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NEUROBEHAVIORAL RESPONSES OF THE FRESHWATER TELEOST, *CYPRINUS CARPIO* (LINNAEUS.) UNDER QUINALPHOS INTOXICATION

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Abstract: Effect of quinalphos on the freshwater fish, *Cyprinus carpio* to elucidate inhibitory effect of quinalphos on acetyl cholinesterase activity associated behavioral changes. Fishes were exposed to one fifth (0.15µg/l) and one tenth (0.75 µg/l) of the lethal concentration (7.5 µg/l) of quinalphos for a period of 1, 7 and 14 days, and were allowed to recover for 7 days. Maximum decrement in acetyl cholinesterase activity of the exposed fish was recorded in brain followed by muscle, gill and liver. Recovery tenures witnessed increment in acetyl cholinesterase activity but significantly differed in comparison with control group. Depression of acetyl cholinesterase activity suggests decreased cholinergic transmission and consequent accumulation of acetylcholine in the tissues leading to cessation of nerve impulses. This has lead to behavioral and morphological changes due to impaired neurophysiology of the fish.

Key words: quinalphos, common carp, neurobehavioral toxicity, acetyl cholinesterase activity, acetylcholine accumulation

Introduction

The frequent uses of pesticides in agriculture practices as well as pest control pollute the soil, hydrosoil and water bodies thus reaching the aquatic ecosystem get enriched in the aquatic food chain organisms like fishes (*Mahira Parveen et al*. 2004). Recent evidence indicates that fish, an extremely valuable resource, are quickly becoming scarce. One consequence of this scarcity is the increasing concern for fish survival and a growing interest in identifying the levels of various chemical pollutants, which are safe for fish and other aquatic life.

Many organophosphates are potent neurotoxins, functioning by inhibiting the action of AChE in nerve cells. Neurotransmitters such as acetylcholine (which is affected by organophosphate pesticides) are profoundly important in the brain's

development, and many OPs have neurotoxic effects on developing organisms even at low levels of exposure. The primary effect of quinalphos and other OPs on vertebrate and invertebrate organisms is the inhibition of AChE activity, the enzyme that degrades the neurotransmitter acetylcholine in cholinergic synapses (*Pan and Dutta,* 1998). Duration of exposure, type of OP, as well as species of fish has an effect on the extent of AChE expression. Acetylcholine (ACh) is the only classical neurotransmitter that after release into the synaptic cleft is inactivated by enzymatic hydrolysis rather than by reuptake. As a consequence, ACh has a turnover rate *in vivo* that is much higher than that of any other transmitter, including catecholamines and amino acids (*Haubrich and Chippendale*, 1977).

The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE-catalyzed hydrolysis of ACh (*Justyna Kopecka et al.* 2004). AChE was identified as the enzyme responsible for termination of cholinergic transmission by cleavage of ACh to acetate and choline; AChE is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction, and the target tissues of the parasympathetic system (*Silman and Sussman,* 2005). The AChE activity is vital to normal behaviour and muscular function and represents a prime target on which some toxicants can exert a detrimental effect. Inhibition of the AChE activity results in a build up of acetylcholine causing prolonged excitatory postsynaptic potential. This results in repeated, uncontrolled firing of neurons leading to hyperstimulation of the nerve/muscle fibres, which leads paralysis, and eventual death.

Quinalphos an extensively used organophosphate in our agricultural fields. Contamination of aquatic ecosystems by sublethal levels of quinalphos is fairly common and had serious impact on non-target fish, *Cyprinus carpio* (Cyprinidae). AChE activity is a biomarker extremely used in aquatic ecotoxicology studies (*Kirby et al.* 2000), and is a fairly sensitive enzyme to low environmental concentrations of organophosphorous compounds.

The objective of the present investigation is to determine the sublethal effect of quinalphos on AChE activity of brain, gill, liver, and muscle in *C. carpio* (Linnaeus) and related effect from this exposure as a way to evaluate the toxicity risk of quinalphos to the test species.

Materials and Methods

Healthy and active *C. carpio* fingerlings were procured from the State Fisheries Department, Dharwad, India. Fish were brought to the laboratory in large aerated crates. Later they were acclimatized for 30 days in large cement tanks (22 x 12 x 5 feet) and fed with commercial dry feed pellets (Nova, Aquatic P. Feed).

The carp (2 ± 0.2 g, 4 ± 0.25 cm) were acclimatized (conditioned) to laboratory conditions for 20 d at 24 ± 1 ºC and are held in 100 l glass aquaria (120

x 45 x 80 cm) containing dechlorinated tap water of the quality used in the test, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA (2005) and found. Water was renewed every day and a 12-12 h photoperiod was maintained during acclimatization and test periods.

Quinalphos (25% EC) was procured from the local market of Dharwad, Karnataka, India, supplied by Hyderabad Chemical Supplies Limited, Hyderabad, India. The expiry date of the test substance checked prior to initiation of the treatment was found suitable for the exposure.

The fish were exposed in batches of ten to varying concentrations of quinalphos with 20 l of water in six replicates for each concentration along with control sets in range finding test. Concentrations of the test compound used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality. Replacement of the water medium was followed by the addition of the desired dose of the test compound.

For LC_{50} calculation mortality was recorded every 24 h and the dead fish were removed when observed, every time noting the number of fish death at each concentration up to 96 h. Duncan's multiple range test (*Duncan,* 1955) was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA (*Winner,* 1971) for arc sine transformed mortality data (dead individuals/initial number of individuals. In addition, LC_{50} were compared by the method of APHA (2005). The LC_{50} with 95% confidence limit for quinalphos were determined/ estimated for 96 h by probit analysis (*Finney,* 1971).

One fifth ($1/5^{th}$, 0.15 µl/l) and one tenth ($1/10^{th}$, 0.75 µl/l) of the acute toxicity value (LC_{50}) was selected as sublethal concentrations for subchronic studies. Fish were exposed to both the sublethal concentrations for 1, 7 and 14 days and allowed to recover in toxicant free medium for seven days (designated as -7) along with the control sets. AChE activities were determined in brain, gill, liver and muscle tissues, besides control in experimental tenures. Behavioral responses were studied in comparison with control (toxicant free media) and treated fish.

Estimation of AChE (E.C. 3.1.1.7) activity: Tissues were excised in physiological saline (0.9% NaCl). Homogenates (4%) of brain, gill, liver and muscle were prepared in cold 50 mM Tris-HCl (pH 6.8) extraction buffer using a glass-teflon homogenizer (Remi Motors Ltd., Mumbai, India) and then centrifuged at 3000 rpm for 15 min. All processes were carried out at 4 ^{o}C and supernatants were used to determine enzyme activity. AChE activity was determined by the method of *Ellman et al.* (1961). Enzymatic activity was determined by measuring the increase in extinction at 412 nm in a spectrophotometer (Systronics, Model No. 169). AChE activity is expressed as nM of acetylthiocholine iodide hydrolyzed/mg

protein/min. Protein contents were measured according to the method of *Lowery et al.* (1951) using bovine serum albumin as standard.

Results and Discussion

Acute toxicity of quinalphos for the freshwater fish, *C. carpio* was found to be 7.5µl/l. The upper and lower 95% confidence limits were found to be 8.33µl/l and 6.75µl/l respectively. It is evident from the results that the quinalphos can be rated has highly toxic to fish. No significant mortality was observed during the sublethal experimental tenures, but the fish were under stress and showed symptoms of dullness, loss of equilibrium, loss of feeding, and erratic swimming.

AChE inhibition: The decrease in AChE activity in fish exposed to one fifth of lethal concentration of quinalphos was recorded maximum in the brain (-75.277%) followed by muscle (-72.455%), gill (-58.280%), and liver (-51.156%) on day 14 of exposure (Table 1). Recovery tenures (7 days) witnessed increment in AChE activity compared to 7 and 14 days but remain slightly high compared to day 1 (Figure 1). Inhibition of AChE activity in recovery tenures was recorded maximum in brain (-39.803%) followed by muscle (-34.615%), gill (-23.708%), and liver (-17.490%). Decrement significantly differed in comparison with control group even under recovery tenures.

Table 1. AChE activity (nM of acetylthiocholine iodide hydrolyzed/mg protein/min) in the tissues of the fish, *C. carpio* following exposure to one fifth (0.15 µl/l) of lethal concentration of quinalphos.

Organ	Control	Sublethal exposure periods in days			
		1	7	14	-7
Brain	328.627^{a}	223.557^{b}	158.913^{d}	81.245^{e}	197.821^{c}
± SD	0.8879	0.8540	0.7858	0.5987	0.7845
Gill	197.216^{a}	153.351^{b}	107.616^{d}	82.278^{e}	150.460^{c}
± SD	0.9145	0.4865	0.5894	0.6565	0.5842
Liver	186.442^{a}	156.509^{b}	113.081^{d}	91.064^{e}	153.832^{c}
± SD	0.7845	0.9561	0.8541	0.6032	0.4912
Muscle	303.280^{a}	227.467^{b}	144.312^{d}	83.536^{e}	198.298^{c}
± SD	0.5486	0.3405	0.8164	0.9642	0.2316

Data are means ± SD (n = 6) for an organ in a row followed by the same letter are not significantly different ($p < 0.05$) from each other according to Duncan's multiple range test.

Depression in AChE activity in fish exposed to one tenth of lethal concentration of quinalphos was recorded maximum in brain (-54.484%) followed by muscle (-52.679%), gill (-43.540%), and liver (-35.884%) on day 14 of exposure (Table 2). Recovery for 7 days witnessed increment in AChE activity compared to day 7 and 14 and remained slightly high compared to day 1 in liver (-8.155%) and gill (-10.517%). Brain (-23.222%) and muscle (-19.878%) remained slightly less in AChE activity compared day 1. Decrement significantly differed in comparison with control group even under recovery tenures.

At sublethal concentrations quinalphos caused greater inhibition of AChE activity in the brain, gill, liver, and muscle tissues. Further these effects are most clearly seen following acute high dose exposure but they can also be observed in lower dose chronic cases as well. The inhibition of AChE results in buildup of acetylcholine within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (*Mileson et al,*. 1998). Results obtained by different workers independently of tissues and species used are quite similar in the AChE inhibitory effects. In accordance with earlier observations made *Rao* (2006) and *Elif and Demet* (2007). Depression of AChE activity in the brain of carp is more sensitive to quinalphos exposure than that in the muscle, gill, and liver. The data reflects that an inhibition of this magnitude may not be lethal to all species but that it may exercise a deleterious impact on important neurobehavioral functions such as swimming and motivation. The behavioral changes observed in the intoxicated fish like erratic, darting, and burst-swimming can be directly related to the impaired neuronal dysfunction of central nervous system due to inhibition of brain AChE activity.

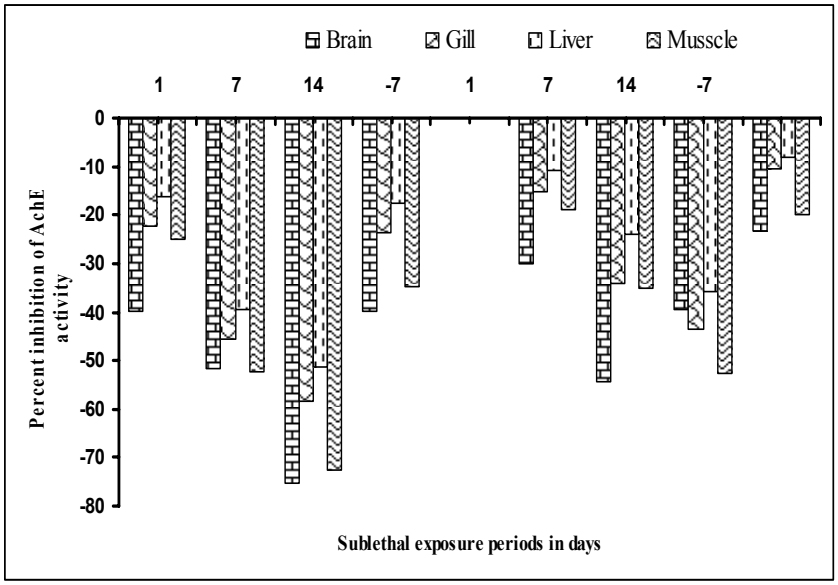
Table 2. AChE activity (nM of acetylthiocholine iodide hydrolyzed/mg protein/min) in the tissues of the fish, *C. carpio* following exposure to one tenth (0.75 µl/l) of lethal concentration of quinalphos.

Organ	Control	Sublethal exposure periods in days			
		1	7	14	-7
Brain	328.627^{a}	256.890^{b}	198.926^{d}	149.575^{e}	252.31^{c}
± SD	0.8879	0.7458	0.9654	0.5976	0.7469
Gill	197.216^{a}	167.155^{c}	130.292^{d}	111.347^{e}	176.474^{b}
± SD	0.9145	0.5241	0.5698	0.8656	0.9568
Liver	186.442^{a}	165.972^{c}	141.718^{d}	119.539^{e}	171.236^{b}
± SD	0.7845	0.8564	0.9564	0.6497	0.6958
Muscle	303.280^{a}	245.867^{b}	197.156^{d}	143.514^{e}	242.991^{c}
± SD	0.5486	0.6569	0.9486	0.7946	0.8679

Data are means ± SD (n = 6) for an organ in a row followed by the same letter are not significantly different ($p < 0.05$) from each other according to Duncan's multiple range test

Caudal bending (left side) was noticed in both the sublethal concentrations with time and persisted even under recovery tenures. The extent of caudal bending was pronounced in higher toxicant concentration. This greatly retarded the normal swimming pattern. Caudal bending may be a sort of paralysis, which is due to the inhibition of muscular AChE activity resulting in blockage of neural transmissions. Bending of caudal region is owing to the fact that caudal portion is the thinnest structure and hence can be conferred any sort of orientation due to paralysis of caudal musculature by inhibition of AChE activity as evidenced in the present study. Further inhibition of AChE activity results in a progressive accumulation of ACh, especially during periods of repetitive stimulation, leading to desensitization of nAChRs (nicotinic acetylcholine receptors) and consequent muscular weakness (*Giniatullin et al*., 1998). Thus quinalphos reduced instinctive behavioural responses and affected morphological features by depression of AChE activity. Quinalphos inhibits AChE activity due to the effects of their active oxygen analog quinalphos-oxon. The ratio between the toxification/detoxification reactions determines the degree of enzyme inhibition and can be used to evaluate metabolism processes (*Timchalk et al*.. 2002).

Figure 1: Percent inhibition of AChE activity in the tissues of the fish, *C. carpio* following exposure to one fifth and one tenth of lethal concentration of quinalphos.



Conclusion

The present study evidenced neurotoxic potential of quinalphos by inhibition of the AChE activity in the tissues of the fish, *C. carpio* at sublethal concentrations. Inhibition of AChE activity in the brain appears to be an early process in response to sublethal exposures, and could be a more sensitive biomarker than inhibition of AChE activity in the muscle, gill, and liver to characterize toxicological impacts.

Neurobihevioralne reakcije slatkovodne ribe *Cyprinus carpio* (Linnaeus.) pod uticajem intoksikacije kvinalfosom

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Rezime

Uticaj kvinalfosa na slatkovodnu ribu, *Cyprinus carpio* je ispitivan kako bi se objasnio inhibirajući efekat kvinalfosa na promene u ponašanju povezane sa aktivnošću acetil-holinesteraze. Ribe su bile izložene jednoj petini (0.15µg/l) i jednoj desetini (0.75 µg/l) letalne koncentracije (7.5 µg/l) kvinalfosa u periodu od 1, 7 i 14 dana, i dozvoljeno im je da se oporave u periodu od 7 dana. Maksimalno opadanje aktivnosti acetil-holinesteraze kod izložene ribe u mozgu, a zatim u mišićima, škrgama i jetri. U oporavku je došlo do povećanja aktivnosti acetil holinesteraze ali se signifikantno razlikovala od kontrolne grupe. Smanjenje aktivnosti acetil holinesteraze upućuje na smanjenje holinergetske transmisije i konsekventne akumulacije acetilholina u tkivima što je dovelo prekida ua nervnim impulsima. To je dovelo do promena u ponašanju i morfoloških promena zbog oštećene neurofiziologije ribe.

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