Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

May 2009, 30(3) 437-440 (2009)
For personal use only
Commercial distribution of this copy is illegal

Acute toxicity bioassay of dimethoate on freshwater airbreathing catfish, *Heteropneustes fossilis* (Bloch)

Rakesh K. Pandey*[1], Ram N. Singh[1], Sarika Singh[1], Narendra N. Singh[2] and Vijai K. Das[1]

[1]Department of Zoology, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur - 228 118, India
[2]Department of Zoology, St. Andrew's College, Gorakhpur - 273 001, India

(Received: June 26, 2007; Revised received: November 10, 2007; Accepted: December 25, 2007)

Abstract: *Pesticides are chemicals used for pest control in the agricultural fields. They finally reach the surrounding water bodies through surface runoff affecting the aquatic fauna. Dimethoate is frequently used organophosphate pesticide due to its high effectiveness and rapid breakdown into environmentally safe products. A 96 hr static acute toxicity test was carried out to determine the LC_{50} value of dimethoate, on the freshwater airbreathing catfish Heteropneustes fossilis (Bloch). The fish were exposed to 7 different concentrations of dimethoate (2.50, 2.75, 3.00, 3.25, 3.50, 3.75 and 4.00 mg l^{-1}) for toxicity bioassay. Control (0.00 mg l^{-1}) was also carried out. The data were subjected to Finney's Probit analysis and processed with Trimmed Spearman-Karber statistical software. The LC_{50} values for dimethoate for 24, 48, 72 and 96 hr were 3.38, 3.23, 3.08 and 2.98 mg l^{-1}, respectively. At higher concentration of dimethoate (3.25 mg l^{-1} and above) the fish showed uncoordinated behaviour such as erratic and jerky swimming, attempt to jump out of water, frequent surfacing and gulping of air, decrease in opercular movement and copious secretion of mucus all over the body.*

Key words: *Acute toxicity, Dimethoate, LC_{50} mortality, Heteropneustes fossilis*
PDF of full length paper is available with authors ***(*rakesh_zoology@yahoo.co.in)***

Introduction

The pesticides are frequently used on crops and animals in the integrated farming practices, for the eradication of a wide range of insect pests, mites and weeds. The indiscriminate use of the pesticides worldwide for agricultural and other activities poses great threat to the environment. According to 1996-97 market estimates (US EPA, 2001), about 5700 million pounds of pesticides were applied throughout the world. Nearly three million cases of pesticide poisoning are reported annually (WHO, 1992).

Organophosphates (OP) are one of the most preferred pesticides due to their high effectiveness and low persistence in the environment. OP pesticides directly inhibit acetylcholinesterase enzyme activity in fishes and invertebrates (Fulton and key, 2001; Rao *et al.,* 2005; Agrahari *et al*., 2006). Dimethoate, an organophosphate insecticide was first described by Hoegberg and Cassaday in 1951 and introduced to the market in 1956. It is absorbed by the organisms following ingestion, inhalation and cutaneous contact. Dimethoate is moderately toxic to birds and mammals (EHC, 1990). The relative less toxicity of dimethoate in birds and mammals is due to its rapid degradation in the liver and elimination of its metabolic products through the urinary route (Begum and Vijayraghavan, 1995). The insects can not hydrolyze dimethoate easily, therefore, they become more susceptible to the toxin (Cope *et al.,* 2004).

The pesticides may finally find their way to the natural water bodies and affect the aquatic environment. The threat of rapidly shrinking genetic base and fast depleting biodiversity is attributed to the indiscriminate use of pesticides. The pesticides enter the food chain and their subsequent bioaccumulation and biotransformation at different trophic levels have disastrous effect to the ecosystem (Grande *et al*., 1994) and consequently disrupt the ecological balance (Heger *et al*., 1995). Aquatic contamination of pesticides causes acute and chronic poisoning of fish and other organisms. The pesticides damage vital organs (Omitoyin *et al*., 2006; Johal *et al*., 2007; Joshi *et al.,* 2007; Velmurugan *et al*., 2007), and skeletal system (Singh *et al.*, 1997) of the exposed fish.

There are reports on the toxicity of different OP pesticides on fish (Dikshith and Raizada, 1981; Verma *et al*., 1982; Srivastava and Singh, 2001; Gul, 2005; Pandey *et al*., 2005). The present study was conducted to determine the static acute toxicity of dimethoate, on a freshwater catfish *Heteropneustes fossilis*.

Materials and Methods

Healthy adult catfish, *Heteropneustes fossilis* (Bloch) of both sexes were collected from local ponds and carefully brought to the laboratory, avoiding any injury during transportation. Fish were treated with 0.05% $KMnO_4$ solution for 2 min to avoid any cutaneous infection. The disinfected fish stock was kept in 500 liter plastic tank for 14 days to acclimatize under laboratory conditions. They were fed on alternate days with wheat flour mixed together with soyabean flour and mustard cake in ratio of 3:1:1. Fish were also fed with goat liver. The left over food and water from the tank was removed and replenished with fresh water in the subsequent morning. Feeding was

Online Copy

Table - 1: Dissolve oxygen (DO) concentration (mg l^{-1}) and percent change in test water during 96 hr acute toxicity bioassay

Concentration (mgl^{-1})	0 hr	24 hr	48 hr	72 hr	96 hr	Percent change in DO after 96 hr
Control (0.00)	7.20	7.15	7.05	6.90	6.75	6.25
2.50	7.20	7.00	6.85	6.70	6.60	8.33
2.75	7.20	6.90	6.65	6.50	6.45	10.41
3.00	7.20	6.85	6.75	6.65	6.60	8.33
3.25	7.20	6.80	6.60	6.55	6.55	9.03
3.50	7.20	6.85	6.65	6.50	6.50	9.72
3.75	7.20	6.80	6.70	6.65	-	7.64
4.00	7.20	6.60	-	-	-	8.33

– : Not measured

Table - 2: LC_{50} values for dimethoate (with 95% Confidence limit) estimated by Trimmed Spearman-Karber method

Test duration (hr)	LC_{50} values (mg l^{-1})	95% lower Confidence limit	95% upper Confidence limit
24	3.38	3.22	3.55
48	3.23	3.08	3.39
72	3.08	2.94	3.22
96	2.98	2.85	3.13

stopped 24 hr prior to the toxicity test. Fish were exposed to natural photoperiod at room temperature, 30.67 ± 0.82 °C.

The physico-chemical parameters such as dissolved oxygen (DO), temperature, pH, and hardness (as $CaCO_3$) of the water were recorded daily for the 96 hr exposure period following the standard methods (APHA, 1998). The experiments were carried out in glass aquaria of 15 liter capacity in static laboratory conditions. The fish approximately of uniform length (17.4 ± 1.14 cm) and weight (27.14 ± 1.95 g) were selected for bioassay. The stock solution was prepared by dissolving dimethoate (Rogor- 30% EC, Rallis India Ltd. Mumbai-India) in absolute alcohol. The range finding tests or exploratory tests prescribed by APHA (1998) was used to determine the concentration range of dimethoate. At concentration 4.00 mg l^{-1}, 100% mortality was found within 24 hr and no mortality was noticed up to 96 hr, at concentrations below 2.50 mg l^{-1}. After determining the test range (2.50, 2.75, 3.00, 3.25, 3.50, 3.75 and 4.00 mg l^{-1}), 8 acclimatized fish in four replicates, for each concentration were released in 15 liter water trough for 24, 48, 72 and 96 hr acute toxicity test. A control set with equal number of fish was simultaneously run. A measured quantity (3.5 ml) of absolute alcohol was also mixed in control.

Fish were not given any food during the experiment (Ward and Parrish, 1982; Reish and Oshida, 1987). Besides mortality, fish behaviour were observed and recorded. LC_{50} values were calculated according to Finney (1971), Hamilton *et al.* (1977).

Results and Discussion

The physico-chemical parameters of the test water used in the experiment were as follows; temperature (27.5 ± 0.10°C), pH (7.3 ± 0.03), DO (7.2 ± 0.2 mg l^{-1}) and hardness as $CaCO_3$ (112.34 ± 0.25 mg l^{-1}). The DO of water was determined in each trough, at different concentration upto 96 hr (Table 1). The data exhibit a decline in DO content of test solution at different concentrations of the toxicant, as compared to control. The DO declines significantly at concentration 2.75 mg l^{-1}, there after due to fish mortality, the oxygen utilization decreased and the decline in DO content became insignificant (Table 1). *Heteropneustes fossilis* being an air breathing fish is more dependant on aerial respiration, therefore the change in DO appears inconspicuous. This indicates that fish utilize more oxygen under stress.

The exposure of fish to different concentrations of dimethoate shows altered behavioural responses, such as restlessness, hyperactivity, abrupt erratic and jerky swimming, decline in opercular movement, frequent surfacing and gulping, avoidance behaviour, increased mucus secretion, discolouration of skin, drooping of fins, loss of balance and finally the death.

The restlessness and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase, leading to accumulation of acetylcholine at synaptic junctions (Fulton and Key, 2001) and stimulation of peripheral nervous system which results in to increased metabolic activities. The higher metabolic rate results into more oxygen utilization (Rao, 1989). The abrupt erratic and jerky swimming may be due to inhibition of the acetylcholinesterase in the brain and neuromuscular junctions (Rao *et al.*, 2005; Agrahari *et al.*, 2006).

The opercular movement in fish has been reported to increase following the exposure of toxins (Pandey *et al.*, 2005; Omitoyin *et al.*, 2006; Yadav *et al.*, 2007). Contrary to it, in this study the opercular movement in fish showed a marked decrease on dimethoate exposure. Chindah *et al.* (2004) reported an initial increase in opercular beat frequency in chlorpyrifos exposed tilapia, followed by a marked decline with exposure time and explained the initial increase as sudden response to shock. *Heteropneustes fossilis*

Online Copy

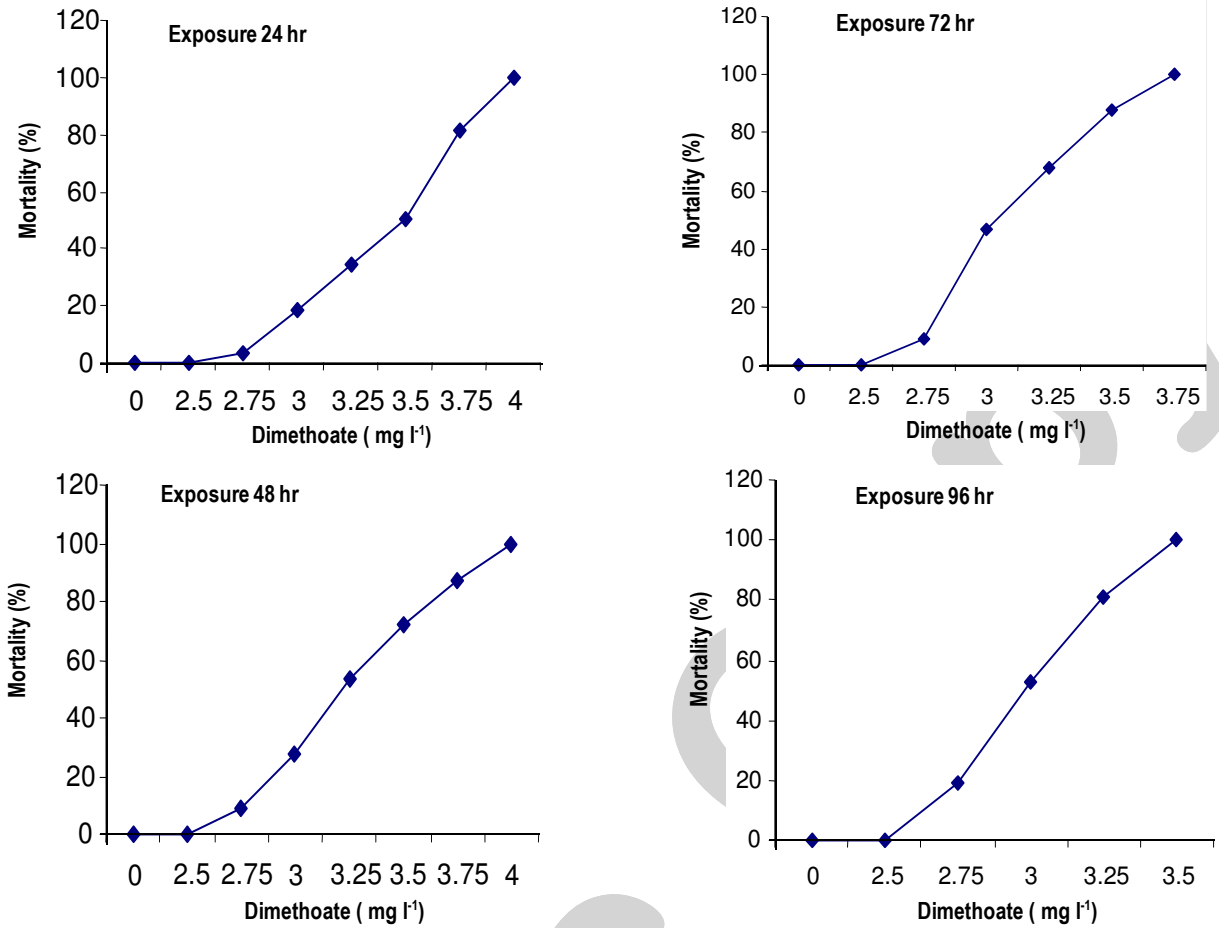

Fig. 1: Percentage mortality of *Heteropneustes fossilis* at different exposure times and different concentrations of dimethoate

exhibit a unique adaptive feature to avoid intake of toxicant by decreasing the opercular movement. Moreover, OP pesticides cause severe damage to the gill membranes (Johal *et al*., 2007; Velmurugan *et al*., 2007), hampering the branchial exchange of gases and leading to asphyxia. To cope with the deficiency of oxygen fish gulp air by frequent surfacing and filling the two lateral highly vascular air sacs with fresh air for accessory respiration. On initial exposure at higher concentration ranges (3.25 mg l^{-1} and onwards) the fish exhibited characteristic avoidance behaviour by rapid swimming, stretching half of their body out of water surface and trying to jump out.

Fish secrete copious amount of mucus, as a defence mechanism to neutralize the effect of insecticide, which gradually covers all over the body, gills and the buccal cavity. Moreover, Bisht and Agarwal (2007) observed hypertrophy and hyperplasia of mucous cells in $CuSO_4$ exposed fish and suggested it as adaptive and defense mechanism to prevent cutaneous entry of toxicant by coagulation through increased mucus production. The skin shows marked decrease in the pigmentation associated with appearance of dark patches and rashes. Pandey *et al*. (1990) also reported depigmentation in toxicant exposed fish and attributed it to reduction in number and size of chromatophores.

The dorsal and pectoral fins which were fully stretched initially, drooped after 48 hr of exposure. In the terminal phase of poisoning, the fish lost their balance and hanged vertically. Finally they fell on to the bottom with their belly upward, leading to death.

LC_{50} values and their corresponding 95% lower confidence and 95% upper confidence limits for 24, 48, 72 and 96 hr are given in the Table 2. The mortality of fish increases with the increase in the concentration of the toxicant, depicting a direct correlation between the mortality and the concentration (Fig.1). The physico-chemical parameters change in static condition affecting the fish behaviour and mortality. Therefore, for a single toxicant, on the same fish species different LC_{50} values have been recorded. In present study, however, the LC_{50} values for 24, 48, 72 and 96 hr for dimethoate were 3.38, 3.23, 3.08 and 2.98 mg l^{-1} respectively. Begum and Vijayaraghavan (1995) reported 96 hr LC_{50} value of dimethoate on another catfish *Clarias batrachus* as 65 mg l^{-1}, which is very high in comparison to the present study on *Heteropneustes fossilis*. Similarly on another airbreathing teleost *Channa punctatus*, Dikshith and Raizada (1981) found LC_{50} value for dimethoate as 47 mg l^{-1} (96 hr), whereas, Srivastava and Singh (2001) reported very low LC_{50} (24 hr) value for dimethoate on *C. punctatus* as 17.9 mg l^{-1}. Similar

variations were reported for another OP pesticide - malathion. Pandey *et al.* (2005) observed 96 hr LC_{50} value for *Channa punctatus* as 6.61 ppm, whereas, Khangarot and Ray (1988) reported a lower LC_{50} value *i.e.* 1.738 ppm for the same species. Earlier, Sprague (1969) reported variable LC_{50} values even for single species and single toxicant, depending on size, age and conditions of test species along with experimental factors.

It is concluded from the present study on dimethoate toxicity that the airbreathing catfish *Heteropneustes fossilis* is very sensitive to the insecticide as evident from the behavioural responses such as erratic swimming, fall in opercular activity and increased gulping of air to meet out the respiratory distress. The fish dies at low concentration of insecticide as compared to other airbreathing teleosts.

Acknowledgments

The financial assistance of University Grant Commission to R.K.P. (F.5.1.3(71)/2004 (MRP/NRCB) and R.N.S. (F.5.1.3(79)/2004 (MRP/NRCB) to carry out the project is gratefully acknowledged. We are thankful to Dr. Gunraj Prasad, Principal, K.N.I.P.S.S., Sultanpur, for providing required research facilities and Sri Prem P. Singh for helping in computer analysis of the data.

References

Agrahari, S., K. Gopal and K.C. Pandey: Biomarkers of monocrotophos in a fresh water fish *Channa punctatus* (Bloch). *J. Environ. Biol.*, **27**, 453-457 (2006).

APHA: Standard methods for the examination of water and wastewater, 20th Edn., Washington D.C. (1998).

Begum, G. and S. Vijayaraghavan: *In vivo* toxicity of dimethoate on protein and transaminases in the liver tissue of fresh water fish *Clarias batrachus* (Linn.). *Bull. Environ. Contam. Toxicol.*, **54**, 370-375 (1995).

Bisht, I. and S.K. Agarwal: Cytomorphological and histomorphological changes in mucous cells of general body epidermis of *Barilius vagra* (Cyprinidae, Pisces) following exposure to herbicide-BLUE VITROL ($CuSO_4$): A statistical analysis. *J. Exp. Zool. India,* **10**, 27-36 (2007).

Chindah, A.C., F.D. Sikoki and I. Vincent-Akpu: Toxicity of an organophosphate pesticide (chlorpyrifos) on a common Niger Delta wetland fish - *Tilapia guineensis* (Blecker 1862). *J. Appl. Sci. Environ. Manage.*, **8**, 11-17 (2004).

Cope, W.G., R.B. Leidy and E. Hodgson: A Text Book of Modern Toxicology. John Wiley and Sons Publication, New Jersey, USA (2004).

Dikshith, T.S.S. and R.B. Raizada: Toxicity Evaluation of Dimethoate Technical in Fish. Report to Shaw Wallace and Co. India Ltd. (1981).

EHC: Environmental Health Criteria World Health Organization. International programme on chemical safety. pp. 1-51 (1990).

Finney, D.J.: Probit Analysis. Univ. Press, Cambridge (1971).

Fulton, M.H. and P.B. Key: Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environ. Toxicol. Chem.*, **20**, 37-45 (2001).

Grande, M., S. Anderson and S. Berge: Effects of pesticides on fish. *Norw. J. Agric. Sci. Suppl.*, **13**, 195-209 (1994).

Gul, A.: Investigation of acute toxicity of chlorpyrifos methyl on Nile tilapia (*Oerochromis niloticus*) larvae. *Chemosphere*, **59**, 163-166 (2005).

Hamilton, M.A., R. Russo and R.V. Thurston: Trimmed Spearman-Karber method for estimating lethal median concentration in toxicity bioassay. *Environ. Sci. Technol.*, **11**, 714 (1977).

Heger, W., S.T. Jung, S. Martin and H. Peter: Acute and prolonged toxicity to aquatic organism of new and existing chemicals and pesticides. *Chemosphere,* **31**, 2702-2726 (1995).

Johal, M.S., M.L. Sharma and Ravneet: Impact of low dose of organophosphate, monocrotophos on the epithelial cells of gills of *Cyprinus carpio communis* Linn. - SEM study. *J. Environ. Biol.,* **28**, 663-667 (2007).

Joshi, Namita, Dharmlata and A.P. Sahu: Histopathological changes in liver of *Heteropneustes fossilis* exposed to cypermethrin. *J. Environ. Biol.,* **28**, 35-37 (2007).

Khangarot, B.S. and P.K. Ray: Studies on toxicity of malathion to freshwater teleost *Channa punctatus* (Bloch) and *Puntius sophore* (Hamilton). *Arch. Hydrobiol.*, **113**, 465-469 (1988).

Omitoyin, B.O., E.K. Ajani, B.T. Adesina and C.N.F. Okuagu: Toxicity of Lindane (Gamma Hexachloro-CycloHexane) to *Clarias gariepinus* (Burchell 1822). *W.J. Zool.*, **1**, 57-63 (2006).

Pandey, A., G.K. Kumar and J.S.D. Munshi: Integumentary chromatophores and mucus glands of fish as indicator of heavy metal pollution. *J. Freshwater Biol.,* **2**, 117-121 (1990).

Pandey, S., R. Kumar, S. Sharma, N.S. Nagpure, S.K. Srivastava and M.S. Verma: Acute toxicity bioassay of mercuric chloride and malathion on airbreathing fish *Channa punctatus* (Bloch). *Ecotoxicol. Environ. Saf.*, **61**, 114-120 (2005).

Rao, D.M.R.: Studies on the relative toxicity and metabolism of endosulfan to the Indian major carp, *Catla catla* with special reference to some biochemical changes induced by the pesticide. *Pest. Bio. Phy.,* **33**, 220-229 (1989).

Rao, J.V., G. Begum, R. Pallela, P.K. Usman and R.N. Rao: Changes in behaviour and brain acetylcholinesterase activity in mosquito fish *Gambusia affinis* in reference to the sublethal exposure of chlorpyrifos. *Int. J. Environ. Res. Public Hlth.,* **2**, 478-483 (2005).

Reish, D.L. and P.S. Oshida: Short term bioassay. *In*: Manual of methods in aquatic environment research part 6. FAO. Fish. Tech. Pap., 247, 1-62 (1987).

Singh, N.N., V.K. Das and A.K. Srivastava: Formothion and propoxur induced ionic imbalance and skeletal deformity in a catfish, *Heteropneustes fossilis*. *J. Environ. Biol.*, **18**, 357-363 (1997).

Sprague, J.B.: Measurement of pollutant toxicity to fish: I. Bioassay methods for acute toxicity. *Water Res.*, **3**, 793-821 (1969).

Srivastava, V.K. and A. Singh: Studies of seasonal variation in toxicity of frequently used commercial organophosphate, carbamate and synthetic pyrethroids pesticides against freshwater fish *Channa punctatus* and behavioural responses to treated fish. *Malaysian Appl. Biol.*, **30**, 17-23 (2001).

USEPA: Pesticide Industry Sales and Usages: 1996 and 1997 Market Estimates, Office of Water. U.S. Environmental Protection Agency, Washington, D.C. (2001).

Velmurugan, B., M. Selvanayagam, E.I. Cengiz and E. Unlu: The effects of Monocrotophos to different tissues of freshwater fish *Cirrhinus mrigala*. *Bull. Environ. Contam. Toxicol.*, **78**, 450-454 (2007).

Verma, S.R., S.K. Bansal, A.K. Gupta, N. Pal, A.K. Tyagi, M.C. Bhatnagar, V. Kumar and R.C. Dalela: Bioassay trials with twenty three pesticides to a freshwater teleost, *Saccobranchus fossilis*. *Water Res.*, **16**, 525-529 (1982).

Ward, G.S. and P.R. Parrish: Toxicity tests. *In*: Manual of methods in aquatic environment research part 6. FAO. Fish. Tech. Pap., 185, 1-23 (1982).

WHO: Our Planet, Our Health: Report of the WHO Commission on Health and Environment, World Health Organization, Geneva (1992).

Yadav, A., S. Neraliya and A. Gopesh: Acute toxicity levels and ethological responses of *Channa striatus* to fertilizer industrial wastewater. *J. Environ. Biol.*, **28**, 159-162 (2007).

Online Copy