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Tributyltin Oxide Induced Physiological and Biochemical Changes in a Tropical Estuarine Clam

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Organotin compounds are widely used as additives in PVC stabilizers, and antifouling paints, and as biocides (fungicides and bactericides). The use of antifouling paints containing organotin compounds in recent years has resulted in enormously elevated levels (Beaumont and Neuman 1986) of these compounds being reported in areas of high pleasure-craft activity. Tributyltin oxide (TBTO) compounds are generally lipophilic in character and have a low solubility in seawater. Since many estuaries are shallow with restricted circulation, it is only natural that estuarine animals would be more impacted by TBT compounds than marine life from other enviroments. Therefore, the biochemical and physiological maasures of toxicant-induced stress would be useful as sensitive, specific predictors of effects of the toxicant at the level of whole animals.

Molluscs have been widely employed in toxicity evaluation and water quality management program in view of their acknowledged role as bioindicators of toxicity levels. Studies referenced in Europe an effects of tributyltin antifouling coatings have shown molluscs to be among the most sensitive non-target groups. TBTO concentrations as low as 0.10 µg L^{-1} have a significant effect on both development and survival of mussels (Valkirs *et al.* 1987).

Although the general awareness of the hazard of environmental pollution had resulted in an upsurge of interest in toxicological studies pertaining to a tropical estuary, the actual metabolic effects of TBTO have received little, if any, inquiry. This motivated the design of laboratory-oriented sublethal chemotoxicity tests to probe into the changes undergone at the molecular level: e.g., metabolic rate (oxygen consumption) and energy content (variations in the glycogen, lipid and lactic acid content). Consequently, the estuarine clam *Villorita cyprenoides* var *cochinensis* was exposed to the stress of tributyltin oxide to study such changes.

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MATERIAL AND METHODS

The clams collected from Cochin backwaters, India, were acclimatized to the laboratory test conditions (salinity = 10 %; pH = 7.2 ± 0.03; dissolved oxygen = 7.8 ± 0.2 mg L^{-1}; temperature =29±2°C). Clams of 20±2 mm were chosen for the toxicity studies. The test animals were fed (with unicellular Synechosistis species) during the acclimation period but were not fed during the course of the experiment.

One g of tributyltin oxide (TBTO; Aldrich, 99%), was dissolved in 1000 ml acetone to obtain a 1000 mg L^{-1} stock solution. The appropriate test concentrations were prepared from the stock solution by diluting with triple distilled water.

The 96-hr LC50 value for TBTO was 0.080 mg L^{-1} (Sujatha 1992). The sublethal concentrations of TBTO chosen for physiological studies were 0.006, 0.008 and 0.010 mg L^{-1}. After the 1-wk acclimation, ten animals each were transferred to separate 10-L prewashed glass troughs filled with the water of experimental salinity and were dosed with the respective test concentrations of TBTO. Triplicate experiments were performed for each concentration of TBTO. Appropriate controls were also maintained. To the central was added the same quantity of acetone that was present in the respective test concentrations of TBTO. The water was changed daily and the biocide added to the the experimental troughs (96 hr) daily. Mortality of the organisms was noted every 12 hr.

During the sublethal tests, no mortality was observed and animals were found to be actively filtering throughout the duration of the experiment. Six sets of animals (control and experimentally exposed) were withdrawn from each of the troughs for the determination of the metabolic rate and biochemical constituents (glycogen, lipid and lactic acid).

0xygen uptake of the animals was measured in Erlenmeyer flasks using Winkler techniques (Strickland and Parsons 1972). The biochemical isolation of glycogen was followed by the method of Hassid and Abraham (1957). Lactic acid was quantitatively converted to aldehyde by heating with concentrated sulfuric acid and complexed with p-hydroxy diphenyl reagent (Barker 1957). the lipid content in the tissue sample was determined by the sulphophospho vanillin method described by Barnes and Blackstock (1973). All spectrophotometric readings were taken on a Hitachi 150-20 UV-VIS spectophotometer. The results were analysed statistically; the significant difference between experimental groups and control groups were determined using Students-t test.

RESULTS AND DISCUSSION

TBTO caused a pronounced decline in the oxygen consumption of the clams (Table 1). TBTO-exposed animals consumed well below a tenth of the oxygen censured by the control animals. The percentage deviation (from control values) of oxygen consumption in animals

exposed to the TBTO is shown in Figure 1.

The respiratory system of the aquatic animals seems to be the prime target of most pollutants, and the respiratory potential is an important physiological parameter that can be used to assess the toxic stress. The oxygen consumption is a product of two important factors, namely ventilation volume and the quantity of gas withdrawn from each liter of water. Therefore, changes in oxygen uptake from water by the animals and variations in the amount of water propelled through the gills resulted in fluctuations in oxygen consumption. Animals exposed to TBTO showed the least uptake of oxygen and this depletion in oxygen consumption was >90% for the three sublethal concentrations of TBTO as compared with the control animals. Decreased oxygen consumption produces metabolic stress which could enhance the vulnerability of the organism to the toxicants. The dscrease in the oxygen uptake has also been attributed to valve closure, decreased ciliary activity and filtration rate or direct impairment of the metabolic activity (Shapiro 1964). The drastic suppression in the rates of oxygen consumption may be due to the considerable - inflicted upon the gill tissue and to the format ion of mucus layers over it, which result in a decreased flow of water onto the gill surface and lead to a reduced oxygen intake. Similarly, the oxygen consumption of *Labeo rohita exposed* to Fenitrothion progressively decreased with increasing concentration of the insecticide (Murty *et al.* 1983).

Table 1. Influence of sublethal concentrations of TBTO on the oxygen consumption rate of *V. cyprenoides* for varying exposure periods* (n=6).

Concentration of TBTO ($mg\ L^{-1}$)	Oxygen consumption, $mL\ O_2/g$ wet wt/hr ; $\bar{x} \pm$ S.D.			
	Exposure period			
	24 hr	48 hr	72 hr	96 hr
Control	2.264 ± 0.294	2.150 ± 0.304	2.213 ± 0.316	2.168 ± 0.295
0.006	0.182 ± 0.026	0.122 ± 0.011	0.120 ± 0.017	0.121 ± 0.024
0.008	0.161 ± 0.023	0.133 ± 0.010	0.152 ± 0.016	0.151 ± 0.021
0.010	0.141 ± 0.020	0.121 ± 0.009	0.113 ± 0.013	0.130 ± 0.019

* All values significantly different from control ($P < 0.005$)

Variations in the glycogen level of the test species exposed to different concentrations of TBTO for varying periods of time are given in Table 2. The glycogen content in the tissue of the clam decreased with progress of time. This reduction was more pronounced in the animals treated with the highest concentration of TBTO (0.01 mg L^{-1}). Deviations of glycogen content (expressed as percentages) from the control values are depicted in Figure 1.

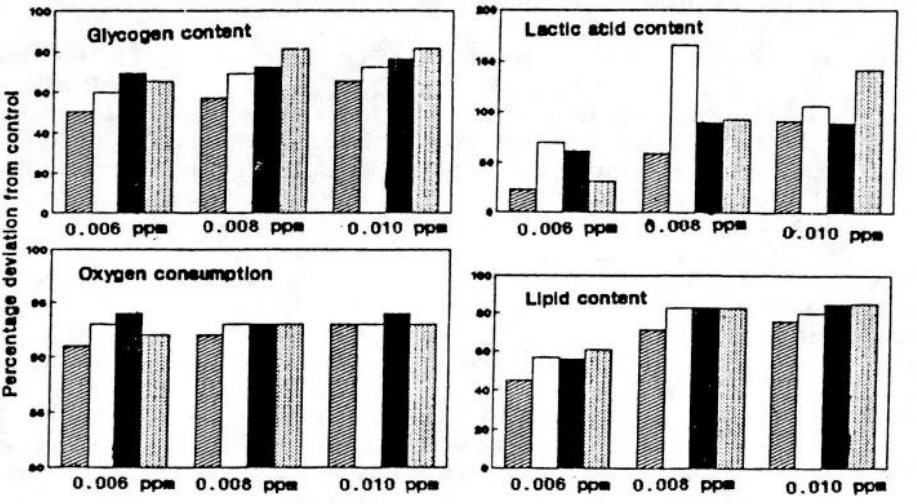

Figure 1. Percentage deviation (from control) of metabolic rate and biochemical constituents to varying sublethal concentrations of TBTO for designated period of time (24 ; 48 ; 72 and 96 hr)

Table 2. Influence of sublethal concentrations of TBTO on the glycogen content of V. *cyprenoids* for varying exposure periods (n=6).

TBTO Concentration ($mg\ L^{-1}$)	Glycogen, mg/g wet wt/h ; $\bar{x} \pm$ S.D.			
	Exposure period			
	24 hr	48 hr	72 hr	96 hr
Control	33.32 ± 0.19	32.51 ± 0.15	35.49 ± 0.35	36.54 ± 0.39
0.006	18.92 ± 0.17	14.03 ± 0.21	13.36 ± 0.19	11.24 ± 0.11
0.008	16.29 ± 0.39	10.72 ± 0.51	10.10 ± 0.19	07.20 ± 0.21
0.010	13.11 ± 0.22	09.53 ± 0.41	06.60 ± 0.27	07.01 ± 0.16

*** All values significantly different from control (P < 0.005)**

All deviations exceeded 50%, and a direct proportionality was observed between exposure concentrations and percentage deviations. The maximum reduction (85%) in oxygen content was detected for the highest sublethal concentration of TBTO.

A decrease in glycogen content appears to be a manifestation of the adaptive response of an organic exposed to biocidal stress. Generally, the increased energy demand associated with the stress

disturbs the carbohybdrate metabolism and causes glycogen depletion which activates glycogenolysis. Thus, the overall increase in glycogen content observed in the present investigation is a clear reflect ion of the above effect and is well supported by the results of several earlier investigations (Tabche *et al.* 1991). In all, the sublethal concentrations of TBTO, the secondary energy source, lipid, was found to be significantly lower in content in animals exposed to toxicants than in control animals (Table 3).

Table 3. Influence of sublethal concentrations of TBTO on the lipid content of *V. cyprenioides* for varying exposure periods (n=6).

TBTO Concentration ($mg\ L^{-1}$)	Lipid, mg/g wet wt; $\bar{x} \pm$ S.D. Exposure period			
	24 hr	48 hr	72 hr	96 hr
Control	4.423 ± 0.122	4.810 ± 0.111	4.553 ± 0.128	4.615 ± 0.121
0.006	2.421 ± 0.125	2.081 ± 0.050	1.998 ± 0.097	1.820 ± 0.082
0.008	1.283 ± 0.062	0.845 ± 0.026	0.782 ± 0.027	0.824 ± 0.016
0.010	1.083 ± 0.038	0.971 ± 0.022	0.704 ± 0.079	0.695 ± 0.008

*All values significantly different from control ($P<0.05$)

This demonstrates the important role of lipid as elementary energy reserves, which are utilized in the absence of or when there is a shortage of the primary metabolite, the carbohydrate. At the low concentration of TBTO, a 24-hr exposure resulted in lowering the lipid content to one half of the control value. Deviations of lipid contents (expressed as percentages from the control values are depicted in Figure 1. At the low TBTO concentration, the percentage deviation observed at 48 hr remained unchanged until 72 hr and recorded a further increase only thereafter. However exposure of the animal to the intermediate concentration for periods longer than 48 hr , did not produce any further variations in its lipid content. High concentration of TBTO, produce changes in the lipid content only during the first half of the experiment, and the percentage deviation from control remained unchanged after 72 hr.

Next to carbohydrates, fats are the best energy source of the body. Therefore under conditions of stress, the bivalves utilise lipid and protein reserves to meet the increased energy demands and consequently entail a depletion of their lipid levels. The considerable decrease in total lipids might be a sequel to the efforts of the organism to replenish any glycogen deficiency (caused by any extraneous reason) and/or to mitigate the multifarious toxic effects of the TBTO present in the animal. The decrease in total phospholipids observed when *Oncorhynchus kisutch* and *Oreochromis mossambicus* were exposed to PCBs and Methyl

parathion (Leatherland *et al.* 1979; Rao and Rao 1984) respectively, could be attributed to their use of energy to resist a stress. Quantitative comparisons of the effects of various sublethal concentrations of TBTO used in the present study disclosed that administration of pesticides in the body of organism has been shown to alter the lipid metabolism and this leads to the depletion of total lipid content (Coglianese and Neff 1982).

Exposure to TBTO, resulted in augmenting the lactic acid concentrations (Table 4). Intermediate and high sublethal concentrations of TBTO (0.008 and 0.010 mg L^{-1}), resulted in

Table 4. Influence of sublethal concentrations of TBTO on the lactic acid content of *V. cyprenoides* for varying exposure periods* (n--6).

TBTO Concentration ($mg\ L^{-1}$)	Lactic acid, mg/g wet wt; $\bar{x} \pm$ S.D. Exposure period			
	24 hr	48 hr	72 hr	96 hr
Control	0.0274±0.0010	0.0236±0.0008	0.0291±0.0027	0.0243±0.0018
0.006	0.0426±0.0034	0.0544±0.0024	0.0630±0.0018	0.0459±0.0018
0.008	0.0552±0.0016	0.0854±0.0027	0.0751±0.0018	0.0671±0.0016
0.010	0.0662±0.0028	0.0664±0.0023	0.0751±0.0018	0.0843±0.0020

*All values significantly different from control ($P<0.02$)

producing maximum elevations in lactic acid content. However, at the low concentration, the increase in lactic acid concentration was exposure dependent up to 72 hr. Although, the percentage deviations from control (Figure 1) generally increased with the increasing concentrations of TBTO, maximum deviations were observed for the 48-hr exposure. The effect of the 96-hr exposure to the low concentration was peculiar in that it resulted in a significant lowering of the lactate content. The 72-hr responses were always less than the 48- and 96-hr responses.

An overall elevation in lactic acid content in tissue is suggestive of the inadequate oxygen supply in the cells which is characteristic of the anaerobic lactate metabolism, hence the high persistence of tissue lactate in bivalves exposed to biocicide–stress. Mohan *et al.* (1987) also attributed the high lactic acid content in the fresh water mussel *Lamellidens marginalis* to pesticide-induced glycolysis. Wedmeyer *et al.* (1984) reported that short term stresses caused rapid depletion of blood glucose and glycogen and an increase in lactic acid content. Elevated concentrations of lactate were reported in the blood of

Indian catfish exposed to a mixture of Aldrin and Farmithion (Srivastava and Sing 1981).

The TBTO-induced lactic acid elevation observed in this investigation pointed out that the bivalves were under respiratory stress and that the anaerobic breakdown of sugars was more effective than the aerobic pathway. A similar inhibition of pyruvate dehydrogenase coupled with enhanced lactic dehydrogenase has been reported by Goel *et al.* (1984) in fish chronically exposed to pesticides.

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