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Evaluation of the Acute Toxicity of Profenofos and Its Effects on the Behavioral Pattern of Fingerling Common Carp (*Cyprinus carpio* L., 1758)

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Abstract Profenofos, an organophosphate insecticide is acetylcholinesterase inhibitor that has the potential to contaminate the ground water. The 96 h LC_{50} value of profenofos was determined in 3-month-old fingerling common carp (*Cyprinus carpio*) with a body weight 1.04 ± 0.25 g and a body length 4.25 ± 0.75 cm at 26 ± 1°C temperature. Trimmed Spearman-Karber (TSK) software was used for the statistical analysis, which calculated the LC_{50} value as 62.4 μg/L for three replicates of the assay. The behavioral responses of fish exposed to profenofos included loss of balance, moving in spiral fashion with sudden jerky movements, lying on their sides and rapid flapping of the operculum with the mouth open.

Keywords Profenofos · Organophosphate · Common carp · *Cyprinus carpio*

Although pesticides are an economical means to control growth of unwanted pests, they are toxic to aquatic organisms, especially fishes. Excessive use of these chemicals results in environmental pollution and toxicity to non target organisms. Thus, the use of pesticides has gained worldwide concern (Venkateswara 2004). In cotton growing areas of the Punjab and Sindh (Provinces of Pakistan) widespread use of pesticides is causing environmental problems including ground water contamination (Tariq et al. 2007) and health problems in exposed populations viz individuals occupationally exposed to pesticides in pesticide production industry (Bhalli et al. 2006a, b) and cotton picking women (Ali et al. 2008). According to the economic survey of Pakistan, 2005–2006, the share of pesticides sprayed on cotton are about 80% of the total pesticides used in these areas (Economic Survey of Pakistan 2006). In last 20 years, the use of pesticides in Pakistan has increased by 1169% (Technical Bulletin 2000). Only 5 to 10% of the cotton growing areas in the Punjab were sprayed with pesticides before 1983, but it has been increased to 100% by 1997 (MinFA 1995; Tariq 2005).

Profenofos [CAS Number: 41198-08-7; Chemical name [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate]; Empirical formula: $C_{11}H_{15}BrClO_3PS$; Molecular weight: 373.63] is a potentially ground water contaminating organophosphorous insecticide, slightly soluble in water (20.0 mg/L) and readily miscible in organic solvents. The substance is hydrolyzed with increasing pH, i.e. 50% loss in 93 days at pH 5, 14.6 days at pH 7 and 5.7 h at pH 9 along with chemically more unstable under alkaline conditions. Moreover, its half life in soil is about one week (Tomlin 1994). This property of Profenofos makes it a better choice for spray as compared to organochlorines, which are more persistent in the environment. Profenofos is of mid range in risk assessment, among the 12 top priority pesticides of cotton (Batley and Peterson 1992).

Profenofos is highly toxic to zooplankton (Immature scud, *Gammarus pseudolimnaeus*, has a 96 h LC_{50} value of 1.30 μg/L), crustaceans (Blue crab, *Callinectes sapidus*, has a LC_{50} value of 33.0 μg/L) and insects (Midge, *Chironomus tentans*, with a LC_{50} value of 86.0 μg/L) It is moderately toxic to birds (LC_{50} for mallard ducks is 150–612 ppm and Japanese quail has more than 1000 ppm) and less toxic for mammals (rat oral acute toxicity is 358 mg/Kg while that of rabbit is 700 mg/Kg (Tomlin 1997).

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However, profenofos is highly toxic to different fish species including Rainbow trout, *Oncorhynchus mykiss*, has 80 µg/L, Crucian carp, *Carassius carassius,* has an acute toxicity of 90.0 µg/L and Bluegill, *Lepomis macrochirus*, is of 300 µg/L (Tomlin 1994).

Common carp (*Cyprinus carpio*) was used as an experimental model for the estimation of LC_{50}, the same species has also been recommended for bioassay experiments by organization for economic cooperation and development (OECD 1992). It is widespread and cultured all over Asia, in most parts of Europe and in some countries of Africa and Latin America (Aydin et al. 2005). The same species is commonly found in Pakistan also.

Objective of the current study was the estimation of acute toxicity of profenofos to common carp which is a recommended fish species for bioassay experiments and abundantly used as a food source in Pakistan. As the fish occur in reservoirs and connecting canals along the fields where profenofos may be sprayed, pesticides could enter water through runoff, drift, soil erosion, accidental and leaching. So it is essential to determine whether profenofos affect non target aquatic organisms. On the basis of this study we can compare toxicity of profenofos to other pesticides and can also use common carp as a model for other fish species. The reported results would be a useful contribution in the ecotoxicity risk assessment studies of profenofos on this fish species.

Materials and Methods

Common carp (*Cyprinus carpio* L., 1758) was obtained from Faisalabad Fish Hatchery and brought to the laboratory in plastic bags with sufficient air. The plastic bags were opened and the fish specimens were shifted to the glass aquaria for 15 days to be acclimatized prior to the pesticide exposure. The specimens were about 3-months-old with an average body length of 4.25 ± 0.75 cm and of body weights 1.04 ± 0.25 g.

Profenofos (50 EC), molecular formula [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate], was supplied by the Ali Akbar Enterprises Lahore, Pakistan. Glass aquaria (with dimensions of 30 cm depth, 30 cm width and 45 cm length) were about 40 L capacity and filled with 30 L of tap water. The temperature of the water was regulated at 26 ± 1°C. Aquaria were continuously aerated, except at the time of feeding, so as the level of dissolved oxygen did not drop below 4.0 mg/L. The electric conductivity and the pH of water were 2.62–2.76 mS and 8.77–9.29, respectively.

Six groups of specimens, each containing 10 individuals, were selected at random and placed in the aquaria. The specimens were fed with rice polish @ 3% of their body weight once in a day and the feeding was stopped 24 h prior to the pesticide exposure to till end of the experiment.

For the estimation of LC_{50}, dosing solutions were prepared from the stock solution by mixing different proportions of stock solution and acetone to get the desired concentrations i.e. 15.6, 31.2, 62.5, 125.0 and 250.0 µg/L. These concentrations were then added to the five different aquaria containing specimens keeping one as negative control receiving no pesticide but maximum acetone that any dosing solution contain. The quantity of dosing solution never exceeds 1 mL throughout the experiment. Mortality of the specimens was recorded after 24, 48, 72 and 96 h of pesticide application. An individual was considered to be dead if it gave no response even to a gentle touch of fish catching net. Dead individuals were removed immediately. LC_{50} and the 95% confidence limits were calculated by a computer program, [TSK (Trimmed Spearman–Karber) program (1991) Version 1.5]. The behavioral changes in the specimens were also noted right after the application of testing dose till the end of experiment. The negative control group was also monitored in the same way for mortalities and change in behavior including loss of balance, moving in spiral fashion with jerks, lying laterally and opened mouth with rapid opercular movements.

Results and Discussion

An increase in number of mortalities with an increase in concentration of the insecticide was observed and has been summarized in Fig. 1. There was no mortality in the control group as well as in the group received 15.6 µg/L (lowest dose) of the insecticide used till the end of experiment (96 h). During the same time span, the mortalities

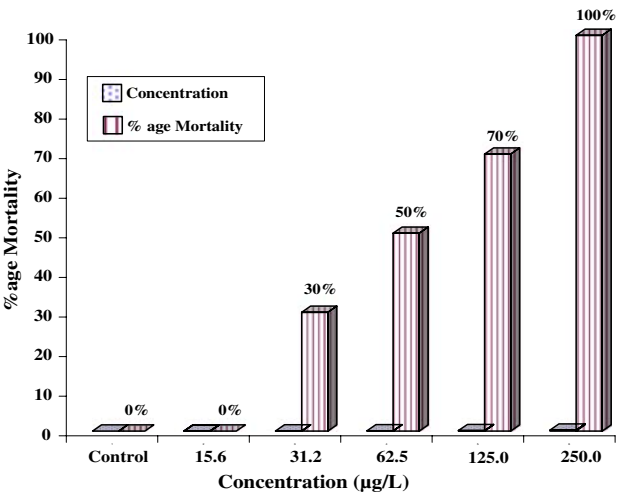

Fig. 1 % Age mortalities of common carp exposed to different concentrations of Profenofos during 96 h

were 100% at the highest concentration (250.0 μg/L) of pesticide used. The estimated 96 h LC_{50} value (95% confidence limits) of profenofos using a static bioassay system for common carp *Cyprinus carpio* was 62.4 μg/L (43.6–89.4) as shown in Table 1. The same table is also showing the other estimated LC_{50} values at different time intervals i.e. 24, 48 and 72 h.

Current study shows that 96 h acute toxicity of profenofos to common carp is 62.4 μg/L which is less toxic as compared to Channel catfish (*Ictalurus punctatus*) (23.5 μg/L), Bluegill (*Lepomis macrochirus*) (25.8 μg/L) or Rainbow trout (*Oncorhynchus mykiss*) (28.1 μg/L) and more toxic as compared to Crucian carp (*Carassius carassius*) (90.0 μg/L) (Tomlin 1994).

LC_{50} value for profenofos in Eastern rainbow trout (*Melanotaenia duboulayi*) and Tilapia (*Oreochromis mossambicus*) are 900.0 μg/L and 272.0 μg/L, respectively (Kumar and Chapman 1998; Venkateswara et al. 2003). While, there is no data available for the acute toxicity of profenofos to the common carp. On comparing the estimated LC_{50} value of profenofos in common carp in this experiment with the available data on different fish species, it is obvious that the common carp is less resistant to profenofos.

For the sake of comparison with the different pesticides on the common carp, it was found that Diazinon, an organophosphate, has a LC_{50} value of 1530 μg/L to the larvae of common carp (Aydin and Koprucu 2005); Dichlorvos (DDVP), a synthetic chemical pesticide, has LC_{50} value of 9410 μg/L to Fingerling Mirror carp, *Cyprinus carpio*, (Ural and Calta 2005) and 2,4-D (2,4-dichlorophenoxyacetic acid), a herbicide, has an acute toxic LC_{50} of 63240 μg/L to common carp (Sarikaya and Yilmaz 2003). These studies revealed that the profenofos is more toxic to common carp than other pesticides.

Different behavioral responses of common carp at different concentrations of the profenofos were observed throughout the experimental period (Table 2). The control group showed the normal behaviour during the whole experiment. The application of lowest concentration (15.6 μg/L) of pesticide to the common carp was observed with normal responses as the control group along with staying motionless in a group at the bottom. In the second lowest concentration (31.2 μg/L) neurotoxic effects of profenofos may be appeared in fish resulting in the loss of balance, moving laterally at the bottom. Loss of balance becomes more evident with swimming in a spiral path with jerks and revolving in water at even higher concentration (62.5 μg/L). Fish in the group applied with 125.0 μg/L solution of pesticide were lying laterally at bottom with loss of balance. At the highest concentration (250.0 μg/L), the more severity of the all these responses was observed including the loss of balance, lying laterally at the bottom, swimming down in a spiral fashion with jerks, rapid opercular movements with opened mouth (Table 2). The appearance of jerks in this case is in accordance with the findings of Fukuto 1990.

Beauvais et al. (2000) and Scholz et al. (2000) reported that behavioral study is one of the important parameter in the assessment of toxicity of pesticides in fish. In the present study fish showed normal behavior in control group but

Table 1 Comparison of acute toxicity of Profenofos on Common carp for 24, 48, 72 and 96 h

Point	24 h Concentration (μg/L)	48 h Concentration (μg/L)	72 h Concentration (μg/L)	96 h Concentration (μg/L)
LC_{50} values	91.8	81.0	71.7	62.4
95% Conf. limits	54.8–153.8	55.1–119.2	50.9–101.1	43.6–89.4

LC_{50} values (with 95% confidence limits) of profenofos to common carp at 24, 48, 72 and 96 h were 91.8 μg/L (54.8–153.8), 81.0 μg/L (55.1–119.2), 71.7 μg/L (50.9–101.1), and 62.4 μg/L (43.6–89.4), respectively. There was significant difference in the LC_{50} values at different time intervals

Table 2 Impact of Profenofos on the behavioral pattern of *Cyprinus carpio* exposed to different concentrations of the profenofos up to 96 h

Parameters	Control	Profenofos (μg/L)				
		15.6	31.2	62.5	125.0	250.0
Activeness	−	−	+	++	++	+++
Loss of balance	−	−	+	++	++	+++
Lateral side Movement	−	−	+	++	++	++
Opercular activity	−	−	+	++	+++	+++
Movement in circular form with jerks	−	−	−	+	++	+++
Rate of swimming	−	−	+	++	++	++

The increase or decrease in the level of behavioral parameters is shown by numbers of (+) sign. The (−) sign indicate normal behavioral conditions

severity in different responses was observed with the increase in insecticide concentration and passage of time. The abnormal behavior includes loss of balance, staying motionless in a group at bottom, lying laterally at bottom, swimming in spiral fashion with jerks, revolving in water, opened mouth and rapid opercular movements. Our results are in agreement with other studies (Sarikaya and Yilmaz 2003; Selvi et al. 2005) applying different pesticides on other fish species.

Though we have not studied here the effect of profenofos on the Acetyl cholinesterase (AChE) activity in the common carp, the previous studies show that profenofos is an acetyl cholinesterase inhibitor. It inhibits the actions of acetyl cholinesterase (AChE) enzyme which in turn inactivates a "neurotransmitter", acetylcholine (AChol). The neurotransmitter is present and necessary in various parts of the nervous system to enable transmission of stimulation either between nerves, or between nerves and various organs. Normally AChE catalyzes the hydrolysis of the acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. In the absence of acetyl cholinesterase, acetylcholine level increased resulting in the failure of transmission of stimuli to the nerves or organs. This leads to the abnormal functioning of the body including loss of balance, moving in circular form (convulsions) and at higher concentrations of insecticides resulting in death of the organism (Fukuto 1990). Profenofos reduced the acetyl cholinesterase (Ach E) activity by 83% in the head of Eastern rainbow trout (*Melanotaenia duboulayi*) and 90% in the head and gills of Tilapia (*Oreochromis mossambicus*) (Kumar and Chapman 1998; Venkateswara et al. 2003). We assumed the same happenings in the case of common carp as well on exposure to profenofos, but it still needs to be investigated in detail.

Current study shows that Profenofos is very highly toxic to the Common Carp fingerlings as compared to other fish species, but further studies, involving the different life stages of this fish along with cytogenetic and other toxicity tests are required to assign a certain level of toxicity to the said pesticide. Owing to high toxicity of profenofos, it is strongly recommended to handle the pesticide carefully using all the precautionary measures so that its harmful effects on aquatic life can be minimized.

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References

Ali T, Bhalli JA, Rana SM, Khan QM (2008) Cytogenetic damage in female Pakistani agricultural workers exposed to pesticides. Environ Mol Mutagen 49:374–380. doi:10.1002/em.20395

Aydin R, Koprucu K (2005) Acute toxicity of diazinon on the common carp (*Cyprinus carpio* L.) embryos and larvae. Pestic Biochem Physiol 82:220–225. doi:10.1016/j.pestbp.2005.03.001

Aydin R, Koprucu K, Dorucu M, Koprucu SS, Pala M (2005) Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. Aquat Int 13:451–458. doi:10.1007/s10499-005-0615-5

Batley GE, Peterson SM (1992) Fate of cotton pesticides in the riverine environment. 38–42. In Impact of pesticides on the riverine environment. Cotton Research and Development Corporation Goondiwindi Australia

Beauvais SL, Jones SB, Brewer SK, Little EE (2000) Physiological measures of neurotoxicity of diazinon and malathion to larval rainbow trout (*Oncorhynchus mukiss*) and their correlation with behavioral measures. Environ Toxicol Chem 19:1875–1880. doi:10.1897/1551-5028(2000)019<1875:PMONOD>2.3.CO;2

Bhalli JA, Khan QM, Haq MA, Khalid AM, Nasim A (2006a) Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in pesticide production industry. Mutagen 21:143–148. doi:10.1093/mutage/gel009

Bhalli JA, Khan QM, Nasim A (2006b) DNA damage in Pakistani pesticide workers assayed using micronucleus assay. Environ Mol Mutagen 47:587–593. doi:10.1002/em.20232

Economic Survey of Pakistan (2006) Finance Division, Government of Pakistan, Islamabad.[http://www.finance.gov.pk/survey/sur_chap_05-06/02-Agriculture]

Fukuto TR (1990) Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect 87:245–254. doi:10.2307/3431032

Kumar A, Chapman JC (1998) Profenofos toxicity to the eastern rainbow fish (*Melanotaenia duboulayai*). Environ Toxicol Chem 17:1799–1806. doi:10.1897/1551-5028(1998)017<1799:PTTTER>2.3.CO;2

MinFA (1995) Agricultural statistics of Pakistan, 1994–95. Islamabad: Ministry of Food and Agriculture, Government of Pakistan

OECD (Organization for Economic Co-operation and Development) (1992) OECD guideline for testing of chemicals. Fish, Acute Toxicity Test 203:1–9

Sarikaya R, Yilmaz M (2003) Investigation of acute toxicity and the effect of 2, 4-D (2, 4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae). Chemosphere 52:195–201. doi:10.1016/S0045-6535(03)00106-1

Scholz NL, Truelove NK, French BL, Berejikian BA, Quinn TP, Casillas E, Collier TK (2000) Diazinon disrupts antipredator and homing behaviors in Chinook salmon (*Oncorhynchus tshawytscha*). Can J Fish Aquat Sci 57:1911–1918. doi:10.1139/cjfas-57-9-1911

Selvi M, Sarıkaya R, Erkoc F, Kocak O (2005) Investigation of acute toxicity of chlorpyrifos-methyl on guppy *Poecilia reticulata*. Chemosphere 60:93–96. doi:10.1016/j.chemosphere.2004.11.093

Tariq MI (2005) Leaching and degradation of cotton pesticides on different soil series of cotton growing areas of Punjab, Pakistan in Lysimeters. Ph. D. Thesis, University of the Punjab, Lahore, Pakistan

Tariq MI, Afzal S, Hussain I, Sultana N (2007) Pesticides exposure in Pakistan: a review. Environ Int 33:1107–1122. doi:10.1016/j.envint.2007.07.012

Technical bulletin (2000) Directorate of pest warning and quality control of pesticides, Punjab

Tomlin CDS (1994) The pesticide manual, incorporating the agrochemicals handbook, 10th edn. The Royal Society of Chemistry and British Crop Protection Council, Cambridge

Tomlin CDS (1997) The pesticide manual, 11th edn. British Crop Protection Council, UK ISBN 1 901396 11 8

TSK (Trimmed Spearman–Karber) program (1991) Version 1.5. Ecological Monitoring Research Division, Environmental

Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, OH 45268, USA

Ural MdS, Calta M (2005) Acute toxicity of dichlorvos (DDVP) to fingerling mirror carp, *Cyprinus carpio* L. Bull Environ Contam Toxicol 75:368–373. doi:10.1007/s00128-005-0763-3

Venkateswara RJ (2004) Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis mossambicus*. Ecotoxicol Environ Saf 59:217–222. doi:10.1016/j.ecoenv.2003.09.015

Venkateswara RJ, Shilpanjali D, Kavitha P, Madhavendra SS (2003) Toxic effects of profenofos on tissue acetylcholinesterase and gill morphology in a euryhaline fish, *Oreochromis mossambicus*. Arch Toxicol 77:227–232