# Toxicity Evaluation of TBTO Exposed to Freshwater Fish *Rasbora Daniconius*.

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#### **ABSTRACT:**

In the present investigation freshwater fish *Rasbora daniconius* exposed to lethal concentrations of TBTO for 24 h, 48, 72h and 96 h exposures were studied in terms of their general behavior, rate of survival and mortality. The activity of fish, *Rasbora daniconius* exposed to lethal concentrations showed minor changes in behavior which were intermediate, continuous and increased respiratory movement, agitated activity of fins and finally autotomy followed by paralysis. Degree of autotomy varied with the time of exposure. The 24 hrs., 48hrs., 72 hrs and 96 hrs LC50 values were found to be 0.55, 0.44, 0.33 and 0.26 ppm respectively. Results show that LC50 values decrease with increase in exposure period.

Keywords: TBTO, Toxicity, Rasbora daniconius

### **INTRODUCTION:**

Rivers are very vulnerable, since waste in effluents from industries, domestic and farms open directly into them. During the past few decades, rising trends of population explosion, development of modern technology, industrialization and dramatic increase in the production and consumption of large variety of new synthetic chemicals and there by high amount of pollutants were released into aquatic environment. In the last two decades ecotoxicology evolved mainly from three different disciplines such as toxicology, applied ecology and environmental chemistry. Ecotoxicology as an interdisciplinary environmental science deals with the interactions between environmental chemicals and biota, thereby focusing on adverse effects at different levels of biological organization. Toxic effects of anthropogenic compounds in biota and ecosystems are investigated in close connection to their environmental chemistry and fate in the environment.

The wide spread use of organotin compounds as stabilizer in manufacturing Polyvinylchloride, as catalyst in the production of synthetic organic polymers and as biocidal agent in wood preservation, crop protection and mainly antifouling system has provided several paints of entry for these compounds into aquatic and terrestrial environments. In the early 1980s the first impact of TBT on nontarget organisms were observed in France, Alzieu et al., (1980). France was the first country to draw up regulations to control TBT emission from antifouling paints and banned the use of TBT-based antifouling on small boats (length <25 m) in 1982. This legislation was adopted later by other countries. Although a consequent drop of aqueous TBT concentrations was expected and reported for some regions, TBT pollution of coastal waters was found to have remained on a high level or even increased further in other areas. Consequently, the International Maritime Organization (IMO) decided in Autumn 2001 to ban the application of TBT based paints on all boats by January 2003 and the presence on ship hulls by January 2008. Meanwhile, the proposed ban has been in acted to law in many countries worldwide. The first adverse effects of TBT on molluscs were observed in Crassostrea gigas at the Bay of Arcachon, one of the centers of oyster aquaculture in Europe with ball-shaped shell deformations in adults, and a dramatically decline of annual spatfall Alzieu et al., (1980). These effects led to a break-down of local oyster production in the bay with marked economic consequences. Much attention on the release of organotin compounds into the environment has found in the form of tributyltin (TBT) which has been widely used as biocide in paints and coating used for their antifouling application. However, in 1980's concern about the apparent toxicity of tributyltin to non-target species led to restricted use among many

industrialized countries. Despite such restriction, tributyltin persists in many areas at the level consider to be chronically toxic.

Among the aquatic organism fishes have been used as biomarkers for assessing the aquatic environmental pollution. Nagabhushanam, *et al.*, (1990) reported the accumulation of tin oxides in fresh water prawn, *Caradina rajadhari*. B. Indira (1989) worked on effect of TBTO on freshwater prawn, *Caradina weberi*. Acute toxicity tests using mysids, copepoda, cranonid shrimp, *Listmata ambilnesis*, Daphnia, Sand crab, *Plalamon paulidens*, Pond snail Mussel, European oyster and common oyster at test organism, and reported by Goodman et *al.*, (1988); Uren (1983); Walsh (1986); and Termink and Everts (1987) respectively. As a result 96 h LC50 was in the range of 1.1317 ng/L in term of organotin tributyltin oxideand the lowest LC50 value was observed for mysids (*Mysidopsis bahia*). Fish is an important food source in India among the local population. In present investigation toxic effect of TBTO on freshwater fish, *Rasbora daniconius* has been evaluated.

## **MATERIALS AND METHODS:**

The freshwater fish, *Rasbora daniconius* were collected from Godavari river near Nanded, Maharashtra. The fishes were maintained in aerated aquarium containing tap water. The physiochemical characteristic of tap water is temperature  $26\pm1^{\circ}$ , pH 7  $\pm$  0.2, dissolve oxygen is  $5.6 \pm 0.4$  ml/lit. They were acclimatized for a week in laboratory conditions. The water in the aquarium was changed every 24 h. After every three days the fishes were fed with green algae. The tributyltin oxide was purchased from Spectrochem Pvt. Ltd .Mumbai. 1-ppm stock solution was prepared in acetone Laughlin *et al.*, (1983). Matured healthy fishes were selected for the experiment. Series of statistic bioassay were conducted under laboratory condition as described by Finney (1971). For each experiment ten healthy fishes of approximately similar size ( $2.5 \pm 1$  cm in length) were exposed to different concentration of tributyltin oxide. The resulting mortality were noted of each concentration for the duration of 24 h, 48 h, 72 h and 96 h. Control group was also maintained in acetone with tap water.

## **RESULTS:**

Freshwater fish, *Rasbora daniconius* exposed to lethal concentrations of TBTO for 24 h, 48, 72h and 96 h exposures were studied in terms of their general behavior, rate of survival and mortality. The fish exposed to zero toxicants were observed to have normal activities such as steady balance, normal surfacing phenomenon non aggressive movement or irregular vertical revolving movements. The activity of fish, Rasbora daniconius exposed to lethal concentrations showed minor changes in behavior which were intermediate. Continuous and increased respiratory movement, agitated activity of fins and finally autotomy followed by paralysis. Degree of autotomy varied with the time of exposure. Higher concentration induced increased autotomy in fishes with in 12 h , where as in chronic concentrations of TBTCl, atomized its find within 16 to 20 h. the results of the bioassay testes are presented in table 1. The fish *Rasbora daniconius* showed approximately identical symptoms in behavioral abnormalities. LC50 values decreases with increase in exposure period. The percentage of mortality increased progressively upto 96 hrs. The 24 hrs., 48hrs., 72 hrs and 96 hrs LC50 values were found to be 0.55, 0.44, 0.33 and 0.26ppm respectively.

Exposure period in hrs.	Concentrations of TBTO	Variance	Regression equation	95% Fiducial limit	
				M1	M2
24 hrs	0.55	0.0006908	Y=1.0923+1.9764x	0.1349291	0.2379604
48 hrs	0.44	0.0001076	Y=2.0024+2.9439x	0.1412287	0.1792702
72 hrs	0.33	0.003307	Y=3.02306+3.2181x	2.3790	2.6044
96 hrs	0.26	0.001744	Y = 5.9414 + 4.5612x	2.3159	2.4797

Table 1: LC50values calculated for fresh water fish Rasbora daniconius<br/>after exposure to TBTO for a period of 24, 48, 72 and 96 h.

#### **DISCUSSION:**

TBT are introduced in aquatic environment are from antifouling paints, PVC industries, paper and pulp industries, as heat stabilizer and biocidal agent in wood preservation, etc. organotin compounds were first developed as mothproofing agents in the 1920s and later used more widely as bactericides and fungicides, Moore *et al.*, (1991). TBT compounds have introduced since late 1940s, Laughlin and Linden (1985), although use of TBT compound in antifouling paints dates only from the 1960s and then initially as a booster biocide in copper based formulations. As results it's of its effectiveness over copper, Wade *et al.*, (1988). At the same time as its explosive increase in the use, the first observation was made of TBT effects on non-target organisms. While toxicity of fouling organism was intentional, its propensity for wider impact on the aquatic environment has been grossly underestimated. An early focus on acute effects especially mortality, Laughlin and Linden (1987), failed to identify sub lethal consequences of prolonged exposure in some aquatic biota. The development of male sexual structure in female (imposex) can be initiated in some gastropod molluscs by TBT in low ng / l.

In the present study, the fishes treated with tributyltin oxide, the acute toxicity level was expressed in terms of LC50 values. The acute value for 24 h for organotin tributyltin oxide was found to be 0.55 ppm, for 48 h found to be 0.44 ppm, for 72 h found to be 0.33ppm (table 1) and the chronic value for 96 h for organotin tributyltin oxide was found to be 0.26 ppm (table 1). The result shows that the LC50 values decreased with increase in exposure period and vice-versa and also the 95 % confidence limits. Mary (1984) has reported that the LC50 values depend on the concentrations of pesticides and also with the time of exposure. The 96 hours LC50 value was the low, however the mortality scored was high. The determination of the LC50 value is of immense importance since it provides fundamental data for the design of more complex disposal model. The values obtained are highly useful in the evaluation of safe level or tolerance level of a pollutant. It is thus significant as a tolerance limit determined here might be useful in conducting chronic studies, since the shrimps are more sensitive to various types of toxicant than fish or molluscs, Couch (1984), and thus may be used as indicator for assaying the water quality.

This finding provides an opportunity to develop tissue TBT concentrations that are directly corelated with observed effects in a wide range of ecologically revalent species. Meador *et al.*, (1996) have reported acute toxicity (LD50) for *Rhepoxynius abronius*, *Eohaustorius* 

*washingtonianus* and *Armandia brevis* at concentrations ranging from 34 - 89 mg TBT / kg body weight (dry weight). Tissue concentrations within or above this range would represent a severe adverse effect and sediments associated with these levels would exceed the level at which cleanup would be required, and would also be inappropriate for open water disposal.

In the present research work the safe concentration for freshwater fish, *Rasbora daniconius* to TBTO was 0.0563 ppm. The death of freshwater fish, *Rasbora daniconius* might be due to toxic stress of TBTO which cause severe physiological and biochemical alteration at cellular as well as organismic level of tested fish. It might be due to the impact of TBTO on gill which ruptured the gill lamellae resulting decline in respiratory rate, an inhibition of the electron-transport system (ETS) or an effect on mitochondrial integrity. The respiration rate of organism is an indicative for the physiological state and changes in the respiration rates may be an indicative for environmental stress. Biological responses of organisms to pesticides in the aquatic environment are usually understood through determining their rate of survival and changes in the levels of various physiological phenomena. Newell (1973) stated that toxicants act as physiological stressors upon the organism. It is well known fact that the rate of oxygen consumption is used as an important tool for understanding the physiological state of metabolic activity of an organism. According to Piver (1973) dialkyltin and trialkyltin compounds are known to be capable of effecting the respiration. Umadevi (1996) studied changes in oxygen consumption of marine fouling dressinid bivalve, *Mytilopsis sallei* exposed to mercury. Thus from present investigation we can say that TBTO is very dangerous and its use should be strictly banned.

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