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RESEARCH ARTICLE

Investigation on acute toxicity and behavioral changes in *Channa punctatus* (Bloch) due to organophosphate pesticide profenofos

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Abstract
Acute toxicity of an organophosphate pesticide profenofos (*O*-4-bromo-2- chlorophenyl-*O*-ethyl *S*-propyl phosphorothioate) to freshwater fish, *Channa punctatus* (Bloch), was studied in a static bioassay. Estimated 96-hour LC_{50} of profenofos was found to be 2.68 μgL^{-1}. On the basis of the obtained LC_{50} values for 96-hour exposure intervals, profenofos can be rated as highly toxic to *C. punctatus*. Fish exposed to profenofos showed hyper excitability, discoloration, erratic swimming, and secretion of excess amounts of mucus on the body and gills with eventual exhaustion and death.

Keywords: Toxicity, behavioral changes, bioassay, *Channa punctatus*, profenofos, mortality

Introduction

Pesticides and related chemicals generated during human activities such as agriculture are discharged directly or indirectly into receiving waters. The effects of these chemicals in the environment has become a global issue (Khan and Law, 2005). In India, as much as 70% of chemical formulations, employed in agricultural practices, are believed to affect nontarget organisms and find their way to freshwater bodies (Bhatnagar et al., 1992).

Alterations in the chemical composition of the natural aquatic environment usually affect behavioral and physiological systems of inhabitants, particularly fish (Radhaiah et al., 1987; Khan and Law, 2005). Fish are very sensitive to a change in their environments and can play a significant role in assessing potential risks associated with contamination in aquatic environment of new chemicals. Fish were reported to respond to mutagens at low concentrations of toxicants in a manner similar to higher vertebrates (Goksoyr et al., 1991; Al-Sabti and Metcalfe, 1995; Lakra and Nagpure, 2009).

Profenofos is commonly used in India for pest control in mango, banana, cotton, and pineapple agriculture (Das et al., 2006; Reddy and Rao, 2008; Kavitha and Rao, 2009). It is one of the most commonly used broad-spectrum organophosphate insecticides of cotton fields (Tejada et al., 1999). This is also a globally used pesticide for the control of various caterpillars, white fly, and mites on cotton and vegetable crops in Egypt (British Crop Protection Council, 1991; Farrag and Shalby, 2007), Australia (Kumar and Chapman, 2001), Japan (Gotoh et al., 2001), Korea (Min and Cha, 2000), Finland (Abass et al., 2007), and China (Hong-Yan et al., 2001). Profenofos is reported to be highly toxic to some aquatic organisms (Shaw, 1995). The acute toxicity studies on zebrafish *(Brachydanio rerio*), tilapia (*Oreochromis mossambicus*), and fingerling of common carp (*Cyprinus carpio L*) have been reported earlier (Min and Cha, 2000; Rao et al., 2003; Ismail et al., 2009). Frequent mass fish kills by profenofos were investigated (U.S. EPA, 1998) and found that the acute risks are of concern for fish (EPA Profenofos Facts,

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2000). Therefore, in view of the ecological impact of this pesticide, the present study aimed at a determination of 96-hour LC_{50} of profenofos to the freshwater fish, *Channa punctatus*, and their behavioral responses during exposure. This species is commonly found in India, Pakistan, Bangladesh, and other countries. It fulfills most of the requirements of a model species, including availability, throughout the year.

Methods

Test animal

The live specimens of freshwater fish *C. punctatus* (Bloch), family: Channidae and order: Perciformes, with an average body size of 12.0 ± 3.0 cm and body weight of 23 ± 2.0 g at the juvenile life stage, were obtained from local sources and acclimated in laboratory conditions for 10 days before study. They were kept in a large holding tank of 1,000 L in capacity during acclimatization. Fish were fed on boiled chicken eggs or poultry wastes at the rate of approximately 4% of fish body weight. The photoperiod was maintained as per normal day and night approximately 12 hours, and every effort was made to provide optimal condition for fish; no mortality occurred during this period.

Test chemical

Before exposure, quality of water was tested according to the APHA, AWWA, WEF (1998). The pesticide for the study, technical-grade profenofos (50% EC), with the product name of CELCRON (EXCEL Crop Care Ltd., Mumbai, India), was purchased from a local market. The test chemical structure and specifications are summarized in Figure 1 and Table 1.

Acute toxicity bioassay

The acute toxicity bioassay was conducted in a static system to determine the 96-hour LC_{50} value of profenofos. Test concentrations were determined on the basis of the range-finding assay, where the lowest value was selected as the highest concentration at which 0% mortality occurred and the highest value as lowest concentration at which 100% mortality occurred. The test specimens were exposed to eight different nominal concentrations (0.5, 1.25, 2.00, 2.75, 3.50, 4.25, 5.00, and 5.75 μg/L), along with one control (without test chemical), keeping 10 fish in each test concentration in 20 L of water in a 50-L plastic tub as an aquarium without change of water. The mean fish-loading ratio in the experimental tanks was 11.5 ± 2.0 g fish/L. No crowding stress was observed during experimentation. Mortality of fish from profenofos exposure was recorded up to 96 hours at every 24-hour interval (Table 2). The experiment was repeated thrice, following the recommendations of APHA, AWWA, WEF (1998). Feeding was stopped 24 hours before exposure, and fish were not fed during the experimentation period, as recommended by Ward and Parish (1982) and Reish and Oshida (1987). No mortality was observed in the control group of fish. For the determination of LC_{50} value of profenofos, the arithmetic method of Karber, as reported by Dede and Kaglo (2001), was considered (Table 3).

Physicochemical properties of test water

Physicochemical parameters were measured before fish loading and during experimentation. The temperature of the test water varied from 21.4 ± 3°C and pH value ranged

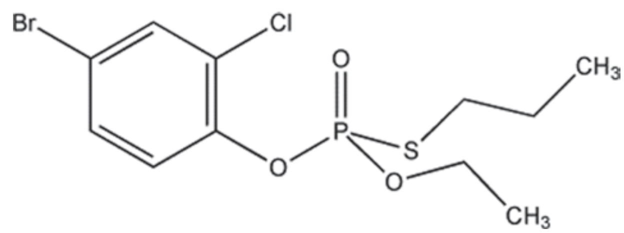

Figure 1. Structure of profenofos.

Table 1. Characteristics/ properties/ specification of the test chemical.

Common name	Profenofos
Chemical/ product name	CELCRON
Chemical Name	(*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl S-propyl phosphorothioate
Grade	Technical (EC_{50})
Supplier	EXCEL crop care Ltd., (Mumbai, India)
WHO and EPA toxicity class	II, Moderately toxic
CAS nNo.	41198-08-7
Alkyl groups	*S*. propyl-*O*. ethyl

Table 2. Data on fish survival at different test concentrations and sampling intervals in *C. punctatus*.

	Number of alive fish at different time intervals (hours)					
Concentration (μg/L)	24	48	72	96	% survival at 96 hours	% mortality at 96 hours
0	30	30	30	30	100	0
0.50	30	30	30	30	100	0
1.25	30	30	27	24	80	20
2.00	30	27	21	18	60	40
2.75	30	21	18	12	40	60
3.50	30	15	12	9	30	70
4.25	27	12	15	6	20	80
5.00	21	15	9	3	10	90
5.75	15	12	3	0	0	100

Table 3. Determination of LC_{50} value of profenofos in *C. punctatus* at 96 hours.

Concentration (μg/L)	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death * concentration difference
0	0	30	0	0	0
0.50	0.50	30	0	0	0
1.25	0.75	24	6	3	2.25
2.00	0.75	18	12	9	6.75
2.75	0.75	12	18	15	11.25
3.50	0.75	9	21	19.5	14.625
4.25	0.75	6	24	22.5	16.875
5.00	0.75	3	27	25.5	19.125
5.75	0.75	0	30	28.5	21.375
Sum (∑)					**92.25**

$$LC_{50} = LC_{100} - \sum \frac{\left(\begin{matrix}\text{Mean death *}\\ \text{Concentration difference}\end{matrix}\right)}{\text{Number of fish per group}} = 5.75 - \frac{92.25}{30} = 2.675\,\mu g/L$$

from 7.5 ± 0.6. The dissolved oxygen (DO) ranged from 7.35 ± 0.8 mgL^{-1}. The conductivity of the water ranged from 278 ± 23 μM cm^{-1}, whereas total hardness ranged from 174.7 ± 8 mgL^{-1} during the experiment.

Results

Acute toxicity

Total mortality observed in different treated groups at 96 hours of exposure is presented in Table 3. An increase in number of mortalities, with an increase in concentration of the insecticide, was observed. The 96-hour LC_{50} value of profenofos was found to be 2.68 μgL^{-1}.

Behavioral effects

Fish exposed to profenofos expressed behavioral abnormalities, such as random swimming, loss of equilibrium, hyperactivity, increased surface activity, opercular activity, and mucus formation. At the beginning of exposure, fish were found to be healthy and very active. During the experiment, they tried to avoid the test water for sometime by swimming fast, jumping, and other random movements in treated groups. At higher test concentrations, fish expressed erratic swimming with jerky movements, along with hyperexcitation. They also secreted excessive amounts of mucus, which covered the buccal cavity, body, and gills. Fish became pale as intensity of body color decreased. Under such conditions, efficiency of oxygen uptake decreased considerably, which was manifested as enhanced breathing rate along with more frequent visits to surface water for gulping fresh air. Eventually, there was loss of balance, exhaustion, and lethargy owing to respiratory incumbency. At last, they assigned a vertical position with mouth near the water surface and tail in downward direction to gulp the air. Soon, they settled down passively at the bottom of the tank; after a while, their bellies turned upward.

Discussion

The present study elucidates the acute toxicity of pesticide profenofos on the Indian freshwater fish, *C. punctatus*. The test result of the 96-hour LC_{50} value of profenofos (50% EC) was determined to be 2.68 μg/L, which indicated profenofos to be very toxic. Previously reported LC_{50} values of profenofos in eastern rainbow trout (*Melanotaenia duboulayi*), tilapia (*Oreochromis niloticus*), and fingerling of common carp (*C. carpio L*) were 0.90 mg/L (Kumar and Chapman, 1998), 0.272 mg/L (Rao et al., 2003), and 62.4 μg/L (Ismail et al., 2009), respectively.

The present study also showed that the pesticide is more toxic in the fish, *C. punctatus*, in comparison to other pesticides, such as LC_{50} of endosulfan at 7.75 μgL^{-1} (Pandey et al., 2006), chlorpyrifos at 8.1198 μgL^{-1} (Ali et al., 2008), cypermethrin and λ-cyhalothrin at 0.4 mgL^{-1} and 7.92 μgL^{-1} (Kumar et al., 2007), carbosulfan, glyphosate, and atrazine at 0.268, 32.540, and 42.380 mgL^{-1}, respectively (Nwani et al. 2010), and carbamate at 17.41 mgL^{-1} (Singh et al., 2007).

The behavioral study gives direct responses of the fish to the pesticides and related chemicals. Radhaiah et al. (1987) and Warner et al. (1966) commented that the behavioral activities of an organism represents the final integrated results of a diversified biochemical and physiological processes. Similar behavioral responses were also reported in *C. punctatus* while exposed to mercuric chloride and malathion (Pandey et al., 2005), hexavalent chromium (Mishra and Mohanty 2008), and cypermethrin and λ-cyhalothrin (Kumar et al., 2007).

The fish from the control group were free from any such type of behavioral changes, so it is clear that only profenofos was responsible for the altered behavior and mortality (Tiwari and Singh, 2003). A thick coat of mucus over the body and dispigmentation are attributed to dysfunction of the endocrine/pituitary gland under toxic stress, causing changes in the number and area of mucus glands and chromatophores (Pandey et al., 1990). The loss of equilibrium status, as recorded in our experiment, may have also been reported in fish exposed to heavy metal and pesticide contaminants (Saxena et al., 1981; Halappa and David, 2009).

Similar results on behavioral responses were also observed in pesticide, heavy metals, and industrial effluents on exposed fish (Ganeshwade et al., 2006; Ramesh and Saravanan, 2008; Pandey et al., 2005; Yadav et al., 2007; Omitoyin et al., 2006). Animal behavior is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances (Sambasiva, 1999). The preceding behavioral abnormalities of the fish and subsequent death imply that the toxic effect is mediated through the disturbed nervous/cellular enzyme system affecting the respiratory function and nervous system, which involves control of almost all vital activities (Desaiah and Koch 1975a, 1975b; Bansal et al., 1985; Pandey et al., 2005). Thus, it can be concluded, from the present study, that fish are highly sensitive to profenofos and that their mortality rate is dose dependent. This study also shows the significance of behavioral parameter in assessing the hazards of the pesticide to fish.

Conclusions

Profenofos is highly toxic to the fish, *C. punctatus*. This type of fish is very sensitive to the presence of even minute quantities of profenofos. Thus, fish can be effectively employed as a model to ascertain the potential hazards of acute exposure of the pesticide to aquatic organisms, especially fish. Moreover, pesticides used on farmlands not only eradicate the target organisms, but could also eliminate other nontarget organisms and also pose a risk to human health. This approach may thus help in the estimation of potential health hazards to nontarget organisms upon exposure to pesticides.

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Declaration of interest

The authors report no financial conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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