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Histopatholocal Changes in Kidney of Freshwater Fish, Nemacheilus Botia, From Nandur Madhmeshwar Dam at Maharashtra, India Exposed to Bis (Tributyltin) Oxide

KEYWORDS: Nemacheilus botia, histopathology, Bis (tributyltin) oxide, TBTO, kidney

S M Nikam
Department of Zoology, Arts, Commerce and Science College, Lasalgaon. Tal- Niphad. Dist. Nashik. (M.S.) India. 422 306.

K B Shejule
Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. (M.S.), India. 431 001.

ABSTRACT *The present study is aimed to assess the histological changes in kidney caused to the fresh water fish, N. botia exposed to sub lethal 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 sever histopathological changes in the kidney. Increase in vacuolization, glomerulus shrinkage, damage to blood vessels, degeneration of tubules and necrosis with pycnotic were the most significant changes observed in fish kidney after Bis (tributyltin) oxide exposure. The effects were more pronounced in 96 hrs sub lethal concentration of Bis (tributyltin) oxide.*

Introduction

Organotin compounds are highly versatile group of organometalics used in a number industrial and agricultural applications, including polyvinyl chloride stabilizers, catalysts, industrial and agricultural biocides, and wood-preserving and antifouling agents (Bock 1981; Jones et al., 1982; Moore et al., 1992). 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 aquatic organism and cause deleterious effect or even death to the aquatic animals. In many countries large scale mortality of fishes have been recorded due to chemicals in water bodies as pollutants. The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century (Rane Minakshi and A. Y. Mahajan, 2013).

High levels of TBT have been detected in several marinas and harbors (Pickston, L., 1988; Alzieu et al., 1989; Daly, H. & Fabris, G., 1993), where damages to aquatic organisms have been described (Gibbs et al., 1988; Byrne et al., 1989; Kingtong et al., 2007). Specific lesions occurring in organ of fish exposed to toxic substances under laboratory condition are helpful as biomarkers of exposures (Ram Nayan Sing. 2012). As a result histopathological is increasingly being recognized as a valuable tool for assessing the impact of environmental pollutant on fishes(Handy, 2002) .The histopathological changes in fish depend on the chemical concentration, exposure time, uptake routes, species and other environmental features (Oliveira Ribeiro et al., 2005; Valdez Domingos et al., 2007). Fish have been shown to bioaccumulate organotins from food chain (Martin et al., 1989; Schwaiger et al., 1992), but sediments and suspended particles might be the main sources of organotin to biota (Tanabe et al., 1994). Several workers investigated the toxicity of Organotin compounds to aquatic animals (Rabbito et al., 2005, Shejule et al., 2006, Kharat P.S., 2007). The morphological effects of TBT in tropical fish Astyanax sp. and more recently in northern fish Salvelinus alpinus were studied (Oliveira Ribeiro et al., 2000; Oliveira Ribeiro et al., 2007). The kidney of teleosts is a multifunctional organ in which four functional aspects can be distinguished. The kidney has an excretory and a hematopoietic function, plays a role in the immune system and produces hormones (Ellis et al., 1989; Zapata et al., 1990). Kidney serves as a major route of excretion of metabolites of xenobiotics and receives the largest proportion of post brachial blood, and therefore, it is more likely to undergo histopathological alteration under stress (Ortiz et al., 2003).

The aim of the present study is to study the histopathological changes in the kidney of freshwater fish N. botia, common, cheap, palatable and easily available exposed to sub-lethal 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, 40 km east of Nashik in Niphad Taluka of Nashik District in Maharashtra State, India 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 market and allowed to acclimatize to laboratory conditions for one week. Stock solution (1ppm) was prepared in tap water (Laughlin et al., 1983). The Series of statistic bioassay were conducted under laboratory condition as described by Finney (1964). The LC50 values for 24, 48, 72 and 96 hours were determined by Finney's probit method and found to be 0.01852, 0.0153, 0.01311 and 0.01099 ppm respectively. For histopathological study, sub-lethal concentration is determined based on one tenth of LC50 values for 24, 48, 72 and 96 hours were used.

Histological study

For histopathological study, sub-lethal concentration is determined based on 1/10th of LC50 values for 24, 48, 72 and 96 hours were used. For each experiment six fishes , N. botia of approximately same weight 1± 0.7 gram (g) and size 3 ± 1 cm were exposed to sub lethal concentrations of TBTO for 24, 48, 72, 96 hours and control was run simultaneously. In the end of experiment periods, fish per treatment was captured and sacrificed. Then, they were dissected and kidneys were removed and washed by buffered normal saline. Tissues were fixed into bouins solution (prepared with saturated picric acid, formaldehyde and

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acetic acid), and then dehydrated through graded alcohol series (70 to 100%), cleared in xylene and embedded in paraffin. 5 to 6 micron thick paraffin sections were cut and stained with haematoxylin and eosin. The slides were examined under a light microscope and photographed for histological studies.

Results and discussion

Control kidney:

The kidney of control fish N. botia comprises number of functional units, coiled uriniferous tubules. Each of which consists of a renal corpuscle and a renal tubule. Columnar epithelial cells with nucleus characterize the proximal segment. The nuclei are rounded and prominent. The distal tubules are surrounded by alkalinement membrane. The interstices of the tubules are enriched with hematopoietic tissue, which contain round to polygonal cells possessing hyper chromatic nuclei (Fig. 1).

Experimental kidney:

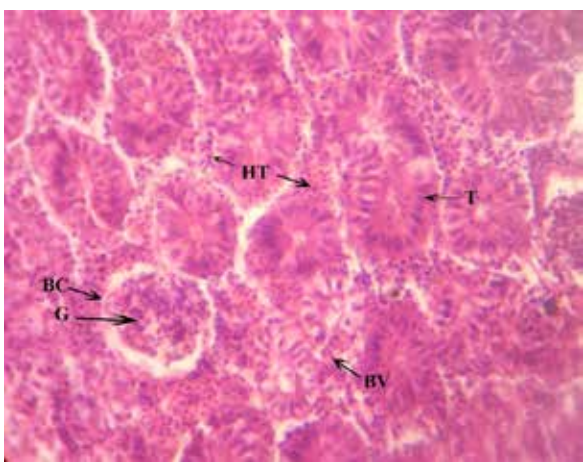

Fig.1: Transverse section of the control kidney (400X) showing Bowman's capsule (BC), glomerulus (G), tubules (T), blood vessels (BV) and hematopoietic tissue (HT)

At 24 hrs of exposure the epithelial cells lining the renal tubules show signs of vacuolation. In the renal tubules, disorganization of cells was seen (Fig. 2). At 48 hrs of exposure more vacuolization in the epithelial cells lining was seen, more disorganization of tubules, damaged blood vessels was seen (Fig. 3). After 72 hrs of exposure the breakages of tubules were prominent, the glomerular region shows shrinkage, necrosis in the area of tubules and the disorganized tubules were seen (Fig. 4).

After 96 hrs of exposure tubular lumen is remarkably widened. Degenerative changes in tubular epithelium are clearly seen (Fig. 5). The nuclei of epithelial cells were lost their shape and look like pyknosis. The necrotic area becomes visible and the damaged blood vessels are clearly seen.

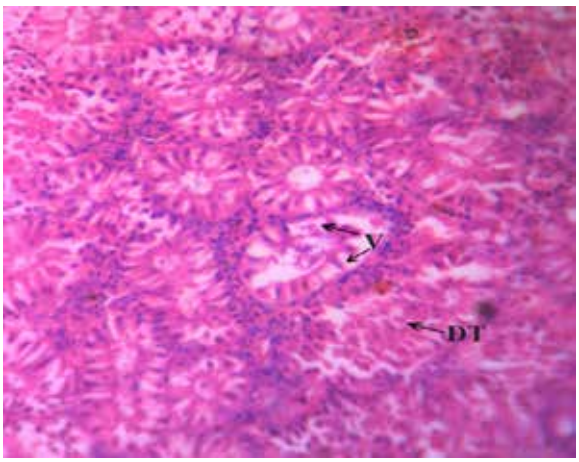

Fig.2: Transverse section of the kidney (400X) after 24 hrs exposure showing vacuolization (V), degenerating tubules (DT).

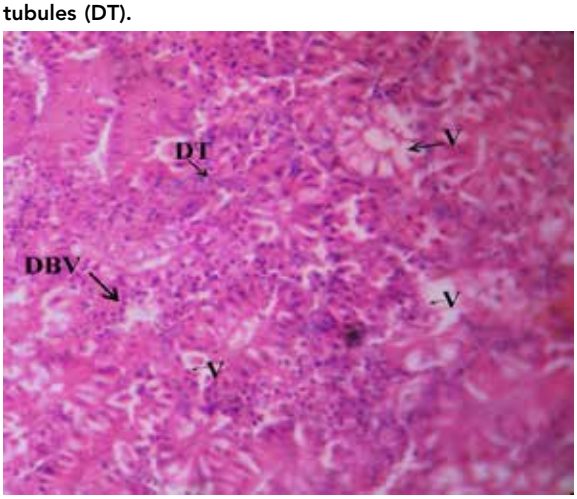

Fig.3: Transverse section of the kidney (400X) after 48 hrs exposure showing damaged blood vessels (DBV), degenerating tubules (DT) and vacuolization (V)

Karl Fent and Willy Meier, (1992) reported degenerative alterations in kidney in early life stages of minnows *Phoxinus phoxinus after exposure of* Tributyltin and they also noticed hydropic vacuolation of the cytoplasm and, in more pronounced cases, irreversible nuclear alterations such as pycnosis, karyorrhexis and karyolysis were also evident. Exposure to TBT also caused histopathological lesions in liver (increased vacuolation) and kidney (hyperplasia of hematopoietic tissue or tubulonephrosis /glomerulopathy) in medaka (Oryzias latipes) and guppy (Poecilia reticulata) (Wester P.W., Canton J.H, 1987; Wester et al., 1990).

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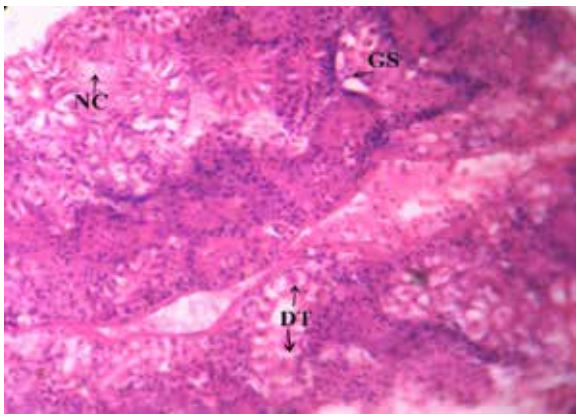

Fig.4: Transverse section of the kidney (400X) after 72 hrs exposure showing glomerulus shrinkage (GS), degenerating Tubules (DT) and necrosis (NC)

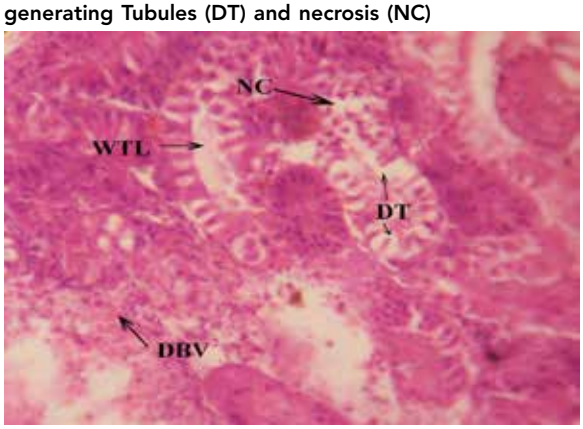

Fig.5: Transverse section of the kidney (400X) after 96 hrs exposure showing Damaged blood Vessels (DBV), Degenerating Tubules (DT), Widen tubular lumen (WTL) and Necrosis (NC)

In a comparative study using BaP and TBT in trout, Oliveira Ribeiro et al., (2007), showed that, despite of the severity of lesions in fish exposed to BaP alone, damages as necrosis in liver and kidney were also observed in individuals exposed to TBT alone. C. A. Oliveira Ribeiro et al., (2009), observed vacuolated cells and cellular deposit within renal tubules in American plaice (Hippoglossoides platessoides) for both TBT and DBT exposed groups. The degenerative changes in tubular epithelium cell within kidney ducts in 96 hrs exposure suggest the occurrence of cell death as described by Alberts et al. (2005).

Conclusion

In conclusion, present study substantiates earlier findings that fish when exposed to lethal concentrations of TBTO, kidney showed structural damage. The study also shows that TBTO is very toxic to fish even at sub lethal and short term exposure. A perusal of data available for aquatic animal exposed to different toxic pollutants, histopathological study showed that therefore the use of TBTO in various industries should be controlled. The present study confirms the toxic potential of this organotins.

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REFERENCE Alberts B., Bray D., Lewis J., Martin R., Roberts K. & Watson J. D., 2005. Molecular Biology of the Cell, Garland Publishing, Inc, New York, 4a ed., 1463p. | Alzieu, C. I., Sanjuan, J., Michel, P., Borel, M. & Dreno, J. P., 1989. Monitoring and assessment of butyltins in Atlantic coastal waters. Mar. Pollut. Bull., 20: 22-26. | Bock R, 1981. Tributyltin compounds and their degradation products. Residue Rev. 79:1–270 | Byrne P, D Speare, HW Ferguson, 1989. Effects of a cationic detergent on the gills and blood chemistry of rainbow trout Salmo gairdneri. Dis. Aquat. Org. 6: 195-196. | C. A. Oliveira Ribeiro1, F. Filipak Neto, A. Siah2, E. Pelletier., 2009. Sub-chronic and Trophic Effects of TBT and DBT in American plaice (Hippoglossoides platessoides). J. Braz. Soc. Ecotoxicol., 4 (1-3):31-36 | Daly, H. & Fabris, G., 1993. An environmental study of Tributyltin in Victorian waters. Report to the State Government of Victoria Environmental Protection Authority | Ellis A.E., Roberts R.J., Tytler P., 1989. The anatomy and physiology of teleosts. In: Roberts, R.J. (Ed.), Fish Pathology, second edition. Balliere Tindall, London, pp. 13-55. | Finney DJ, 1964. Probit analysis second edition: Cambridge University Press, London. | GibbS P. E., Pascoe P. L. & Burt G. R., 1988, Sex change in the female dog-whelk, Nucella lapillus, induced by Tributyltin from antifouling paint. J. Mar. Biol. Assoc., 68: 715-731. | Handy R.D., Runnats T. and Russell P.M., 2002. Histopathologica biomarkers in three spined sticlebacks, Gasterosteus aculetus from several rivers in southern England that meet the fresh water fisheries directives. Ecotoxicology , 11:467-476. | Jones P A, M F Millson, C C McBain and J A Ruddick, 1982. Organotins in the Canadian environment: A synopsis. EPS3-EC-82- 1. Environment Canada, Environmental Protection Service. | Karl Fent, Willy Meier, 1992. Tributyltin-induced effects on early life stages of minnows Phoxinus phoxinus. Archives of Environmental Contamination and Toxicology 22(4): 428-438 | Kharat P.S., 2007. Some physiological aspects of freshwater prawn, Macrobrachium kistnensis exposed to organotin tributyltin chloride. Thesis of Dr. B.A.M.U. Aurangabad, pp: 1-185. | Kingtong, S., Chitramvong, Y. & Janvilisri, T., 2007. ATP-binding cassette multidrug transporters in Indian rock oyster Saccostrea forskali and their role in the export of an environmental organic pollutant tributyltin. Aquat. Toxicol., 85: 124-132. | Laughlin, R.B., J.R.W. French and H.E. Guard, 1983. Acute and sublethal toxicity of tributyltin oxide (TBTO) and its pu- tative environmental product, tributyltin sulfide (TBTS) to zoeal mud crabs, Rhithropanopeus harrisii. Water, Air and Soil Pollution, 20: 69-79. | Martin R. C., Dixon D. G., Maguire R. J., Hodson P. V. & Tkacz R. J., 1989. Acute toxicity, uptake, depuration and tissue distribution of tri-n-butyltinin rainbow trout, Salmogairdneri. Aquat. Toxicol., 15: 37-52. | Moore D R J, D G Noble, S L Walker, D M. Trotter, M P Wong, and R C Pierce, 1992. Canadian water quality guidelines for organotins. Sci. Ser. No. 191.Environment Canada, Ecosystem Sciences and Evaluation Directorate, Eco-Health Branch, Ottawa | Oliveira Ribeiro C. A., Padros J., Valdez Domingos F.X., Akaishi F. M. & Pelletier E., 2007. Histopathological evidence of antagonistic effects of tributyltin on benzo[a]pyrene toxicity in the Arctic charr (Salvelinus alpinus). Sci. Total Environ., 372: 549-553 | Oliveira Ribeiro C. A., Pelletier E. & Rouleau C., 2000. Comparative gill damages and bioaccumulation of inorganic mercury on tropical and nordic fish. Environ. Res., 83: 286-292. | Oliveira Ribeiro, C. A. Vollaire, Y. Sanchezchardi A. & Roche H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (Anguilla anguilla) at the Camargue Nature Reserve, France. Aquat. Toxicol., 74: 53-69. | Ortiz J.B., De Canales M.L.G. and Sarasquete C., 2003. Histopathological changes induced by lindane (gamma-HCH) in various organs of fishes. Sci Mar, 67(1):53-61. | Pickston, L., 1988. Review of the use of antifoulants containing triorganotins in New Zealand. Report to the Ministry for the Environment, New Zealand. | Rabbito, I.S., J.R. Alyes Costa, H.C. Silva De Assis, E.E.Pelletier, F.M. Akaishi, A. Anios, M.A. Randi 23.. C.A. Oliveira Ribeiro, 2005. Effect of dietary Pb(II) and tributyltin on neotropical fish, Hoplias malabaricus, Histopathological and Biochemical Findings. J. Ecotoxicol Environ Saf., 60(2): 147-56 | Ram Nayan Sing. 2012. Histopathological alternations in the kidney of Cyprinus carpio after exposure to dimethonate (EC 30%).Ind.J.Sci.Res., 3(1):127-131. | Rane Minakshi and A. Y. Mahajan, 2013. Effect of thiamethoxam on oxygen consumption of the freshwater bivalve, Lamellidens marginalis (lamarck). The bioscan 8(2): 469-472, 2013 | Schwaiger J., Bucher F., Ferling H., Kalbfus W. & Negele R. D., 1992. 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 myskiss). Aquat. Toxicol., 23: 31-48. | Shejule, K. B., P.S. Kharat and R.S. Kale, 2006.Toxicity of organotin tributyltin chloride to Freshwater prawn, Macrobrachium kistensis, J.Aquacult., 7(1): 141-144 | Tanabe S., Iwata, H. & Tatsukawa R., 1994, Global contamination by persistent organochlorines residues in harbours and their ecotoxicological impact on marine mammals. Sci. Total Environ., 154: 163-177. | Valdez Domingos, F. X, Azevedo, M. Silva, M. D. Randi, M. A. F., Freire, C. A., Silva, De Assis H. C. & OLiveira Ribeiro C. A., 2007. Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ. Res., 105: 350-363. | Wester P.W., Canton J.H, 1987. Histopathological study of Poecilia reticulata (guppy) after long-term exposure to bis(tri-n-butyltin)oxide (TBTO) and di-n-butyltinchloride (DBTC). Aquat. Toxicol. 10, 143-165. | Wester P.W., Canton J.H., Van lersel A.A.J., Krajnc E.I., Vaessen, H.A.M.G., 1990. The toxicity of bis(tri-n-butyltin)oxide (TBTO) in small fish species Oryzias latipes (medaka) and Poecilia reticulate (guppy). Aquat. Toxicol. 16, 53-72. | Zapata A.G., Cooper E.L., 1990. The Immune system: Comparative Histophysiology. Wiley, Chichester. |