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# Acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to herbicide pretilachlor

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## Abstract

Pretilachlor is a systemic herbicide belonging to chloroacetamide group which is being used in rice fields for the control of annual weeds. The acute toxicity bioassay of pretilachlor was carried out by probit analysis method and the safe level was estimated by multiplying the 96 h  $LC_{50}$  with different application factors. The 12, 24, 48, 72 and 96 h  $LC_{50}$  values were obtained as 11.12, 9.55, 8.57, 7.11 and 5.84 mg  $L^{-1}$  respectively while the safe values found ranges 0.05 to 0.58 mg  $L^{-1}$ . Buccal movements as well as the number of burst swimming movements in fishes exposed to pretilachlor were found to be increased significantly in response to all concentrations of the herbicides tested. Number of feeding attempts reduced significantly while irregular responses in fishes were observed regarding their attempt to form group after exposure to herbicide. As a whole, exposure of fishes to pretilachlor showed significant effects on all studied behaviours. So it can be concluded that the herbicide pretilachlor is toxic to fishes and its excess use should be avoided.

Keywords: Ecology, Zoology

## 1. Introduction

During the last decade continuous use of herbicides into the agricultural activities has resulted in frequent detection of these chemicals in both surface and ground water which can impose adverse effect on non-target organisms (Gilliom, 2007). Among these non target organisms, fishes are the most important and played significant roles in assessing potential risk associated with contamination in aquatic environment (Lakra and Nagpure, 2009). They served as bio-indicators of environmental pollution and permit early detection of aquatic environmental problems (Lopez-Barea, 1996; Van Der Oost et al., 2003).

The contamination of aquatic ecosystems by chloroacetamide herbicides like acetochlor, butachlor, metolachlor, alachlor and pretilachlor has gained increased attention in last few years after they have been identified as probable carcinogens. Acetochlor and alachlor can cause tumors in the nasal turbinates, butachlor causes stomach tumors, and metolachlor causes liver tumors (Coleman et al., 2000; Dearfield et al., 1999). Most of the studies have demonstrated the toxicity of chloroacetamide herbicides to fish and other aquatic organisms (Geng et al. 2005, 2010; Ateeq et al., 2002; Peebua et al., 2007; Chang et al., 2011). Butachlor disrupt the reproductive process and affect the thyroid and sex steroid hormones in Zebra fish (Chang et al., 2011). Studies have demonstrated the presence of chloroacetamide herbicides in surface water (Hladik et al., 2008), soil (Chao et al., 2007), and sediment (Xue et al., 2005). It has been reported that butachlor is moderately toxic and irritating for *R. rutilus caspicus* and *S. lucioperca* (Mohammad and Hedayati, 2017). Although various works have been done so far to evaluate the toxicity of butachlor, metachlor, acetochlor and alachlor, the literature regarding the toxic effects induced by pretilachlor is relatively scarce. Pretilachlor (2-chloro-2',6'-diethyl-N-[2-propoxyethyl] acetanilide) is a systemic herbicide which is being used extensively in rice fields for control weeds. This herbicide inhibits cell division via preventing the synthesis of long chain fatty acids (Kaushik et al., 2006). Ali and Mohamad (2013) reported that the toxicity of pretilachlor on *Gambusia* increases with increasing concentration and exposure time. The investigations on pretilachlor have focused on removal of pretilachlor from the environment and the effect of pretilachlor on soil microorganisms (Saha et al., 2012; Toan et al., 2013).

Behavioural changes resulting from exposure of chemicals provide more sensitive early warnings than standard test methods (Smith and Logan, 1997). In fishes, behavioural responses to herbicides remain relatively scarce. Measurements on the swimming-oriented responses, opercular movement, feeding attempt, jerk swimming, and schooling are among the most common tests used in behavioural toxicology of fish.

Like other chloroacetamide herbicides pretilachlor may be toxic for fishes and various assays are required for its toxicity evaluation. For short term lethality assays many different fish species have been used based on their ease of culture, ecological relevance and economic importance. The fish species *Clarias batrachus* was selected for the present study because of wide distribution in the freshwater environment, non-invasive, availability throughout the season and easy acclimation to laboratory conditions. In addition to above, the fish is a bottom dweller which makes it to be in contact with xenobiotics in water as well as in sediment.

For the studies on toxicity of chemicals on animals most of the test guidelines recommend use of measured concentrations of the test chemical (OECD, 1992; USEPA, 1996; ASTM, 2007). But ecotoxicological models generally have large data requirements so must be based on use of both measured and nominal concentrations of testing chemical. As per ecotox database, 66% of such studies are based on nominal concentrations (ECOTOX; <http://www.epa.gov/ecotox/>). Raimondo et al. (2009) compared toxicity data on fishes reported as nominal and measured concentrations and developed an interspecies correlation estimation model for 12 pairs of fish species. They found that they were not significantly different for any species pairs and models developed from one concentration type predicted the reported toxicity of the other with high certainty. They concluded that acute toxicity test results reported as either measured or nominal concentrations may be included in ecotoxicological models. As the reports regarding the acute toxicity of pretilachlor on *C. batrachus* is lacking in available literature first we had chosen the nominal concentration of herbicide in the present study which will be followed by measured concentration in near future.

In view of the above the present study has been carried out to evaluate the acute toxicity of herbicide pretilachlor on fresh water teleost *Clarias batrachus* (Linnaeus) and to observe four behavioural activities i.e number of buccal movement per minute, number of feeding attempt per 5 minute, number of burst swimming per 5 minute and number of occurrence of grouping per 5 minute after herbicide exposure.

## 2. Materials and methods

### 2.1. Ethics statement

In the present study test fishes were obtained from local markets. Experiments were conducted according to guidelines approved by the relevant authorities in India (Indian National Science Academy and Indian Council of Medical Research) and all the experimental protocols were approved by the departmental research committee of department of Zoology, Guru Ghasidas University, Bilaspur (India).

## 2.2. Experimental fish and herbicide

The fishes of an average weight  $10 \pm 251$  gm and length  $8 \pm 557$  cm were selected to carry out acute toxicity bioassay. Selected fish specimens were bathed in 0.05% potassium permanganate ( $\text{KMnO}_4$ ) to avoid skin infections. Before commencement of the experiments the fishes were kept for one month in glassy aquarium ( $60 \text{ cm} \times 30 \text{ cm} \times 30 \text{ cm}$  size) filled with dechlorinated tap water for their acclimatization to laboratory conditions. During this period they were fed with boiled eggs. The physico-chemical characteristics of water were determined as per [APHA guidelines \(2012\)](#) and was replaced every 24 h to remove waste materials. The aquarium water was maintained with temperature =  $28 \text{ }^\circ\text{C} \pm 1.0 \text{ }^\circ\text{C}$ , pH =  $6.5 \pm 0.2$  units, total hardness =  $28 \pm 0.05$  mg/l, dissolved oxygen =  $6.6 \pm 0.01$  mg/l, salinity =  $0.6 \pm 0.02$  ppt. Day and light period maintained through the entire experiment was 12:12 h. Commercial formulation of pretilachlor (CAS No. 51218-49-6) with trade name Rifit (50 % EC), manufactured by Syngenta India limited, India was used for toxicity testing.

All chemicals used in the present study were at least of analytical grade.

## 2.3. Acute toxicity bioassay and determination of sub lethal concentration

The  $\text{LC}_{10-90}$  value of pretilachlor was determined through acute toxicity bioassay using *C. batrachus* in a semi-static laboratory system according to the OECD guideline No 203. The solution was added to the aquarium ( $60 \times 30 \times 30$  cm in size with 40 L capacity) following the method of Pluta (1989). The aquarium water containing fresh herbicide mixed was replaced after every 24 h. A group of 10 fish specimens were randomly exposed to each of the predefined pretilachlor concentrations (1.0, 3.9, 5.9, 7.9, 9.9 and  $11.9 \text{ mg L}^{-1}$ ). Another set of 10 fish in aquarium water without herbicide were used a control. The experiments were set in triplicate to obtain the various LC values. Probit analysis method was used to determine the  $\text{LC}_{10-90}$  values of pretilachlor for 12, 24, 48, 72 and 96 h to *C. batrachus* ([Finney, 1971](#)). The safe level estimates of the herbicides were drawn according to Sprague (1971), CWQC (1972), NAS/NAE (1973), IIC (1977), CCREM (1991) and [Hart et al. \(1948\)](#). Herbicide in water was determined by Liquid chromatography Equipment (C18 reverse phase, ultraviolet detector with  $5 \text{ } \mu\text{m}$  granulometry and 30 cm equivalent length, LOD:  $0.05 \text{ } \mu\text{g L}^{-1}$  with recovery % of 99%).

## 2.4. Behavioural responses

For study of behavioural response the 15 minute test was conducted after each exposure. The behaviour end points chosen were number of buccal movement per

minute, number of feeding attempt per 5 minute, number of burst swimming per 5 minute and number of occurrence of grouping per 5 minute.

The data obtained were subjected to one way analysis of variance (ANOVA) by statistical package SPSS (Version 16).

### 3. Results

The LC<sub>50</sub> values (with 95% confidence limits) of different concentrations of pretilachlor were determined and are represented in Table 1. Significant differences in ( $p < 0.05$ ) in LC<sub>10–90</sub> values were observed for different times of exposures. As the exposure time increases the median lethal concentration (LC<sub>50</sub>) decrease.

#### 3.1. Estimation of safe level

The safe level of herbicide pretilachlor to fish *C. batrachus* is presented in Table 2. The values of safe level varied from 0.05 to 0.58 mg L<sup>-1</sup>

#### 3.2. Behavioural response of fishes

The observed behavioural responses of fishes are shown in Figs. 1, 2, 3, and 4. The buccal movements in fishes exposed to pretilachlor were found to be increased significantly at all tested concentrations (Fig. 1). Number of feeding attempts was reduced significantly in exposed fishes (Fig. 2). Similarly a significant increase in the number of burst swimming reactions was observed which increase with increasing concentration of the herbicide (Fig. 3). However no particular trend in tendency to form group was observed in exposed fishes (Fig. 4).

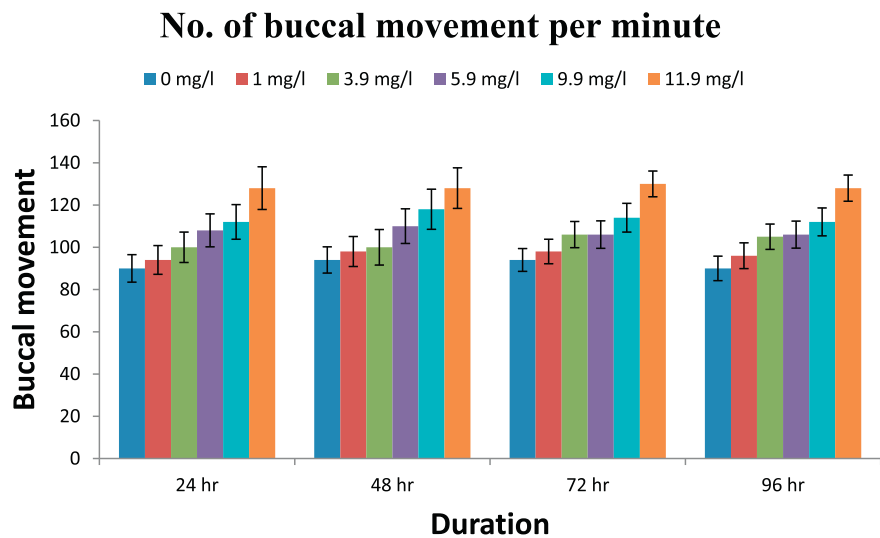
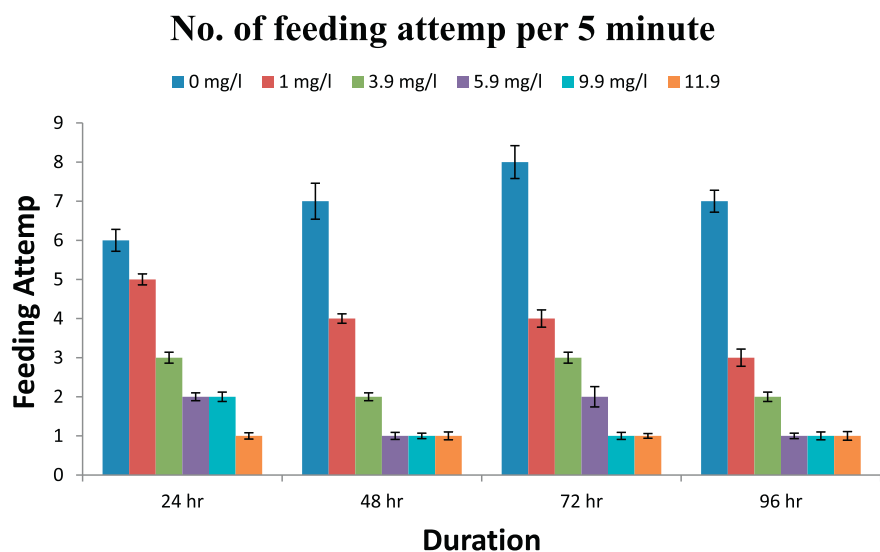
**Table 1.** Lethal concentrations of pretilachlor at various exposure time for *C. batrachus*.

Point	Concentrations (mg L <sup>-1</sup> ) at various exposure times (95% confidence intervals)				
	12 h	24 h	48 h	72 h	96 h
LC <sub>10</sub>	4.91 <sup>a</sup>	4.65 <sup>a</sup>	2.34 <sup>b</sup>	1.31 <sup>c</sup>	1.21 <sup>c</sup>
LC <sub>20</sub>	5.59 <sup>a</sup>	5.46 <sup>a</sup>	3.48 <sup>b</sup>	2.24 <sup>c</sup>	1.18 <sup>d</sup>
LC <sub>30</sub>	7.47 <sup>a</sup>	5.25 <sup>b</sup>	3.56 <sup>c</sup>	2.34 <sup>d</sup>	1.78 <sup>e</sup>
LC <sub>40</sub>	09.48 <sup>a</sup>	07.89 <sup>b</sup>	5.88 <sup>c</sup>	3.98 <sup>d</sup>	2.45 <sup>e</sup>
LC <sub>50</sub>	11.12 <sup>a</sup>	9.55 <sup>b</sup>	8.57 <sup>c</sup>	7.11 <sup>d</sup>	5.84 <sup>e</sup>
LC <sub>60</sub>	11.18 <sup>a</sup>	9.25 <sup>b</sup>	7.98 <sup>c</sup>	5.58 <sup>d</sup>	4.08 <sup>e</sup>
LC <sub>70</sub>	13.22 <sup>a</sup>	11.49 <sup>b</sup>	11.47 <sup>b</sup>	9.12 <sup>c</sup>	6.28 <sup>d</sup>
LC <sub>80</sub>	15.56 <sup>a</sup>	13.12 <sup>b</sup>	13.21 <sup>b</sup>	11.01 <sup>c</sup>	9.45 <sup>d</sup>
LC <sub>90</sub>	16.00 <sup>a</sup>	14.27 <sup>b</sup>	12.03 <sup>c</sup>	12.24 <sup>c</sup>	10.09 <sup>d</sup>

Lethal concentration values in rows with different letters significantly differ at  $p < 0.05$ .

**Table 2.** Safe level estimates of pretilachlor to *C. batrachus*.

96 h LC <sub>50</sub> (mg. L <sup>-1</sup> )	Method	Calculation/application factor	Safe level (mg L <sup>-1</sup> )
5.84	Hart et al.	$48 \text{ h LC}_{50} \times 0.03 \times S^2$ where $S = 24 \text{ h LC}_{50}/48 \text{ h LC}_{50}$	0.316
	Sprague	0.1	0.584
	CWQC	0.01	0.0584
	NAS/NAE	0.1–0.00001	0.5839
	CCREM	0.05	0.292
	IJC	5% of 96 h LC <sub>50</sub>	0.292

**Fig. 1.** Buccal movements in fishes exposed to different concentration of herbicide pretilachlor.**Fig. 2.** Feeding attempt in fishes exposed to different concentration of herbicide pretilachlor.

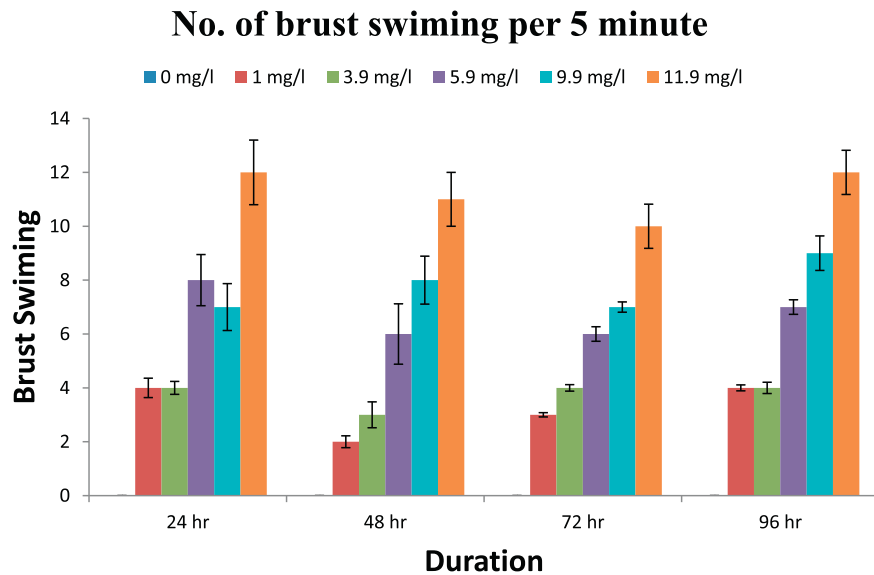


Fig. 3. Burst swimming in fishes exposed to different concentration of herbicide pretilachlor.

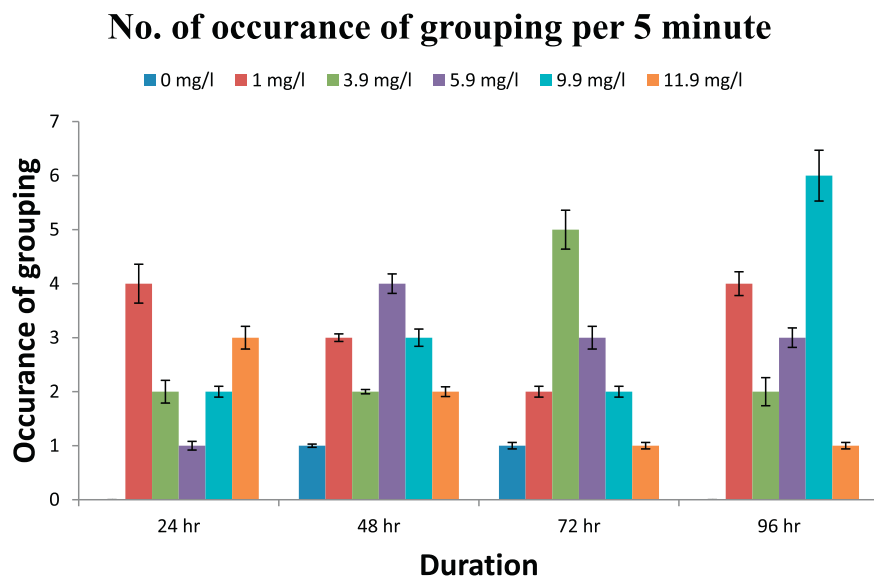


Fig. 4. Group formation in fishes exposed to different concentration of herbicide pretilachlor.

## 4. Discussion

The present study was carried out to evaluate the acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to herbicide pretilachlor. The results clearly indicated that exposure of fishes to pretilachlor based herbicide resulted in increased mortality with increasing concentration of the herbicide. The 96 h LC<sub>50</sub> value reported in the present study for commercial formulation of

pretilachlor is lower than the value reported by Ali and Mohammad in gambusia (2013). However it is higher than the value obtained by Maryam et al. (2013) in grass carp. These differences in obtained values of LC<sub>50</sub> for different species may be due to age, size and health (Abdul-Farah et al., 2004) or the existing physiological parameters of water (Eaton and Gilbert, 2008). A large difference in the values of safe level (ranged from 0.05 to 0.58 mgL<sup>-1</sup>) was obtained which indicates that it is difficult to decide the acceptable concentration of pretilachlor for *C. batrachus* based on the present study.

Behavioural changes are one of the most sensitive indicators of potential toxic effect in fishes (Banaee et al., 2011) but very few works have been done so far regarding the behavioural sensitivity of the fish to the pesticides used in agricultural fields. Behavioural alterations were observed in fishes exposed to glyphosate-based herbicides (Langiano and Martinez, 2008; Lushchak et al., 2009; Nwani et al., 2010; Shigiri et al., 2012), and butachlor (Chang et al., 2011). Similar to the observations of previous workers the present study demonstrates that fish *C. batrachus* display immediate and various behavioural responses to herbicide pretilachlor. Four behavioural endpoints, related to burst swimming reactions, buccal movements, feeding attempts, and grouping were studied because these endpoints are easily accessible and thus helpful in assessment of sub-lethal effects of contaminants in fish and can also be used as an indicator of acute changes in the chemical environment.

The buccal movements in fishes exposed to pretilachlor were found to be increased significantly in response to all concentrations of the herbicides tested. Buccal movements are directly associated with respiratory movement so it can be concluded that sub acute exposure to herbicide adversely affects the process of respiration in fishes similar to the conclusions made by Saglio and Trijasse (1998) in case of atrazine. Burst swimming reactions in fishes are result of stressful condition (Fuiman, 1986). A significant increase in the number of burst swimming reactions was observed in fishes exposed to all concentration of herbicides. Similarly number of feeding attempts in fishes reduced significantly in exposed fishes. This decreased feeding attempt may be directly related to gustatory sensitivity of fishes to contaminants. Very few literatures is available in this regard however it has been reported in *Cyprinus carpio* that the nerve innervating terminal buds in the lip region was sensitive to stimulation by high concentration of benthocarb, isoprothiolane and fenitrothion (Ishida and Kobayashi, 1996). Irregular responses in fishes were observed regarding their attempt to form group after their exposure to herbicides which need further studies to for periods longer than the present study. On the basis of results obtained it can be concluded that the herbicide pretilachlor is toxic to fishes and its use should be in controlled manner to avoid its long term harmful effects in the environment.



## Declarations

### Author contribution statement

Sushant Verma: Conceived and designed the experiments; Analyzed and interpreted the data.

Rakesh Soni: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

### Funding statement

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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