DOI: 10.2478/cjf-2025-0017

CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

sciendo

ACUTE TOXICITY IMPACT OF INDOXACARB ON GILL NEUROENDOCRINE SYSTEM AND BRAIN OF STINGING CATFISH *Heteropneustes fossilis*

Ajeet Kr. Mishra, Anita Gopesh, K.P. Singh*

Neurobiology Lab., Department of Zoology, University of Allahabad, Prayagraj, India

*Corresponding Author: kpsald29@gmail.com

ARTICLE INFO

Received: 21 February 2025
Accepted: 25 September 2025

ABSTRACT

The recent study aimed to evaluate the impact of acute toxicity of indoxacarb (IDC) on pseudobranchial neurosecretory cells (PNSCs), biochemical assays and histopathological changes in the brain, and accompanying behavioral abnormalities in the air-breathing stinging catfish *Heteropneustes fossilis*. The 96-hour LC_{50} of IDC was ascertained at a dose of 0.075 mg/L. Live catfishes were exposed to LC_{50} dose of IDC for 24, 48, 72 and 96 hrs, and the respective parameters were studied at predetermined intervals. After 96 hrs of exposure to IDC, histopathology of PNSCs demonstrates degenerative changes, reduced neurosecretory material, significantly decreased number of PNSCs along with nucleo-cytoplasmic (N/C) ratio, as compared to the control group. The exposed fishes displayed detachment of stratum marginale (SM) and stratum opticum (SO), mild necrosis in stratum periventricular (SPV), and compact neuroarchitecture of stratum fibrosum et grisium superficiale (SFGS), stratum griseum centrale (SGC) and stratum album centrale (SAC), whereas degeneration of mononuclear cells and Purkinje cells in the cerebellum was observed. The acetylcholinesterase (AChE), superoxide dismutase (SOD), and catalase (CAT) activities were significantly decreased, whereas malondialdehyde (MDA) level was significantly increased, as compared to the control group. The total distance traveled, swimming speed, mobility time, absolute turn angle, head: distance traveled, and maximum speed were significantly decreased, whereas immobility time, maximum inactive episode, and time freezing were significantly increased in comparison with the control group. This study concludes that acute toxic concentrations of IDC may cause physiological dysregulation, biochemical changes in brain tissues and mild necrotic changes in PNSCs and neuronal cells in fish, resulting in substantially associated behavioral disturbances.

Keywords:
Indoxacarb
Pseudobranchial neurosecretory system
Fish brain
locomotor activity
AChE
Anti-oxidative enzyme

How to Cite

Mishra, Kr. A., Gopesh, A., Singh, K.P. (2025): Acute toxicity impact of indoxacarb on gill neuroendocrine system and brain of stinging catfish *Heteropneustes fossilis*. Croatian Journal of Fisheries, 83, 157-166. DOI: 10.2478/cjf-2025-0017.

INTRODUCTION

During the last few decades, one of the major threats to the biodiversity of non-target flora and fauna has been attributed to the extensive use of organophosphate (OP) insecticides, which present challenges for the preservation of global biodiversity. Due to excessive use of organophosphates in agricultural fields, AChE and other potent biomarkers in non-target organisms develop resistance through sudden mutations or alterations in amino acid residues (Villatte et al., 2000; Bourne et al., 2003). Hence, there is a need to develop alternatives to conventional biomarker inhibitors. The foremost alternative to organophosphates is indoxacarb (IDC), a moderately hazardous, non-systemic and reduced-risk insecticide (USEPA, 2000) which kills lepidopteran larvae in tomato, lettuce, bean, and other crops as a result of binding to sodium ion channel sites and blocking the influx of sodium ions into nerve cells (Ghelichpour et al., 2017; Mirghaed and Ghelichpour, 2015). The lethal concentration of IDC is moderately to highly toxic to freshwater and estuarine fishes due to its low water solubility and short aqueous photolysis half-life (Moncada and Branch, 2003). In surface water, the lower and higher concentrations of IDC ranged from 3.7 µg/L to 13.7 µg/L (0.0037 to 0.0137 mg/L), respectively (Monteiro et al., 2019). In lettuce crops, IDC concentration was up to 7.76 µg/L (0.0077 mg/L) (EFSA et al., 2018b). Moreover, due to surface runoff in aquatic environments, IDC concentration was detected as high as 50 µg/L (0.05 mg/L) in different seasons in the Jiulong River, China (Zheng et al., 2016).

The scholarly reports on the toxicity of IDC in fishes as non-target aquatic organisms are available for histopathological alterations of the gill and kidney (Mirghaed et al., 2018), biochemical and molecular characterization, including thyroid disruption, protein synthesis inhibition (Ghelichpour et al., 2017; Mirghaed and Ghelichpour, 2015), osmotic stress tolerance, antioxidant and stress gene expression, and oxygen consumption efficiency (Ghelichpour et al., 2018, 2019; Bantu et al., 2017). Furthermore, the toxicity of IDC on the excitatory amino acid transporter-related (EAAT) gene (maintaining homeostasis in the central nervous system) was observed in the brain of female zebrafish (Wang et al., 2020).

Recently, effects of acute exposure to chlorpyrifos (CPF) on degenerative changes in pseudobranchial neurosecretory cells (PNSCs), present in the gill region of both sides of Indian air-breathing catfishes, were reported by our laboratory (Mishra et al., 2022, 2024), but the data on the effects of acute toxicity of IDC on histopathological changes in PNSCs of air-breathing catfishes are almost absent. However, PNSCs are likely part of the complex neuroendocrine or chemosensory system in the gill region of fish (Yadav et al., 2023; Mishra et al., 2022, 2024), which coordinate different functions of respiratory physiology (Evans et al., 2005; Zaccone et al.,1996; Munshi and Hughes, 1981), and play a significant role in hypoxia, pollution, and other stressors. To the best of our knowledge, the investigation of IDC-induced toxicity on histopathological changes and secretory cycle of PNSCs has not yet been studied.

Keeping the above views in mind, we hypothesized that acute IDC toxicity could induce histopathological and quantitative changes in the PNSCs, biochemical changes in the brain, and associated respiratory and swimming behaviors in experimental fishes, which may coordinate to regulate stress physiology in fishes. Therefore, the present study focused on investigating the acute toxic effects of IDC on histopathological changes in the PNSCs of the gill region, optic tectum and cerebellum areas of the brain, biochemical estimation of AChE and oxidative stress in the fish brain, respiratory frequency of opercular movements, and locomotor/swimming behavior patterns in a facultative air-breathing catfish *H. fossilis*.

MATERIALS AND METHODS

Live specimens of *H. fossilis* (length, 15.53 ± 0.42 cm; weight, 33.09 ± 0.78 g) were collected from unpolluted local ponds in Prayagraj, Uttar Pradesh, India (25°.60′ N and 81°.84′ E at an elevation of 98 m). These fish were placed in a large aquarium (121 × 45 × 50 cm) and acclimatized under standard laboratory conditions with dechlorinated tap water at a normal water temperature (23 ± 2 °C), and a natural photoperiod (13L: 11D) for 15 days. During acclimatization and the experimental period, the fish were fed *ad libitum* with minced goat liver and commercial fish pellet (Optimum fish food, Samut Prakan, Thailand). The total amount of feed provided was not less than 2–3% of their body weight per day. Dissolved oxygen (5.10 ± 0.220 mg/L), total hardness (126 ± 1.85 mg/L), chlorine (104 ± 1.23 mg/L), alkalinity (350 ± 2.10 mg/L), and pH (7.4 ± 0.03) were monitored daily during the experiment (APHA, 1995).

The oxidiazin insecticide, indoxacarb (IDC) (14.5% suspension concentration), was used as the test compound for the determination of median lethal concentration (LC_{50}). The LC_{50} for 96 hrs of IDC was found to be 0.075 mg/L, determined according to the arithmetic method of Karber as adopted by Dede and Kaglo (2001), using the daily renewal bioassay system (Table 1). The mortality of fish was observed daily during 96 hrs of exposure. There was no distinction between the sexes in experiments. Lethal concentration (LC_{50}) was calculated by the equation:

$$LC_{50} = \frac{LC_{100} - (\text{Mean death} \times \text{Concentration difference})}{\text{Number of fish per group}}$$

Table 1. Determination of LC_{50} dose of indoxacarb at 96 hrs for *H. fossilis*

Conc. (mg/l)	Conc. difference	No. of live fishes	No. of dead fishes	Mean death	Mean death × Conc. difference
0	0	12	0	0	0
0.04	0.01	12	0	0	0
0.05	0.01	11	1	0.5	0.005
0.06	0.01	9	3	2.0	0.02
0.07	0.01	7	5	4.0	0.04
0.08	0.01	5	7	6.0	0.06
0.09	0.01	2	10	8.5	0.085
0.1	0.01	1	11	10.5	0.105
0.11	0.01	0	12	11.5	0.115
					∑ = 0.43

The fish were divided into two groups, the control group and IDC treatment group (n = 12 per group). The fish were exposed under semi-static conditions for 4 days (96 hrs), where IDC-exposed water was replaced every day with freshly prepared pesticide solution. At the end of the experiment, at selected time points (24, 48, 72 and 96 hrs), the fish were anesthetized by cold anesthesia, and pseudobranchial neurosecretory mass and brain were excised following decapitation (Mishra et al., 2022).

During experiments, the locomotory or swimming behavior of the control and IDC-exposed fishes was recorded for 5 min in a specially designed aquarium (24 × 12 × 12 cm) at regular intervals, 24 hrs (n = 12), 48 hrs (n = 10), 72 hrs (n = 8), and 96 hrs (n = 6) by an automated video tracking system, ANY-maze software (Stoelting Co., USA). As per study design, selected indices of fish swimming behavior, including total distance traveled, swimming speed, time mobile, absolute turn angle, maximum inactive episodes, head: distance traveled, maximum speed, and time freezing, were recorded by ANY-maze during experimentation.

Furthermore, the frequency of opercular movement of the control and exposed fishes was quantified manually for 1 min only during the stable state of the fish (Gupta and Dua, 2010).

After preparation of paraffin blocks of the brain and pseudobranchial neurosecretory mass (NSM) of both control and exposed groups, the paraffin blocks were cut into 8 µm thick sections by a rotary microtome. After stretching and deparaffinization of slides through different grades of ethanol, the brain sections were stained with hematoxylin and eosin (H&E) stain; whereas NSM sections were stained with a neurosecretion-specific stain, acid violet (Takasugi and Bern, 1962; Mishra et al., 2022). The respective images of selected areas of control and exposed groups were identified and captured by a Nikon photomicroscope.

For quantitative estimation of pseudobranchial neurosecretory cells (PNSCs), ImageJ software (NIH, Bethesda, MD) was used for counting cell size, cell number, and the nucleus/cytoplasmic (N/C) ratio of PNSCs. The cells of control and experimental groups were randomly selected at higher magnification (400X). The total area of the cytoplasm with nucleus (A_{nc}) and areas of all nuclei (A_n) were measured in each cell, and the equation

$$N/C = A_{nc} / (A_{nc} - A_n)$$

was used for the determination of the N/C ratio of PNSCs. After amputation, the fish brain was quickly removed and homogenized (10%v/v) in a 0.1 M phosphate buffer containing Triton X-100 using a homogenizer. The crude homogenates were centrifuged at 10,000 rpm for 30 min at 4 °C. The supernatants have enzyme sources that were used for the estimation of acetylcholinesterase (AChE) activity (Topal et al., 2017; Ellman et al., 1961) and protein quantity (Lowry, 1951). Enzyme activity was determined by reading the changes in absorbance over 5 min at a wavelength of 412 nm in a UV1800 spectrophotometer (Molecular Devices, Shimadzu, Japan). The superoxide dismutase (SOD) activity in brain tissues was analyzed by Marklund and Marklund (1974). The unit of SOD activity is expressed as 50% inhibition of pyrogallol autoxidation per min/mg protein. The increase in absorbance was recorded at 412 nm for 3 min at 30-second intervals. Catalase (CAT) activity in brain homogenates was measured by Sizer and Beer (1952). The decomposition rate of the substrate H_2O_2 at a final concentration of 10 Mm was monitored at 240 nm and 25 °C after adding the sample directly to a cuvette. Lipid peroxidation was measured according to the method of Esterbauer and Cheeseman (1990). The concentration

of MDA in brain tissue was determined from a standard plot and expressed in nmol/mg tissue.

All data represent the mean ± SEM value. The level of significance between control and IDC-treated fish was tested using an unpaired Student's t-test. A probability level of $P < 0.05$, $P < 0.01$, and $P < 0.001$ was set as statistically significant.

RESULTS

In IDC-exposed fishes, behavioral indices such as total distance traveled, swimming speed and mobility time were significantly decreased ($P < 0.001$, $P < 0.01$, $P < 0.05$) in subsequent days (24, 48, 72, and 96 hrs), whereas time immobile phases were significantly increased ($P < 0.001$, $P < 0.05$) in subsequent time interval (24, 48, 72, and 96 hrs) (Fig.1). Alternatively, absolute turn angle, maximum inactive episodes and time immobile phases were significantly increased ($P < 0.001$, $P < 0.01$, $P < 0.05$) at 24 hrs, whereas these indices were not significant ($P > 0.05$) at 48, 72 and 96 hrs, as compared to the control group of randomly selected fishes (Table 2).

In IDC-exposed fishes, opercular movements were significantly decreased ($P < 0.001$) as compared to the control group at 24, 48, 72, and 96 hrs (Table 3).

After exposure to an acute toxic concentration of IDC for 96 hrs, detachment of stratum marginale (SM) and stratum opticum (SO), mild necrosis in stratum periventricular (SPV), and compact neuroarchitecture of stratum fibrosum et grisium superficiale (SFGS), stratum griseum centrale (SGC) and stratum album centrale (SAC) of optic tectum were observed. The degeneration of mononuclear cells, vacuolization in SFGS layers, and mild necrosis in granular cells of SPV layers were noticed, as compared to compact neuroarchitecture with normal cells in all distinct layers of optic tectum in the control group (Fig. 2 (A and B)). In the cerebellum, degeneration of mononuclear cells and Purkinje cells was observed after 96 hrs of exposure to IDC, as compared to the control (Fig. 2 (C and D)).

In IDC-exposed fishes at 96 hrs, degenerative changes (necrosis) were observed in some PNSCs with reduced neurosecretory material (NSM), whereas PNSCs of the control group were of normal shape and size, and filled with NSM in the cytoplasm [Fig. 3 (A & B)].

Student's t-test analysis revealed that the number of PNSCs significantly decreased ($P < 0.001$) after exposure to IDC for 96 hrs. Similarly, the N/C ratio of PNSCs was also significantly reduced ($P < 0.05$) (Table 4), whereas the cell size increased insignificantly ($P > 0.05$), as compared to the control group.

The acetylcholinesterase (AChE) activity in the IDC-exposed brain was significantly decreased ($P < 0.001$), as compared to the control brain. The AChE activity in the control brain was recorded at 0.028 ± 0.0003 µmol of acetylcholine hydrolyzed min^{-1} mg^{-1} of protein, whereas it was substantially decreased to 0.0079 ± 0.00034 µmol

Table 2. Effects of an acute toxic concentration of IDC on different indices of locomotory/swimming behavior at different time intervals

	24 hrs (n = 12)		48 hrs (n = 10)		72 hrs (n = 8)		96 hrs (n = 6)	
	Control	0.075 mg/L	Control	0.075 mg/L	Control	0.075 mg/L	Control	0.075 mg/L
Total distance traveled (m)	229.5 ± 15.38	143.2 ± 7.29***	221.1 ±17.3	119.1 ± 8.15***	219 ± 20.2	132 ± 13.9**	196 ± 21.2	131 ± 12.3*
Swimming speed (m/s)	0.780 ± 0.056	0.370 ± 0.036***	0.709 ± 0.054	0.361 ± 0.46***	0.532 ± 0.042	0.380 ± 0.049*	0.658 ± 0.050	0.348 ± 0.057**
Time mobile (sec)	285 ± 6.84	185 ± 20.1***	276 ± 8.90	226 ± 20.8*	255 ± 15.4	186 ± 32.8	257 ± 14.8	177 ± 27.9*
Time immobile (sec)	48.3 ± 8.88	90.5 ± 16.5*	47.8 ± 11.0	124 ± 30.1*	36.6 ± 4.89	139 ± 24.0***	51.2 ± 11.4	126 ± 31.1*
Absolute turn angle (degree)	58500 ± 5330	43800 ± 3870*	37900 ± 2780	33100 ± 4250	34400 ± 4480	31100 ± 3690	40300 ± 2700	31100 ± 4440
Maximum inactive episode (m/s)	63.7 ± 11.4	159 ± 25.8**	70.4 ± 24.8	117 ± 33.9	31.4 ± 9.59	88.6 ± 34.3	47.0 ± 16.3	98.4 ± 37.8
Head: distance traveled (m)	172 ± 19.2	146 ± 10.7	148 ± 11.8	134 ± 9.48	120 ± 17.0	114 ± 19.8	146 ± 5.26	86.0 ± 15.2**
Maximum speed (m/s)	11.5 ± 0.805	8.45 ± 1.87	4.35 ± 1.18	2.76 ± 0.30	13.37 ± 1.627	7.17 ± 1.90*	10.1 ± 1.53	8.33 ± 2.61
Time freezing (sec)	77.4 ± 22.6	86.8 ± 21.1	98.7 ± 15.6	130 ± 22.3	141 ± 27.0	158 ± 23.8	118 ± 4.69	222 ± 34.8*

All data represent mean ± SEM value at 24 hrs (n = 12), 48 hrs (n = 10), 72 hrs (n = 8), and 96 hrs (n = 6). *, **, and *** represent the level of significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, determined by Student's t-test.

in the IDC-exposed brain after 96 hrs. The SOD and CAT activity was significantly decreased ($P < 0.001$) in the IDC-exposed group, in comparison with the control group at 96 hrs. The MDA level in the control brain was 0.016 ± 0.0001 nmol of MDA formed mg^{-1} of tissue, although the MDA level was substantially increased ($P < 0.001$) to 0.019 ± 0.00015 µmol in the IDC-exposed brain after 96 hrs (Table 5).

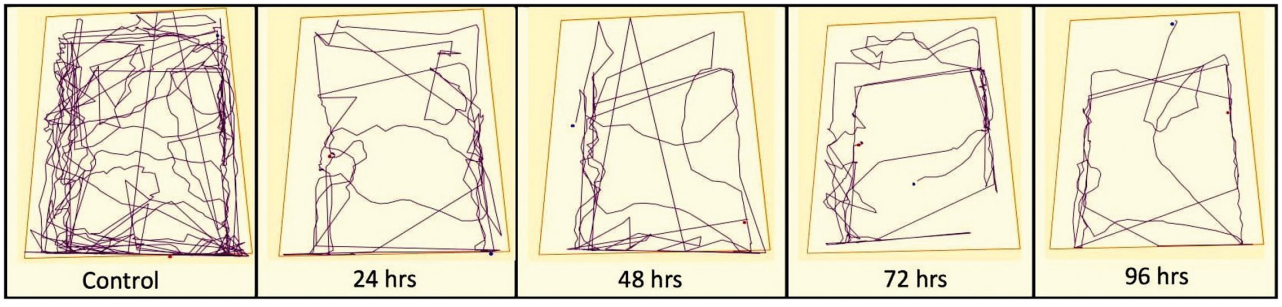


Fig 1. Automated representative track records of locomotory activity of control and IDC-exposed fishes at 24, 48, 72, and 96 hrs

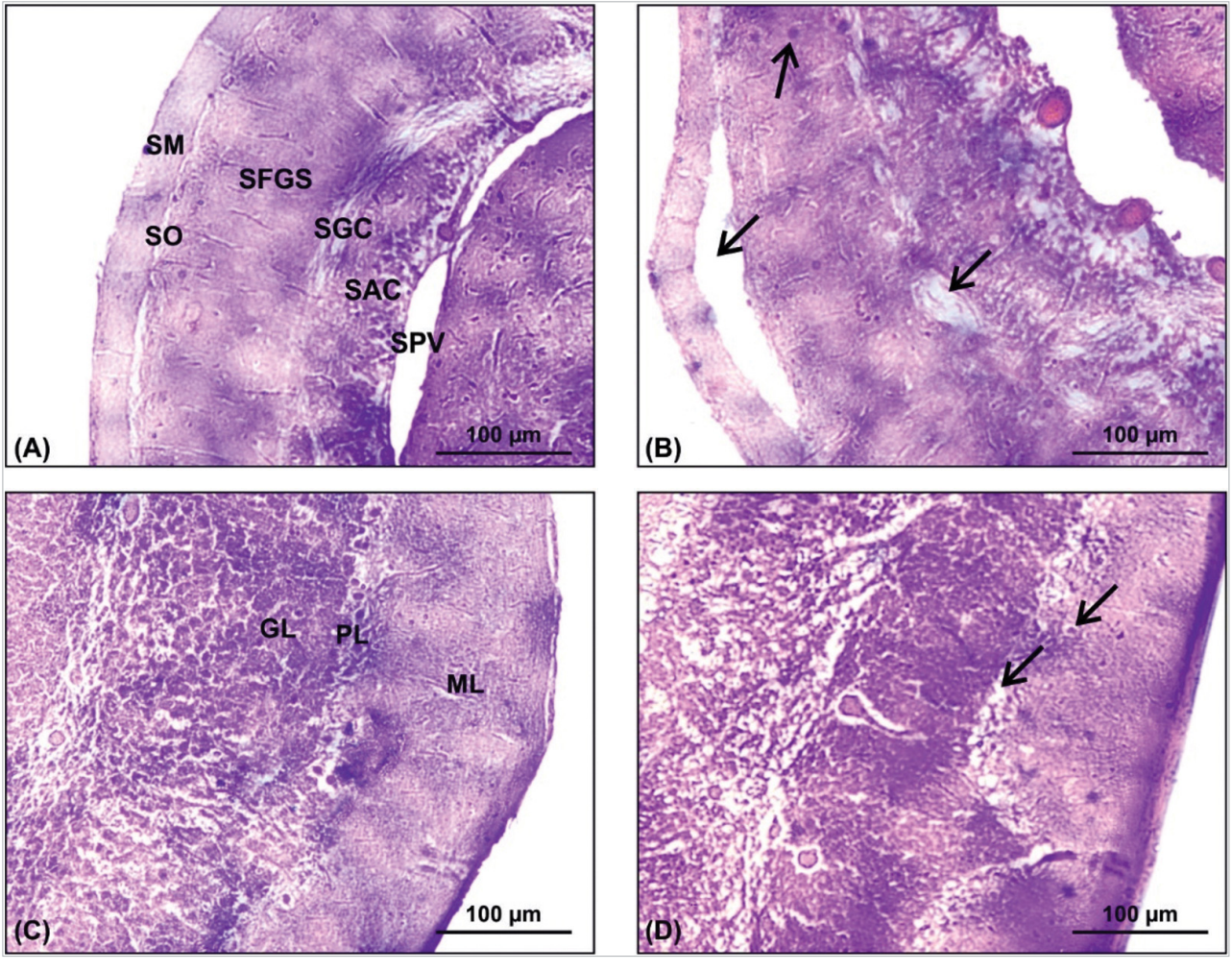
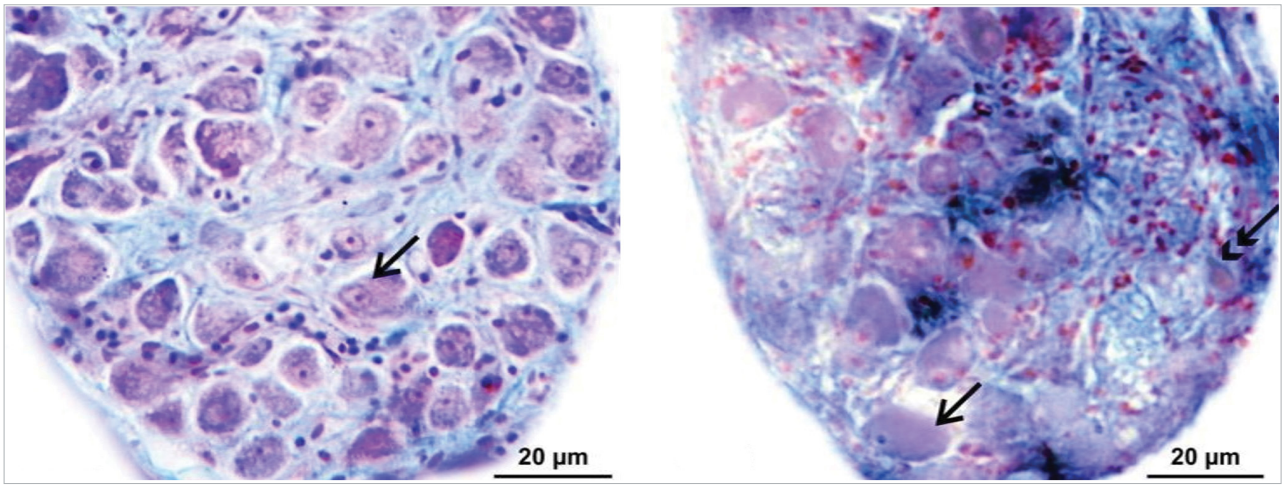


Fig 2. Effects of an acute toxic concentration of IDC on optic tectum and cerebellum of *H. fossilis* at 96 hrs. Microphotographs: 3A shows intact zones of all layers of the optic tectum of control fish; 3B shows detachment of SO and SM layer necrosis in the mononuclear cells with vacuolization; 3C shows a normal structure of neurons in the cerebellar lobe in the control group; 3D shows degeneration of Purkinje cells and loss of granule cells after IDC exposure (H&E staining) (magnification = 100X; scale bar = 100µm), (ML = molecular layer, PL = Purkinje layer, GL = granular layer).

Table 3. Effects of an acute toxic concentration of IDC on opercular movements

	24 hrs (n = 12)		48 hrs (n = 10)		72 hrs (n = 8)		96 hrs (n = 6)	
	Control	**0.075 mg/L**	**Control**	**0.075 mg/L**	**Control**	**0.075 mg/L**	**Control**	**0.075 mg/L**
Opercular movements (breath/min)	95.8 ± 1.14	70.8 ± 1.91***	105 ± 3.45	75.4 ± 5.07***	102 ± 1.13	87.5 ± 2.65***	94.0 ± 1.18	67.0 ± 2.1***
	t = 11.3, $P < 0.001$		t = 4.86, $P < 0.001$		t = 4.86, $P < 0.001$		t = 11.2, $P < 0.001$	

All data represent mean ± SEM value at 24 hrs (n = 12), 48 hrs (n = 10), 72 hrs (n = 8), and 96 hrs (n = 6), respectively. *** indicate level of significance at $P < 0.001$, determined by Student's t-test.



(A) (B)

Fig 3. Effects of IDC on pseudobranchial neurosecretory cells of the gill region. Figure 3 A shows mature pseudobranchial neurosecretory cells (PNSCs) with a distinct nucleus, nucleolus, and cytoplasm filled with dense neurosecretory material (NSM). Figure 3 B shows necrosis in some cells with reduced NSM. (Scale bar = 20µm, magnification = 400x, acid violet staining)

Table 4. Effects of acute toxic exposure to IDC on cell number, cell diameter, and N/C ratio of PNSCs

Parameters	**Control**	**0.075 mg/L**
Number of PNSCs	46.83 ± 2.30	23.66 ± 0.669***
Cell diameter of PNSCs (µm)	128.72 ± 11.29	130.98 ± 17.83
N/C ratio of PNSCs (µm)	1.71 ± 0.133	1.33 ± 0.0579*

All data represent mean ± SEM value for evaluating the effects of an acute toxic concentration of IDC on cell numbers (n = 6), cell diameter (n = 20), and N/C ratio (n = 20) of PNSCs. Symbols * and *** indicate a level of significance at $P < 0.05$ and $P < 0.001$, respectively, determined by Student's t-test.

Table 5. Effects of acute toxic exposure to IDC on AChE, SOD, CAT, and MDA levels in the fish brain

Parameters	**Control**	**0.075 mg/L IDC**
AChE activity (µmol /min/mg protein)	0.028 ± 0.00037	0.0079 ± 0.00034***
SOD activity (µmol /min/ mg protein)	0.75 ± 0.013	0.675 ± 0.0094***
CAT activity (µmol H_2O_2 / min / mg protein)	3.56 ± 0.14	2.11 ± 0.23***
MDA level (nmol/mg tissue)	0.016 ± 0.0001	0.01917 ± 0.00015***

All data represent mean ± SEM value (n = 6 per group) for assessing the effects of an acute toxic concentration of IDC on AChE, SOD, CAT activity and MDA level of the fish brain. *** indicates a level of significance at $P < 0.001$, determined by Student's t-test.

DISCUSSION

In the current study, acute IDC toxicity induced cytomorphological, necrotic, and quantitative changes in PNSCs with reduced neurosecretory material; however, the functional study regarding the synthesis of neurosecretory material remains uncertain. It is speculated that signaling molecules (pesticides or other stressors) may trigger PNSCs to release NSM, leading to increased and overt surfacing activity in fish to avoid low oxygen concentrations in aquatic environments. The functional status of the neurosecretory activity is linked with changes in the size of the nucleus and N/C ratio, and may be considered as an index of cell regulation. Previously, our laboratory reported that exposure to acute and sub-lethal concentrations of CPF leads to a hypoxia-like environment in the aquarium, resulting in NSM release and vacuolization in PNSCs (Mishra et al., 2022, 2024).

The rationale for selecting the LC-50 dose of IDC (0.075 mg/L) was in consonance with the doses found in the Jiulong River in China (0.05 mg/L) (Zheng et al., 2016). Other researchers reported a wide range of lower and higher IDC concentrations (0.0037 – 0.0137 mg/L) in fresh and marine/estuarine waters, and the acute toxicity of IDC ranges from 0.029 to 2.94 mg/L (USEPA, 2000). Even in these water bodies, chronic toxicity of IDC ranges from 0.004 to 0.15 mg/L (USEPA, 2000). Thus, we selected an environmentally relevant concentration of IDC in the freshwater tank as an acute dose (0.075 mg/L) in the present study.

The functional layers of the optic tectum coordinate visual perception with body movements and motor activity (Perez-Perez et al., 2003). The current results indicate that IDC causes histopathological lesions in the neuroarchitecture of different layers, especially SM and SO layers of the optic tectum and necrotic changes in neuronal cells of the cerebellum. The mild inhibitory effects of IDC on optic tectum and cerebellum may be due to reversible inhibition of AChE activity in the synapse or apoptosis of mononuclear cells and granular cells which retarded the function of neuronal cells (Tripathi et al., 2013; Mishra and Devi, 2014; Arslan et al., 2017). These results are in agreement with other studies which have reported histopathological, biochemical, and molecular changes in the brain due to the toxicity of indoxacarb in common carp *Cyprinus carpio* (Taheri Mirghaed et al., 2018).

Fish AChE activity has been widely used as a sensitive biomarker to ascertain the freshwater pollution load (de la Torre et al., 2002). Due to more neuronal networking in the different areas of the brain, the highest level of AChE inhibition was noticed in the brain, followed by the liver and kidneys. In our study, decreased AChE activity was recorded in brain tissue after 96 hrs, and the parallel results agreed with the reduction of AChE activity in rats exposed to indoxacarb (Nassar, 2016). The range of AChE activity was 52.94 – 368.45 nmol thiocholine min^{-1} mg^{-1} protein in the different regions of the fish brain, but the highest activity was recorded in the optic tectum of *H. fossilis* under stress-free conditions (Roy et al., 2006; Tripathi et al., 2013).

The molar mass of indoxacarb is 527.8 g/mol, hence it does not cross the blood-brain barrier swiftly due to its higher molecular weight (>500 g/mol), thus causing fewer neurodegenerative changes in the brain.

In aquatic toxicology, the brain tissue of fish is damaged as a result of oxidative stress conditions produced by ROS (Livingstone, 2001). As previously shown in the literature, it is well documented that xenobiotic agents may induce ROS production, which deviates antioxidant defense mechanisms of the organism and causes oxidative stress (Klotz and Steinbrenner, 2017; Slaninova et al., 2009). In the present study, anti-oxidative enzymatic assays were altered, including SOD and CAT levels, reflecting the development of a compensatory mechanism in response to increased oxidative stress. An earlier study demonstrated that indoxacarb and other toxicants might change the expression of antioxidant-related genes, resulting in an alteration of antioxidant enzyme activity (Ghelichpour et al., 2019; Hedayati et al., 2014; Jin et al., 2010). Another study also indicates that SOD and CAT enzymatic gene expression is dysregulated in gill, kidney and liver tissue after exposure to different concentrations of indoxacarb (Ghelichpour et al., 2019). The increased level of other biomarkers, including TNF-α, IL-1β and IL-8, due to activating macrophages, counteracts the deleterious effects of xenobiotic exposure (Sakamoto et al., 2003). The increased cortisol level due to IDC toxicity indicates increased stress condition and glucose anabolism (Miraghed et al., 2018). The increased MDA level in the present study indicates increased lipid peroxidation in brain tissues by enhancing oxidation of polyunsaturated fatty acids. Similar results were reported for increased MDA levels due to ATR exposure in liver tissue of common carp (Jin et al., 2010) and brain tissue due to CPF exposure in *H. fossilis* (Mishra et al., 2024). Thus, homeostasis of oxidative stress and antioxidant defense systems in the brain tissues could not be maintained due to exposure to IDC in the present study.

Behavioral changes are the first indicators to assess the effects of toxicants on the internal physiological homeostasis of aquatic animals in a toxicological study, and this study was highly acknowledged for the accuracy of a user-friendly tool; hence, individual fish activity was recorded by an automated tracking software (Kavitha and Rao 2008; Bonifecio et al., 2016). The decreased opercular movement and locomotory performances were observed in our study on subsequent days of exposure. Other symptoms, such as hyperactivity, erratic movements, increased mucus secretion and settlement at the bottom of the aquarium before death, were also observed but not included in the current study. These behavioral changes may be due to the increased metabolism and inhibition

of the acetylcholinesterase enzyme (Mishra et al., 2022; Mirghaed and Ghelichpour, 2015). The abnormality in the opercular movement was due to lethargy, secretion of mucus, and decreased lamellar respiratory surface (Velmurugan et al., 2007; Johl and Sharma, 2007). On the other hand, IDC may cause a reduction of basal metabolism or voluntary locomotion and consequently reduced production of ATP (Miraghed et al., 2018). Toxic substances increase metabolism and oxygen demand in fish, and elevated temperature results in increased toxicity of IDC and other pesticides (Mirghaed et al., 2015; Osterauer and Köhler, 2008).

CONCLUSION

This study concludes that acute exposure to IDC may cause physiological and biochemical disturbances, and behavioral changes due to mild necrotic changes, discharge of NSM in PNSCs and neuronal cell damage in the optic tectum and cerebellum of the fish brain. These findings suggest that the regulated scales of alternative organophosphates may also be recommended for controlling insect pests with lesser toxic effects on the non-target organisms such as fish.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of Zoology, University of Allahabad, Prayagraj, India for providing laboratory facilities and Botanical Survey of India (BSI), Prayagraj for photomicrography.

UTJECAJ AKUTNE TOKSIČNOSTI INDOKSAKARBA NA ŠKRŽNI NEUROENDOKRINI SUSTAV I MOZAK SOMA *Heteropneustes fossilis*

SAŽETAK

Cilj istraživanja bio je procijeniti utjecaj akutne toksičnosti indoksakarba (IDC) na pseudobranhijalne neurosekretorne stanice (PNSC), biokemijske testove i histopatološke promjene u mozgu te prateće abnormalnosti u ponašanju kod soma *Heteropneustes fossilis*. 96-satni LC_{50} IDC-a utvrđen je pri dozi od 0,075 mg/L. Živi somovi bili su izloženi LC_{50} dozi IDC-a tijekom 24, 48, 72 i 96 sati, a odgovarajući parametri proučavani su u unaprijed određenim intervalima. Nakon 96 sati izloženosti IDC-u, histopatologija PNSC-a pokazuje degenerativne promjene, smanjeni neurosekretorni materijal, značajno smanjen broj PNSC-a zajedno s nukleocitoplazmatskim (N/C) omjerom u usporedbi s kontrolnom skupinom. Izložene ribe pokazale su odvajanje stratum marginale (SM) i stratum opticum (SO), blagu nekrozu u stratum periventrikularnom (SPV) i kompaktnu neuroarhitekturu stratum fibrosum et grisium superficiale (SFGS), stratum griseum centrale (SGC) i stratum album centrale (SAC), dok je uočena degeneracija mononuklearnih stanica i Purkinjeovih stanica u malom mozgu. Aktivnosti acetilkolinesteraze (AChE), superoksid dismutaze (SOD) i katalaze (CAT) bile su značajno smanjene, dok je razina malondialdehida (MDA) bila značajno povećana u usporedbi s kontrolnom skupinom. Ukupna prijeđena udaljenost, brzina plivanja, vrijeme pokretljivosti, apsolutni kut okretanja, prijeđena udaljenost glava i maksimalna brzina značajno su smanjeni, dok su vrijeme nepokretnosti, maksimalna neaktivna epizoda i vrijeme zamrzavanja značajno povećani u usporedbi s kontrolnom skupinom. Ova studija zaključuje da akutne toksične koncentracije IDC-a mogu uzrokovati fiziološku disregulaciju, biokemijske promjene u moždanom tkivu i blage nekrotične promjene u PNSC-ima i neuronskim stanicama kod riba, što rezultira značajno povezanim poremećajima u ponašanju.

Ključne riječi: indoksakarb, pseudobranhijalni neurosekretorni sustav, mozak ribe, lokomotorna aktivnost, AChE, antioksidativni enzim

REFERENCES

American Public Health Association (1995): Standard Methods for the Examination of Water and Wastewater, 19th ed. APHA, Washington, DC.

Arslan, H., Ozdemir, S., Altun, S. (2017): Cypermethrin toxication leads to histopathological lesions and induces inflammation and apoptosis in common carp, *Cyprinus carpio* L. Chemosphere, 180, 491-499.

Authority, E.F.S., Arena, M., Auteri, D., Barmaz, S., Brancato, A., Brocca, D., Bura, L., Cabrera, L.C., Chiusolo, A., Marques, D.C. and Crivellente, F. (2018): Peer review of the pesticide risk assessment of the active substance spinosad. European food safety authority Journal, 16 (5), e05252.

Bantu, N., Kumar, R.K., Vakita, V. (2017): Acute toxicity and respiratory responses in freshwater fish, *Labeo rohita* exposed to an agrochemical indoxacarb. Algerian Journal Environmental Science Technology, 2349–7041.

Bonifacio, A.F., Cazenave, J., Bacchetta, C., Ballesteros, M.L., de los Ángeles Bistoni, M., Amé, M.V., Bertrand, L., Hued, A.C. (2016): Alterations in the general condition, biochemical parameters and locomotor activity in *Cnesterodon decemmaculatus* exposed to commercial formulations of chlorpyrifos, glyphosate and their mixtures. Ecological Indicators, 67, 88-97.

Bourne, Y., Taylor, P., Radić, Z., Marchot, P. (2003): Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site. The EMBO journal, 22, 1-12.

De la Torre, F.R., Ferrari, L., Salibián, A. (2002): Freshwater pollution biomarker: Response of brain acetylcholinesterase activity in two fish species. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 131 (3), 271-80.

Dede, E.B., Kaglo, H. D. (2001): Aqua-toxicological effects of water soluble fractions (WSF) of diesel fuel on *Oreochromis niloticus* fingerlings. Journal of Applied Science and Environmental Studies, 5, 93– 96.

Ellman, G.L., Courtney, K. D., Andres, J.V., Featherstone, R.M. (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7 (2), 88-95.

Esterbauer, H., Cheeseman, K. H. (1990): Determination of aldehydic lipid peroxidation products: Malondialdehyde and 4-Hydroxynonenal, in: Packer L, A.N. Glazer (Eds.): Methods in Enzymology, 186, 407-421.

Evans, D.H., Piermarini, P.M., Choe, K.P. (2005): The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiological Reviews, 85 (1), 97-177.

Ghelichpour, M., Mirghaed, A.T., Hoseinifar, S.H., Khalili, M., Yousefi, M., Van Doan, H., Perez-Jimenez, A. (2019): Expression of immune, antioxidant and stress related genes in different organs of common carp exposed to indoxacarb. Aquatic Toxicology, 1, 208-16.

Ghelichpour, M., Mirghaed, A.T., Mirzargar, S.S., Joshaghani, H., Mousavi, H.E. (2018): Modification of saltwater stress response in *Cyprinus carpio* (Linnaeus, 1758) pre-exposed to pesticide indoxacarb. Ecotoxicology and Environmental Safety, 147, 139-43.

Ghelichpour, M., Taheri Mirghaed, A., Mirzargar, S. S., Joshaghani, H., Ebrahimzadeh Mousavi, H. (2017): Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquaculture Research, 48 (11), 5666-76.

Gupta, N., Dua, A. (2010): Mercury induced behavioral alterations in *Channa punctatus*. Pollution Research, 29 (4), 721-723.

Hedayati, A., Hoseini, S.M., Ghelichpour, M. (2014): Acute toxicity of waterborne manganese to *Rutilus caspicus* (Yakovlev, 1870) – gill histopathology, immune indices, oxidative condition, and saltwater resistance. Toxicological & Environmental Chemistry, 96 (10), 1535-45.

Jin, Y., Zhang, X., Shu, L., Chen, L., Sun, L., Qian, H., Liu, W., Fu, Z. (2010): Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*): Chemosphere, 78 (7), 846-52.

Johal, M. S., Sharma, M. L. (2007): Impact of low dose of organophosphate, monocrotophos on the epithelial cells of gills of *Cyprinus carpio communis* Linn.-SEM study. Journal of Environmental Biology 1, 28 (3), 663-7.

Kavitha, P., Rao, J. V. (2008): Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis*. Environmental Toxicology and Pharmacology, 26 (2), 192-8.

Klotz, L.O., Steinbrenner, H. (2017): Cellular adaptation to xenobiotics: Interplay between xenosensors, reactive oxygen species and FOXO transcription factors. Redox Biology, 13, 646-54.

Livingstone, D.R. (2001): Contaminant- stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution bulletin, 42, 656-666.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. Journal of biological Chemistry, 193 (1), 265-75.

Marklund, S., Marklund, G. (1974): Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47 (3), 469-74.

Mirghaed, A.T., Ghelichpour, M. (2015): Determination of lethal concentrations of indoxacarb in fingerling *Cyprinus carpio* at two temperatures. International Journal of Aquatic Biology, 3 (5), 339-45.

Mishra, A., Devi, Y. (2014): Histopathological alterations in the brain (optic tectum) of the fresh water teleost *Channa punctatus* in response to acute and sub-chronic exposure to the pesticide chlorpyrifos. Acta Histochemica, 11 6(1), 176-81.

Mishra, A.K., Gopesh, A., Singh, K. P. (2022): Acute toxic effects of chlorpyrifos on pseudobranchial neurosecretory system, brain regions and locomotory behavior of an air-breathing catfish, *Heteropneustes fossilis* (Bloch 1794): Drug and Chemical Toxicology, 45 (2), 670-679.

Mishra, A.K., Gopesh, A., Singh, K. P. (2024): Effects of chlorpyrifos toxicity on brain, pseudobranchial neurosecretory system and swimming performance of a catfish, *Heteropneustes fossilis*. Drug and Chemical Toxicology, 47 (1), 67-80.

Moncada, A., Branch, E.M. (2003): Environmental fate of indoxacarb. Environmental Monitoring Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

Monteiro, H.R., Pestana, J.L., Novais, S.C., Soares, A.M., Lemos, M.F. (2019): Toxicity of the insecticides spinosad and indoxacarb to the non-target aquatic midge, *Chironomus riparius*. Science of the Total Environment, 666, 1283-91.

Munshi, J.S.D., Hughes, G.M. (1981): Gross and fine structure of the pseudobranch of the climbing perch: *Anabas testudineus* (Bloch), Journal of Fish Biology, 19, 427–438.

Nassar, A.M.K. (2016): Acetylcholinesterase: A universal toxicity biomarker. Journal of Agricultural and Environmental Sciences, 15, 28.

Osterauer, R., Köhler, H.R. (2008): Temperature dependent effects of the pesticides thiacloprid and diazinon on the embryonic development of zebrafish (*Danio rerio*): Aquatic Toxicology, 86, 485-494.

Perez-Perez, M.P., Luque, M.A., Herrero, L., Nunez-Abades, P.A., Torres, B. (2003): Afferent connectivity to different functional zones of the optic tectum in goldfish. Visual Neuroscience, 20 (4), 397-410.

Roy, S., Chattoraj, A., Bhattacharya, S. (2006): Arsenic-induced changes in optic tectal histoarchitecture and acetylcholinesterase–acetylcholine profile in *Channa punctatus*: amelioration by selenium. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 144 (1), 16-24.

Sakamoto, Y., Harada, T., Horie, S., Iba, Y., Taniguchi, F., Yoshida, S., Iwabe, T., Terakawa, N. (2003): Tumor necrosis factor-α-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-κB activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. The Journal of Clinical Endocrinology & Metabolism, 88 (2), 730- 735.

Slaninova, A., Smutna, M., Modra, H., Svobodova, Z. (2009): Oxidative stress in fish induced by pesticides. Neuroendocrinology Letters, 30 (1), 2.

Taheri Mirghaed, A., Ghelichpour, M., Mirzargar, S.S., Joshaghani, H., Ebrahimzadeh Mousavi, H. (2018): Toxic effects of indoxacarb on gill and kidney histopathology and biochemical indicators in common carp (*Cyprinus carpio*): Aquaculture Research, 49 (4), 1616-27.

Takasugi, N., Bern, H. A. (1962): Experimental studies on the caudal neurosecretory system of *Tilapia mossambica*. Comparative Biochemistry and Physiology, 6 (4), 289-303.

Topal, A., Alak, G., Ozkaraca, M., Yeltekin, A.C., Comaklı, S., Acıl, G., Kokturk, M., Atamanalp, M. (2017): Neurotoxic responses in brain tissues of rainbow trout exposed to imidacloprid pesticide: Assessment of 8-hydroxy-2-deoxyguanosine activity, oxidative stress and acetylcholinesterase activity. Chemosphere, 175, 186-91.

Tripathi, A., Rahman, M., Chakraborty, B. (2013): Distribution pattern of acetylcholinesterase in the optic tectum of two Indian air breathing teleosts. Annals of Neurosciences, 20 (1), 7.

USEPA (2000): United States Environmental Protection Agency, Pesticide Fact Sheet: Indoxacarb.

Velmurugan, B., Selvanayagam, M., Cengiz, E.I., Unlu, E. (2007): The effects of monocrotophos to different tissues of freshwater fish *Cirrhinus mrigala*. Bulletin of Environmental Contamination and Toxicology, 78, 450-454.

Villatte, F., Ziliani, P., Marcel, V., Menozzi, P., Fournier, D. (2000): A high number of mutations in insect acetylcholinesterase may provide insecticide resistance. Pesticide Biochemistry and Physiology, 67 (2), 95-102.

Wang, J., Jia, B., Li, Y., Ren, B., Liang, H., Yan, D., Xie, H., Zhang, X., Liang, H. (2020): Effects of multi-walled carbon nanotubes on the enantioselective toxicity of the chiral insecticide indoxacarb toward zebrafish (*Danio rerio*): Journal of Hazardous Materials, 397, 122724.

Yadav, L., Sengar, M., Zaccone, D., and Gopesh, A. (2013): Paraneuronal pseudobranchial neurosecretory cells in scorpion catfish, *Heteropneustes fossilis*: An environment scanning electron microscope and transmission electron microscope study. Acta Zoologica, 94 (1), 58-65.

Zaccone, G., Mauceri, A., Fasulo, S., Ainis, L., Cascio, P.L., Ricca, M.B. (1996): Localization of immunoreactive endothelin in the neuroendocrine cells of fish gill. Neuropeptides, 30 (1), 53-57.

Zheng, S., Chen, B., Qiu, X., Chen, M., Ma, Z., Yu, X. (2016): Distribution and risk assessment of 82 pesticides in Jiulong River and estuary in South China. Chemosphere, 144, 1177-92.