



## Quinalphos induced alteration in respiratory rate and food consumption of freshwater fish *Cyprinus carpio*

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### Abstract

Acute toxicity of commercial grade organophosphate insecticide, quinalphos (25% emulsified concentration) to common carp (*Cyprinus carpio*) was tested through bioassay. The acute toxicity of quinalphos to the fingerlings exposed for 96hr was found to be 2.75ppm. For sub lethal toxicity study, the fish were exposed to two concentration viz.,  $1/10^{\text{th}}$  of  $LC_{50}$  (0.275 ppm) and  $1/5^{\text{th}}$  of  $LC_{50}$  (0.55 ppm) along with lethal concentration (2.75 ppm) as reference for 48 hr. The carps were under stress and mortality was insignificant in both sub lethal and lethal concentrations. However, considerable variation in respiration rate and food consumption rate was observed in both lethal and sublethal concentrations. The alteration observed in the physiological condition may be a consequence of impaired oxidative metabolism and elevated physiological stress by fish against quinalphos

### Key words

Acute toxicity, Common carp, Food consumption, Quinalphos, Respiratory rate,

### Introduction

Contaminants such as petroleum hydrocarbons, heavy metals and pesticides can cause direct toxic effect when released into aquatic environment (Fleeger *et al.*, 2003). Pesticides used for crop protection in agriculture and horticulture may enter ditches, ponds, lakes and rivers in numerous ways such as direct overspray, spray drift, leaching to surface and ground water, run off from land, and/ or accidental spills (Capri and Trevisan, 1998). As a result of the pollutants transport from industrial areas into the environment and their chemical persistence, many freshwater ecosystems are faced with spatially or temporally alarming high levels of xenobiotic chemicals (Brack *et al.*, 2002; Diez *et al.*, 2002).

Consequently, these hazard chemicals may affect the non- target biotic communities of aquatic ecosystem (Hann and Goldsborough, 1997). It has been widely documented that pesticide concentration in the natural environments are often high enough to kill certain organisms (Hatakeyama *et al.*, 1994) and affect the structure and function of the invertebrate communities (Liess and Ohe, 2005). Now, there is a growing concern worldwide over the indiscriminate use of such chemicals,

resulting in environmental pollution and toxicity risk to aquatic organisms (Khan, 1996). Pesticides exert their impact at multiple levels, including molecules, tissues, organs, individuals, populations and communities and a variety of ecotoxicological tests have been designed to assess these effects (Cairns and Niederlehner, 1995). Pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centres of brain or the tissue involved in breathing. The effect of toxicants on the respiration of fishes and invertebrates has received wide spread attention and were reviewed by Hughes (1976) and Wright (1978).

Among different classes of pesticide, organo phosphorous insecticides represent one of the most widely used classes of pesticide with high potential for human exposure in both rural and residential environments (Ngoula *et al.*, 2007). Quinalphos is an organophosphate extensively used in agriculture for pest eradication (Das and Mukherjee, 2000). Quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothiate) is a synthetic organo-phosphate, 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. It is a hard insecticide, which has become a matter of concern because of its potential and hazardous effect. The fish *Cyprinus carpio* was selected as experimental model because of its wide availability in local tanks and ponds. It also serves as a cheap protein rich source of food for the vast population of India. Hence, the present study was undertaken to determine the effect of quinalphos (25% EC: emulsifiable concentrate) on the oxygen consumption and food consumption in common carp exposed to different concentrations of quinalphos and period.

### Materials and Methods

**Animal collection and maintenance :** Common carp (2-3cm) fry acquired from Bhadra fish farm, Shimoga, Karnataka were transported to the fish farm of College of Fisheries, Mangalore in well oxygenated polythene bags containing clean pond water. The fry were reared to fingerlings (4.5-5.0 cm) in a pond (5×5×1) for a period of 1 month using formulated fish feed.

The fingerlings were transferred to 1000 l fiber reinforced plastic tank in the laboratory after proper acclimation. Vigorous aeration was provided in the tanks with natural photoperiod of 12 hr. The fish were fed twice a day with biogold floating feed. The walls of the holding tank were thoroughly cleaned and excreta were siphoned off on daily basis to prevent the building of ammonia in the medium. Fish were conditioned in holding tank for 10 days before utilizing them for the experiments.

The optimum temperature ( $28\pm 1$  °C), dissolved oxygen (7-7.5 ppm) and pH (6.9-7.3) of the water were maintained throughout the experiment. Individuals measuring  $4.5\pm 0.5$ cm in total length and weighing  $4\pm 0.5$ g were selected for the present study. A mixed population of one hundred individuals was used to exclude the possibilities of influence of sex of the individuals on the parameters studied.

**Bioassay test :** Lethal toxicity study of quinalphos (25% EC) was carried out by using static renewal system. Laboratory conditioned fish of uniform size were selected to assess the lethal concentration of the toxicant. Experimental glass containers of 20 l capacity were used for the experiment. Ten fish each were accommodated in 18 l of test solution. The stock solution of 1000 ppm quinalphos was prepared using distilled water. From this stock solution, different concentrations (2.5 to 3.2 ppm) were prepared. The experiments were conducted in triplicate with appropriate controls. The animals were not fed during the experiment and water was never aerated. The test solution was replenished every 24 hr. Care was taken to leave the animals with minimum disturbance. Dead fish were removed immediately from the test medium.

The percentage mortality of fish for each concentration was taken into account for 96 hr. The 96 hr  $LC_{50}$  value of quinalphos was calculated by probit analysis (Finney, 1971).

**Estimation of oxygen consumption :** The experiments on the oxygen consumption of common carp were carried out in a glass aquarium of 20 l capacity. The lethal ( $LC_{50}$  at 96 hr i.e. 2.75 ppm) and two sub lethal concentrations ( $1/10^{\text{th}}$  of  $LC_{50}$  and  $1/5^{\text{th}}$   $LC_{50}$  at 96 hr) were selected to study the oxygen consumption rate for 48 hr in static system with 12 hr interval. Ten fish each were accommodated in 18 l of test solution. The surface water of the control and test chamber was covered with a thin film of liquid paraffin, to prevent diffusion of atmospheric air into test medium. The amount of dissolved oxygen in water for every 12 hr was estimated by Winkler method (Golterman and Clymo, 1969). The difference in dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. The oxygen consumption examination was based on the method described by Chinni et al. (2000).

**Estimation of food consumption :** For determining the food consumption rate, carps were fed once in 12 hr with "bio gold" dry feed pellet. After 15 min, the remaining food was removed. It was dried overnight at 60°C and weighed to compare mean food consumption Broek et al. (1997).

**Statistical analysis :** The data were subjected to statistical analysis employing ANOVA and Duncan's multiple range test at  $P < 0.05$  (Duncan, 1995; Snedecor and Cochran, 1968).

### Results and Discussion

The  $LC_{50}$  value of quinalphos for common carp was 2.75 ppm at 96 hr (Table 2). Fish mortality increased with increase in concentration of Quinalphos. The 16 %,83% and 100% of fish death were observed at 2.5 ppm, 3.1 ppm and 3.2 ppm respectively for 96 hr of exposure to Quinalphos, however no deaths of fish were observed in control.

Gulping air, swimming at the water surface, disrupted shoaling behavior was observed during exposure period. Similar observations were made by Ural and Simsek (2006) and Chebbi and David (2010) when they exposed fingerlings of European catfish to dichloro-vos and common carp to quinalphos respectively.

A variation in respiration rate is an indicator of stress and is frequently used to evaluate the changes in metabolism under environmental deterioration (Chebbi and Muniswamy, 2010). It is clearly evident from the results that quinalphos affected the oxygen consumption rate of fish under both sub lethal concentrations (Table 1). There was a significant decrease in oxygen consumption in common carp exposed to sub lethal concentration 0.275ppm (0.302- 0.426) and 0.55 ppm (0.342-0.366) compared to control (0.392-0.499). However, exposure to lethal concentration (2.75ppm), oxygen consumption significantly increased throughout the experimental period and highest oxygen consumption rate was attained during 12 hr (0.618) and 36 hr (0.572). While in sublethal concentration of 0.275 and

**Table 1 :** Oxygen consumption ( $\text{mg l}^{-1} \text{gm}^{-1} \text{h}^{-1}$ ) and Food consumption ( $\text{gm feed gm}^{-1} \text{body weight}$ ) of common carp at different concentration of Quinalphos

| Hour Conc.(ppm) | 12  | 24            | 36             | 48            |
|-----------------|---|---------------|----------------|---------------|
|                 | <b>Oxygen consumption (<math>\text{mg l}^{-1} \text{g}^{-1} \text{h}^{-1}</math>)</b> |               |                |               |
| Control         | 0.4990±0.042  | 0.4186±0.061  | 0.3923±0.022   | 0.3699±0.022  |
| 0.275           | 0.4263±0.029  | 0.3960±0.041  | 0.3036±0.028   | 0.3020±0.047  |
| 0.55            | 0.3663±0.026  | 0.3546±0.053  | 0.3423±0.042   | 0.3500±0.037  |
| 2.75            | 0.6180±0.087  | 0.5233±0.032  | 0.5723±0.029   | 0.5340±0.028  |
|                 | <b>Food consumption (g feed g<sup>-1</sup> body weight)</b>                           |               |                |               |
| Control         | 0.01705±0.015   | 0.01441±0.018 | 0.01821±0.0041 | 0.01488±0.011 |
| 0.275           | 0.01626±0.015   | 0.00716±0.010 | 0.01241±0.010  | 0.00731±0.012 |
| 0.55            | 0.01031±0.007   | 0.00615±0.001 | 0.00989±0.004  | 0.00514±0.012 |
| 2.75            | 0.00827±0.007   | 0.00448±0.010 | 0.00788±0.006  | 0.00426±0.011 |

P<0.05 significant difference

**Table 2 :** Determination 96hr LC<sub>50</sub> of Quinalphos in common carp

| Concentration (ppm) | No of fishes used | Mean % mortality |
|---------------------|-------------------|------------------|
| 2.5                 | 10                | 16               |
| 2.6                 | 10                | 36               |
| 2.7                 | 10                | 43               |
| 2.8                 | 10                | 56               |
| 2.9                 | 10                | 66               |
| 3.0                 | 10                | 76               |
| 3.1                 | 10                | 83               |
| 3.2                 | 10                | 100              |

0.55ppm, oxygen consumption reached minimum during 36<sup>th</sup> and 48<sup>th</sup> hr respectively.

A decrease in the respiratory rate in both the sub lethal concentration due to toxicant induced stress, avoidance and biotransformation. If gills or membrane functions are destroyed due to xenobiotic chemicals or the membrane functions are disturbed by a change in permeability the oxygen uptake rate would rapidly decrease (Grinwis *et al.*, 1998, Hartl *et al.*, 2001). Further, the metabolic rate in relation to respiration of fish could be increased under chemical stress (Chebbi and David, 2010).

Numerous studies have shown that fish such as *Cirrhinus mrigala* (Mushgeri and David, 2003), *Labio rohita* (Patil and David, 2008), *Oreochromis mossambicus* (Logaswamy and Remia, 2009) *Ctenopharyngodon idella* (Tilak and Swarnakumari, 2009), *Oreochromis niloticus* (Barbieri and Ferriera, 2010) and *Cyprinus carpio* (Singh *et al.*, 2010) may either increase or decrease their respiration rate in response to variety of pesticides.

The data on food consumption, calculated per gram body weight in sublethal and lethal concentrations of Quinalphos for common carp is given in Table 1. There was a significant decrease in food consumption of common carp exposed to both lethal (0.0042-0.0082) and sub lethal concentration (0.0071-

0.0162) and (0.0051-0.010) as compared to control (0.0144-0.182). The highest food consumption was attained during 36<sup>th</sup> hr in control (0.0182) and lower food consumption was attained during 48<sup>th</sup> hr in lethal concentration (0.0042).

*Oreochromis mossambicus* exposed to sub lethal level of phosphamidon and methyl parathion significantly affected the rates of feeding, absorption, metabolism and conversion (Singh *et al.*, 2010). Fish experiencing acute exposure to sub lethal concentrations of the insecticide exhibited significant feeding impairment with potentially severe consequences for their ecological fitness (Floyd *et al.*, 2008) presumably, the decrease in food intake may be due to increase in metabolic rate associated with tissue repair and development of defense and copper excreting metabolisms (Broeck *et al.*, 1997).

From the present study, it is evident that quinalphos (25% EC) was highly toxic and had a profound impact on the feeding behavior and respiratory rate of *Cyprinus carpio* exposed to sub-lethal concentrations of quinalphos.

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