BEHAVIORAL AND RESPIRATORY RESPONSES OF THE FRESHWATER FISH, *CYPRINUS CARPIO* (LINNAEUS) UNDER QUINALPHOS INTOXICATION IN SUBLETHAL TENURES

By

Sameer G. Chebbi and Muniswamy David

Karnatak University's Research Laboratory, Toxicology and Molecular Division, Department of Zoology, Karnatak Science College, Dharwad-580 001, Karnataka, India

Abstract

Carp fingerlings were exposed to different concentrations (6.64 to 7.88 µl/l) of quinalphos for 96 h. Behavioral patterns and oxygen consumption were studied in lethal (1, 2, 3 and 4 d) and sub lethal concentrations (1, 5, 10 and 15 d). One fifth (1/5th, 1.5 µl/l) and one tenth (1/10th, 0.75 µl/l) of the acute toxicity value was selected as sublethal concentrations for subchronic studies. The fish were exposed to both the sublethal concentrations for 1, 5, 10 and 15 days. Behavioral responses and respiratory rate were studied in experimental tenures. 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 behavioral and morphological changes might be due to inhibition of acetylcholinesterase (AChE) activity. Inactivation of AChE results in excess accumulation of acetylcholine in cholinergic synapses leading to hyperstimulation and cessation of neuronal transmission (paralysis). The carp were found under stress but mortality was insignificant in both the sublethal concentrations. Considerable variation in respiratory rate was observed in both one fifth and one tenth sublethal concentrations of quinalphos respectively. An alteration in respiratory rates is due to the respiratory distress. This may be a consequence of impaired oxidative metabolism and elevated physiological response by the fish against quinalphos stress.

Introduction

- Insecticides are extensively used to protect agricultural crops against the damages caused by pests. However, these chemicals may reach other ecological compartments as lakes and rivers through rains and wind, affecting many other organisms away from the primary target.
- The significant increase of chemical in the water resources has lead to deleterious effects for aquatic organisms. Many of such chemicals can induce, besides death of exposed organisms, other effects, like genetic disorders and physiologic alterations.
- Quinalphos is a synthetic organophosphate, non-systemic, broad spectrum insecticide and acaricide, acting as a cholinesterase inhibitor, with contact, stomach, and respiratory action.

- Fish are able to uptake and retain different xenobiotics dissolved in water via active or passive processes.
- Research in fish demonstrated that mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress.
- 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.
- Any change in the behavior and physiology of fishes indicates the deterioration of water quality, as fishes are the biological indicators.

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 and fed with commercial dry feed pellets.
- The carp (2 ± 0.2 g, 4 ± 0.25 cm) were acclimatized to laboratory conditions for 20 d at 24 ± 1 ºC and are held in 100 l glass aquaria containing dechlorinated tap water of the quality used in the test, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA.
- Quinalphos (25% EC) was procured from the local market of Dharwad, Karnataka, India, supplied by Hyderabad Chemical Supplies Limited, Hyderabad, India.
- 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 LC50 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 (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, LC50 were compared by the method of APHA (2005). The LC50 with 95% confidence limit for quinalphos were determined/estimated for 96 h by probit analysis (Finney 1971).
- One fifth (1/5th, 1.5 µl/l) and one tenth (1/10th, 0.75 µl/l) of the acute toxicity value (LC50) was 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. Behavioral 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.
- Respiratory rate (oxygen consumption) of quinalphos exposed fish was measured besides control by following the method of Welsh and Smith (1953) as described by Shivakumar (2005). The values are expressed as ml of oxygen consumed/g wet wt. of fish/h.

RESULTS AND DISCUSSION

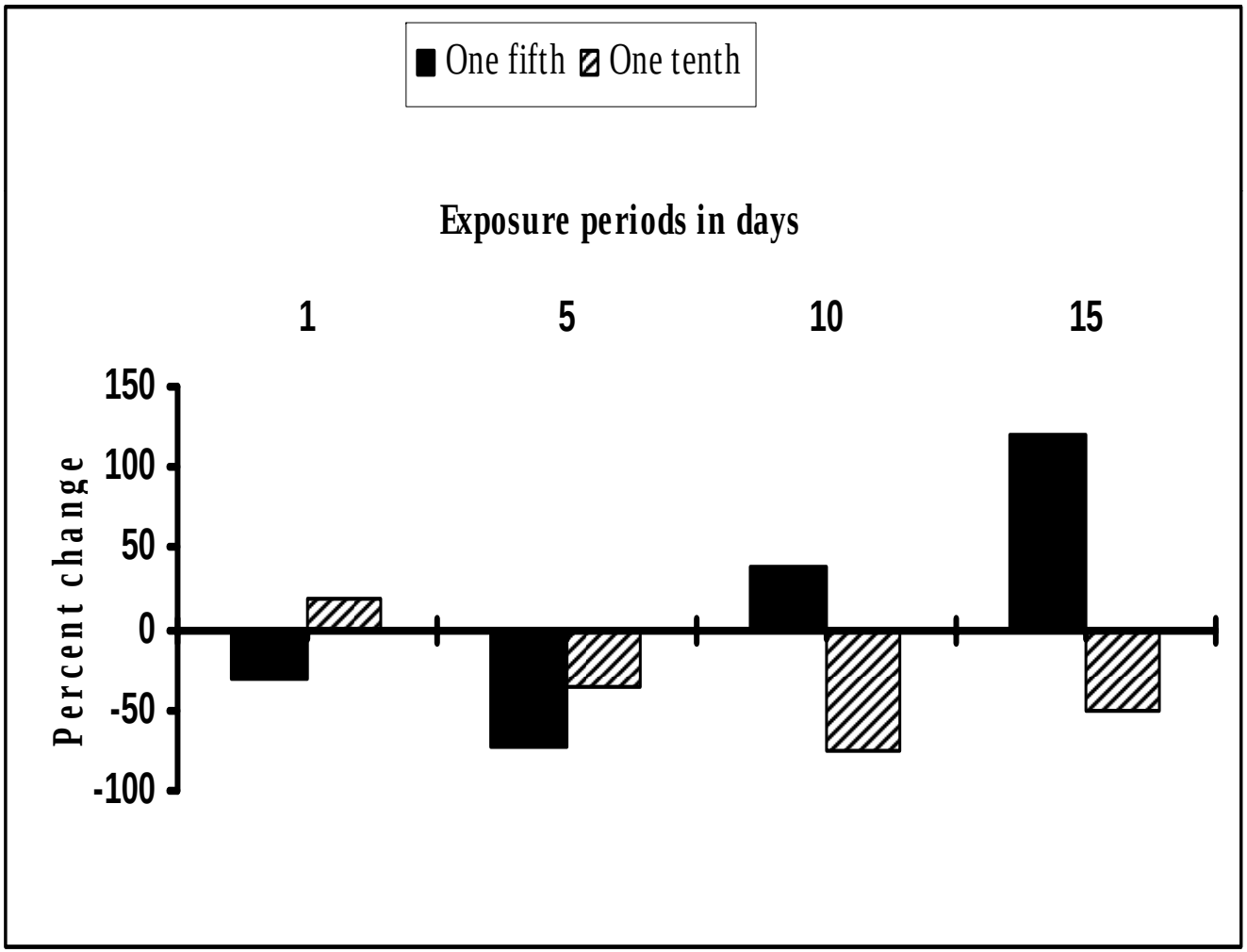
- Experiments were done to assess the behavior of carp without any toxicant and with two sublethal concentrations of quinalphos. In the present study the control fish behaved in a natural manner i.e., they were active for feeding and alert to slightest of disturbance with their well synchronized movements therefore, the results of these non-exposure series were taken as standards for the whole test periods.
- In toxic media carp exhibited disrupted shoaling behavior, localization to bottom of the test chamber and independency (spread out) in swimming. Subsequently fish moved to the corners of the test chambers, which can be viewed as avoidance behavior 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 AChE activity leading to accumulation of acetylcholine in cholinergic synapses ensuing hyperstimulation. And inhibition of AChE activity is a typical characteristic of organophosphate compounds (Holmstedt 1963; Padilla et al. 1996; Timchalk et al. 2002).
- They slowly became lethargic, restless, and secreted excess mucus all over the body. Intermittently some of the carp were hyper excited resulting in erratic movements. An excess secretion of mucous in fish forms a non-specific response against toxicants, thereby probably reducing toxicant contact. It also forms 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 (2006) following RPR-V exposure to euryhaline fish, *Oreochromis mossambicus*.

- Disrupted shoaling behavior, easy predation, gulping air, and swimming at the water surface (surfacing phenomenon) were observed on the first day itself in both the sublethal exposure periods. This situation further continued intensely throughout the test tenures, which is in accordance with the observations made by Ural and Simsek (2006). Gulping of air may help to avoid contact of toxic medium and to ease respiratory stress. Surfacing phenomenon i.e., significant preference of upper layers in exposed group might be a demand for higher oxygen level during the exposure period (Katja et al. 2005).
- 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 in higher toxicant concentration. Caudal bending may be a sort of paralysis, which might be due to the inhibition of muscular AChE resulting in blockage of neural transmissions. Bending of caudal base 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 acetylcholinesterase inhibition. Thus quinalphos reduced instinctive behavioral response and affected morphological features.
- Hyper extension of fins, dullness in body color 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 so throughout the test tenures. Leaning of fish indicates reduced feeding behavior and diversion of fish metabolism towards adaptability to the toxic media.

- 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 (Rice 1990).
- A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations. Fish in one tenth of LC50 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.
- 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 respiratory rate of *C. carpio* under one fifth and one tenth of sublethal concentrations .
- Studies on respiratory rate depicted neither time nor dose dependency. Overall alterations are simply random .
- Fish exposed to one fifth of lethal concentration showed drastic decrement in respiratory rate on day 1 to day 5 and also exhibit a drastic increase in the same, during subsequent exposure tenures recording a maximum of 119.526% on the fifth day of recovery.
- In one tenth of lethal exposure the respiratory rate increased on day 1 and decreased significantly on day 5 and 10 but on seventh day of recovery there is increment as compared to day 10. The respiratory rate in one fifth and one tenth of lethal media were found in the order of $1 > 5 < 10 < 15$ 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 would be destroyed due to xenobiotic chemicals (Grinwis et al. 1998) or the membrane functions are disturbed by a changed permeability (Hartl et al. 2001), oxygen uptake rate would even rapidly decreased. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. On the contrary 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. Two possible strategies may be; to get rid of the toxicant through excretion, sequestration of the toxicant in inactive tissues, and or moving away from the area containing the toxicant.
- Additional energy expenditure is also required for the metabolism, excretion and deposition of the toxicant, physiological compensation and avoidance behavior. The increase in protein synthesis and energy expenditure will eventually be reflected in an increase in respiration rate (Connell et al. 1999). Furthermore, physiological reactions, such as activation of biotransformation enzyme systems in the presence of xenobiotic substances enable the organisms to survive in subacute exposures.

Respiratory rate (ml of oxygen consumed/g wet wt. of fish/h) of the freshwater fish, *C. carpio* following exposure to one fifth (1.5 µl/l) and one tenth (0.75 µl/l) sublethal concentrations of quinalphos.



Conclusion

- The analysis evidenced that quinalphos is highly toxic and had profound impact on the behavior and respiratory rate in both the sublethal concentrations to *C. carpio*.
- Deviation in the respiratory rate is due to impaired oxidative metabolism and altered respiratory physiological response to quinalphos stress.
- To conclude, quinalphos depicted severe critical impact on behavior of the carp. It reduced/decreased the animals' ability to adapt to its environment by 1) increasing the time required to learn to escape or to avoid external noxious stimuli, 2) decrease the animal sensitivity to subtle changes in the environment, or 3) interfere with the animals' ability to retain previously learned behavior.
- Thus quinalphos reduced instinctive behavioral response and affected morphological features. The impairments in fish respiratory physiology and behavioral response even under recovery tenures may be due to slow release of sequestered quinalphos from the storage tissues.

Thank you