EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
464-468
Year: 2018

TRIBUTYLTIN OXIDE (TBTO) INDUCED HISTOLOGICAL ALTERNATIONS IN GILL OF THE FRESHWATER FISH, *NEMACHEILUS BOTIA*.

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Article Received on 05/01/2018 **Article Revised on 25/01/2018** **Article Accepted on 15/02/2018**

ABSTRACT
The present study is aimed to assess the histological changes in the gill of freshwater fish, *N. botia* exposed to sublethal concentration of Bis (tributyltin) oxide (TBTO). In this study, sub lethal concentration is determined based on one tenth of lethal concentration. Light microscopic studies exhibited severe histopathological changes in the gill. The histopathological changes in gills were characterized by the hyperplasia of epithelial cells lining, mucus cells, bulging of mucus cells, dilation of blood vessels, increase in sub epithelial space and disorganization of secondary filament were noticed after short term exposure to a sublethal concentration of TBTO.

KEYWORDS: *Nemacheilus botia*, Toxicity, Bis (tributyltin) oxide, TBTO, Gill.

INTRODUCTION
Organotin compounds are a highly versatile group of organometallics used in industrial and agricultural applications, including polyvinyl chloride stabilizers, catalysts, industrial and agricultural biocides, and wood-preserving and antifouling agents (Moore *et al.*, 1992[1]). These chemical through surface runoff reaches to the unrestricted areas like ponds and rivers which alters the physicochemical properties of water and is toxic to non target aquatic organism. The environmental destruction caused by anthropogenic factor lead to further destruction of aquatic fauna and especially the fishes (Vidyarani *et al.*, 2010[2]). Organotin compounds are readily incorporated into the tissues of filter feeding zooplankton, grazing invertebrates and eventually higher organisms such as fish, water birds, and mammals where it accumulates (Hussein *et al.*, 2011[3]). TBTO is a xenobiotic Organotin compound that may affect the internal vital organs and alter behavior (Triebskon *et al.*, 1994[4]).

Several workers investigated the toxicity of Organotin compounds to aquatic animals (Rabbito *et al.*, 2005[5], Shejule *et al.*, 2006[6]). In fish, gill is a main vital organ for their respiratory and osmoregulatory functions. They have generally considered a good tissue indicator of the water quality and are appropriate for the assessment of environmental impact (Mazon Fanta *et al.* 2002[7], Fanta *et al.* 2003[8]). Histopathological studies are performed to evaluate the direct effects of contaminants on fish in laboratory bioassays (Ortiz-Delgado *et al.*, 2007[9]). Some studies have reported the toxic effects of TBT on morphological and functional alterations of teleost gills in aquatic media (Schwaiger *et al.* 1992[10], Tsuda *et al.* 1992[11], Wang and Huang 1998[12]). Exposure to TBT cause histopathological lesions in liver, kidney, eye and gill epithelium in medaka *Oryzias latipes* and guppy *Poecilia reticulata* (Wester and Canton, 1987[13]; Wester *et al.*, 1990[14]), and masculinization in Japanese flounder *Paralichthys olivaceus* (Shimasaki *et al.*, 2003[15]).

Changes in the respiratory activity of fish have been used by several investigators as indicators of repose to environmental stress (Gopal and David, 2010[16]; Magar and Afsar Shaikh, 2012[17]; Jothinarendiran, 2012[18]; Maharajan *et al.*, 2013[19]; Ram Nayan Singh, 2014[20]).

The aim of the present study is to study the histopathological alternations in the gill of freshwater fish *N. botia*, common, cheap, palatable and easily available exposed to sublethal concentration of Bis (tributyltin) oxide for 24, 48, 72 and 96 hours.

MATERIALS AND METHODS
The fish *Nemacheilus botia* were netted from Nandur Madhmeshwar Dam, in Niphad Taluka of Nashik District in Maharashtra State, India located on the coordinates of 19°59’ to 20°4'N and 74°2’ to 74°10’E. The fishes were brought to laboratory and release in glass aquaria (size 0.909 X 0.303 X 0.303 m.), where a continuous and gentle flow of tap water was maintained. The fishes were fed on fishmeal procured from the

market and allowed to acclimatize to laboratory conditions for one week. Water was aerated twice a day to prevent hypoxic conditions. Stock solution (1ppm) was prepared in tap water (Laughlin *et al*., 1983[21]). The LC_{50} values for 24, 48, 72 and 96 hours were determined by Finney's probit method[22]). For the histopathological study, sublethal concentration is determined based on $1/10^{th}$ of LC_{50} values for 24, 48, 72 and 96 hours were used. For each experiment six fishes, *N. botia* of approximately same weight and size were exposed to sublethal concentrations of TBTO for 24, 48, 72, 96 hours and control was run simultaneously. At the end of the experiment, fish per treatment was captured and sacrificed. Gills were removed and washed with buffered normal saline and fixed into Bouins solution for 48 hrs. They were processed through graded series of alcohols. Then they were cleared in xylene and processed for cold and hot impregnation before embedded in paraffin wax. Sections were cut to 6 microns thickness. Stained with Ehrlich hematoxylin and Eosin (dissolved in 70% alcohol) and were mounted in DPX for permanent slide preparation. Slides were viewed under a microscope. The possible changes in tissues of fish, *N.botia* treated with TBTO were observed and photographs were taken.

RESULTS

The evaluation of Lc_{50} concentration of pollutants is an important step before carrying out further studies on physiological changes in animals. In the present study the *N. botia* exposed to TBTO, the acute toxicity level was expressed in terms of Lc_{50} values. The LC_{50} values were found to be 0.01852, 0.0153, 0.01311 and 0.01099 ppm at 24, 48, 72, and 96 hours respectively.

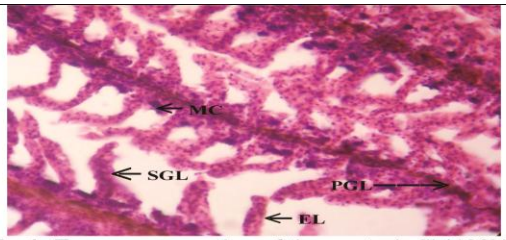

Fig.1: Transverse section of the control gill (400X) showing primary gill filament (PGL), secondary gill filament (SGL) and epithelial lining (EL).

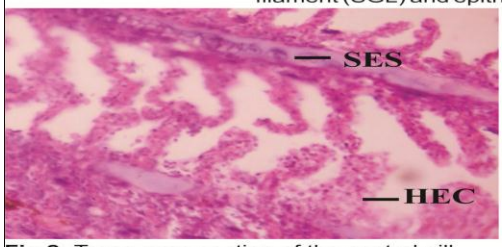

Fig.2: Transverse section of the control gill (400X) showing hyperplasia of epithelial cells (HEC) and sub epithelial space (SES) after 24 hrs exposure of TBTO

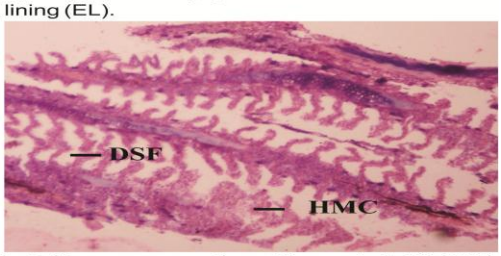

Fig.3: Transverse section of the control gill (400X) showing hyperplasia of mucus cell (HMC) and disorganization of secondary filament (DSF) after 48 hrs exposure of TBTO.

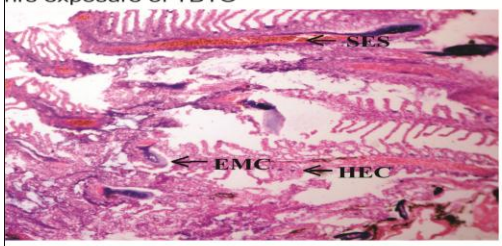

Fig.4: Transverse section of the control gill (400X) showing enlargement of mucus cell (EMC) and hyperplasia of epithelial cells (HEC)) after 72 hrs exposure of TBTO.

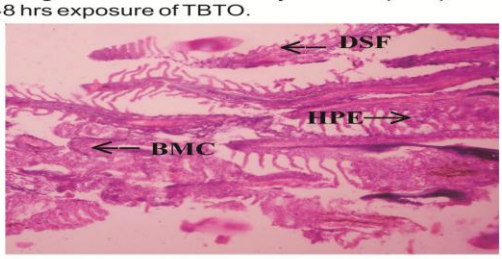

Fig.5: Transverse section of the control gill (400X) showing bulging of mucus cell (EMC), disorganization of secondary filament (DSF) and hyperplasia of epithelial cells (HEC)) after 96 hrs exposure of TBTO.

In the result gill of control freshwater fish, *Nemacheilus botia* is composed of filaments of primary lamellae arranged in double rows and secondary lamellae arise from these filaments. The secondary lamellae are lined by a simple squamous epithelium which rests on basement membrane covering the pillar cell-blood channel system and which constitutes the main vascular area of the gills. Large numbers of mucous cells are present on the epithelial gill rackers, whereas primary lamellae had comparatively small and less number of mucous cells. No recognizable changes were observed in the gills of the control fish (Fig.1).

After exposure to sub-lethal concentrations of TBTO at 24 hrs of exposure, hyperplasia of epithelial cells lining and sub epithelial space was noticed (Fig. 2). Bulging of mucus cells and disorganization of the secondary filament was seen in the treatment after 48 hrs (Fig. 3).

At 72 hrs of exposure, enlargement of mucus cell and hyperplasia of epithelial cells were noticed (Fig. 4).

Significant damage to gill lamellae, bulging of mucus cell, disorganization of secondary filament and hyperplasia of epithelial was seen at the treatment after 96 hrs exposure of TBTO (Fig.5).

DISCUSSION

Chemical pollution in aquatic ecosystems, especially freshwater systems, is a major environmental concern. In the present investigation the freshwater fish, *Nemacheilus botia* was exposed to different sublethal concentration of TBTO.

The gills, which play an important role in many metabolic functions in the fish, such as respiration, osmoregulation, and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants (Camargo and Martinez, 2007[23]). Considerable interest has been shown in recent years in the histopathological study while conducting sub-lethal tests in fish. Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant are a functional response of organisms which provides information on the nature of the toxicant. The mucus cells react instantaneously to the pollutants and secrete copious mucus to form a thick protective layer over the entire exposed surface (Jeebu Kumar Jha *et al.,* 2014[24]). A large amount of mucous secretion acts as a defense mechanism against several toxic substances (Handy and Eddy, 1991[25]; Mazon *et al.,* 1999[26]). Due to TBTO intoxication the hyperplasia of epithelial cells lining, mucus cells, bulging of mucus cells, dilation of blood vessels, increase in sub epithelial space and disorganization of the secondary filament was observed after exposure to sublethal concentration of test toxicants.

The presence of necrosis is, in fact, one of the most visible damages in tissues affected by a pollutant (Rabitto *et al.,* 2005[27]). In the guppy (*Poecilia reticulata*) and medaka (*Oryzias latipes*), lesions in gill epithelium were found (Wester and Canton, 1987[13]; Wester *et al.,* 1990[14]). Schwaiger *et al.,* 1994[28] noticed degeneration and necrosis of epithelial cells and chloride cells in the gills in rainbow trout after exposure to TBTO in concentrations ranging from 0.6 to 4.0 pg/l for 28 days. Marine teleosts such as *Cyprinodon variegatus* (sheepshead minnow) showed 40% necrosis in gill tissue after 28 days exposure to 4 µg/L TBTO (Bryan and Gibbs, 1991[29]).

A number of histopathological changes have been reported in fish exposed to different chemical compounds are on these lines are Das and Mukherjee, 2000[30]; Rodrigues *et al.,* 2001[31]; Tilak et al., 2001a[32]; Tilak *et al.,* 2001b[33] and Anita susan and Tilak, 2003[34]; Ortiz *et al.,* 2003[35]; Cengiz and Unlu, 2003[36]; Machado and Fanta, 2003[37]; Altinok and Capkin, 2007[38]; Velmurugan *et al.,* (2007)[39], which are in agreement with the observed histopathological changes under tributyltin oxide exposure.

CONCLUSION

The gills, which participate in many important functions in fish, such as respiration, osmoregulation, and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants (Mazon *et al.,* 2002[7]; Fernandes *et al.,* 2003[40]). In conclusion, present study substantiates earlier findings that in freshwater fish, *N. botia* when exposed to sublethal concentrations of TBTO, gills showed structural damage. Based on present results, it can be concluded that tributyltin oxide is able to cause various severe damages in gill as reported by Zhang *et al.* (2008).[41] Therefore the application of TBTO in various industries should be control.

ACKNOWLEDGMENT

Authors are thankful to the Head Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.) India for providing laboratory facilities during experimentations. We also sincerely thanks to Shri. Govindrao Holkar, General Secretary N.V.P Mandals, and principal, Arts, Commerce and Science College, Lasalgaon. Tal- Niphad. Dist. Nashik. (M.S.) India. 422 306 for providing fullest cooperation.

REFERENCES

1. Moore D R J, D G Noble, S L Walker, D M. Trotter, M P Wong, and R C Pierce: Canadian water quality guidelines for organotins. Sci. Ser. No. 191. Environment Canada, Ecosystem Sciences and Evaluation Directorate, Eco-Health Branch, Ottawa (1992).
2. W. Vidyarani, H. Sunita and S. Sanayaima: Toxic effect of selected pesticides on an endemic loach *Lepidocephalichthys irrorata* (fam. cobitidae). *The bioscan*., 2010; 3: 635-641.
3. Hussein K Okoro, Olalekan S Fatoki, Folahan A Adekola Bhekumusa J Ximba and Reinette G Snyman: Sources, environmental levels and toxicity of Organotin in marine environment-a review, *Asian J. Chem*, 2011; 23(2): 473-482.
4. Triebskon R, Kohler H D, Flemming J, Braunbeck T, Negele R D, Rahmann H: Evaluation of bis(tri-n-butyltin) oxide (TBTO) neurotoxicity in rainbow trout (*Oncorhynchus mykiss*) behavior, weight, increase and tin content. *Aquatic Toxicology*., 1994; 30: 189-197.
5. Rabbito, I.S., J.R. Alyes Costa, H.C. Silva De Assis, E.E.Pelletier, F.M. Akaishi, A. Anios, M.A. Randi C.A. Oliveira Ribeiro: Effect of dietary Pb(II) and tributyltin on neotropical fish, *Hoplias malabaricus*, Histopathological and Biochemical Findings. *J. Ecotoxicol Environ Saf*., 2005; 60(2): 147-56.
6. Shejule, K. B., P.S. Kharat and R.S. Kale: Toxicity of organotin tributyltin chloride to Freshwater

prawn, *Macrobrachium kistensis*, *J.Aquacult.,* 2006; 7(1): 141-144.
7. Mazon, A.F., G.H.D. Pinheiro and M.N. Fernandes: Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, Prochilodus scrofa. Brazilian J. Biol., 2002; 62: 621-631.
8. Fanta, E., Rios, F. S., Romão, S., Vianna, A. C. C. & Freiberger, S: Histopathology of the fish *Corydoras paleatus* contaminated with sub lethal levels of organophosphorus in water and food. *Ecotox Environ Safe*, 2003; 54(2): 119-130.
9. Ortiz-Delgado, J. B., Segner, H., Arellano, J. M. & Sarasquete, C: Histopathological alterations, EROD activity, CYP1A protein and biliary metabolites in gilthead seabream *Sparus aurata* exposed to Benzo(a)pyrene. *Histol Histopathol.*, 2007; 22(4): 417-432.
10. Schwaiger, J., Bucher, F., Ferling, H., Kalbfus, W. & Negele, R.D: A prolonged toxicity study on the effects of sublethal concentrations of bis(tri-n-butyltin)oxide (TBTO): histopathological and histochemical findings in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology.*, 1992; 23(1): 31-48.
11. Tsuda T, S Aoki, M Kojima, T Fujita: Accumulation and excretion of tri-*n*-butyltin chloride and tributyltin chloride by willow shiner. *Comp. Biochem. Physiol.*, 1992; 101: 67-70.
12. Wang DY, BQ Huang: Toxic effects of tributyltin (TBT) on early life stages of thornfish (*Terapon jarbua* Forsskål). *J.Fish. Soc. Taiwan*, 1998; 25: 15-25.
13. Wester, P.W. & Canton, J.H.: Histopathological study of *Poecilia reticulate* (guppy) after long-term exposure to bis (trin-butyltin) oxide (TBTO) and di-n-butyltindichloride (DBTC). *Aquat. Toxicol.*, 1987; 10: 143-65.
14. Wester, P.W., Canton, J.H., Van Iersel, A.A.J., Krajnc, E.I., Vaessen, H.A.M.G.: The toxicity of bis(tri-n-butyltin)oxide (TBTO) in small fish species *Oryzias latipes* (medaka) and *Poecilia reticulata* (guppy). *Aquat. Toxicol*., 1990; 16: 53–72.
15. Yohei Shimasaki, Takeshi Kitano, Yuji Oshima uguru Inoue, Nobuyoshi Imada, Tsuneo Honjo: Purification and identification of a tributyltin-binding protein from serum of Japanese flounder, *Paralichthys olivaceus*, 2003; 22(1): 141-144.
16. Chebbi, S.Gopal. and David, M.: Respiratory responses and behavioural anomalies of the carp *Cyprinus carpio* under quinalphos intoxication in sublethal doses. *Science Asia*., 2010; 36: 12-17.
17. Magar, R. S., and Afsar Shaikh: Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sublethal treatment with the insecticide malathion. *Treds in life sciences*, 2012; 1(3): 2319–4731 (Print); 2319–5037.
18. Jothinarendiran, N. Effect of Dimethoate Pesticide on Oxygen Consumption and Gill Histology of the Fish, *Channa punctatus. Current Biotica.*, 2012; 5: 500-507.
19. Maharajan, A., Usha r., P.S. Paru Ruckmani, B.S. Vijaykumar, V. Ganapiriya and P. Kumarasamy: Sublethal effect of profenofos on oxygen consumption and gill histopathology of the Indian major carp, *Catla catla* (Hamilton). *Int. J. Pure appl. zool*., 2013; 1(1): 196-204.
20. Ram Nayan Singh: Effects of Dimethoate (EC 30%) on Gill Morphology, Oxygen Consumption and Serum Electrolyte Levels of Common Carp, *Cyprinus Carpio* (Linn). International Journal of Scientific Research in Environmental Sciences, 2014; 2(6): 192-198.
21. Finney DJ: Probit analysis second edition: Cambridge University Press, London (1964).
22. Laughlin, R.B., J.R.W. French and H.E. Guard: Acute and sub lethal toxicity of tributyltin oxide (TBTO) and its pu- tative environmental product, tributyltin sulfide (TBTS) to zoeal mud crabs, *Rhithropanopeus harrisii. Water, Air and Soil Poll*, 1983; 20: 69-79.
23. Camargo M.M. and Martinez, C. B.: Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotrop. Ichthyol.*, 2007; 5: 327-336.
24. Jeebu Kumar Jha, Ranjana, Pankaj kumar and A. P. Mishra: Histopathological changes in the gills of *Channa Gachua*, an air breathing teleost after short term exposure of hostathion. *The bioscan*, 2014; 9(3): 925-929.
25. Handy, R. D. and Eddy, F. B: The absence of mucous on the secondary lamellae of unstressed rainbow trout, *Oncorhynchus mykiss. J. Fish Biol.*, 1991; 38: 153-155.
26. Mazon, A. F., Cerqueira, C. C. C., Monteriro, E. A. S. and Fernandes, M. N: Acute copper exposure in freshwater fish: Morphological and physiological effects. In: Biology of Tropical Fishes, (Val, A. L., Almieidaval, V. M. F., Eds.) INPA, Manaus., 1999; 263-275.
27. Rabitto I.S., Alves Costa JR, Silva de Assis HC, Pelletier EE, Akaishi FM, Anjos A, Randi MA, Oliveira Ribeiro CA.: Effects of dietary Pb (II) and tributylin on neatropical fish, *Hoplias malabaricus* histopathological and biochemical findings. *Ecotoxicol. Environ. Saf*., 2005; 60: 147-156.
28. Schwaiger,J., H.F.Falk, F.Bucher, G.Orthuber, R.Hoffmann, and R.D.Negele: Prolonged exposure of rainbow trout *(Oncorhynchus mykiss)* to sublethal concentration of bis(tri-n-butyltin)oxide: effect on lecocyte, lymphatic tissues and phagocytosis activity. In: Sublethal and chronic effects of pollutants on fresh water fish, edited by R.Muler and R.Lloyed. Blackwell Scientific, Cambridge M.A., 1994; 113-123.
29. Bryan, G. W., and Gibbs, P. E.: Impact of low concentrations of tributyltin (TBT) on marine organisms: A review. In *Metal ecotoxicology:*

Concepts and applications, eds. M. C. Newman and A. W. McIntosh, pp. 262–323. Chelsea, MI: Lewis (1991).

30. Dass B K, Mukherjee, S C: A histopathological study of carp [*Labeo rohita*] exposed to hexachlorocyclohexane. *Vet. Arhiv.*, 2000; 70: 169-180.
31. Rodrigues, E.D.L., Ranzani-Paiva, M.J.T., Pacheco, F.J. and Veiga, M.I.D.: Histopathologic lesions in the liver of *Prochilodus lineatus* (Pisces, Prochilodontodae) exposed to a sublethal concentration of the organophosphate insecticide Dipterex 500 (Trichlorfon). *Maringa*, 2001; 23(2): 5003-505.
32. Tilak, K.S., Veeraiah, K. and Ramana Kumari, G.V.: Biochemical changes induced in freshwater fish *Labeo rohita* (Hamilton) exposed to pesticide mixture. *Asian J. of Microbiol. Biotech. & Eng. Sci*., 2001a; 3(4): 315-319.
33. Tilak, K. S., Veeraiah K. and Yacobu K.: Studies on histopathological changes in the gill, liver and kidney of *Ctenopharyngodon idella* (Valenciennes) exposed to technical fenvalerate and EC20%. *Pollu. Res.*, 2001b; 20(3): 387-393.
34. Anita, Susan, T., and K.S. Tilak: Histopathological changes in the vital tissues of the fish *Cirrhinus mrigala* exposed to fenvalerate technical grade. *Pollut. Res*., 2003; 22: 179-184.
35. Ortiz J.B., De Canales M.L.G., and Sarasquete C.: Histopathological changes induced by lindane (gamma-HCH) in various organs of fishes. *Sci., Mar*. 2003; 67(1): 53-61.
36. Cengiz, E. I.; Unlu, E. (2003): Histopathology of gills in mosquitofish, *Gambusia affinis* after long-term exposure to sublethal concentrations of malathion. *J. Environ. Sci. Heal.* B, 2003; 38: 581-589.
37. Machado, M.R. and Fanta, E: Effects of the organophosphorous methyl parathion on the branchial epithelium of fresh water fish *Metynnis roosevelti. Braz. Arch. Boil. Technol.*, 2003; 46(3): 361-372.
38. Altinok, I. and Capkin, E.: Histopathology of rainbow trout exposed to sublethal concentrations of methiocarb or endosulfan. *Toxicol Pathol*., 2007; 35: 405-410.
39. Velmurugan, B., Selvanayagam, M., Cengiz, E. I., and Unlu, E.: The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *J Environ Sci Health Part (B)*., 2007; 42(2): 157-163.
40. Fernandes, M.N., Mazon, A.F., Val, A.L., Kapoor, B.G.: Environmental pollution and fish gill morphology. In: (Eds.), Fish Adaptation. *Science Publishers, Enfield*, 2003; 203-231.
41. Zhang, J., Zuo, Z., Chen, R., Chen, Y. and Wang, C.: Tributyltin exposure causes brain damage in *Sebastiscus marmoratus*. *Chemosphere*, 2008; 73: 337-343.