Respiratory responses and behavioural anomalies of the carp *Cyprinus carpio* under quinalphos intoxication in sublethal doses

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ABSTRACT: A static renewal bioassay was conducted to determine the acute toxicity (LC_{50}) of commercial grade organophosphate insecticide, quinalphos (25% emulsified concentration) to common carp, *Cyprinus carpio*. The acute toxicity of quinalphos to carp fingerlings exposed for 96 h was found to be 7.5 µl/l. The fish were exposed to sublethal concentrations ($0.1LC_{50}$ and $0.2LC_{50}$) for up to 15 days. Fish in toxic media exhibited irregular, erratic, and darting swimming movements, hyper excitability, and loss of equilibrium and sinking to the bottom. Caudal bending was the chief morphological alterations during the exposure tenures. The carp were found to be under stress but mortality was insignificant in both sublethal concentrations. Considerable variation in respiratory rate was observed in both sublethal concentrations. The observed alteration in respiratory rates may be a consequence of impaired oxidative metabolism and elevated physiological response by the fish against quinalphos stress. The impairments in fish respiratory physiology and behavioural response even under recovery tenures may be due to slow release of sequestered quinalphos from the storage tissues.

KEYWORDS: organophosphate pesticide, common carp, acute and chronic toxicity, oxygen consumption, behavioural distress

INTRODUCTION

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. As a result of the pollutants transported from industrial areas into the environment and their chemical persistence, many freshwater ecosystems are faced with high levels of xenobiotic chemicals[1,2]. Pesticides used in agriculture are among the most hazardous chemicals. Such chemicals may reach lakes and rivers through rains and wind, affecting many other organisms away from the primary target. It is estimated that generally only about 0.1% of the pesticide reaches the specific target[3,4]. The detrimental effect of insecticides on aquatic environments is incontestable[5,6]. Many of such chemicals can induce genetic disorders and physiological alterations, if not death of the exposed organisms.

Organophosphates have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds. Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important because they are highly toxic to aquatic organisms.

Quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothiate) is a synthetic organophosphate, non-systemic, broad spectrum insecticide and acaricide, acting as a cholinesterase inhibitor with contact, stomach, and respiratory action. The major use of quinalphos in farming is to protect corn, cotton, and fruit trees against insects.

Fish are able to take up and retain chemicals dissolved in water via active or passive processes. They can be used to detect and document pollutants released into their environment. Mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress[7]. The interest in understanding the physiological mechanisms associated with fish responding to environmental stress has been growing. Sublethal concentrations of pesticides in aquatic environments cause structural and functional changes in aquatic organisms and this is more common than mortality[8]. Since quinalphos is extensively applied in agriculture for pest eradication

in India, it is pertinent to study its hazardous effect on the aquatic system as it is assumed that the residue might affect the fish[9].

The problem of pollutant bioaccumulation and subsequent physiological trauma is often reflected in the rate of oxygen uptake, either through disrupted metabolism or in the mobilization of compensatory homeostatic mechanisms. Any change in the behaviour and physiology of fish indicates the deterioration of water quality, as fish are biological indicators.

The common carp, *Cyprinus carpio* L. (Cyprinidae), is a very important staple fish generally found in rivers, ponds, and reservoirs. Quinalphos is an extensively used organophosphate insecticide for agricultural crops. Hence the current study was undertaken to evaluate the aquatic toxicity of quinalphos with special emphasis on behavioural responses and respiratory performance of the carp exposed to sublethal concentrations of commercial grade quinalphos.

MATERIALS AND METHODS

Sample collection, maintenance and acute toxicity test

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

The carp (2 ± 0.2 g, 4 ± 0.25 cm) were acclimatized to laboratory conditions for 20 days at 24 ± 1 °C and were held in 100-l glass aquaria (120 cm × 45 cm × 80 cm) containing dechlorinated tap water of the quality used in the test. Its physico-chemical characteristics were analysed following the methods of the APHA[10] and found to be as follows: temperature 24 ± 2 °C; pH 7.0 ± 0.2 at 24 °C; dissolved oxygen 6.6 ± 0.8 mg/l; carbon dioxide 6.3 ± 0.4 mg/l; total hardness 23.4 ± 3.4 mg/l of $CaCO_3$; phosphate 0.39 ± 0.002 μg/l; salinity 0.3 ppm, specific gravity 1.001, and conductivity less than 10 μS/cm. Water was renewed every day and a 12 h:12 h photoperiod was maintained during acclimatization and test periods. The fish were fed regularly with commercial fish food pellets during acclimatization and test tenures but feeding was stopped two days prior to exposure to the test medium for the acute toxicity test.

Quinalphos (25% EC) was obtained from the local market of Dharwad, Karnataka, India, supplied by Nagarjuna Fertilizers and Chemicals Limited, Hy-

Table 1 Acute toxicity (96 h LC_{50}), slope, and 95% confidence limits of quinalphos to *Cyprinus carpio*.

Pesticide	96 h LC_{50} (μl/l)	Slope	95% Confidence limits	
			Upper	Lower
Quinalphos	7.5 ± 0.0236	1.095	8.33	6.75

derabad, India. The expiry date of the test substance checked prior to initiation of the treatment was found to be suitable for the exposure. The required quantity of quinalphos was drawn directly from this emulsifiable concentrate using a variable micropipette.

The fish were exposed in batches of 10 to varying concentrations of quinalphos with 20 l of water in 6 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 the LC_{50} calculation, mortality was recorded every 24 h and the dead fish were removed when observed, every time noting the number of fish deaths at each concentration up to 96 h. Duncan's multiple range test[11] was used to compare mean mortality values after estimating the residual variance by repeated measures ANOVA[12] for arcsine transformed mortality data (dead individuals/initial number of individuals). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. In addition, LC_{50} values were compared by the method of APHA[10]. The LC_{50} values with 95% confidence limit for quinalphos were determined for 96 h by probit analysis[13] (Table 1).

Study periods and toxicant concentrations

One fifth (1.5 μl/l) and one tenth (0.75 μl/l) of the acute toxicity value (LC_{50}) were selected as sublethal concentrations for subchronic studies. Fish were exposed to both the sublethal concentrations for 1, 5, 10, and 15 days along with the control sets. Behavioural responses and respiratory rate were studied in experimental tenures. The control (toxicant free medium) and quinalphos exposed fish were kept under continuous observation during experimental periods.

Assay of respiratory rate

Oxygen consumption was studied in sub lethal concentrations for several days (1, 5, 10, and 15 days). In the present study, the respiration rate of the same

fish was measured from 1 to 15 days with a 5-day interval for sublethal concentrations. At the end of 24 h exposure, each fish was transferred from the test chamber (5 l capacity) to a respiratory chamber of 1 l capacity. The fish were allowed to stabilize for 5 min and then the experiment was run for a period of 1 h. After the experiment, the fish were replaced in their respective test chambers. The same procedure was repeated for day 5, day 10, and day 15. Any dead fish during the course of the experiment were removed and the test repeated so as to obtain the response of at least 3 fish. Controls were also run simultaneously to obtain information on the oxygen consumption of the fish in the normal state. At the end of 96 h, the fish were sacrificed, blotted dry, and weighed to the nearest mg to calculate the metabolic rate. Respiratory measurements were made by the method adopted by Welsh and Smith[14] as described by Shivakumar[15] and the dissolved oxygen was estimated adopting Winkler's iodometric method. The oxygen consumed by the fish is expressed as ml of oxygen consumed/g wet weight of fish/h. The respiratory measurements were made in diffused daylight and the time of the experiment was kept constant (11.00 am to 3.00 pm) to avoid the effect of time of day on the respiration of the fish. The temperature and pH during the course of the experiments were $24 \pm 2\,°C$ and pH 7.1 ± 0.2 at $24\,°C$, respectively.

Statistical analysis

Data correspond to the average of six replicates. The data obtained were analysed statistically by following Duncan's multiple range tests.

RESULTS AND DISCUSSION

Quinalphos toxicity

Acute toxicity of quinalphos for *C. carpio* was found to be 7.5 µl/l. The upper and lower 95% confidence limits were found to be 8.33 µl/l and 6.75 µl/l, respectively (Table 1). It is evident from the results that the quinalphos can be rated as highly toxic to fish. No significant mortality was observed during the experimental tenures in both the sublethal concentrations.

Behaviour of normal and exposed fish

Experiments were done to assess the behaviour of carp without any toxicant and with two sublethal concentrations of quinalphos. In the present study the control fish behaved in a natural manner, i.e., they were active for feeding and alert to slightest of the disturbance with their well synchronized movements. The behaviour did not vary significantly between the control groups. Therefore, the results of these non-exposure series were taken as standards for the whole test period. In toxic media, carp exhibited disrupted shoaling behaviour, localization to the bottom of the test chamber, and independency (spreading out) in swimming. The above symptoms followed the loss of co-ordination among individuals and occupancy of twice the area of that of the control group were the early symptoms of quinalphos exposure in both the sublethal concentrations. Subsequently, fish moved to the corners of the test chambers, which can be viewed as avoidance behaviour of the fish to the quinalphos. In the toxic environment fish exhibited irregular, erratic, and darting swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms are due to inhibition of acetylcholine esterase (AChE) activity leading to accumulation of acetylcholine in cholinergic synapses ensuring hyperstimulation. Inhibition of AChE activity is a typical characteristic of organophosphate compounds[16–18]. The fish slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the carp were hyperexcited resulting in erratic movements. An excess secretion of mucous in fish is a non-specific response against toxicants, forming a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucus. Similar observations were made by Rao[19] following RPR-V exposure to euryhaline fish, *Oreochromis mossambicus*.

Disrupted shoaling behaviour, easy predation, gulping air, and swimming at the water surface (surfacing phenomenon) were observed on the first day in both the sublethal exposure periods. This continued throughout the test tenures, which is in accordance with the observations made by Ural and Simsek[20]. Gulping of air may help to avoid contact of toxic medium and to ease respiratory stress. Surfacing phenomenon in the exposed group might be a demand for higher oxygen level[7]. Surfacing phenomenon and easy predation continued even under recovery tenures of 5 days in both the test concentrations. This reflects the catastrophic impact posed by the toxicant. Of all, easy predation phenomenon is one of the most critical damages caused by a pollutant on sensitive species like fish, which ultimately decide the species survival in a given ecosystem. Caudal bending (left side) was noticed in both the toxicant concentrations with time, which greatly retarded the normal swimming pattern. The extent of caudal bending was pronounced with higher toxicant concentration. Caudal bending may be a type of paralysis, which might be due to the

inhibition of muscular AChE resulting in blockage of neural transmissions. The bending of the caudal base results from 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 AChE inhibition. Thus quinalphos reduced instinctive behavioural response and affected morphological features.

Hyperextension of fins, dullness in body colour and fish body becoming lean towards abdominal position and being under stress in later sublethal exposure periods was observed. There was a slight swelling of the abdominal region that remained throughout the test tenures. Leaning of fish indicates reduced feeding behaviour and diversion of fish metabolism towards adaptability to the toxic media. Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. This was pronounced and, in the case of $0.2LC_{50}$ exposure, continued even under recovery tenures. For these animals, it might be profitable to decrease their food uptake under toxic environmental conditions to lower the energetic costs of digestion. Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods can have a clear impact on growth and reproduction[21]. A substantial growth reduction caused by toxicant stress has important implications for survival. Fish in one tenth of LC_{50} were alert and feeding actively. Intermittently some of the fish sank to the bottom with their least opercular movements, failing to fight against quinalphos stress in both sublethal exposure tenures.

Respiratory toxicity of quinalphos

A variation in the respiratory rate is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is clearly evident from the results that quinalphos affected the respiratory rate of *C. carpio* under both sublethal concentrations (Table 2). Fish exposed to $0.2LC_{50}$ showed a drastic decrease in respiratory rate on day 1 to day 5 and also exhibited a drastic increase in the same, during subsequent exposure tenures recording a maximum of 56.45% on the first day of exposure. In the $0.1LC_{50}$ case, the respiratory rate increased on day 1 and decreased significantly on days 5 and 10. The respiratory rates of the fish from day 1–15 in the $0.2LC_{50}$ and $0.1LC_{50}$ media were found to be in the order $1 > 5 < 10 > 15$ and $1 > 5 > 10 < 15$, respectively. A steep decline and drastic increment in respiratory rate in both the sublethal concentrations are due to toxicant induced stress, avoidance, and biotransformation.

If gills are destroyed due to xenobiotic chemicals[22] or the membrane functions are disturbed by a changed permeability[23], the oxygen uptake rate would rapidly decrease. On the other hand, the metabolic rate in relation to respiration of fish could be increased under chemical stress. Altered respiratory rate can be correlated with the altered opening and closing of opercular coverings and mouth observed in the study.

Numerous studies have shown that animals may either increase or decrease their respiration rate in response to a variety of toxicants such as metals, phenol, and pesticides. Behavioural abnormalities have been attributed to nervous impairment due to blockage of nervous transmission between the nervous system and various effector sites[24], the enzyme dysfunctions that may cause paralysis of respiratory muscles and/or depression of the respiratory centre, and disturbances in energy pathways which result in depletion of energy[25].

Two possible strategies may be (1) to get rid of the toxicant through excretion, sequestration of the toxicant in inactive tissues, and (2) to move away from the area containing the toxicant. As discussed above, synthesis of specific stress proteins may be initiated or increased to detoxify the toxicant, to sequestrate the toxicant in inactive tissues, to transform the toxicant to a more excretable form, or to repair

Table 2 Respiratory rate (ml of oxygen consumed/g wet weight of fish/h) of *C. carpio* following exposure to $0.2LC_{50}$ and $0.1LC_{50}$ of quinalphos.

Parameter	Control	Sublethal exposure periods in days							
		$0.2LC_{50}$				$0.1LC_{50}$			
		1	5	10	15	1	5	10	15
Respiratory rate	0.5654	0.9844	0.8565	0.9449	0.9185	0.7894	0.6734	0.6271	0.6854
± SD	0.0288	0.2597	0.1183	0.1646	0.1391	0.0823	0.0590	0.0333	0.0758

Data are means ± SD ($n = 6$). All respiratory rates are significantly different from one another ($p < 0.05$), according to Duncan's multiple range test.

damage. The increase in the protein synthesis in the above processes is expected to be accompanied by an increase in respiration. Additional energy expenditure is also required for the metabolism, excretion and deposition of the toxicant, physiological compensation, and avoidance behaviour. The increase in protein synthesis and energy expenditure will eventually be reflected in an increase in respiration rate[26]. Furthermore, physiological reactions, such as activation of biotransformation enzyme systems in the presence of xenobiotic substances, enable the organisms to survive in subacute exposures.

CONCLUSIONS

The analysis evidenced that quinalphos is highly toxic and had a profound impact on the behaviour and respiratory rate in both sublethal concentrations to *C. carpio*. Deviation in the respiratory rate is due to impaired oxidative metabolism and altered respiratory physiological response to quinalphos stress. Quinalphos decreased the ability of animals to adapt to its environment by (1) increasing the time required to learn to escape or to avoid external noxious stimuli, (2) decreasing the animal sensitivity to subtle changes in the environment, or (3) interfering with the ability of animals to retain previously learned behaviour. Thus quinalphos reduced instinctive behavioural response and affected morphological features. The impairments in fish respiratory physiology and behavioural response exposure tenures may be due to slow release of sequestered quinalphos from the storage tissues.

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