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Laboratory Assays of Tributyltin Toxicity to Some Common Marine Organisms

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The behavior of seven representative marine organisms towards tributyltin oxide (TBTO) was studied under controlled laboratory conditions. It was observed that the sensitivity of marine organisms varies from species to species. The highest tolerance to TBTO was shown by a fouling species *Mytilopsis sallei* (LC–50/28 days = 13.0 µg/l TBTO) followed by Indian rock oyster *Saccostrea cucullata*. The most sensitive species seemed to be the green mussel *Perna viridis* (LC–50/28 days = 0.28 µg/l). The environment quality target for TBTO in India is proposed to be 0.03 µg/l as an advisory recommendation.

INTRODUCTION

The development of a self-polishing antifouling paint based on copolymer, containing chemically bound tributyltin oxide (TBTO) is a quantum leap in the pursuit of better coatings, and has been recognized as a technological achievement in a true sense of the word. Lately, however, the deleterious effects of TBTO on marine life of commercial value have been reported from several maritime countries in Europe and the United States. The leachates from the toxic paint applied on small mechanical crafts and pleasure boats anchored in shallow coastal waters and estuaries are feared to inflict catastrophic damage to young growth stages of a variety of edible shellfish and finfish. The Arcachon Bay in France, the Roach and Crouch estuaries in England, and the South Slow estuary in Oregon, all of which produce rich harvests of oysters and other shellfish, are the worst TBTO affected areas. The more dramatic effects were observed on adult oysters that developed deformities in their shells. According to Alzieu and his coworkers (1982) the affected individuals formed cavities between the calcified layers of their shells that were filled with a 'protein gel' that differed chemically from the protein normally formed during shell growth. The presence of TBTO even in parts per billion concentration was found to be detrimental to marine productivity. The ability of a marine snail *Nucella lapillus* to produce eggs was seriously jeopardized in areas of popular yachting activity and marinas where tributyltin concentrations were in less than detectable amounts (Bryan et al. 1986).

If the potential of TBTO-based coating is to be fully exploited, it is necessary that concerted efforts are made to establish the extent of its harmful effects, if any, on marine life such as edible finfish and shellfish. The present paper containing the results involving the assessment of toxicity of TBTO to both fouling organisms and edible organisms is one such attempt.

METHODS

Test organisms

In this study, marine organisms were selected on the basis of guidelines provided by the U.S. Environmental Protection Agency (EPA) (Anonymous 1986). At least one species each from five different phyla was selected for the study. In barnacles and tube worms, the two major fouling species, both adult and one-day old juveniles, were used. The marine species selected as test organisms were *Hydroides elegans, Balanus amphitrite, Perna viridis, Saccostrea cucullata, Mytilopsis sallei, Ascidia* sp., and *Therapon jarbua*.

Bioassay studies

The bioassay studies involved both short-duration (acute toxicity) and long-duration (chronic toxicity) exposures of the test organisms to graded concentrations of TBTO. The methods used are standard testing procedures (Anonymous 1981).

Acute Toxicity Assays: The short-term toxicity studies were carried out under static seawater conditions. The exposure period of the test organisms to TBTO concentrations was 96 hours, with daily renewal of toxic solution (25 liters per tank). The organisms were not fed during the 96-hour exposure period. After exposure to TBTO, the organisms were transferred to tanks containing fresh running seawater for their revival. During this period, the animals were provided with food. Except for finfish, which were fed on 'fish-food', all other test organisms were fed on unicellular alga *Dunaliella primolecta*. Mortality was recorded after the 48-hour revival period. From the mortality values obtained at different graded concentrations of TBTO, a concentration rendering 50% of the population dead, i.e., LC–50/96 hour, was computed adopting a method described by Litchfield and Willicoxon (1949).

Chronic Toxicity Assays: These long-term tests were carried out in flowing seawater as recommended by the U.S. EPA. The flow rate of water in bioassay tanks containing 25 liters of TBTO solution was maintained at five liters per hour; 120 liters of test solution were used in 24 hours. The testing tanks (aquaria) were also provided with a continuous supply of air at a regulated rate. Test solutions were prepared daily. In this study, the organisms were fed twice every day ('fish food' for finfish and algal food for the other test organisms). The organisms were transferred to tanks containing fresh seawater after 28 days exposure and the final mortality counts were recorded after a 48-hour revival period. All experiments were carried out in duplicate. The lethal concentrations required for achieving 50% mortality (LC 50) were computed from the data obtained on mortalities recorded at graded concentrations.

Analytical methods

Preparation of Test Solution: TBTO solutions for bioassay studies were prepared in acetone according to the method described by Laughlin et al. (1983).

Estimation of TBT in Tissues of Test Organisms: The method described by Bryan et al. (1986) was followed for the preparation of tissues for estimation of total organic tin. The inductively coupled plasma (ICP) technique was used for the estimation of tin in the tissues. The bioconcentration factor (BCF) was computed by using the formula:

$$BCF = \frac{\mu g\ TBTO/kg\ dry\ wt\ of\ tissue}{\mu g\ TBTO/l\ in\ seawater}$$

RESULTS

Bioassay studies

Results of bioassays on TBTO toxicity obtained for different groups of test organisms are tabulated in two tables. Table 1 gives the acute toxicity values (96-hour exposure) as well as chronic toxicity values (28-day exposure) obtained for the adult organisms. Table 2 shows the effects of low concentrations of TBTO on the one-day-old juvenile barnacle *Balanus amphitrite* and on the tube worm *Hydroides elegans* (figure 1).

Table 1. Toxic concentrations of tributyltin oxide to various marine species.

Organism	LC 50/96 hours (µg/l)	LC 50/28 days (µg/l)
Mytilopsis sallei	53.0	13.0
Saccostrea cucullata	25.0	10.0
Hydroides elegans	—	3.8
Balanus amphitrite	—	3.5
Ascidia sp.	10.1	2.8
Therapon jarbua	4.6	0.4
Perna viridis	4.8	0.3

Table 2. Effects of chronic toxicity of tributyltin oxide to one-day-old juveniles of two fouling species.

Organism	TBTO Concentration (µg/l)	Results
Balanus amphitrite	0.56	no mortality, growth almost normal
	5.60	90% mortality
Hydroides elegans	0.56	no mortality, growth almost normal
	5.60	90% mortality

Bioconcentration of TBTO

TBTO accumulated in the tissues of *S. cucullata* and *P. viridis* subjected to various concentrations for a shorter period of 96 hours was determined. The results are given in tables 3 and 4. It was observed that the amounts of TBTO, determined as inorganic tin, accumulated in the soft tissues of these two bivalve species were inversely proportional to the concentrations of TBTO to which they were exposed.

DISCUSSION

The selection of the test organisms for the study was made according to the guidelines provided by the U.S. EPA (Anonymous 1986). The criteria used for the selection of the organisms were that the test species were

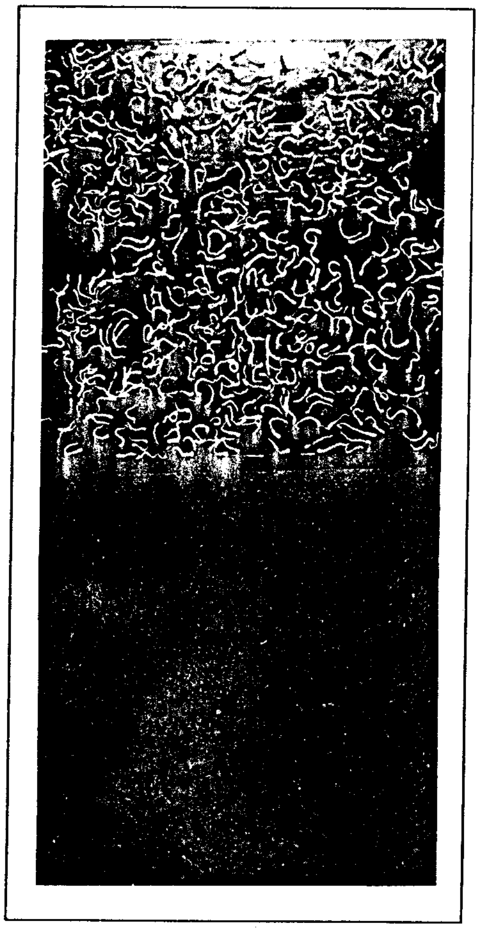

Figure 1. Behavior of one-day-old juveniles of *Hydroides elegans* exposed for 28 days to t ityltin oxide. The worms show 100% mortality in 5.6 µg/l of TBTO (bottom) and normal g. wth, in terms of size, in 0.56 µg/l of TBTO (top).

Table 3. Accumulation of tributyltin oxide (TBTO) in tissues of the oyster *Saccostrea cucullata*.

TBTO Concentration in Test Solution (µg/l)	Dry Wt. of Tissues (gms)	Inorganic Tin Estimated (µg)	Calculated TBTO (µg)	Bioconcentration Factor (BCF)
5.6	0.7085	27.5	68.8655	x 17,356
7.5	0.6279	29.5	73.8739	x 15,686
10.0	0.7505	40.0	100.1680	x 13,346
15.5	0.4983	39.0	97.6638	x 12,644
Control	0.6532	nil	–	x nil

Table 4. Accumulation of tributyltin oxide (TBTO) in tissues of the shellfish *Perna viridis*.

TBTO Concentration in Test Solution (µg/l)	Dry Wt. of Tissues (gms)	Inorganic Tin Estimated (µg)	Calculated TBTO (µg)	Bioconcentration Factor (BCF)
2.8	1.0007	36.0	90.1512	x 32,174
4.0	0.9993	34.0	85.1428	x 21,300
5.6	1.0003	34.0	85.1428	x 15,199
10.0	1.0002	30.0	75.1260	x 7,503
Control	0.9983	nil	–	x nil

indigenous to local waters, at least one species each from a minimum of five animal phyla was studied, at least one species belonged to vertebrate animals, and at least one species was a sedentary form. In addition, sturdiness was also considered. A marine species of mussel, namely *M. sallei*, highly tolerant to pollutants, was therefore included as the test organism.

Results (table 1) showed that whereas the fouling organisms, particularly *M. sallei*, withstood higher TBTO concentrations (53.0 µg/l), two species, namely *T. jarbua* and *P. viridis*, were highly sensitive to very low TBTO concentrations (4.6 and 4.8 ug/l, respectively). *M. sallei*, which had been earlier reported as a most tolerant species to free chlorine (Karande et al. 1982) thus proved to be highly tolerant to TBTO as well. The selection of *M. sallei* as a most sturdy species turned out to be helpful and underlined the importance of practicing caution in selection of the test organisms in this type of assay experiments. Organisms displaying extreme responses to experimental conditions need to be avoided because they do not represent average behavior of a local community.

A trend in tolerance limits noted in different organisms during chronic exposures was generally the same as that observed in acute toxicity experiments. The highest tolerance to TBTO was again shown by the fouling mussel *M. sallei* (LC 50 = 13.0 µg/l) and the least tolerance was noted in the edible green mussel *P. viridis* (LC 50 = 0.28 µg/l). Cardwell and Sheldon (1986) listed chronic LC 50 values for different organisms reported by various workers. These TBTO LC-50 values ranged from 0.025 µg/l to 10 µg/l. *M. sallei*, as evident from the present study, thus seems to have the highest degree of tolerance to TBTO compared to any marine species studied.

Sensitivity of marine organisms to TBTO varies from species to species. Sensitivity, even in the same species, may differ. Wild populations particularly have been found to be more tolerant than the laboratory reared species (Salazar et al. 1987). Gastropods and bivalves are reported to be the most sensitive organisms (0.02 to 0.46 ppb) followed by crustaceans

(0.09 to 0.14 ppb). Algae too were found to be more tolerant, showing tolerance in the range of 0.1 to 3.5 ppb (Rextrode 1987). Our results showed that the tolerance of endemic species, namely the green mussel as well as fish (0.28 and 0.34 ppb, respectively) was comparable to the tolerance reported in temperate species.

It was suggested by Waldock et al. (1987) that oysters and mussels may be regarded as indicators of the presence of TBTO in seawater because of their ability to respond readily to this chemical species by showing changes either in morphological or in physiological behavior. The choice of the oyster as a TBTO indicator was made on the basis of thickening of the shell displayed in the presence of TBTO (Thomas 1967, Key et al. 1976, Alzieu et al. 1982, Thain et al. 1987). In temperate waters, the oyster *Crassostrea gigas* is recommended as an indicator species. The present study has shown that the Indian oyster species *S. cucullata* shows relatively higher tolerance to TBTO and, therefore, its value as the sentinel organism for TBTO presence is reduced. The green mussel *P. viridis* may be identified as the indicator species for Indian waters since it shows lower tolerance (higher sensitivity) to the presence of TBTO in seawater.

A wide variety of marine organisms exposed to chemical pollutant species are reported to accumulate the chemicals in their body tissues (Salazar 1989). Abilities to bioaccumulate and depurate these chemical compounds vary from species to species. In the present study, the BCF of two species, the oyster and green mussel, were determined (tables 3, 4).

The oysters, when exposed to a concentration of 5.6 µg/l of TBTO, showed a BCF of 17,356. On the other hand, when they were exposed to a higher concentration of 15.5 µg/l of TBTO, BCF was found to be 12,644. In the green mussels, also, the BCF was highest, i.e., 32,174, at the lowest exposure concentration of 2.8 µg/l of TBTO.

Salazar et al. (1987) had observed an inverse relationship between concentration of TBTO and the bioconcentration factor in the organisms they had studied. Their results on *Mytilopsis edulus* mussel follow, table 5.

Table 5. Relationships between concentration of tributyltin oxide and the bioconcentration factor in *Mytilis edulus* reported by Salazar et al. (1987).

TBTO Concentration (µg/l)	Body Burden (µg/TBT/gm tissue)	BCF
0.542	10.38	23,000
0.204	5.40	26,500
0.079	2.96	37,500
0.006	0.42	70,000

On the basis of this first assay study carried out in India, it is not possible to recommend any ultimate permissible concentration limit of TBTO in our waters. However, it is possible to recommend guidelines by comparing the present data to that generated elsewhere. For instance, it is noted by Cardwell and Sheldon (1986) that LC 50 (chronic) for most organisms falls within a range of 0.025 to 10.0 ppb. In our study where seven representative marine organisms were used in the assays, the LC 50 values varied between 0.28 and 13.0 ppb. These values are thus within the range reported by Cardwell and Sheldon (1986). Presently, therefore, for our waters it is prudent to adopt the guidelines suggested by the U.S. EPA for American waters. The environment quality target for TBTO in Indian waters is thus proposed to be placed around 0.03 µg/l. It is also suggested that a concentration of 0.24 µg/l of TBTO be permitted for short durations of time in certain situations, such as during hosing of hulls of tributyltin-coated ships in dry dock or in the event of any episode of it being accidentally present in coastal waters.

The water quality criteria, which need periodical updating, are provisional, as in other maritime countries. In order to evolve national guidelines with regard to stipulation of acceptable concentrations of TBTO, a broad data base on the toxicity of TBTO to representative organisms is required and needs to be created. This needs to be followed by a sustained program of ecological predictions in specific key areas of the coastal zones of the country.

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