



Short-term exposure to quinalphos induced biochemical and haematological changes in freshwater fish, *Oreochromis mossambicus*

K.C. Chitra*, P. Nikhila and K.P. Asifa

*Department of Zoology, University of Calicut, Malappuram-673635, Kerala, India.

Abstract: The toxic impact of quinalphos, an organothiophosphate, on the biochemical as well as haematological parameters was studied in the adult freshwater fish, *Oreochromis mossambicus*. In the present study, 0.5µl/ L quinalphos was chosen to represent sublethal concentration for 48 and 96 hours as short-term exposure and respective control animals were maintained. Quinalphos induced toxic stress to the exposed fishes, which is obvious by the reduction in the oxygen consumption of the fishes at the time of exposure and this could be due to shrinkage of the respiratory epithelium or possibly due to mucus accumulation on gills. Decrease in the haemoglobin content was observed and this may be due to either an increase in the rate at which the haemoglobin is destroyed or decreased rate of haemopoietic potential of the fish. In the present study, the significant increase in WBC count indicates hypersensitivity of leucocytes to quinalphos and these changes may be due to immunological reactions to produce antibodies to cope up with the stress. Decrease in the level of RBC count indicated decrease in erythropoietic activity or severe anemic state. Reduction in the plasma and tissue protein of quinalphos exposed fishes may be due to its utilization to mitigate the energy demand when the fishes are under stress. Increase of plasma glucose and the total glucose content in tissues like liver, muscle and gill might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by quinalphos stress. Thus the biochemical and hematological alterations due to acute short-term exposure to quinalphos were due to the toxic stress of the toxicant.

Keywords: Quinalphos, Haemoglobin, Fish, Oxygen Consumption, Glucose.

1. Introduction

The use of insecticides are being increased in the recent years to control the pest, in which only 1% of the pesticide applied hits the target pest while, the remaining 99% of the pesticide drifts into the environment contaminating soil, water and biota. This poses a constant threat to the non-target organisms especially to fishes; because pesticides are known to alter their behavior pattern, growth, nutritional value and physiology. Therefore, it becomes a matter of great concern when aquatic pollution due to pesticides are discussed. Quinalphos is one of the organophosphate insecticides extensively used in agriculture, which has become a matter of concern today because of its potentiality and hazardous effect. The recommended dose of quinalphos for field application is 0.188mg of

quinalphos per liter of water on rice field. Among this 50% of concentration goes into the water where fish is cultivated and ultimately lead to deleterious effects on aquatic organisms.

One of the early symptoms of acute pesticide poisoning is failure of respiratory metabolism where some pesticides decrease the oxygen uptake of fish. The rate of oxygen consumption can be used as a bio-detector in monitoring the physiological effects of toxicants and the oxygen consumption pattern will indicate the possible mapping of metabolic pathways influenced by the toxicant stress (Mushigeri and David, 2002). Apart from this haemolysis of red blood cells provides simple and rapid way of understanding the effect of pollutants on biological membranes (Harington *et al.*, 1971).

Blood is the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues of the body, the physiological state of an animal at a particular time is reflected in its blood. Thus blood parameters are considered as a pathophysiological indicator of the entire body and therefore important in diagnosing the structural and functional status of fishes exposed to toxicants (Sarkar *et al.*, 2004; Maheswaran *et al.*, 2008). So fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations. Fish live in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. Alteration in haematological and biochemical parameters of toxicant treated fish has recently emerged as an important tool for water quality assessment in the field of environmental toxicology. These studies could be used to indicate the health status of fish as well as water quality. Thus the objective of the present investigation is to determine the short-term effect of quinalphos on some selected haematological and biochemical parameters in freshwater fish, *Oreochromis mossambicus* and related effect from this exposure as a way to evaluate the toxicity risk of quinalphos to the test species.

2. Material and methods

2.1 Chemicals

Quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothioate; 70% EC), was a generous gift from Hikal Laboratory, Gujarat, India. All other chemicals were of analytical grade and obtained from local commercial sources.

2.2 Treatment

The lethal concentration for 50% killing of animals, (LC₅₀) values was computed on the basis of probit analysis (Finney, 1971) for 96 h, which was 5µl/L (Chitra *et al.*, 2012). In the present study, one-tenth of the dosage (0.5µl/L) quinalphos was chosen to represent sublethal concentration for 48 and 96 hours as short-term exposure and respective control animals were also maintained. Fishes of control and treatment groups were continuously monitored for acute toxicity testing. Oxygen consumption of fishes was measured to test the energy metabolism of the test animal. The body weights of fishes as well as the weights of organs as gill and liver from control and treatment groups were also measured at the end of the treatment. Biochemical and haematological examinations was performed at the end of each treatment.

2.3 Measurement of oxygen consumption

Oxygen consumption of fish was measured using modified Winkler's method (Kunemann and

Bashamohideen, 1978). To the collected sample of water in a 300ml BOD bottle 2ml MnSO₄ solution was added and followed by 2ml alkaline-iodide-azide reagent. The bottle was stopper carefully to exclude air bubbles and mixed by inverting bottle a few times. When precipitate has settled completely 2ml concentrated H₂SO₄ was added, recapped and mixed by inverting several times until the solution was completely mixed, then it was titrated against sodium thiosulphate solution. When it becomes light yellow, 2 or 3 drops of starch was added till it reaches colorless endpoint. The oxygen consumption of fish was then expressed in ml of oxygen consumed/g wet wt. of fish/h.

2.4 Tissue processing and biochemical analysis

At the end of each exposure period, fishes were caught very gently using a small dip net, one at a time with least disturbance. Each fish was held and wrapped with a clean, dry filter paper and the posterior half of its body was blotted with another clean coarse filter paper. Caudal peduncle of the fish (control and experimental group) was severed at a single stroke using a sharp blade. After discarding the first drop of blood, the freely oozing blood was collected in a small watch glass containing a sufficient quantity of anticoagulant (2% ethylenediaminetetraacetic acid - EDTA). The blood was thoroughly mixed with the anticoagulant using a thin, blunt glass rod, during the process of collection itself.

The whole blood was used for the estimation of erythrocyte and leucocytes counts (Rusia and Sood, 1992) and haemoglobin (Drabkin, 1946) in the control and experimental groups. The remainder of the blood sample was centrifuged at 4000 rpm for 3 minutes to separate the plasma, which was then used for the biochemical estimation of plasma protein (Lowry *et al.*, 1951) and carbohydrate (Trinder, 1969). Tissues such as gill, liver and muscle from control and treatment groups were also removed to analyze the total protein and carbohydrate.

2.5 Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) and the means were compared by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p < 0.05$ against control groups. Data are presented as mean \pm SD for ten animals per group. All biochemical estimations were carried out in duplicate.

3. Results

3.1 Effect of quinalphos on body weights

Exposure to quinalphos at the dose of 0.5µl/L for 96 h showed a significant decrease in the body weight. However, no significant changes in the body weights were observed in 48 h exposed fishes (Fig. 1).

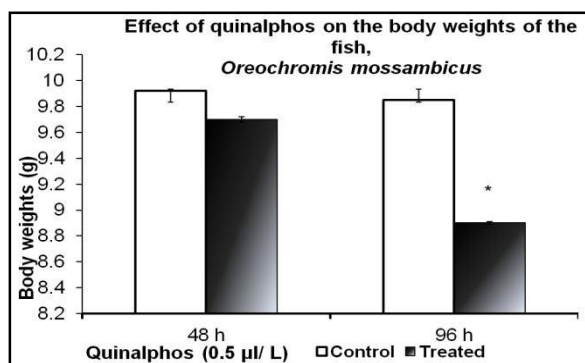


Fig. 1. Effect of quinalphos on the body weights of the fish, *Oreochromis mossambicus*.

3.2 Effect of quinalphos on organ weights

The weights of organs as liver and gill decreased significantly at the end of 48 and 96 hours of quinalphos exposure when compared with those of control fishes (Figs. 2 and 3).

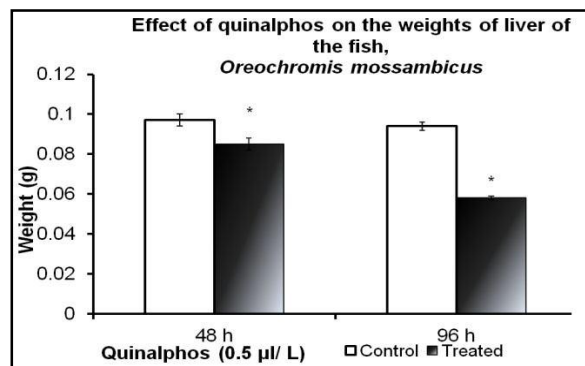


Fig. 2. Effect of quinalphos on the weights of liver of the fish, *Oreochromis mossambicus*.

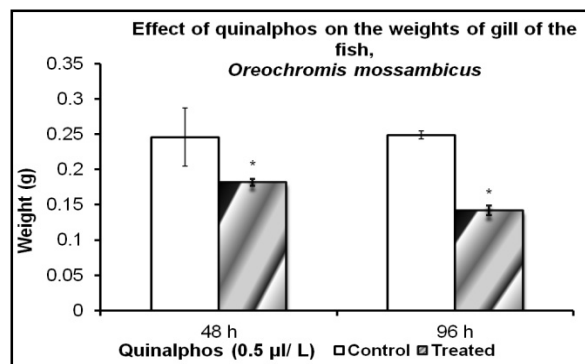


Fig. 3. Effect of quinalphos on the weights of gill of the fish, *Oreochromis mossambicus*.

3.3 Acute toxicity

In the toxic environment, fish exhibited irregular, erratic, and darting swimming movements, and loss of equilibrium followed by hanging vertically in water. They slowly became lethargic, restless, and secreted excess mucus all over the body. Body surface darkening was noticeable in this phase of poisoning,

mainly on the dorsal part. The surface of the body was opaque with slightly increased amount of mucus and with expressive pigmentation mainly on the dorsal part. 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.

3.4 Effect of quinalphos on oxygen consumption

A reduction in oxygen consumption was observed after the fish exposed to quinalphos at the dose of 0.5µl/L for 48 and 96 hours (Fig. 4).

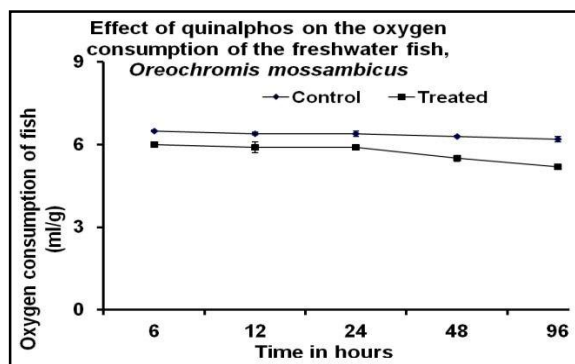


Fig. 4. Effect of quinalphos on the oxygen consumption of the freshwater fish, *Oreochromis mossambicus*.

3.5 Effect of quinalphos on haematological parameters

Quinalphos at the dose of 0.5µl/L for 48 and 96 hours showed a significant decrease in the level of Hb (%) and total RBC whereas WBC count was increased significantly ($p < 0.05$) when compared to the control animals (Figs. 5, 6 and 7).

Treatment of quinalphos at the dose of 0.5µl/L for 48 and 96 hours showed a significant ($p < 0.05$) increase in the level of blood glucose whereas it significantly decreased the blood protein in the treated groups as compared with the control fishes (Figs. 8 and 9).

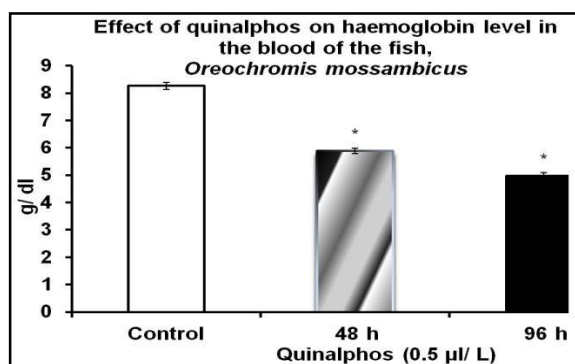


Fig. 5. Effect of quinalphos on haemoglobin level in the blood of the fish, *Oreochromis mossambicus*.

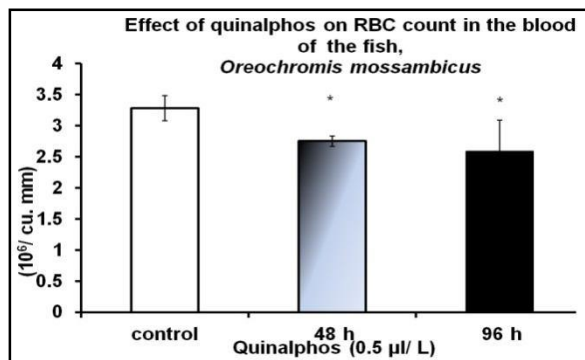


Fig. 6. Effect of quinalphos on RBC count in the blood of the fish, *Oreochromis mossambicus*.

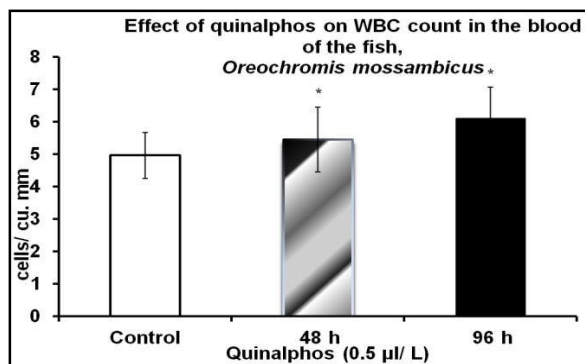


Fig. 7. Effect of quinalphos on WBC count in the blood of the fish, *Oreochromis mossambicus*.

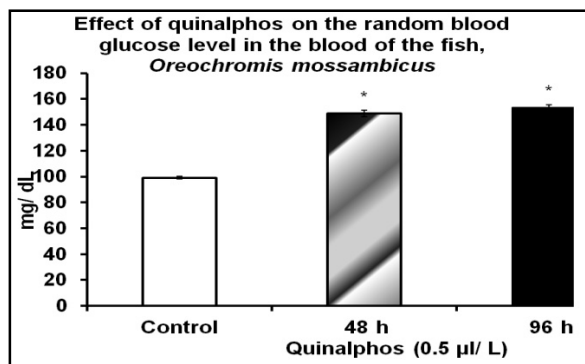


Fig. 8. Effect of quinalphos on the random blood glucose in the blood of the fish, *Oreochromis mossambicus*.

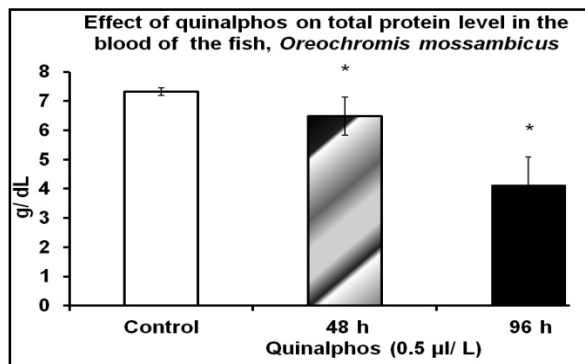


Fig. 9. Effect of quinalphos on total protein level in the blood of the fish, *Oreochromis mossambicus*.

3.6 Effect of quinalphos on biochemical parameters

Exposure to quinalphos at the dose of 0.5 $\mu\text{l/L}$ for 48 and 96 hours showed a significant decrease in the protein whereas increased the level of glucose in liver, muscles and gills of the quinalphos-treated fishes than that of the control group (Figs. 10 and 11).

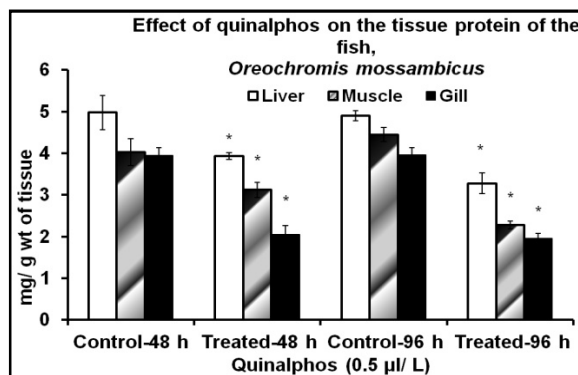


Fig. 10. Effect of quinalphos on the tissue protein of the fish, *Oreochromis mossambicus*.

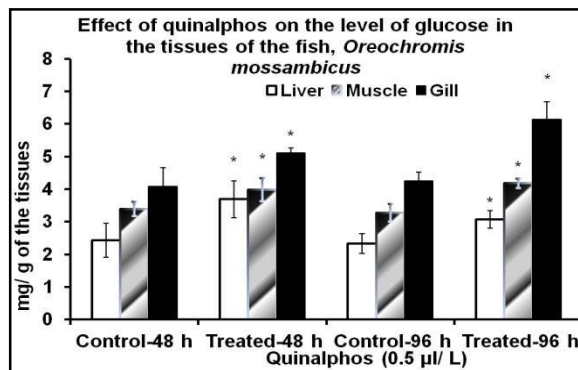


Fig. 11. Effect of quinalphos on the level of glucose in the tissues of the fish, *Oreochromis mossambicus*.

4. Discussion

Aquatic ecosystems that run through agricultural areas have high probability of being contaminated by run-off and groundwater leaching by a variety of pollutants. A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes. In India as much as 70% of the chemical formulations employed in agricultural practices are believed to affect non-target organisms and find their way to freshwater bodies, ultimately polluting them. In the present study, one of the organophosphorus pesticides quinalphos was used to evaluate its short-term toxicity in freshwater fish, *Oreochromis mossambicus*.

The body weights of fishes were monitored throughout the study to ensure the effect of toxic compounds on the general health status of the animals. Exposure to quinalphos at the dose of 0.5 $\mu\text{l/L}$ for 96 h showed a slight decrease in the body weight. However,

no significant changes in the body weights were observed in 48 h exposed fishes. Reduction in body weight gain may reflect a variety of responses, including internal and external environmental factors such as rejection of feed, treatment-induced anorexia or systemic toxicity.

In the present study, the weights of gill and liver were significantly decreased after 48 and 96 hours of quinalphos treatment. The decrease in the weights of liver might be due to the result of atrophy or necrosis of hepatocytes (Busacker *et al.*, 1990). Reduction in the weights of gill could be due to degeneration of gill filament and lamellar epithelium which was confirmed by the histopathological study of gill in quinalphos exposed fish (Chitra *et al.*, 2012). In the present study of acute toxicity testing an increased secretion of mucus by the skin was observed which is considered as a defensive mechanism of the fish that make its body slippery for quick movement in the test solution and avoids the absorption of the toxicant by the general body surface. Similar observation was reported in the toxic effect of two pesticides, rogor and endosulfan to the air-breathing fish, *Heteropneustes fossilis* (Borah and Yadav, 1995).

One of the early symptoms of acute pesticide poisoning is failure of respiratory metabolism. The respiratory potential and the oxygen consumption of the animal are the important physiological parameters to assess the toxic stress because it is a valuable indicator of energy expenditure during metabolism (Proser and Brown, 1973). It was clearly evident from the present results that quinalphos affected respiratory rate of *O. mossambicus*. In the present study, decrease in oxygen consumption uptake from quinalphos-exposed water may be mainly due to shrinkage of the respiratory epithelium (Chitra *et al.*, 2012) or possibly due to mucus accumulation on gills.

In such a case, because of decrease in oxygen consumption also reported to cause pronounced haematological changes (Tilak and Satyavardhan, 2002). According to Blaxhall and Daisley (1973), the determination of haemoglobin concentration can be a good indicator of detecting anemic conditions in fish. In the present study quinalphos at the dose of 0.5µl/L for 48 and 96 hours showed a significant decrease in the level of Hb (%) and total RBC whereas WBC count was increased significantly when compared to the control animals and this could be due to haemodilution, a mechanism that reduces the concentration of the pollutants in the circulatory system (Smit *et al.*, 1979). The other possible reason for the decrease in the haemoglobin concentrations of fishes under toxic stress might be due to either an increase in the rate at which the haemoglobin is destroyed or due to decreased rate of haemoglobin synthesis (Reddy and Bashamohideen, 1989) or might be attributed to depression/exhaustion of haemopoietic potential of the fish (Seth and Saxena, 2003).

In the present study decrease in total red blood cells (RBC) count during quinalphos treatment suggesting a decrease in erythropoietic activity or severe anemic state. White blood cells (WBC), or leukocytes, are important component of the immune system involved in defending the body against both infectious diseases and foreign materials. The increased total leucocytes count of the quinalphos-exposed fish in the present study indicates increased defensive reaction against the stressors. The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to pesticide (Joshi *et al.*, 2002). In the present study, the significant increase in WBC count indicates hypersensitivity of leucocytes to quinalphos and these changes may be due to immunological reactions to produce antibodies to cope up with the stress induced by quinalphos. Increased WBC count has also been reported in fishes exposed to other certain xenobiotics like endosulfan (Abidi and Srivastava, 1988).

In fish, proteins are the primary energy source and are involved in regulating physiological and metabolic processes in the body and play a vital role as energy precursors in fishes exposed to stress conditions. In the present study, decrease in blood protein and tissue protein after 48 and 96 hours of quinalphos exposure could be attributed to the enhanced activities of proteases, lower protein synthesis at the transcriptional level and increased proteolysis due to utilization for metabolic processes under quinalphos toxicity. The decrease in the protein content may also be due to its utilization to mitigate the energy demand when the fishes are under stress (Chandravathy and Reddy, 1994; Soyngbe *et al.*, 2012).

Carbohydrates are the first substrates to be utilized in metabolism more under toxic stress conditions. Changes in blood glucose have been suggested as useful general indicator of environmental stress in fish (Nemcsok and Boross, 1982). In the present study, the significant increase of plasma glucose level might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by quinalphos stress, particularly in osmoregulation which may contribute to the restoration of plasma osmolarity.

In the present study exposure to quinalphos at the dose of 0.5µl/L for 48 and 96 hours showed a significant increase in the level of glucose in liver, muscles and gills than that of the control group. This could be due to increased glycogenolysis or decreased glycogen synthesis. Depletion in the level of glucose in gill tissue might be due to damage to the surface of gill filaments when the freshwater fishes were exposed to quinalphos.

5. Conclusions

It can be concluded from the present study that quinalphos shows high toxicity on the freshwater fish *Oreochromis mossambicus* even at a short-term

exposure as the fishes are very sensitive to the presence of even minute quantities of toxicant in their environment causing severe metabolic stress. Thus haematological and biochemical parameters serve as one of the good biotest for assessing the toxic hazards to fish.

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