



## A study on the renal histopathology of snake head fish, *Channa punctata* (Bloch, 1793), due to chronic lindane exposure

Debashish  
Bhattacharjee

Aquatic Toxicology and Remediation Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar-788011, India

Suchismita Das

Aquatic Toxicology and Remediation Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar-788011, India.  
Corresponding author

### ABSTRACT

*The histopathological effects of 0.0255 g/L lindane on kidney tissue architecture of the snake head fish *Channa punctata* were determined by light microscopy at 7, 14 and 21 days of exposure durations. The result of this work clearly indicated that lindane adversely affected the kidney tissues. Lesions in the kidney tissues were characterized by degeneration in the epithelial cells of renal tubule, narrowing of the tubular lumen, necrosis of glomeruli, degeneration and atrophy of the renal tubule, and loss of haemopoietic tissues. The results of this study indicated that lindane had the potential to disrupt renal functions.*

**KEYWORDS :** toxicity, lindane, histopathology, renal tubule, glomeruli

### Introduction

Lindane is an organochlorine pesticide, classified by the World Health Organization (WHO, 2003) as 'moderately hazardous'. It has been widely used in public health and agriculture in India (Geetha et al. 2008; Bhattacharjee and Das, 2013). Lindane has been associated with pollution due to its long persistence and quick accumulation in fatty tissues (Pesce et al. 2008). Lindane has been detected in soil, drinking water, industrial effluent and sewage (Amorim et al. 2002; WHO, 2003). Most human exposure to lindane is from eating food and water contaminated with the pesticide. Acute exposure to lindane has been reported to cause irritation, dizziness, headaches, diarrhoea, nausea, vomiting and in some cases convulsion and death (Zucchini-Pascal et al. 2009; Hrnčić et al. 2011). Lindane exerts its toxicity mainly by stimulation of the central nervous system subsequent to inhibition of GABA channels (Ogata et al. 1988; Bist et al. 2009; Hfaiedh et al. 2011). It is very stable in both fresh and salt water environments. It will disappear from the water by secondary mechanisms such as adsorption on sediment, biological breakdown by microflora and fauna and adsorption by fish through gills, skin and food (Ulman, 1972). Lindane is highly toxic to fish, bees and aquatic invertebrates (EXTOXNET, 1996). In fish, absorption of lindane occurs by different ways through gills and skin or by ingestion of contaminated water and food and may lead to high mortality rate or cause many biochemical and histological alterations in survived fish (Coetzee, 1998).

Evaluation of histopathology as a monitoring tool can provide an assessment of the effects of environmental stressors on fish population. Also, it is one of the most reliable indicators for health impairment of aquatic animals by anthropogenic activities (Hinton and Laurén 1990). In fish, as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen-containing waste products from the metabolism such as ammonia, urea and creatinine. Following exposure of fish to toxic agents such as pesticides, tissue alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al. 1997). Histopathological biomarkers are very effective measure of stress since many pollutants either toxic or non toxic have to undergo metabolic activation in order to be able to culminate cellular change in the affected organism. Thus, the present study aims at estimating kidney histopathology as a monitoring tool for sub-lethal lindane toxicity. The study also evaluates the effects of durations (7, 14 and 21 days) on intensity of nephro toxicity.

### Materials and methods

Healthy specimens of *Channa punctata* (length, 16.58 ± 0.3cm, and weight, 25.7 ± 0.5 g) were procured from a local fishery near Assam University campus with no reported pollution load. They were acclimatized for 3 weeks in a large cement tank (1000 L capacity) under laboratory conditions. During the acclimatization period, fish were fed

ad libitum with minced goat liver twice daily along with commercially available fish food 'Tokyu'. (composition: crude protein-32% ; crude fat - 4% ; crude fibre- 6% ; Crude ash-10% ; moisture - 9% ; nitrogen free extract-31%) at the rate of 3% body weight. The insecticide lindane 6.5% W.P. (trade name- KunaheX) was used to prepare the stock solution by dissolving 50 g of lindane in 1 litre of tap water. 10 fish were selected from the stock and placed in different 10 L glass aquaria where they are exposed to sublethal concentration of 0.0225 g/L lindane (96 h LC50 was reported elsewhere to be 2.25 g/L) for 7, 14 and 21 days. For the experiment, five replicates were maintained. The fish were dissected at 7, 14 and 21 days intervals; the kidney tissues were fixed in 10% formaldehyde and embedded in paraffin for microscopic examination. Various sections (5µ) were obtained, stained with haematoxylin and eosin and examined under a light microscope. A search was carefully conducted to check for the presence of alterations. The experiments were performed as per the guidelines recommended by Assam University Ethical Committee for treatments of experimental animals.

### Results

No histopathological changes were observed in the kidney of the control fish and they showed normal architecture throughout the study period (Fig 1). The histopathological results indicated that kidney tissues were affected adversely by exposure to 0.0255g/L lindane. The most remarkable changes the kidney showed was degeneration in the epithelial cells of renal tubule, vacuolation, narrowing of tubular lumen, and glomerular contraction, and after 21 days of exposure, necrotic areas were evident (Fig 1). At 7d of exposure duration, shrinkage of tubular lumen was observed. At 14d, the severity intensified as vacuolation of tubular epithelium appeared along with disintegration and necrosis of glomerulus. During the same duration, some of the haemopoietic tissues started to degenerate. At 21 d, most of the renal tubules almost degenerated or atrophied; while, the glomerulus atrophied and necrosed (Fig 1).

### Discussion

Telest kidney consists of the anterior, head kidney and the posterior, tail kidney. Head kidney consists of lymphoid tissue and the tail kidney consists of many nephrons and interstitial lymphoid tissue. The interstitial tissue is the major haemopoietic tissue in the body. Each nephron consists of two parts, the glomerulus and the urinary tubule. The glomerulus capsule consists of an inner and outer layer of single flattened epithelia. Renal tubules consist of single layer of epithelial cells (Takashima and Hibiya, 1995). In *C. punctata*, histological changes in the kidney after exposure to lindane were noticed: degeneration in the epithelial cells of renal tubule, degeneration of haemopoietic tissue, degeneration of glomerulus, intracytoplasmic vacuoles in epithelial cells of renal tubules with narrowing of the tubular lumen. Dilatation of the lumina of the kidney tubules, necrosis of tubules, shrinkage of glomerular tuft, vacuolation, blood cells in the

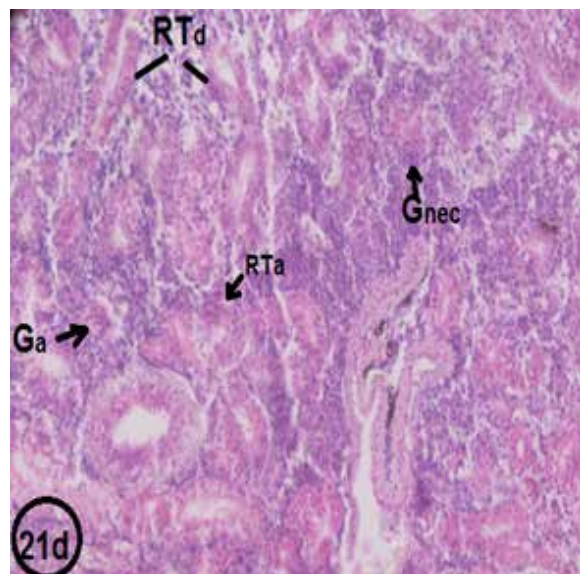
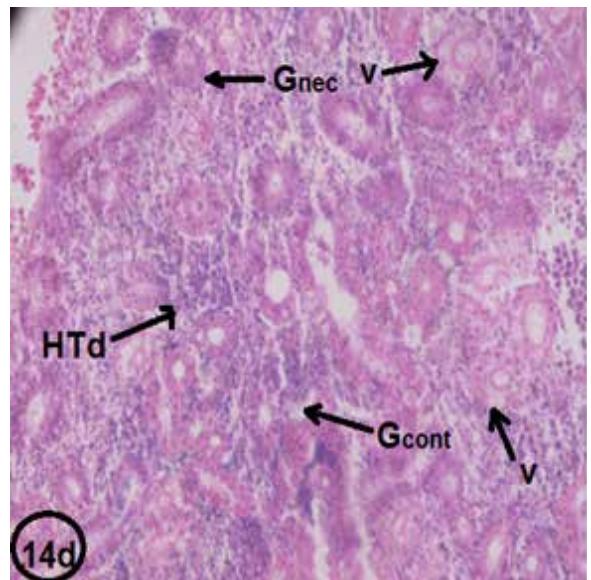
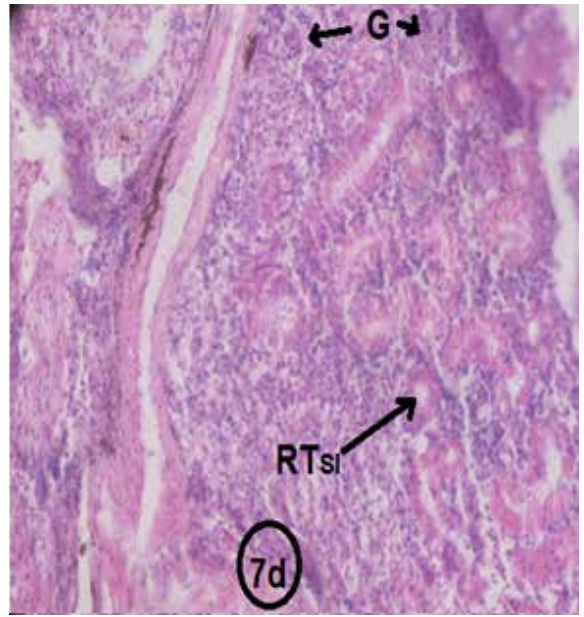
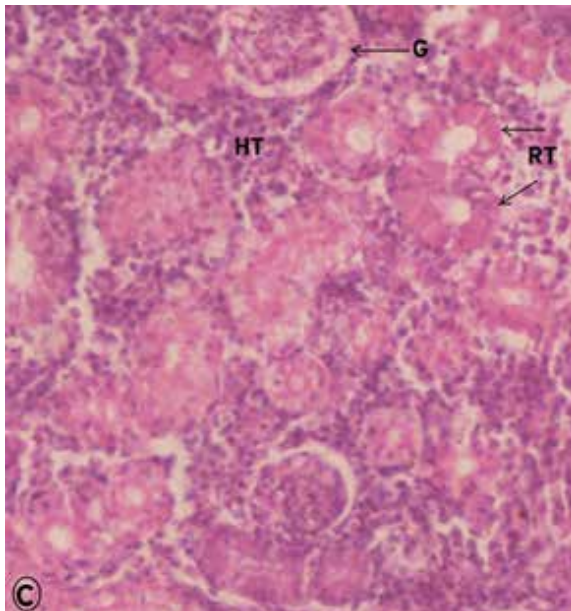
glomerular tuft have also been reported in *Heteropneustes* fossils exposed to chlorpyrifos (Srivastava et al. 1990). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995). Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman's space (Takashima and Hibiya, 1995). Following exposure of fish to toxic agents such as pesticides, histological alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al. 1997; Thophon et al. 2003). Similar alterations were found in fish exposed to organic contaminants (Veiga et al. 2002) and mixed environmental contaminants (Schwaiger et al. 1997; Pacheco and Santos, 2002).

The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme activity that causes changes in metabolism, further leading to cellular intoxication and finally death. These manifests as necrosis, as well as from chemical insult, lesions may arise and other degenerative alterations to which the organism responds with an inflammatory, defensive reaction (Roganovic- Zafirova et al. 2003). In a study carried out after an accidental discharge of lindane in river water, histopathological analyses performed in the study suggested a positive relationship between accidental discharge of pesticide and the occurrence of histological alterations in *Barbus* sp. The kidney of fish showed a disintegration of convoluted tubules along with large intracytoplasmic vacuoles in epithelial cells of these tubules (González de Canales et al. 2003). In lindane exposed fish, Ortiz et al. (2003) found disintegration of the convoluted tissues, large intracytoplasmic vacuoles, shrinkage of glomerulus and increased space within the Bowman's capsule. Such types of structural changes in kidney are indicative of toxicity, which might subsequently compromise the renal functions.

**Conclusion**

Lindane, even in highly diluted form, as in this study, had the potential to incite toxic insults in the renal tissues of fish, which is indicative of serious toxicity.

Fig 1: Kidney of control *Channa punctata* (H&E 400x); (7d, 14d, 21d) kidney of fish exposed to 0.0255 g L<sup>-1</sup> lindane (H&E 400x) for 7, 14 and 21d respectively. Notice the loss of normal renal architecture (H&E 400). G-glomerulus, HT-haemopoietic tissue, RT-renal tubule, RTsl-shrinkage of tubular lumen, RTd- renal tubule degeneration, RTa-renal tubule atrophy, V- vacuolation of renal epithelium, HTd-haemopoietic tissue disintegration, Gc- glomerular contraction, Ga- glomerular atrophy, Gnec- glomerular necrosis.



## REFERENCES

- Amorim, MJDB Sousa, JP Nogueira, AJA & Soares, AMVM 2002, 'Bioaccumulation and elimination of 14C-lindane by *Enchytraeus albidus* in artificial (OECD) and a natural soil.' *Chemosphere*, vol. 49, pp. 323-329. | Bhattacharjee, D & Das, S 2013, 'Toxicity of Organochlorine pesticide, Lindane to fish- A review.' *Journal of Chemical and Pharmaceutical Research*, vol. 5, pp. 90-96. | Bist, R & Bhatt, DK 2009, 'The evaluation of effect of alpha-lipoic acid and vitamin E on the lipid peroxidation, gamma-amino butyric acid and serotonin level in the brain of mice (*Mus musculus*) acutely intoxicated with lindane.' *Journal of Neurological Science*, vol. 276, pp 99-102. | Coetzee, L 1998, 'Bioaccumulation of metals in selected fish species and the effects of pH on aluminum toxicity in a cichlid *O. mossambicus*.' 1st Edn, Randse Afrikanase Universitets, South Africa. | EXTOWNET, 1996. Extension Toxicology Network, 'A pesticide information project of cooperative extension offices'. USDA/Extension Service/National Agricultural Pesticide Impact Assessment Program. | Geetha, M & Fulekar, MH 2008, 'Bioremediation of pesticides in a developed reactor for conserving aquatic ecosystem.' In: *Proceedings of Taal 2007: The 12th World Lake Conference*: 933-935. | Gonz'alez de Canales, ML Ortiz, JB & Sarasquete, C 2003, 'Histopathological changes induced by lindane in several organs of fishes.' *Scientia Marina*, vol. 67, pp. 53-61. | Hfaiedh, N, Murat, JC & Elfeki, A 2011, 'Protective effects of garlic (*Allium sativum*) extract upon lindane-induced oxidative stress and related damages in testes and brain of male rats.' *Pesticide Biochemistry and Physiology*, vol. 100, pp. 187-192. | Hinton, DE & Lauren, DJ 1990, 'Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure'. In: *Biomarkers of Environmental Contamination* (McCarthy, JF & Shugart, LR Eds). Boca Raton, Lewis Publishers. pp.17- 57. | Hrnčić, D Rašić-Marković, A Djuric, D Šušić, V & Stanojlović, O 2011, 'The Role of nitric oxide in convulsions induced by lindane in rats.' *Food and Chemical Toxicology*, vol. 49, pp. 947-954. | Ogata, N Vogel, S & Narahashi, T 1988, 'Lindane but not deltamethrin blocks a component of GABA-activated chloride channels.' *FASEB J*, vol. 2, pp. 2895-2900. |