

# Impact of Sublethal Propoxur Exposure on Kidney Acetylcholinesterase Activity in *Tilapia mossambica* (Peters)

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## ABSTRACT

The present study examined the detrimental effects of propoxur (2-isopropoxyphenyl N-methyl carbamate) on the kidneys of *Tilapia mossambica* (Peters) to assess the acetylcholinesterase (AChE, EC 3.1.1.7) enzyme and its toxicity on behaviour. Propoxur is extremely toxic to *Tilapia mossambica*, with a median lethal concentration (LC) of  $0.52 \pm 0.01$  mL/L. The parameters of kidney AChE were analysed after exposure to a sublethal concentration (0.065 ml/L) for periods of 15 and 30 days. Propoxur significantly ( $P < 0.001$ ) inhibited kidney AChE, with reductions of 25.78% and 44.73% observed after 15 and 30 days of exposure, respectively. The changes in behavioural responses underscore the adverse effects of propoxur on fish. These results indicate that propoxur poses a risk to the environment and, by extension, to humans.

**Key Words:** Propoxur, kidney, *Tilapia mossambica*, Acetylcholinesterase inhibition

## INTRODUCTION

Aquatic ecosystems are under constant pressure from the influx of anthropogenic contaminants, primarily sourced from domestic, agricultural, and industrial effluents. Among these, the persistence of synthetic pesticides is particularly concerning; their bioaccumulation triggers a cascade of physiological, biochemical, and histological impairments in freshwater biota. These substances ultimately compromise organismal health by disrupting fundamental metabolic pathways and inhibiting critical enzymatic functions (Cope, 2004; Wang et al., 2009). Biological responses serve as indicators to evaluate the toxic effects of pollutants.

Carbamates are favoured as pesticides due to their short duration and effectiveness against pests and insects. These chemicals act as anticholinergic agents by binding to the esteric site of acetylcholinesterase (AChE) in the body. This specific interaction makes AChE inhibition a recognised biomarker for these insecticides (Fukuto, 1990; Hualing Fu, 2018). Propoxur, a carbamate pesticide, is extensively used to protect crops from various insects and pests and as an alternative to DDT for controlling malaria vectors. However, its widespread use has led to significant environmental pollution issues. The primary impact of propoxur is the inhibition of AChE, resulting in the accumulation of acetylcholine (ACh) at nerve synapses, which disrupts nerve function (Fernandes et al., 2008; Fernandes et al., 2015; Srivastava and Singh, 1982). Propoxur poses ecotoxicological risks to non-target organisms, such as fish and mammals. The toxicological profile of propoxur (2-isopropoxyphenyl N-methyl carbamate) in freshwater teleosts has been characterised by significant enzymatic and structural disruptions (Sharma et al., 2020). Studies have shown that propoxur can reduce blood and brain AChE activity by 50% following oral administration of 10 mg/kg body weight (Yadav et al., 2010). At a concentration of 0.0538 ppm, propoxur significantly decreased brain AChE activity in the fish species *Tor tambroides* (Ahmad et al., 2016). Comprehensive research on the toxic effects of propoxur on AChE in aquatic species is limited. Therefore, this study aimed to assess the impact of propoxur on AChE inhibition in the kidney of *Tilapia mossambica*, and fish behaviour to explore the potential effects of propoxur exposure.

## MATERIALS AND METHODS

### Fish and Treatment

20% EC propoxur, (C<sub>11</sub> H<sub>15</sub> O<sub>3</sub> N) 2-isopropoxyphenyl N-methyl carbamate, mol. Wt. 209.29, a light-yellow liquid, was selected for this study. Fishes (*Tilapia mossambica*, length: 10±2 cm, weight: 10±2 gm ) were collected from Patra fish seed farm, which is located in Bhopal, Madhya Pradesh, India and were acclimatised to the laboratory conditions for 15 days in glass aquaria. Fishes were fed daily with palletised supplementary feed, and water was renewed daily. The physico-chemical characteristics of water were determined ( APHA, 1995 ), and were found as: temperature- 25 ± 3° C, pH- 7.2 ± 0.2, dissolved oxygen- 6.9 ± 0.5 mg/L, total alkalinity- 173 ± 10 mg/L and total Hardness- 16 ± 1 mg/L. The 96 h LC<sub>50</sub> of propoxur was determined (Duodoroff, 1951) and estimated to be 0.52 ml/L. A sublethal concentration (0.065 ml/L or 65 ppm), which was 1/8 of the 96 h LC<sub>50</sub> value of propoxur for the *Tilapia mossambica*, was selected for 15 and 30 days of exposure. The control was maintained for the same duration. The behaviour of the fish was observed during the experiment.

### Enzyme Assay

At the end of the experiment, the fish were sacrificed and dissected. A 5% kidney homogenate was prepared in an ice-cold 0.25 M sucrose solution and centrifuged at 12,000 rpm for 7 min. AChE activity was measured spectrophotometrically at 540 nm using the method described by Metcalf (1951). Acetylcholine iodide (AChI) was used as the substrate. Protein estimation was done according to Lowry et al. (1951) using Bovine serum albumin as a standard.

## RESULTS

### Behavioural Changes

At a sublethal concentration of 0.065 ml/L of Propoxur for 48 hours, the treated fish displayed erratic, rapid, and jerky movements. After prolonged exposure of 15 and 30 days, the fish showed reduced activity, struggled to breathe, had limited swimming ability, and eventually became unbalanced and lethargic.

### AChE Activity and Inhibition

The activity of acetylcholinesterase in the kidney was assessed after the fish were exposed to 0.065 mL/L of propoxur for 15 and 30 days. A notable reduction in AChE activity was detected in all samples tested after exposure to 0.065 ml/L propoxur in the kidney of *T. mossambica*, with the decrease dependent on exposure duration. Specifically, the kidney AChE activity of *T. mossambica* dropped significantly by 25.78% after 15 days and by 44.73% after 30 days compared to the control group. The AChE activity levels fell to 1.41±0.15 (15 days) and 1.05±0.20 (30 days), in contrast to the control level of 1.90±0.22 (micromoles of AChI hydrolysed/mg protein/hour) at 0.065 ml/L of propoxur (Table-01).

Table-1 AChE Activity in the Kidney of *T. mossambica* after propoxur Exposure

Exposure Duration	AChE Activity (µmoles of AChI hydrolysed/mg protein/hour) (Mean ± SD)	% Reduction (Relative to Control)	Statistical Significance
Control	1.90 ± 0.22	-	-
15 Days	1.41 ± 0.15	25.78%	P< 0.001
30 Days	1.05 ± 0.20	44.73 %	P< 0.001

## DISCUSSION

Propoxur, a kind of carbamate, is recognised for its neurotoxic effects by inhibiting the enzyme acetylcholinesterase (AChE). This enzyme primarily functions to end impulse transmission by breaking down the cationic neurotransmitter acetylcholine. Inhibition of AChE results in an accumulation of ACh at synapses and neuromuscular junctions, causing overstimulation of ACh receptors (Gupta, 1994), preventing normal function (Dutta & Arends, 2003). Previous studies have established the utility of acetylcholinesterase (AChE) as a critical biomarker for pesticide-induced stress in aquatic environments. This study found that the kidney AChE activity in *T. mossambica* was significantly reduced by 25.78% after 15 days and 44.73% after 30 days when exposed to 0.065mL/L of propoxur, compared to the control group. Similar AChE inhibition in various fish tissues has been documented by several researchers (Kumar & Chapman, 2001; Rao et al., 2003; Joseph & Raj, 2011). Longer exposure times correlate with more severe negative effects. The observed AChE activity inhibition aligns with findings from other studies (Das & Mukherjee, 2003; Rao, 2004; Crestani et al., 2007; Joseph & Raj, 2011; Sharma et al., 2020). However, the potential for physiological recovery following toxicant withdrawal is a vital aspect of aquatic toxicology. Sharma et al. (2020) noted that while propoxur caused significant hepatic and enzymatic damage in *T. mossambica*, the inhibited AChE showed a substantial recovery of approximately 69.3% after 30 days of leaching in toxicant-free water. This suggests that while carbamate-induced damage is severe, it may be reversible if the environmental stressor is removed, though the associated histological alterations, like cellular vacuolization, may require longer periods for total repair (Sharma et al., 2020).

Behavioural changes have been linked to the toxic effects of the environment. In our current research, we noted behavioural changes in test fish, such as increased activity, erratic, rapid and jerky movements, with short-term propoxur exposure for 48 hrs., while prolonged exposure resulted in slow breathing, reduced swimming activity, lethargy, and loss of balance. These changes in fish movement may be due to acetylcholine accumulation at the synapse, which disrupts coordination between the nervous system and muscles (Rao et al., 2005; Rao, 2006).

The urgent need to transition from synthetic organophosphates and carbamates to sustainable alternatives is underscored by the high environmental and health risks associated with irreversible enzyme inhibition. Biopesticides, particularly those derived from botanical sources, offer a promising solution due to their biodegradable nature and multi-targeted neurotoxic effects on pests with lower long-term toxicity to non-target species. For instance, research on *Annona squamosa* leaf extracts has demonstrated significant anticholinesterase activity, effectively inhibiting AChE in aquatic models like *Labeo rohita* during exposure, yet allowing for a rapid and substantial recovery once the toxicant is removed (Sharma, 2026). This capacity for enzymatic recovery—reaching nearly 80% within 120 hours—contrasts sharply with the persistent and often permanent damage caused by synthetic chemical pesticides. Therefore, it may be suggested that integrating the natural alternatives into Integrated Pest Management (IPM) is essential for sustainable and responsible agricultural practices.

## CONCLUSION

The current research demonstrates that propoxur is highly toxic, significantly impacting the AChE activity of the fish kidney. The kidney biochemical parameters can serve as rapid and sensitive indicators of pesticide stress in fish. The study concludes that Propoxur can pose a significant threat to environmental health. Therefore, integrating natural anticholinesterase alternatives into Integrated Pest Management (IPM) strategies not only maintains agricultural productivity but also ensures the preservation of aquatic biodiversity by minimising the permanent physiological disruptions typically observed with conventional chemical interventions.

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