioma cingulata	•	X	X	X	•	•		X		X	X	•	X	•	•	•	•	X
Hydroides dianthus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pillsbrya rubitorio/ pseudomilitaris (undif).	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ARTHROPODA																		
Cirripedia (Barnacles)																		
Balanus spp.	X	•	X	•	•	•			X	•	•	•			•	•	•	•
TUNICATA (Sea squirts)																		
Ciona intestinalis				X	X				X	X	X	X			•	•	•	•
Polyclinum sp.[1]	•	•	•	•	•	•				•	•	X		X	X	•	X	•
Didemnum candidum	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X
Didemnum albanyense										X						X		
Diplosoma macdonaldi	X	X	X	X	X	X					X		X	X	X			
Didemnidae (unid.)				X						X								
Perophora sp.		•		X	•	•		X		X	X	X		X		X	X	X
Ascidia spp.[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pyuridae[3]	X	X	X	X	X	X		X	X	X	X	•	X	X	X	X	X	•
Botryllioides sp. A (brown/tan)	X	X	X	X	X	X				X	•	X	X	•	•	•	•	•
Botryllioides sp. B (red/orange)	X	X	X	X	X	X						X	X	•	•	•	•	•
Symplegma viride												X						

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

[1] *Polyclinum vasculosum* (?)

[2] Includes *Ascidia nigra*, *A. interrupta*, etc.

[3] Includes *Herdmania momus*, *Microcosmus exasperatus*

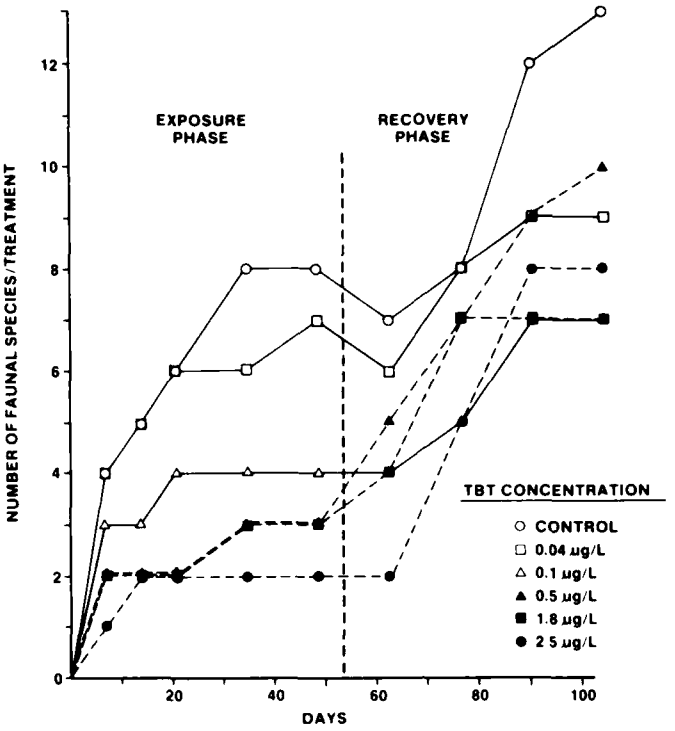


Figure 5. Total number of faunal species on settlement panels plotted versus time for bioassay control and leachate treatments.

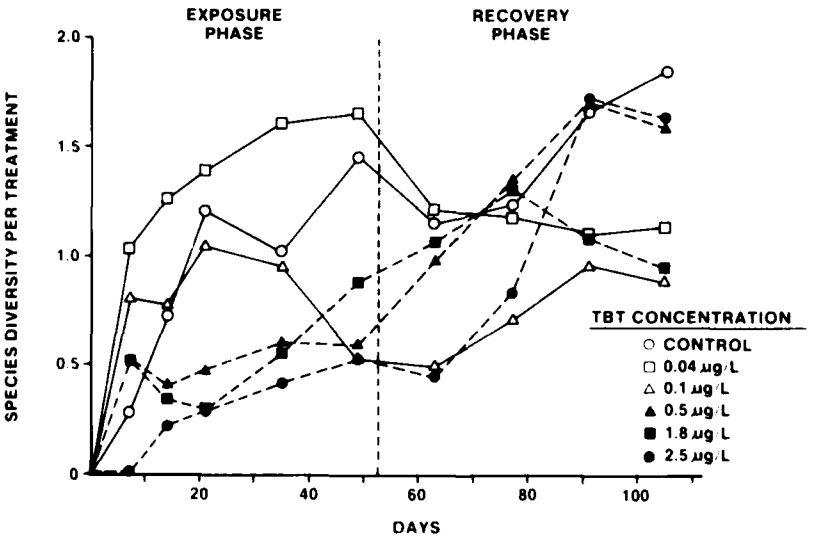


Figure 6. Species diversity (modified Shannon-Wiener index) of fauna on settlement panels plotted versus time for bioassay control and leachate treatments.

Table 6. Significant differences between bioassay treatments for mean total number of faunal species on settlement panels at ends of treatment and recovery phases.

	TBT in µg/L				
	Control	0.04	0.01	0.05	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-percent level. Any two treatments underscored by the same bar are not significantly different.

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 7). Organisms least affected were those capable of complete closure in hard, tubular shells, such as *Hydroides* and *Pileolaria* spp, or in thick body walls, and solitary tunicates such as *Ascidia* spp. 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.

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.

Introduced Fauna

Survival of oysters exposed to TBT concentrations $\leq$ 1.8 µg/L was similar to the controls (figure 7). Slightly enhanced survival of oysters in the three low-TBT treatments was probably due to reduced predation of oysters by flatworms. This was attributable 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-µg/L TBT was 50 percent after 30 days of exposure (figure 7).

Table 7. 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 ng/L						TBT in ng/L					
	2.5	1.8	0.5	0.1	0.04	CTRL	2.5	1.8	0.5	0.1	0.04	CTRL
PORIFERA (Sponges)												
Calcarea (Calcareous sponges)												
Leucosia kaiana							X	X	X	X	X	X
CNIDARIA (Coelentrates)												
Anthozoa (Anemones)												
Aiptasia pulchella										X	X	X
BRYOZOA												
Bugula neretina										X		X
MOLLUSCA												
Pelecypoda (Bivalves)												
Anomia nobilis				X	X	X		X	X			
Gastropoda												
Crepidula aculeata						X						
ANNELIDA												
Polychaeta												
Sabellidae (unid.)												X
Hydroides elegans	X	X	X	X	X	X	X	X	X	X	X	X
Pileolaria militaris/ P. pseudomilitaris (undif).	X	X	X	X	X	X	X	X	X	X	X	X
TUNICATA (Sea squirts)												
Ciona intestinalis							X	X	X			
Polyclinum cf. vasculosum					X	X			X		X	X
Didemnum candidum				X	X	X	X	X	X	X	X	X
Diplosoma macdonaldi							X	X	X			X
Perophora sp.										X		X
Ascidia spp.[1]		X	X		X	X	X	X	X		X	X
Pyuridae[2]						X	X	X	X			X
Botryllioides sp. A (brown/tan)							X	X	X	X	X	X

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

[1]Includes *Ascidia nigra, A. interrupta,* etc.

[2]Includes *Herdmania momus, Microcosmus exasperatus*

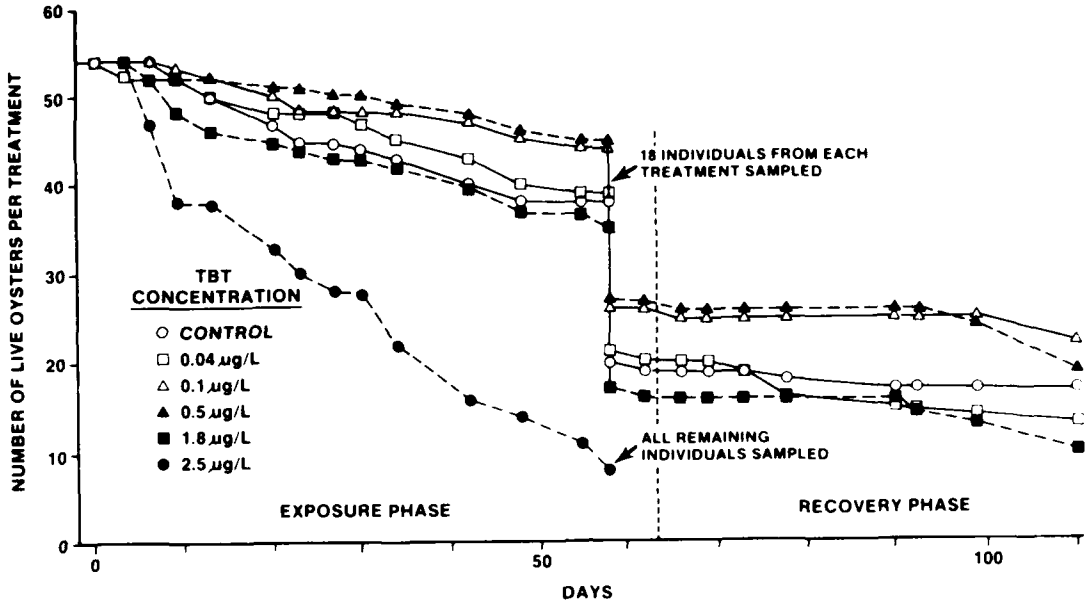
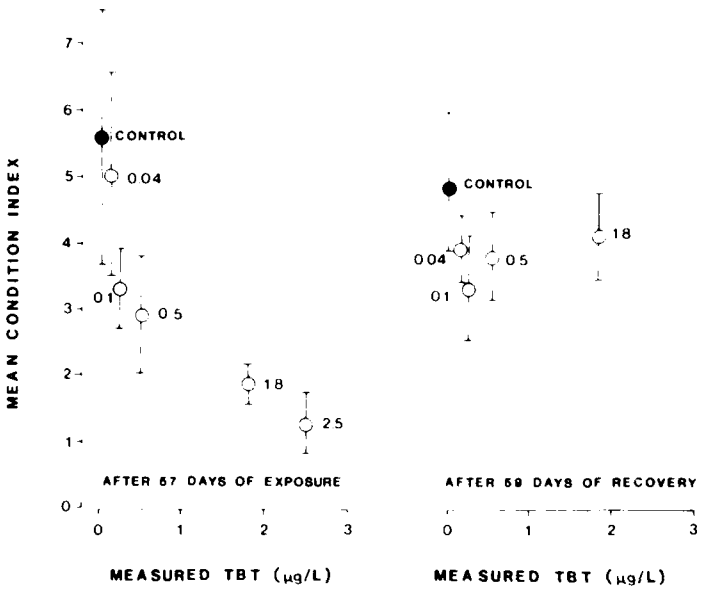


Figure 7. Total number of live oysters (Crassostrea virginica) plotted versus time for bioassay control and leachate treatments.

Condition indices for oysters collected after 57 days of exposure were significantly lower in oysters exposed to TBT concentrations of 0.1 µg/L and greater (figure 8). 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 indices for control oysters is very similar to the mean condition index of 5.3 obtained by Sakuda (1966) for oysters collected from West Loch, Pearl Harbor. Note that all condition indices of the present study and of Sakuda's study are substantially lower than the generally accepted minimum level of about eight for quality marketable oysters. Additionally, the pattern of variability in condition indices seen in the present study matches trends documented by Westly (1961) 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 use of oysters in condition index studies, variability could probably be reduced by using dry tissue weights instead of wet weights.

After 2 months of recovery, condition indices of surviving treatment oysters had returned to near-control levels (figure 8). This relatively rapid recovery to normal conditions indicates that oysters had resumed normal feeding and toxic effects from TBT were apparently short-lived. As of this writing, analyses of oyster tissue content of organotins 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.



Vertical bars are 95% confidence limits

Figure 8. Mean condition indices of oysters (Crassostrea virginica) after 57 days' exposure to control or leachate treatment and 59 days' recovery.

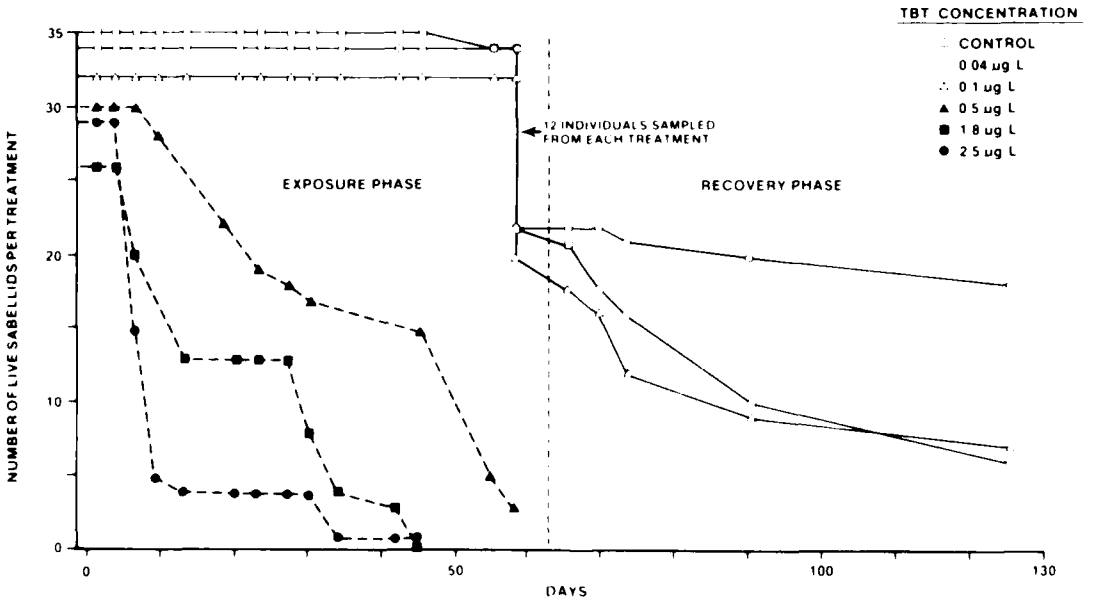


Figure 9 Total number of live sabellid feather-duster worms (Sabellastarte sanctijosephi) plotted versus time for bioassay control and leachate treatments.

Survival of sabellid feather-duster worms exposed to TBT showed that these organisms are highly sensitive (figure 9). Ninety-five percent of all individuals exposed to TBT concentrations of 0.5 to 2.5 μg/L were dead after 60 days of exposure. Sabellids in the 0.04 and 0.1 μg/L treatments sustained no significant mortality during exposure. However, sabellids in those same treatments experienced a pulse of mortality during the first half of the recovery phase. Also, several individuals in the 0.1 μg/L treatment shed their tentacle crowns during and after exposure as an apparent response to TBT stress.

Posttreatment sabellid mortality in lower level TBT exposures suggested that those individuals may have accumulated lethal amounts of TBT over the 60-day treatment interval and were unable to depurate enough TBT to affect their recovery. Actual TBT content of sabellid tissues will be reported when those analyses are completed.

Swimming crabs (*Thalamita admete*) were observed in quantities of at least one per three-tank treatment for the duration of the experiment. The crabs were inadvertently recruited to the tanks either as small juveniles living within growth on the pre-fouled panels or as larvae in the supply seawater. Growth rates of crabs seen in all tanks were rapid. When individuals attained a size of 3 to 4 cm they were netted from the tanks and frozen for later TBT-content analysis. There were no discernable between-treatment differences in overall abundances and growth rate of crabs during treatment or recovery phases. Clearly, neither juvenile nor adult swimming crabs were affected by TBT concentrations as high as 2.5 μg/L.

Precise numbers of glass shrimp (*Palaemon* spp.) per tank could not be obtained because of the difficulty of regularly finding all individuals hidden in the irregular substrates such as filamentous algae, fouling communities, and sabellid worm clusters. However, frequent approximate counts showed their abundance stabilized at about 12 per tank several days after introduction to the tanks. For the remainder of the experiment, their average abundance and behavior did not vary between treatments. Additionally, female shrimp with normally developing eggs were spotted in all treatments. As was the case with the crabs, shrimp were obviously not affected by TBT concentrations encountered in this experiment.

Anemones

Anemones (*Aiptasia pulchella*) entered the bioassay tanks in larval form and occurred in patchy distribution on various hard substrates. Although relatively common, reliable quantification of anemone populations was difficult because of their mobility and frequent cryptic habits. Observations on their health and presence/absence, however, were useful in assessing their susceptibility to TBT.

Five days after beginning leachate treatment, all anemones in 2.5 μg/L TBT tanks were contracted; whereas, all individuals in other treatments were normally expanded. After 12 days of treatment, anemones in the 0.5, 1.8, and 2.5 μg/L TBT tanks were estimated to be less than 50 percent as abundant as in control and lower concentration treatments. Individuals remaining in high-level treatments exhibited signs of stress such as persistent tentacle retraction and abnormally profuse expulsion of acontia (nematocyst-laden filaments). No *A. pulchella* were observed in the three high-level treatments on the 26th day of exposure. Based on these general observations, an estimated 50 percent

mortality level for *A. pulchella* had occurred after 12 days at a TBT concentration of 0.5 µg/L.

A less common species of anemone, *Haliplanella luciae*, was observed in low abundance on oyster shells and fouling panels in the bioassay tanks. Live and apparently unstressed individuals of this species were found in all treatments during the experiment. The anemone's high tolerance to TBT leachates is in strong contrast to the low tolerance exhibited by *A. pulchella*.

Haliplanella luciae is thought to have been accidentally introduced to Hawaii on imported oysters. From its presumed zone of origin along the Pacific coast of Asia, it has now spread over much of the northern hemisphere. Several studies of this actinian have shown it to be exceptionally tolerant of environmental extremes (Shick, 1976). Contraction and profuse mucus production are two strategies used by *Haliplanella luciae* to protect itself from threatening conditions.

Infauna

Inventory of infauna recovered from fine sediments on the tank bottoms at the end of the experiment showed a mean of 13 species from all tanks with no significant differences in the numbers of species between treatments. Statistical between treatment comparisons of diversity indices and total numbers of individuals and specific taxa also revealed no significant differences. Residual toxicity of sediment bound organotin leachates was, therefore, minimal as evidenced by complete recovery of infauna.

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 high nutrient, high organic seawater. The results obtained here are directly applicable to predicting potential paint leachate effects in harbor environs. 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 water (Henderson, 1985). Furthermore, concentrations of dissolved organotin were not reduced by 0.7 hour residence time in the high organic water of the Ford Island site, as mean measured TBT concentrations were generally at or above predicted levels.

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 the 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 (Alzien et. al., 1982; Waldock & Thain, 1983; Beaumont & Budd, 1985). 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.

Direct effects of leachates on the mortality and health of the crab and shrimp species monitored were nil over the entire exposure range of 0.04 to 2.5 μg/L TBT. Although long-term survival of most crustaceans would apparently not be affected even by relatively high environmental concentrations of organotins, measurements of chronic bioaccumulation of organotins by "food" crustaceans should be accomplished to assess potential human health impact.

In nearly all aspects of this bioassay, 0.5-μg/L TBT 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, 90-percent mortality of feather-duster worms, and 100-percent mortality of taxa such as colonial tunicates, an encrusting bryozoan (*Schizoporella errata*), and a bivalve mollusk (*Anomia nobilis*). Thus, these results reinforce the Department of the Navy's environmental assessment determination (1985) 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 for 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 corroborate 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, the condition index of oysters, or other introduced species in long-term 0.04-μg/L TBT exposures.

REFERENCES

Alder, H. L. and E. B. Roessler (1972). *Introduction to Probability and Statistics*. W. H. Freeman and Co., San Francisco. 373 pp.

Alzieu, C., M. Heral, Y. Thibaud, M. J. Dardignac, and M. Feuillet (1982). Influence des peintures antisalissures a base d'organostanniques sur la calcification de la coquille de l'huitre *Crassostrea gigas*. *Revue des Travaux de l'ISTPM*, Vol. 45(2). pp. 100-116.

Beaumont, A. R. and M. D. Budd (1985). High mortality of the larvae of the common mussel at low concentrations of tributyltin. *Marine Pollution Bulletin* (in press).

Department of the Navy (1985). Fleet-wide implementation of organotin antifouling hull paints: interim finding of no significant impact. *Federal Register*, Vol. 50(120). pp. 25748-25750.

Evans, E. C. (ed.) (1974). *Pearl Harbor biological survey - final report*. Naval Undersea Center Technical Note 1128. 718 pp.

Galtsoff, P. S. (1964). The American oyster, *Crassostrea virginica* Gmelin. *Fishery Bulletin of the Fish and Wildlife Service*. Vol. 64. 480 pp.

Henderson, R. S. (1985). *Marine microcosm experiments on effects of copper and tributyltin-based antifouling paint leachates*. (NOSC TR 1060) San Diego: Naval Ocean Systems Center.

Sakuda, H. M. (1966). Condition of the American oyster, *Crassostrea virginica*, in West Loch, Pearl Harbor, Hawaii. *Transactions of the American Fisheries Society*. Vol. 95(4). pp. 426-429.

Shick, J. M. (1976). Ecological physiology and genetics of the colonizing actinian, *Haliplanella luciae*. In: *Coelenterate Ecology and Behavior*. G. O. Mackie (ed.). Plenum Press, New York. pp. 137-146.

Sokal, R. R. and F. J. Rohlf (1969). *Biometry*. W. H. Freemen and CO., San Francisco. 776 pp.

Strickland, J. D. H. and T. R. Parsons (1972). *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Bulletin 167, Ottawa. 310 pp.

Valkirs, A. O., P. F. Seligman, G. Vafa, V. Homer, S. H. Lieberman, and P. Stang (1985). *Speciation of butyltins and methyltin in seawater and marine sediments by hydride derivatization and atomic absorption detection*. (NOSC TR 1037) San Diego: Naval Ocean Systems Center.

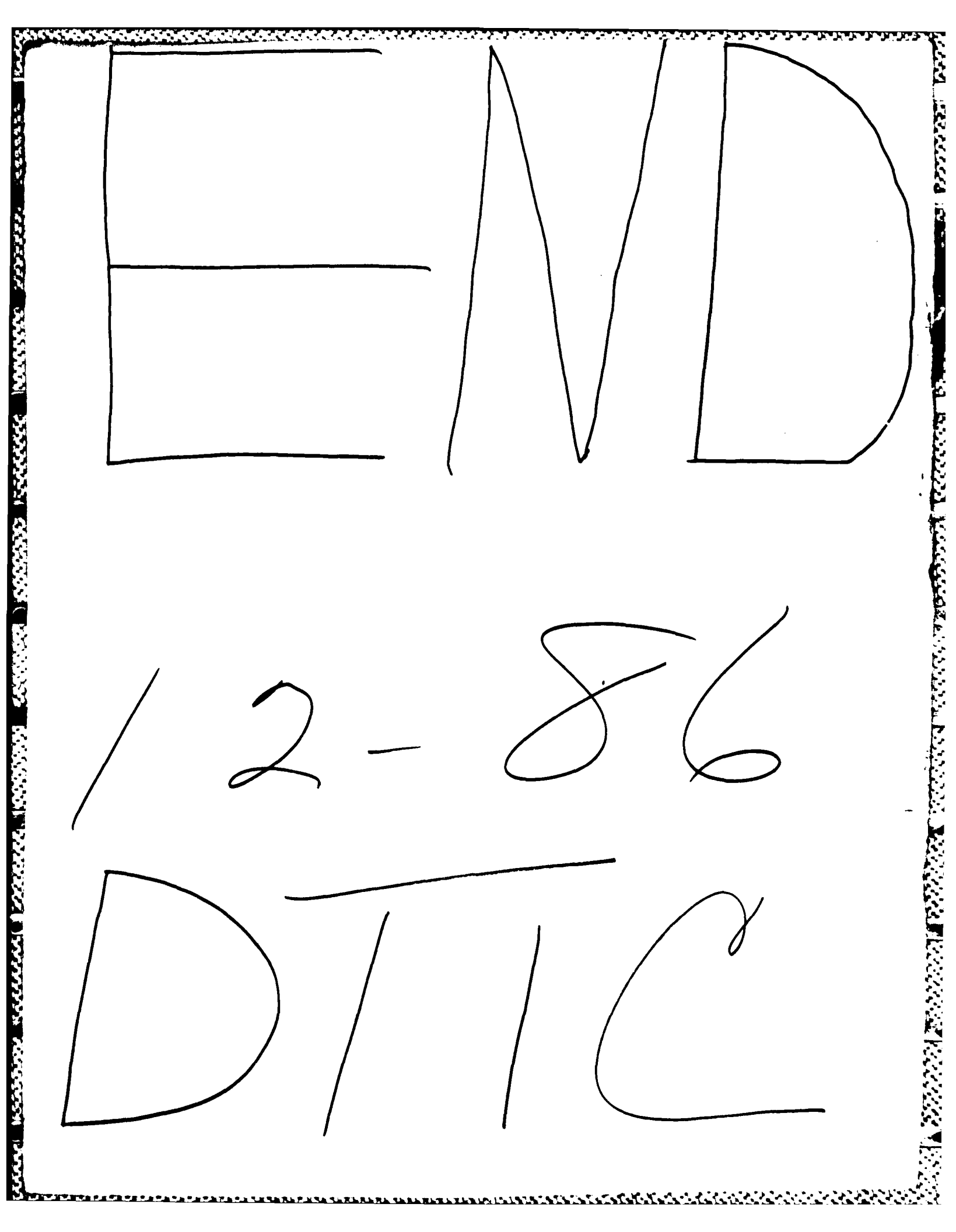
Waldock, M. J. and J. E. Thain (1983). Shell thickening in *Crassostrea gigas*: organotin antifouling or sediment induced? *Marine Pollution Bulletin*, Vol. 14(11). pp. 411-415.

Westly, R. E. (1961). Selection and evaluation of a method for quantitative measurement of oyster condition. *Proceedings of the National Shell-fisheries Association*, Vol. 50. pp. 145-149.

Wihlm, J. L. and J. C. Dorris (1958). Biological parameters for water quality criteria. *Bioscience*, Vol. 18. pp. 477-481.

ABBREVIATIONS

TBT	=	Tributyltin
TBTO	=	Bis (tri-n-butyltin) oxide
L	=	Liter
ml	=	Milliliter
m	=	Meter
cm	=	Centimeter
mm	=	Millimeter
in.	=	Inch
g	=	Gram
μg	=	Microgram
ppb	=	Parts per billion
ppt	=	Parts per thousand
°C	=	Degrees centigrade
°F	=	Degrees Farenheit
N	=	Nitrogen
P	=	Phosphorus
LC_{50}	=	The concentration of a particular toxin at which 50 percent of a population of organisms is killed within a specified time interval.


END
12-86
DTIC