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Risk Assessment of Tributyltin Oxide in Aquatic Environment: A. Toxicity and Sublethal Effects on Brain AChE and Gill ATPases Activity of Tilapia Fish, *Oreochromis niloticus*

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Abstract: The present study was carried out to investigate the acute toxicity of TBTO and the effects of its sublethal concentration on brain AChE and gill ATPases activity of tilapia fish, *Oreochromis niloticus* during acute (3-96 h) and subchronic (7-28 d) exposure periods. The results showed that TBTO is extremely toxic to *O. niloticus* with 96 h LC_{50} of 16.3 μg L^{-1} using static renewal bioassay test. Sublethal concentration of 4.1 μg L^{-1} (25% of 96 h LC_{50}) of TBTO inhibited brain AChE activity by 24.01 and 24.06% of control at 96 h and 7 d, respectively. Gill Na^+, K^+-ATPase activity was inhibited by 51.8 and 54.5% at 96 h and 7 d, respectively. The activity of gill Mg^{2+}-ATPase was decreased by 26.9 and 24.28% at 96 h and 21 d, respectively. After 7 d of recovery, AChE and Mg^{2+}-ATPase activites were completely recovered. The activity of Na^+, K^+-ATPase was partially recovered but the inhibition was still significant. The inhibition percentages were higher in acute exposure than in subchronic exposure in both AChE and Na^+, K^+-ATPase, but the opposite result was recorded in Mg^{2+}-ATPase. It can be concluded that there is a need for more evaluation and international regulation to minimize the organotin input to aquatic environment.

Key words: TBTO, toxicity, AChE, ATPase

INTRODUCTION

Tributyltin Oxide (TBTO) has been used as a molluscicide for schistosomiasis control in tropical areas[1]. It is currently used as an antifouling agent in marine paints. Paints containing up to 20% TBT compound is slowly released from the painted surface with a leaching rate of 1-5 μg cm^{-2} d^{-1} into fresh or sea water providing protection against encrustations for submerged parts of boats, docks, fishing nets and marine structures[2]. TBTO is also used in cooling water pipes for electric power plants and other industries at the rate of 7-10 μg L^{-1} active ingredient of TBTO in the water flow which may range from 10,000 to 200,000 m^3 h^{-1} to protect the inside surface of pipes against fouling organisms. So we can notice that from these treated pipes; an amount of 1.7 to 48 kg d^{-1} of TBTO could be discharged into the sea[3]. Thus the use of marine paints results in significant inputs of biocides in the aquatic environment[4].

TBT compounds have been cited as one of the most toxic substances ever introduced in the marine environment[5]. It is extremely hazardous to some aquatic organisms, its 96 h LC_{50} is 6.9 μg L^{-1} for rainbow trout[3]. The lethal threshold concentration of TBTO for *Tilapia mosambica* is measured between 8 and 16 μg L^{-1}[6]. The toxicity of TBTO to fresh water fish carp (*Cyprinus carpio*) is measured with LC_{50} of 75 and 32 μg L^{-1} at 24 h and 96 h holding times, respectively[7]. The sensitivity of different life stages of fish to TBTO may be varied. Larval stages are more sensitive to TBTO than adults. The 48 and 96 h LC_{50} of TBTO to the adult sole (*Solea solea*) were 88 and 36 μg L^{-1}, whereas to the larval stage, they were 8.5 and 2.1 μg L^{-1}, respectively[8]. TBT compound may show no interaction with AChE but organophosphorus compounds and carcbamate exhibit high potency for inhibiting AChE activity of fresh water fish[9]. On the other hand, mitochondrial ATPases could be the target for TBTO action[10] and may cause osmoregulatory disturbance.

The objective of the present study was to asses the risk of TBTO in aquatic environment concerning its toxicity and sublethal effects on brain AChE and gill ATPases activity of tilapia fish.

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MATERIALS AND METHODS

Tested compound: Bis (tri-n-butyltin) Oxide (TBTO) formulated antifoulant MA1161 (15% EC) was purchased from Borman, Italiana.

Experimental animal: Fresh water fish tilapia, *Oreochromis niloticus,* with an average length and weight of 12 cm and 25 g, respectively were collected from Barsik Farm, Agriculture Ministry. Fish were acclimatized in dechlorinated tap water under the natural conditions of photoperiod and temperature according to the method of APHA[12].

Acute toxicity study: Static renewal bioassay test according to APHA[12] was carried out to evaluate the acute toxicity of TBTO. Tested concentrations were selected to be (10-90 μg L^{-1}) to produce mortality range from 5 up to 95%. Mortalities were recorded at 24, 48, 72 and 96 h in treatment and control. The data were analyzed according to the method of Finney[13] to determine the median lethal concentrations (LC_{50}).

Biochemical studies: Fish were exposed for every day-renewal protocol to sublethal concentration of 4.1 μg L^{-1} (25% of 96 h LC_{50}) for 28 days. Samples of four animals were taken from treatment and control at different time intervals, 3, 6, 24, 48,72 and 96 h for acute study and 7, 14, 21 and 28 d for subchronic study. The remained fish after 28 d in treatment was transferred to clean renewal water for 7 d to study the recovery from TBTO effect. Brain Acetylcholinesterase (AChE) activity was determined according to the method of Ellman *et al.*[14]. Branchial Adenosintriphosphatase (ATPase) activity was determined according to the method of Koch[15] .

RESULTS AND DISCUSSION

Acute toxicity study: Bioassay is considered as one of the most useful techniques available for predicting the potential hazard of environmental pollutants. The acute toxicity of TBTO to *O. niloticus* at different times of exposure (24-96 h).LC_{50} values were ranged from 31.5 to 16.33 μg L^{-1} at 24 h and 96 h of exposure, respectively. Also LC_{95} values ranged from 49.19 to 26.91 μg L^{-1} at 24 h and 96 h of exposure, respectively (Table 1). Various environmental pollutants revealed the same trend to fresh and sea water fishes[16]. The slope functions showed the homogeneity of fish to the toxicity of TBTO at different times of exposure (Table 1). According to the ranking scheme for assessing the acute toxicity of chemicals to fish described by Hodgson and Levi[17], TBTO could be classified as extremely toxic to fish (24 h LC_{50} < 1 mg L^{-1}). This finding coincides with that recorded by Matthiessen[6] who found that the 24 h LC_{50} of TBTO to *Tilapia mossambica* was 28 μg L^{-1}. Chliamovitch and Kuhn[18] measured the 24 h LC_{50} of TBTO for rainbow trout (*Salmon gairdneri*) and tilapia (*Tilapia rendelli*) as 30.8 and 53.2 μg L^{-1}, respectively. In addition, Assem[11] found that the 96 h LC_{50} of TBTO to euryhaline red tilapia (*Oreochromis* sp.) was 15.1 μg L^{-1}.

Biochemical studies: Inhibition of AChE activity was regarded as a significant parameter in assessing toxicological effects of various toxicants. It is well known that organophosphorus compounds produce their toxic action by inhibiting AChE[19,20]. On the other hand there are another different toxicants such as synthetic pyrethroids had an inhibitory effect on AChE of fish[21]. The present study showed the effect of TBTO on brain AChE. TBTO caused inhibition of brain AChE activity at all exposure times in a time dependent manner (inhibition percentages were increased when the time of exposure increased). The percentage inhibitions were 1.48, 3.59, 14.32, 18.75, 22.22 and 24.01% of the control at 3, 6, 24, 48, 72 and 96 h, respectively (Table 2). The percentages of inhibition were decreased when exposure time increased. The inhibition percentages were 24.06, 16.76. 4.81 and 2.19 at 7, 14, 21 and 28 d, respectively. After 7 d of recovery (Table 3), AChE activity was completely recovered. On the contrary, Ibrahim[9] found that the organotin compound triphenyltin acetate TPTA did not show any interaction with AChE of cat fish. The activity of AChE can be used as a tool for demonstrating the possible cause of fish death[22]. Brain AChE activity in aquatic organisms can be used in aquatic organisms as a good diagnostic tool for chronic pollution in biomonitoring programs[23].

The effect of acute exposure of fish to sublethal concentration of TBTO caused significant inhibition of Na^+, K^+-ATPase. The inhibition percentages were increased with the time of exposure. The maximum inhibition percentage was 51.84% at 96 h (Table 2). The inhibition percentages ranged from 6.21-51.84% during the acute exposure intervals (3-96 h).

TBTO decreased the activity of the enzyme at all tested time intervals. The maximum percent of inhibition recorded after 7 d of exposure was 54.49% (Table 3). The percentages of inhibition were decreased with the time of exposure to be 14.27% at 28 d. After 7 d of recovery, the activity of the enzyme partially recovered but the inhibition was still significant (12.39%).

Table 1: The acute toxicity of TBTO to fish tilapia *Oreochromis niloticus* at different times of exposure

Exposure time (h)	LC_{50} (μg L^{-1})	95% Confidence limits of LC_{50} (μg L^{-1})	LC_{95} (μg L^{-1})	Slope±SE
24	31.15	30.28-32.04	49.19	10.17±0.62
48	26.70	25.80-27.63	42.48	7.60±0.49
72	22.57	21.80-23.36	33.73	9.42±0.60
96	16.33	15.60-17.09	26.91	7.58±0.50

Table 2: *In vivo* effects of acute exposure to TBTO on brain AChE, gill Na^+, K^+-ATPase and Mg^{2+}-ATPase specific activity of *O.niloticus* at different times of exposure

	AChE (OD 412/mg protein/min		Na^+,K^+-ATPase (μmol Pi/mg protein/h)		Mg^{2+}-ATPase (μmol Pi/mg protein/h)	
Exposure time (h)	Control	Treatment	Control	Treatment	Control	Treatment
3	1.9±0.04	1.87±0.16	4.86±0.70	4.65±0.34	15.17±0.60	15.45±2.72
6	1.92±0.14	1.86±0.09	4.70±0.22	3.76±0.18	15.33±0.76	14.25±1.51
24	1.86±0.05	1.59±0.08	4.69±0.90	2.67±0.50	15.38±1.71	14.23±2.21
48	1.85±0.06	1.50±0.12	4.68±0.28	2.80±0.38	15.76±0.33	12.57±1.33
72	1.86±0.06	1.45±0.15	4.66±0.50	2.46±0.12	15.26±1.06	12.95±2.15
96	1.87±0.07	1.42±0.16	4.63±0.42	2.23±0.38	15.68±0.70	11.46±1.39

$LSD_{0.05}$ values for time are 0.08, 0.38 and 1.06 for AChE, Na^+, K^+-ATPase and Mg^{2+}-ATPase, respectively
$LSD_{0.05}$ values for treatment are 0.06, 0.31 and 0.87 for AChE, Na^+, K^+-ATPase and Mg^{2+}-ATPase, respectively

Table 3: *In vivo* effects of sub chronic exposure to TBTO on brain AChE and gill Na^+, K^+-ATPase and Mg^{2+}-ATPase specific activity of *O.niloticus* at different times of exposure

	AChE ODλ412/mg protein/min		Na^+,K^+-ATPase (μmol Pi/mg protein/h)		Mg^{2+}-ATPase (μmol Pi/mg protein/h)	
Exposure time (d)	Control	Treatment	Control	Treatment	Control	Treatment
7	1.87±0.06	1.42±0.04	4.77±0.43	2.17±0.10	15.73±0.79	12.48±1.72
14	1.85±0.13	1.54±0.11	4.70±0.28	2.87±0.37	15.54±0.57	12.40±2.34
21	1.87±0.13	1.78±0.04	4.85±0.63	3.47±0.37	15.52±0.21	12.13±0.64
28	1.83±0.08	1.79±0.07	4.87±0.70	3.92±0.44	15.59±0.82	12.54±2.23
Recovery	1.84±0.05	1.86±0.06	4.86±0.75	4.26±0.31	15.66±0.52	15.76±1.46

$LSD_{0.05}$ values for time are 0.05, 0.42 and 0.99 for AChE, Na^+, K^+-ATPase and Mg^{2+}-ATPase, respectively
$LSD_{0.05}$ values for treatment are 0.05, 0.37 and 0.88 for AChE, Na^+, K^+-ATPase and Mg^{2+}-ATPase, respectively

The acute and subchronic effects of TBTO on Mg^{2+}-ATPase in Table 2 and 3 showed that no relationship exists between inhibition percentages and times of exposure. The maximum percentage of acute and subchronic inhibition was 26.91 and 24.28% at 96 h and 21 d of exposure, respectively. Mg^{2+}-ATPase activity was increased to the control level after 7 d of clean water exposure.

The present study illustrated that TBTO caused significant inhibition of branchial Na^+, K^+-ATPase and Mg^{2+}-ATPase activcites in *O. niloticus* at all times of exposure. Triphenyltin acetate (TPTA) also reduced the activity of ATPase in brain and muscle of catfish, *Ictalurus punctatus*[9]. Veiga *et al.*[10] reported that the target for TBTO action is the mitochondrial ATPase. Assem *et al.*[11] showed that Na^+, K^+-ATPase of red tilapia was inhibited by 5 μg L^{-1} TBTO. An opposite response was found by Pinkney *et al.*[24] when stripped bass, *Morone saxalilis* exposed to TBT, showed increase in Na^+, K^+-ATPase activity by 48% at 0.1 μg L^- and no significant changes in gill Mg^{2+}-ATPase activity at different concentrations were detected. In the present study, it is noticed that TBTO is a more potent inhibitor for Na^+, K^+-ATPase than Mg^{2+}-ATPase. Assem *et al.* [11] noticed that gill Na^+, K^+-ATPase of red tilapia was more sensitive than Mg^{2+}-ATPase at comparable concentrations of TBTO.

TBT compounds are lipophilic as indicated by their octanol/water partition coefficient[25]. The potent effect of TBTO on gill Na^+, K^+-ATPase may be explained by the fact that Na^+, K^+-ATPase is a lipoprotein and requires phospholipids for optimal activity[26]. It is therefore possible that TBTO or its lipophilic metabolites may bind to the lipid moity *in vivo* which may result in altering the allosteric characteristics of the enzyme. Thus leading to the inhibiton of its activity. The inhibitory effect of TBTO on ATPase can be considered as an alarming symptom for an adverse health effect because improper functioning of the Na^+, K^+-ATPase may disturb the transport of sodium and potassium[27] as well as uptake and release of certain neurotransmitters in brain synaptosomes[28]. This can lead to central nervous system dysfunction.

It is recommended that paints containing TBT should be restricted to boats >25 m according to the international regulations. Moreover, the use of TBTO in cooling systems must be banned.

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