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INVESTIGATION OF ACUTE TOXICITY OF PHORATE (AN ORGANOPHOSPHATE) ON FRESH WATER FISH *CYPRINUS CARPIO*

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ABSTRACT

Acute toxicity of Phorate, a broad-spectrum organophosphorus insecticide for numerous crops throughout the world and potential toxic pollutant contaminating aquatic ecosystems, was investigated on the fingerlings of *Cyprinus carpio* using static bioassays over a period of 96 hours. The mortality rate of *Cyprinus carpio* was monitored under laboratory conditions for the period of 96 hrs. Bioassays were repeated three times and the data obtained was evaluated using Finney's Probit Analysis Statistical Method. The 96 hours LC_{50} of the exposed fish to Phorate was determined to be 0.71 ppm/lit. The behavioural changes observed against Phorate intoxication were increased opercular movement, respiratory distress such as gasping for air, loss of equilibrium and erratic swimming prior to mortality. In the treated fishes significant change in the colour of the gills from dark red to brownish black, high mucus films over surface of gills was also observed.

KEY WORDS: **Acute toxicity, Phorate, *Cyprinus carpio,* LC_{50}, mortality**

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INTRODUCTION

Environment is the representative of the physical components of the earth where man is the important factor in influencing it. Environmental pollution is the biggest menace to the human race on this planet today. Environment pollution is a worldwide problem and its potential to influence the health of human populations is great [1, 2]. Human exposure to pollution is believed to be more intense now than at any other time in human existence [3]. Aquatic environment is the ultimate sink for all pollutants where they are going to affect the zoans living in the water.

The dependence of modern agriculture on agrochemicals like pesticides heavily is emerging as a threat to the ecological balance of aquatic ecosystems. Synthetic pesticides like organophosphates used for controlling pests in agriculture are one of the major causes of aquatic pollution. The residues of these pesticides mostly reach into aquatic ecosystems through surface run off and affect the health of non target organisms including fish. Agricultural runoff, effluents from pesticide manufacturing industries, or accidental spillage pose a serious threat to the aquatic environment, affecting aquatic lives including that of fish as well as other organisms [4, 5].

Organophosphates are widely used throughout the world as an impotant group of pesticides because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment [6]. Phorate is a soil and systemic insecticide and miticide, used for the control of sucking and chewing insects, mites and soil dwelling pests in sugarcane, rice, beetroot, carrots, maize, sorgum, potatoes, tomatoes, soya beans, wheat, chillies, onion, sunflower, cotton, groundnut, coffee, some ornamental plants, herbaceous plants and bulbs.

Fishes are aquatic vertebrates that are members of the largest and most diverse vertebrate taxon which are the trophic level connection in aquatic ecosystems. Hence, fish bioassay experiments are indices to determine the acute toxicity and possible effect on metabolism due to the toxicant stress [7, 8]. Hence in the present investigation, an organophosphate insecticide Phorate is selected, which is widely used in the local area to combat pests, to investigate the acute toxicity on a fresh water edible fish *Cyprinus carpio*, a representative of the aquatic environment.

MATERIALS AND METHODS

The Indian major carp *Cyprinus carpio* (Linnaeus, 1758) is selected as the ideal experimental model for the present investigation. It is popularly known as common carp, widely present in temperate regions of the world, which is extensively cultivated in freshwater ecosystems. *Cyprinus carpio* fish were collected from the department of fisheries, Anantapur, Andhra Pradesh and were immediately transported in big 20 Lts fish containers each with 50 fish to the laboratory. Then they were released into large cement tanks with sufficient dechlorinated tap water and allowed to acclimatize for 15 days. The fish were fed with commercial fish pellets daily. Then the fish were separated into the batch of having the size 10 ± 2 gm and were maintained in static water without any flow [9]. Water was renewed every day to provide fresh water, rich in oxygen.

As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of animals, size, sex etc., [10], precautions were taken throughout this investigation to control all these factors. As a part of its water from the same source has been used for maintenance of fish. Physico, chemical factors of water used for the experiment are pH 7.5 to 7.7, salinity 0.193 gm/liter, dissolved O_2 6-8 ml/ liter, alkalinity 88 ppm (as Ca CO_3), chlorinity 0.112 gm/liter, sodium 1.23 m.moles/liter, potassium 30.6 m.moles/liter, calcium 4.32 m.moles/liter, CO_2 2.09 mg/liter, O_2 % saturation 8, hardness of water 160 ppm (as Ca CO_3). The animals were starved for 24 hours prior to each estimation, to avoid any influence of

differential feeding. The size of the animals selected was also maintained strictly throughout the investigation.

Pesticide selected for this study is phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate) an organophosphorus insecticide which is widely used on numerous crops including paddy. Commercial grade of phorate (10% CG) was selected to study its effect on fish *Cyprinus carpio* . The pesticide was obtained from the local market, which was manufactured by Hyderabad Chemical Products Ltd. Commercial formulation of this pesticide is used because only commercial preparation is used in agriculture. Stock solutions were prepared with tap water. The quantity of tap water used to be non-toxic to non-target animals and it was biologically safe in preparation of stock solution of pesticides [11].

Lethal concentration (LC_{50}) of phorate to *Cyprinus carpio* was determined by "Probit method" of Finney [12]. The percent mortality of the fish in different concentrations of phorate was determined immediately after 96 hours of exposure. For this the experimental fish were divided into batches of thirty each, and were exposed to different concentrations of phorate and each batch to one concentration, ranging from 0.4 ppm to 1.1 ppm. This range was obtained on trial and error basis. Toxicity evaluation was carried out in static water and mortality rate was observed in all concentrations of phorate immediately after 96 hours of the exposure period. A batch of fish separately maintained alongside in fresh water medium without phorate served as control. The experiment was repeated thrice for accuracy. The mortality rate at each concentration, derived from the mean of three replications was converted as percent mortality value. From this, the probit mortality value was obtained [12].

The percent mortality values as well as probit mortality values were plotted separately against the pesticide concentration and LC_{50}s at 96 hours were derived from these two curves. For subsequent verification the LC_{50}s obtained by graphical methods, Dragstedt and Beheren's method as given by Carpenter [13] was employed. As per this method the animals were exposed to log 2 concentrations of pesticide for the same exposure period. The percent mortality values were calculated from the cumulative mortalities, with them LC_{50} was derived. Finally the LC_{50} at 96 hours of phorate was obtained by taking the mean of LC_{50} s derived from percent and probit mortality curves and Dragstedt and Beheren's method.

RESULTS AND DISCUSSION

The data on the percent and probit mortality of the fish, *Cyprinus carpio* in different concentrations (ppm/l) of phorate at 96 hours of exposure are presented in Table-I. From the data it is clear that there was a linear relationship between the percent or probit mortality of the fish and the concentration of phorate. Thus the percent and/or probit mortality of the fish increased with the increase in phorate concentration. The percent mortality plotted against log concentrations of pesticide gave sigmoid curve (Graph-I), whereas the probit mortality plotted against log concentrations gave straight line (Graph-II). The 96 hours LC_{50} of phorate to fish was obtained by taking the means of LC_{50}s derived from the percent and probit mortality curves, as well as the value calculated by Dragstedt and Beheren's method [13]. The LC_{50}s obtained by these three methods along with their mean are presented in Table-II for comparison. The mean of these three methods was considered as LC_{50} in evaluating the level of phorate toxicity to the fish *Cyprinus carpio* (*C.carpio*).

Table-I

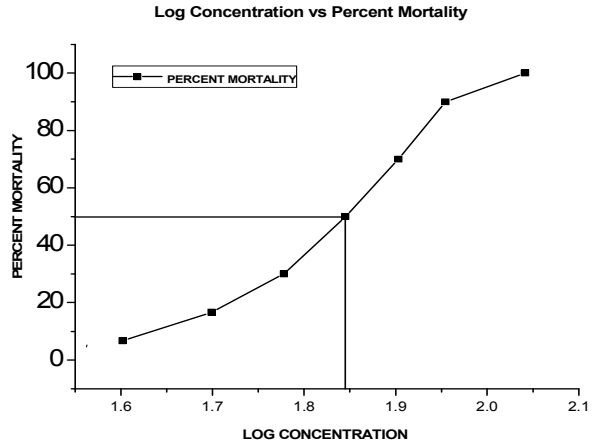
96 hours percent and probit mortality of C.carpio in different concentrations of phorate.

Concentration (ppm)	Log Concentration	Number of Fish exposed	Number of Fish dead	Percent Mortality	Probit Mortality
0.4	1.6020	30	2	6.7	3.52
0.5	1.6989	30	5	16.6	4.05
0.6	1.7781	30	9	30	4.48
0.7	1.8451	30	15	50	5.0
0.8	1.9031	30	21	70	5.52
0.9	1.9542	30	27	90	6.28
1.1	2.0414	30	30	100	7.33

Each value is a mean of three replicants.

Graph-I

Percent mortality of the fish C.carpio against log concentration of phorate (sigmoid curve).



Graph-II

Probit mortality of the fish C.carpio against log concentration of phorate (linear curve).

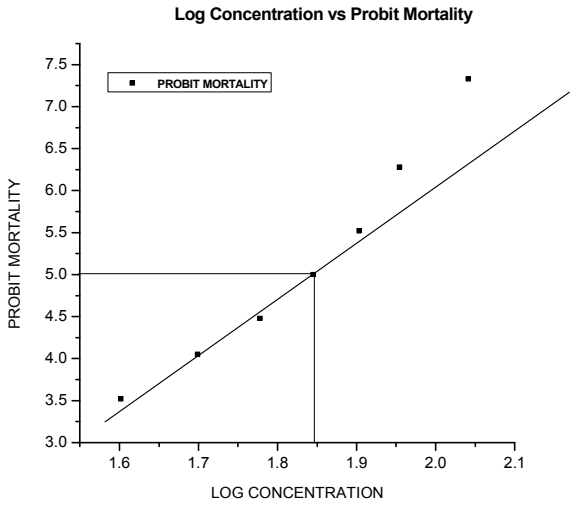


Table-II
LC_{50} of phorate to the fish, C. carpio after 96 hours exposure

Name of the method	LC_{50}/96 hours (ppm/lit)
Percent mortality (sigmoid curve)	0.70
Probit mortality (linear curve)	0.70
Dragstedt and Behren's method	0.75
Mean	0.71
S. D. ±	0.029

S. D. = Standard Deviation

Research in the area of toxicology on the effects of phorate on fish was scarcely done. However, some work was carried out by Saxena and Sarin [14, 15] on desert gerbil *Meriones hurrianae*; Mohssen Morowati [16, 17, 18] on the male swiss albino mouse, *Mus musculus*, Jyothi and Narayan [19] on fresh water fish *Clarias batrachus* and Anand Pratap Singh et al., [20] on snake headed fish *Channa punctatus*, about the toxic effects of phorate. Reported 96-hours LC_{50} values of phorate are 0.8 ppm in *Clarias batrachus* [19] and 0.3mg/L in *Channa punctatus* [20].

The level of toxicity of pesticides may differ from one type to the another, and from one species to the another [21, 22, 23]. The differences could be attributed to the chemical nature of the toxicant, interaction of the chemical with biological system, resistance capacity of the animal, detoxification mechanisms involved, assay techniques, purity of the pesticide and the additives or emulsifiers present in the commercial grade formulations. Different metabolic pathways occurring among fish species may result in different patterns of biotransformation, leading to more or less toxic metabolites [24]. Magnitude of toxic effects of pesticides also depends on length and weight, corporal surface/body weight ratio and breathing rate [25, 26]. This is in agreement with Sprague [27] who observed variation in LC_{50} values for the same species and toxicant depending on size, age and condition of test species along with experimental factors.

In the present study the fish maintained in freshwater without phorate behaved normally i.e., they were very active and movements were well coordinated. They were alert and at any site disturbance they swam faster. But in lethal and near lethal concentrations of phorate they became highly irritable and hyper excitable. Jumping movements were observed, with profuse mucus secretion and loss of equilibrium. Examination of gills revealed significant change in their colour from dark red to brownish black. High mucus films over the surface of gills were also observed. Some of the other behavioral changes observed in the fish exposed to phorate include increased opercular movement, dullness, loss of equilibrium, stop of food intake, erratic and hysteric swimming, swimming at the water surface, circling movement, and gasping. Similar type of behavioral changes were also observed in *Heteropneustes fossilis* on exposure to an organophosphate insecticide, dimethoate [28]. Prior to death, the fish became less active or generally inactive, remained hanging vertically in the water or lay down on their sides at higher concentrations. Under lethal conditions the fish slowly became sluggish with short jerky movements and erratic opercular activity; finally turned upside down and died.

Behavioral changes observed in the present study in phorate exposed carp, *Cyprinus carpio* appear to be a manifestation of phorate toxicity. Upon exposure to this pesticide, increase in the surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in poisoned water. Similar observations have been reported in *Anabas testudineus* after exposure to monocrotophos [29]. Moreover, the hypoxic condition also contributes to increase surfacing as reported by Radhaiah and Jayantha [30]. Hypoxic condition arises primarily due to damage of gills of pesticide exposed fish which hampers oxygen uptake [31]. Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to the accumulation of acetylcholine in synaptic

and muscular junctions [32]. Paralytic movements and suffocation caused by the mucus film over gills could be a few reasons for the death of fish at lethal concentrations.

CONCLUSION

From the present study it is very clear that phorate is highly toxic to freshwater fish, *Cyprinus carpio*, as the LC_{50} of this compound to the fish is obtained at very low concentration. It may be concluded that the results of this study are highly useful in evaluating the phorate toxicity in a fresh water environment. On the whole, with the knowledge of toxicity studies and behavioral observations, it could be possible to establish limits of tolerance and susceptibility of the fish to the toxicity of phorate in the fresh water environment.

REFERENCES

1. Fereidoun H, Nourddin MS, Rreza NA, Mohsen A, Ahmad R and Pouria H, The Effect of Long-Term Exposure to Particulate Pollution on the Lung Function of Teheranian and Zanjanian Students, Pakistan Journal of Physiology, 3(2): pp. 1-5, (2007).
2. Progressive Insurance, Pollution Impact on Human Health, Retrieved from Pryde LT, 1973,"Environmental Chemistry", An Introduction, (2005).
3. Schell LM, Gallo MV, Denham M and Ravenscroft J, Effects of Pollution on Human Growth and Development: An Introduction, Journal of Physiological Anthropology, 25(1):103-112, (2006).
4. Dabrowski JM, Peall SKC, Reinecke AJ, Liess M and Schulz R, Runoff-related pesticide input into the lourens river, South Africa: basic data for exposure assessment and risk mitigation at the catchment scale. Water, Air, Soil Pollut. 135, 265-283, (2002).
5. Anasco N, Uno S, Koyama J, Matsuoka T and Kuwahara N, Assessment of pesticide residues in freshwater areas affected by rice paddy effluents in Southern Japan. Environ. Monit. Assess, 160, 371-383, (2010).
6. Singh RN, Pandey PK, Singh NN and Dass VK, Acute toxicity and behavioral responses of common carp *Cyprinus carprio* (Linn.) to an Organophosphate (Dimethoate). World Journal of Zoology. 5 (3): 183-188, (2010).
7. Murty AS, Toxicity of pesticides to fish, Vol. I and II, CRC Press Inc., Bocaraton. pp. 483 and 355, (1986).
8. Subramanian, M.A, Toxicology MJP Publishers, Chennai. p. 202, (2004).
9. Doudoroff P, Anderson BG, Bordwic GE, Galtsolf BS, Hart NB, Patrick R, Strong ER, Surber EW and Vanfforn WH, Bio-assay method for evaluation of acute toxicity of industrial wastes to fish sewage. Indi. Wastes, 23:1380-1397, (1951).
10. Sivaramakrishna B, Suresh A and Radhakrishnaiah K, Assessment of mercury toxicity by the changes in oxygen consumption and ion levels in the fresh water snail *Pila clobosa* and the mussel *Lamellidens marginalis*. Bull. Environ. Contamn. Toxicol, 46:913-920, (1991).
11. Jagannatha Rao KS, Solvent validity test models. Action Committee Report FRAME, Committee Royal Society Building, London, U.K.120, (1981).
12. Finney DJ, Probit Analysis, 3rd edition, Cambridge University press, Cambridge, pp.333, (1971).
13. Carpenter PL, In: Immunology and Serology, 3rd edition, W.B.S.Saunder company, Philadelphia, London, Toronto, p.254, (1975).
14. Saxena AK and Sarin K, Effect of repeated administration of thimet (phorate) on testes of the desert gerbil *Meriones hurrianae* (Jerdon): biochemical and histopathological studies. Indian Journal of Experimental Biology., 17, 863-865, (1979).
15. Saxena AK and Sarin K, Pathological and biochemical changes in the liver and testis of the desert gerbil *Meriones hurrianae* (Jerdon). Effect of single intraperitonial injection of phorate (thimet). Indian Journal of Experimental Biology,18, 1001-1004, (1980).

16. Mohssen Morowati, Inhalation toxicity studies of Thimet (Phorate) in male swiss albino mouse, *Mus musculus* I. Hepatotoxicity. Environmental Pollution, Vol. 96, No. 3, pp. 283-288, (1997).
17. Morowati Mohssen, Biochemical and Histopathological Changes in Serum Creatinine and Kidney Induced by Inhalation of Thimet (Phorate) in Male Swiss Albino Mouse, *Mus musculus* . Environmental Research., Vol 87 (1), 31-36, (2001).
18. Morowati M, Inhalation toxicity studies of Thimet (Phorate) in the male swiss albino mouse, *Mus musculus*: II. Lung histopathology, pseudocholinesterase level and haematological studies. Environmental Pollution, 103: 309-315, (1998).
19. Jyothi B and Narayan G, Certain pesticide-induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus*. Food and Chemical Toxicology., 37: 417-421, (1999).
20. Anand Pratap Singh, Surendra Singh, Prabhat Bhartiya and Khushbu Yadav, Toxic effect of Phorate on the Serum Biochemical Parameters of Snake Headed Fish *Channa punctatus* (Bloch) . Advances in Bioresearch , Vol 1 (1) : 178 – 182, (2010).
21. Pickering QH, Henderson C and Lemke A, The toxicity of organic phosphorus insecticides to different species of warm water fishes. Trans. Amer. Fish. Soc. 91(2): 175-184, (1962).
22. Macek KJ and Mc. Allister WA, Insecticide susceptibility of some common fish family representatives. Trans. Amer. Fish. *Soc*., 99:20, (1970).
23. Chambers JE and Yarbrough JD, Parathion and methyl parathion toxicity to insecticide resistant and susceptible mosquito fish (*Gambusia affinis).* Bull. Environ. Contam Toxicol., 11: 315-320, (1974).
24. Johnsson CM and Toledo MCF, Acute toxicity of endossulfan to the fish *Hyphessobrycon bijassciatus* and *Brachydanio rario*. Archiv environ. Cuta Toxicol. 24: 151-155, (1993).
25. Singh BB and Narain AS, Acute toxicity of thiodane to cat fish, *Heteropneustes fossilis*. Bull Environ. Conta Toxicol, 28: 122-127, (1982).
26. Murty AS, Toxicity of pesticides to fish. CRC Press Inc., Boca Raton, F.*L*.,143p (1986).
27. Sprague JB, Measurement of pollutant toxicity to fish: Bioassay methods for acute toxicity water, Res. 3: 793-821, (1969).
28. Anoop Kumar Srivastava, Diwakar Mishra, Shilpee Shrivastava, Sunil kumar Srivastav and Ajai K Srivastav, Acute toxicity and behavioural responses *of Heteropneustes fossilis* to an organophosphate insecticide, dimethoate. Int. J. of Pharma and Bio Sciences, 1(4): B 359-363, (2010).
29. Santhakumar M and Balaji M, Acute toxicity of organophosphorus insecticide monorotophos and its effect on behaviour of an airbreathing fish, *Anabas testudineus* (Bloch). J. Environ Biol. 21(2):121-123, (2001).
30. Radhaiah V and Jayantha KR, Behavioral response of fish *Tilapia mossambica* exposed to fenvalerate. Environ. Ecol. 6(2): 2-23, (1998).
31. Velmurugan B, Selvanayagam M, Cangiz E and Unlu E, The effects of monocrotophos to different tissues of freshwater fish *Cirrhnus mrigala.* Bull. Environ. Contam toxicol. 78(6): 450-454, (2007).
32. Rao JV, Begum M, Pallela M, Usman PK. and Rao RN, Chnages in behavior and brain acetylcholinestarase activity in mosquito fish, *Gambusia affinis* in relation to sublethal exposure of chlorphyrifos. Intl. j. Environ Res. Public health. 2(3-4): 478-483, (2005).