International Journal of Pharma and Bio Sciences

ACUTE TOXICITY AND BEHAVIOURAL RESPONSES OF *HETEROPNEUSTES FOSSILIS* TO AN ORGANOPHOSPHATE INSECTICIDE, DIMETHOATE

ANOOP KUMAR SRIVASTAVA*, DIWAKAR MISHRA†, SHILPEE SHRIVASTAVA**, SUNIL KUMAR SRIVASTAV** AND AJAI K. SRIVASTAV**[1]

*Department of Zoology, D.A.V. Post Graduate College, Azamgarh (U.P.), India
†Department of Zoology, Government Girls Post Graduate College, Ghazipur (U.P.), India
**Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273 009 (U.P), India

[1]*Corresponding Author* ajaiksrivastav@hotmail.com

ABSTRACT

Static renewal evaluation of the acute toxicity of an organophosphate pesticide, dimethoate against the freshwater fish *H. fossilis* was conducted in the laboratory. *H. fossilis* showed behavioural changes against dimethoate intoxication. There were increased opercular movement, sluggish, lethargic and abnormal swimming, loss of buoyancy and muscular tetany. The treated fishes also showed fading of their body colour. The LC_{50} values of dimethoate to the freshwater catfish, *Heteropneustes fossilis* at various exposure periods are 15.92 mg/l for 24 h; 13.42 mg/l for 48 h; 12.39 mg/l for 72 h and 11.34 mg/l for 96 h. The upper confidence limits were 16.59, 14.72, 13.54 and 12.86 mg/l for 24, 48, 72 and 96 h and lower confidence limits were 15.32, 12.18, 11.23 and 9.79 mg/l, respectively. From the present study it seems that the freshwater catfish, *H. fossilis* is more susceptible to dimethoate toxicity as the LC_{50} value for this organophosphate is less than other reported fish species. These results indicate that dimethoate exposure to the fish caused toxic effects.

KEY WORDS

LC_{50}, dimethoate, fish, toxicity, organophosphate

INTRODUCTION

Application of pesticides has contributed greatly in enhancing agricultural yields and also for the control of insect-borne diseases. Excessive use of broad-spectrum or non-selective pesticides damages the ecosystem, sometimes irreversibly, contaminates soil surface and ground water as well as food chains and thus compromises the health and well being of the inhabitants of aquatic and terrestrial environment. Organophosphate compounds comprise insecticides currently used worldwide for agricultural and household applications. These insecticides produce toxicity by inhibition of the enzyme acetylcholinesterase which accumulates in the synapses of the central and peripheral nervous system. This in turn results into overactivation of postsynaptic cholinergic receptors and signs of cholinergic neurotoxicity. Among various groups of pesticides, organophoshates are more frequently used, due to their high insecticidal property, low mammalian toxicity, less persistence and

rapid biodegradability in the environment. Dimethoate [IUPAC name- O,O-dimethyl-S-methyl carbamoyl methyl phosphoro-dithioate] is an organophosphate (trade name Rogor) possessing contact and systemic properties.
Fish serves as a bioindicator species as it responds with great sensitivity to changes in the aquatic environment and thus, has an important role in the monitoring of water pollution. The purpose of this investigation was to evaluate the acute toxicity of an organophosphate pesticide -- dimethoate for the catfish, *Heteropneustes fossils* in static renewal bioassay. *H. fossilis* was selected because it is hardy, readily available, easy to handle and can be kept alive for longer duration in the aquaria. This is a common edible freshwater fish of great economic connotation and forms an important species in many water resources mainly ponds, ditches, swamps, marshes and sometimes occurs in muddy rivers[1]. The insecticide dimethoate was selected for study because it is a widely used organophosphate insecticide to kill mites and aphids among other insects and is applied on citrus, cotton, fruit, olives, potatoes, tea, tobacco and vegetables. It is also used on flies in home gardens and on livestock. Dimethoate is highly soluble in water and can leach into nearby water sources and affect aquatic organisms[2]. This is a low persistence pesticide possessing half-life of 4-16 days but can last longer depending on the conditions[3].The concentration of dimethoate has been detected in 11.3% of 2061 California surface water samples from 1991-2005, with a maximum concentration of 11.31 μg/L[4].

MATERIALS AND METHODS

Live specimens of adult freshwater catfish *Heteropneustes fossilis* (both sex, body wt 30-42 g) were collected locally during the pre-spawning phase from Ramgarh Lake (Gorakhpur). They were kept into large plastic tanks containing 500 liters of dechlorinated tap water for acclimatization for 15 days. The physicochemical characteristics of the tap water used are as follows: pH 7.42 ± 0.08, dissolved oxygen 8.24 ± 0.18 mg/l, temperature 20.26 ± 1.32 C, hardness 160.8 ± 5.32 mg/l, and electrical conductivity 286.36 ± 60.42 μmho/cm, respectively.
Four day static renewal toxicity test[5] was performed to determine the LC_{50} values of dimethoate. Stock solution of dimethoate was prepared in acetone and the desired concentration was obtained by diluting with tap water for preparation of test solution. Five replicates, each containing ten fish were subjected to dimethoate at concentration of 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19 mg/l. Control groups, each having ten fish kept in tap water containing acetone (0.4 ml per liter) was run concurrently. All experiments were carried out in cylindrical glass aquaria containing 30 liters of test solution. All solution (control and test) were renewed daily and dead fishes were immediately removed.
At different exposure periods (24, 48, 72 and 96 h), the behavioural changes and mortality of the fish was observed. The mortality of the fish was subjected to Probit analysis with the POLO-PC software (LeOra Software) to calculate the LC_{50} and upper and lower confidence limits at 95% level.

RESULTS AND DISCUSSION

The untreated fish *Heteropneustes fssilis* kept in the glass aquaria tend to move together. They come to the surface at intervals to gulp air. A number of changes were observed in the behaviour of *H. fossilis* exposed to dimethoate. Fishes came to the surface of water much more frequently. They also occasionally tried to jump out of water. Moreover, dimethoate treated fish exhibited increased opercular movement, increased mucous secretion and progressively became sluggish and lethargic. Prior to death in contaminated medium, the fishes mostly showed abnormal swimming movements including loss of orientation and a tendency of muscular tetany. The dimethoate treated fish also showed fading of their body colour when compared to controls.
The per cent mortality of *H. fossilis* after exposure to various concentrations of dimethoate for 24, 48, 72 and 96 h has been depicted in Fig. 1. The LC_{50} values at various exposure periods

are 15.92 mg/l for 24 h; 13.42 mg/l for 48 h; 12.39 mg/l for 72 h and 11.34 mg/l for 96 h. The upper confidence limits were 16.59, 14.72, 13.54 and 12.86 mg/l for 24, 48, 72 and 96 h and lower confidence limits were 15.32, 12.18, 11.23 and 9.79 mg/l, respectively.

H. fossilis showed behavioural changes against dimethoate intoxication. These were increased opercular movement, sluggish, lethargic and abnormal swimming, loss of buoyancy and muscular tetany. The treated fishes also showed fading of their body colour. These behavioural changes can be considered as symptoms of stress on account of the toxicological nature of the environment. The behavioural changes showed by the fishes after dimethoate intoxication are similar to those observed in other fishes exposed to organophosphate pesticides[6,7,8,9]. Altered movement of *Channa gachua* at different concentrations of dimethoate exposure has been reported by Bayne *et al.*[10] and Verma *et al.*[11]. The hyperactivity and erratic movements were observed, especially during the first 48 h of exposure with dimethoate to the *Cyprinus carpio* fry[12]. In the present study *H. fossilis* showed skin discoloration. Such types of changes were also observed in zebrafish after toxaphene intoxication[13]. *H. fossilis* were also found to secrete mucous along their opercular region, prior to death following dimethoate poisoning. This phenomenon is, evidently, a symptom of the inflammatory reaction of the gills towards the pollutant.

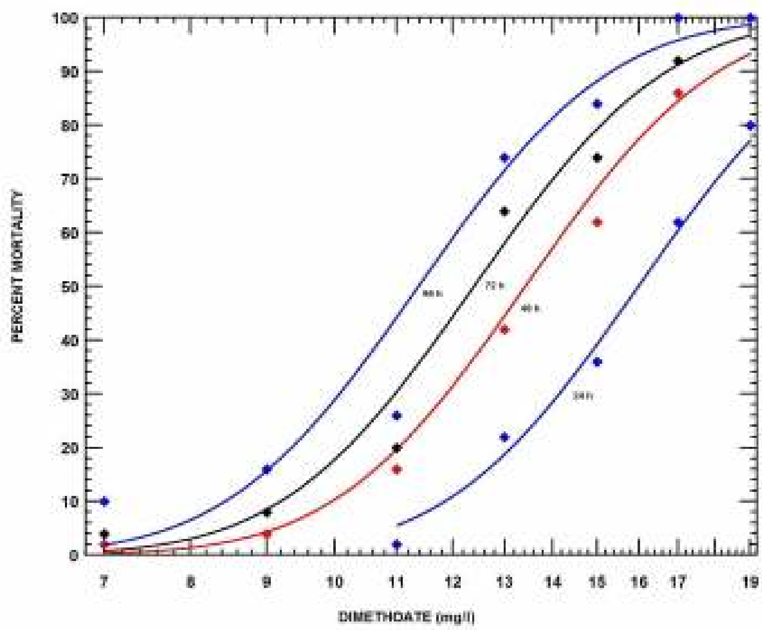

Figure 1
Percent mortality of the fish Heteropneustes fossilis after 24, 48, 72 and 96 h exposure to different concentrations of dimethoate (mg/l).

The LC_{50} values of dimethoate for the freshwater fish, *H. fossilis* at 24, 48, 72 and 96 h were 15.92, 13.42, 12.39 and 11.34 mg/l, respectively. Shukla[9] reported the LC_{50} value of dimethoate for *Colisa fasciatus* as 13.0 mg/l for 24 h, 11.4 mg/l for 48 h, 10.0 mg/l for 72 h and 9.3 mg/l for 96 h. Vittozzi and Angelis[14] reported 0.78 mg/l and 0.79 mg/l as 96 h LC_{50} values of dimethoate for bluegill and trouts respectively. The 96 h LC_{50} value of dimethoate for *Lebistes reticulates* has been reported as 19 mg/l[15]. The 96 h LC_{50} value for dimethoate to the fish *Cyprinus carpio* has been reported as 26.11 mg/l[12]. The median lethal concentration (LC_{50}) of dimethoate to freshwater food fish, *Clarias batrachus* is 65 ppm [16]. Acute toxicity values of dimethoate (96 h LC_{50}) for fish species found in Canada ranged from 6 mg/l for bluegill

(*Lepomes macrochirus*) to 22.4 mg/l for carp (*Cyprinus carpio*, 7 days LC_{50})[17]. The LC_{50} of dimethoate for 96 h exposure to the fish Nile tilapia (*Oreochromis niloticus*) is 40 mg/l[18]. Schimmel *et al.*[19] concluded that it is difficult to compare the toxicity of individual insecticides to different species of fish because they are influenced by several factors like temperature, hardness, pH, and dissolved oxygen content of the test water.

Interrelationship between ambient temperature and susceptibility of fish to toxicants appears to be a common feature. A wide range of insecticides have been found to increase the toxicity at higher temperature[20,21].

The mechanism involved in the increase of susceptibility of fish to toxicants with rise in temperature is not well understood[22], though effect on general metabolism and respiration rate could largely be involved[20,23,24]. Rise in water temperature reduces the solubility of oxygen in water which could affect fish physiololgy. It could increase the metabolic rate (oxygen demand) of fish[25], limiting the affectivity of blood oxygenation and hemoglobin affinity for oxygen (Bohr effect), thus resulting in low dissolved oxygen levels and greater accumulation of waste products and lowering the resistance of fish to stress. Reduced solubility of oxygen in water at higher temperatures could also increase the ventilation at gills and the respiration rate[26], causing a larger quantity of water to move across the gill epithelium, thus increasing the possibility of greater uptake of contaminants from the medium and intensifying the stress.

CONCLUSION

From the present study it seems that the freshwater catfish, *H. fossilis* is more susceptible to dimethoate toxicity as the LC_{50} value for this organophosphate is less than other reported fish species. These results indicate that dimethoate exposure to the fish caused toxic effects. Further such fishes (having accumulated dimethoate in their body) may affect the human being after consuming the fish as food.

REFERENCES

1. Rainboth WJ, Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome, p. 265, (1996).
2. Tuduri L, Harner T, Blanchard P, Li Y-F, Poissant L, Waite DT, Murphy C, and Belzer W, A review of currently used pesticides (CUPs) in Canadian air and precipitation: Part 2: Regional information and perspectives. Atmospheric Environment 40: 1563-1578, (2006).
3. Athanasopoulos P, Kyriakidis NB, and Georgitsanakou I, Effect of storage temperature on the degradation of dimethoate in fortified orange and peach juices. Journal of Agriculture and Food Chemistry 48:4896-4899, (2000).
4. CDPR, Surface Water Database. California Environmental Protection Agency, Department of Pesticide Regulation (2007). Available on line at: http://www.cdpr.ca.gov/docs/sw/surfcont.htm
5. APHA (American Public Health Association), Standard methods for the examination of water and waste waters. 15th edition, APHA, Washington, DC, (1985).
6. Ahmed AM, Acute toxicity of four organophosphorus insecticide to a freshwater teleost, *Channa punctatus* (Bloch). Pakistan Journal of Zoology, 7: 135-140, (1975).
7. Ghose, TK, Nuvan induced physiological, biochemical and behavioural changes in *Barbus stigma*. Pollution Research, 5: 3, (1986 a).

8. Ghose, TK, Comparative toxicogenic evaluation of two commonly used organophosphate pesticides, Ekalux (EC 25%) and Rogor (Dimethoate) on the ovarian recrudescence in the freshwater teleost, *Sarotherodon mossambicus*. Utter Pradesh Journal of Zoology, 6: 2, (1986b).
9. Shukla M, Toxicological assessment of some common pollutants on a freshwater fish. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, India, (1995).
10. Bayne BL, Widows L, and Worral C, Physiological responses of marine biota to pollutants. Academic Press, New York, U.S.A, (1977).
11. Verma SR, Bhatnagar MC, and Dalela RC, Biocides in relation to water pollution. Part II. Bioassay studies of a few biocides to a freshwater fish, *Channa gachua.* Acta Hydrochimica et Hydrobiologica, 6: 137-144, (1978).
12. De Mel GWJLMVTM, and Pathiratne A, Toxicity assessment of insecticides commonly used in rice pest management to the fry of common carp, *Cyprinus carpio*, a food fish culturable in rice fields. Journal of Applied Ichthyology, 21: 146-150, (2005).
13. Ree GE, and Paney JF, Effect of toxaphene on reproduction of fish. Chemosphere, 34: 855-867, (1997).
14. Vittozzi L, and Angelis GD, A critical review of comparative acute toxicity data on freshwater fish. Aquatic Toxicology, 19: 167-204, (1991).
15. Gupta PK, Studies on acute toxicity of some insecticides to a freshwater teleost *Lebestes reticulatus* (Peters). Acta Hydrochimica et Hydrobiologica, 12: 629, (1984).
16. Begum G, In vivo toxicity and accumulation of dimethoate in freshwater fish *Clarias batrachus* (Linn.). Ph.D. thesis, Osmania University, Hyderabad A.P., India, (1993).
17. C.C.M.E. (Canadian Council of Ministers of the Environment), Canadian water quality guidelines for the protection of aquatic life: Dimethoate. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg (1999).
18. Sweilum MA, Effect of sublethal toxicity of some pesticides on growth parameters, haematological properties and total production of Nile tilapia (*Oreochromois niloticus* L.) and water quality of ponds. Aquaculture Research, 37: 1079, (2006).
19. Schimmel SC, Patrick Jr. JM, and Forester J, Heptachlor-toxicity to and uptake by several estuarine organisms. Journal of Toxicological and Environmental Health, 1: 955-965, (1976).
20. Macek KJ, Hutchinson C, and Cope OB, The effects of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. Bulletin of Environmental Contamination and Toxicology, 4: 174-183, (1969).
21. Muirhead-Thomson RC, Pesticide and freshwater fauna. Academic Press Inc., London, pp 248, (1971).
22. Singh BB, and Narain AS, Acute toxicity of thiodon to catfish *Heteropneustes fossilis.* Bulletin of Environmental Contamination and Toxicology, 28: 122-127, (1982).
23. Wedemeyer GA, Mayer FP, and Smith L, Environmental stress and fish diseases. Diseases of fishes, Book 5 (eds.: Srieszko SF, Axelrod HR). T.H.F. Publication, Neptune, pp. 192, (1976).
24. Gordon MR, and McLeay DJ, Sealed-jar bioassays for pulpmill effluent toxicity: Effects of fish species and temperature. Journal of Fish Research Board Canada, 34: 1389-1396, (1977).
25. Davis JC, The exchange of oxygen at the gills of fish in response to oxygen availability. In: Chemistry and Physics of Aqueous Gas Solutions (ed.: Adams WA). Electrochemical Society, Princeton, N.J., pp. 293, (1975).
26. Jones DR, Randall DJR, and Jarman GM, A graphical analysis of oxygen transfer in fish. Respiratory Physiology, 10: 285-298, (1970).