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Behaviour and Respiratory Dysfunction as an Index of Malathion Toxicity in the Freshwater Fish, *Labeo rohita* (Hamilton)

Vineetkumar K. Patil[1], M. David[1,*]
[1] Karnatak University's Research Laboratory, Environmental and Molecular Toxicology Division, Department of Zoology, Karnatak Science College, Dharwad 580 001, Karnataka, India.

* Corresponding Author: Tel.: +91.836 2446436; Fax: +91.836 2747884, 2771275;
E-mail: davidma941964@rediffmail.com

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Abstract

Short-term definitive test by static renewal bioassay method was conducted to determine the acute toxicity (LC_{50}) of commercial grade organophosphate insecticide, malathion (50% EC) in the freshwater edible fish, *Labeo rohita*. Carp fingerlings were exposed to different concentrations (6.0 to 10.1 µl/L) of malathion for 96 h. The acute toxicity value was found to be 9.0 µl/L and one tenth of LC_{50} (0.9 µl/L) was selected for sub acute studies. Behavioural patterns and oxygen consumption were studied in lethal (1, 2, 3 and 4 d) and sublethal concentrations (1, 5, 10 and 15 d). Carp in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom which might be due to inactivation of AChE activity which results in excess accumulation of acetylcholine in cholinergic synapses leading to hyperstimulation. Variation in oxygen consumption (70.39 to 80.50%, -4.45 to 21.35%) was observed in both lethal and sublethal concentrations of malathion respectively. Alterations in oxygen consumption may be due to respiratory distress as a consequence of impairment in oxidative metabolism. Fish in sublethal concentration were found under stress, but that was not fatal.

Key words: Malathion toxicity, behaviour, oxygen consumption, *Labeo rohita.*

Introduction

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. 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). Some of these chemicals are biodegradable and quickly decay into harmless or less harmful forms, while others are non-biodegradable and remain dangerous for a long time. 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). Thus, ecological damage of the environment caused by anthropogenic factors as well as the presence of hazardous agents may affect fish.

Malathion is a non-systemic, wide spectrum organophosphate insecticide. It was one of the earliest organophosphate insecticides developed (Introduced in 1950). This is used for agricultural and non-agricultural purposes. Once malathion is introduced into the environment, usually from spraying on crops or in wide urban or residential areas, droplets of malathion in the air fall on soil, plants, water or man-made surfaces. While most of the malathion will stay in the areas where it is applied, some can move to areas away from where it was applied by rain, fog and wind. In water, malathion breaks down quickly by the action of water and bacteria in the water. Malathion is broken down in air by reacting with other chemicals formed naturally in the air by sunlight to form a more toxic product called malaoxon. If malathion is present on dry soil or on man-made surfaces such as sidewalks, pavements, or playground equipment, it usually does not break down as fast as it would in moist soil.

Once malathion is introduced into the environment, it may cause serious intimidation to the aquatic organisms and is notorious to cause severe metabolic disturbances in non-target species like fish and fresh-water mussels (USEPA, 2005). The physiological condition of the organism during toxic impact must be considered to understand the influence of pesticide. *Labeo rohita* is edible freshwater fish of great economic connotation and it is used in the composite fish culture.

Behaviour is considered a promising tool in ecotoxicology (Drummond and Russom, 1990; Cohn and MacPhail, 1996) and these studies are becoming prominent in toxicity assessments in unicellular organisms (Tadehl and Häder, 2001), insects (Jensen *et al.*, 1997) and fish (Little and Finger, 1990). Most physiologic and environmental changes can induce variations in fish behavior (Israeli-Weinstein and Kimmel, 1998; Almazán-Rueda *et al*., 2004). Hence the present study was under taken to evaluate the aquatic toxicity of malathion with special emphasis on behavioural and oxygen consumption of the

freshwater teleost, *L. rohita* exposed to lethal and sublethal concentrations of commercial grade malathion.

Material and Methods

A total of 130 healthy and active *L. rohita* fingerlings weighing 5±2 g and average length of 7.5±0.23 cm were collected from the State Fisheries Department, Dharwad, Karnataka and were transferred to large cement tanks. Carps were acclimatized to laboratory conditions for 15 d at 24±1°C and are held in large glass aquaria containing dechlorinated tap water of the quality used in the test and its physico-chemical characteristics were analyzed following the methods mentioned in APHA (2005) and found as follows: water temperature: 24±2°C, pH: 7.1±0.2 at 24°C, dissolved oxygen: 9.3±0.8 mg/L, carbon dioxide: 6.3±0.4 mg/L, total hardness: 23.4±3.4 mg as $CaCO_3$/L, phosphate: 0.39±0.002 µg/L, salinity: nil, specific gravity: 1.0030 and conductivity less than 10 μScm^{-1}. Water was renewed everyday and 12-12 h of photoperiod was maintained daily during acclimation and test periods. Fish were fed regularly with oil cake and rice bran during acclimation and feeding was stopped three days prior to exposure to test medium.

Concentrations of the test compounds used in short term definitive tests were between the highest concentration at which there was no mortality and the lowest concentration at which there was 100% mortality in the range finding tests. Replacement of the water medium was followed by the addition of the desired dose of the test compound. Fish were exposed in batches of ten to varying concentrations of malathion with 10 L of water in six replicates for each concentration, along with a control.

Malathion (50% EC) was procured from the scientific fertilizer company Pvt. Ltd. Tiruchirapalli, Tamil Nadu, India. The expiry date of the test substance checked prior to initiation of the treatment was found suitable for the exposure. Required quantity of malathion was drawn directly from this emulsified concentrate using variable micropipette.

For LC_{50} calculation, mortality was recorded every 24 h and dead fish were removed when observed, every time noting the number of fish deaths at each concentration up to 96 h. Duncan's multiple range (DMR) test was employed for comparing mean mortality values after estimating the residual variance by repeated measures ANOVA (Winner, 1971) for arc sine transformed mortality data (dead individuals/ initial number of individuals). Time of exposure was the repeated measure factor, while treatment (concentration and control) was the second factor. In addition, LC_{50} were compared by the method of APHA (2005). The LC_{50} with 95% confidence limits for malathion were determined for 96 h by probit analysis (Finney, 1971).

One tenth of the LC_{50} (0.9 µl/L) was selected as sublethal concentration for chronic study (1, 5, 10 and 15 d). The control and malathion exposed fish were kept under continuous observation during experimental periods. The whole animal oxygen consumption was measured for lethal and sublethal concentrations besides control by following the method of Welsh and Smith (1953) as described by Saroja (1959).

Each experiment was repeated six times and the mean value was calculated. The data obtained were analyzed statistically by following DMRT (Duncan, 1955).

Results and Discussion

Acute toxicity of malathion for the freshwater fish, *L. rohita* was found to be 9.0 µl/L. The upper and lower 95% confidence limits were found to be 9.9828 µl/L and 8.1139 µl/L respectively (Table 1). It is evident from the results that the malathion can be rated as moderately toxic to fish.

In the present study, the control fish behaved in natural manner i.e. they were active with their well-coordinated movements. They were alert at the slightest disturbance, but in the toxic environment , fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium which is due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyperstimulation (Mushigeri and David, 2005). They slowly became lethargic, hyper excited, restless and secreted excess mucus all over the body. Mucus secretion in fish forms a barrier between body and toxic media thereby probably reduces contact of toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by Rao *et al.* (2003) and Parma de Croux *et al.* (2002). Opercular movements increased initially in all exposure periods but decreased further steadily in lethal exposure compared to sublethal exposure periods. Increased gill opercular movements observed initially may possibly compensate the increased physiological activities under stressful conditions (Shivakumar and David,

Table 1. 96 h LC_{50}, slope and 95% confidence limits of malathion to the freshwater fish, *Labeo rohita*

Pesticide	96 h LC_{50} value (µl/L)	Slope	95% Confidence limits	
			Upper limit	Lower limit
Malathion	9.0	1.0944	9.9828	8.1139

2004).

Gulping air at the surface, swimming at the water surface, disrupted shoaling behaviour and easy predation was seen on the first day itself in lethal and sublethal exposure period and continued the same further intensely which is in accordance with the observations made by Ural and Simsek (2006). Gulping of air may helps to avoid contact of toxic medium. Surfacing phenomenon i.e., significant preference of upper layers in exposed group might be a demand of higher oxygen level during the exposure period (Katja *et al*., 2005). Finally fish sunk to the bottom with the least opercular movements and died with their mouth opened.

In sublethal exposure, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Leaning of fish indicate reduced amount of dietary protein consumed by the fish at pesticide stress, which was immediately utilized and was not stored in the body weight (Kalavathy *et al*., 2001).

A change in respiration rate is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is clearly evident from the studies (Table 2) that malathion affected oxygen consumption of *L. rohita* under lethal and sublethal concentrations. Fish exposed to lethal concentration depicted increased oxygen consumption on day 1 to day 3 and decreased on day 4. In sublethal exposure, oxygen consumption decreased on day 1 and increased on day 5 and 10 but day 15 witnessed decrement as compared to control (Figure 1). However, it is interesting to note that sublethal day 5 depicted extremely high oxygen consumption. The high value recorded on 5th day of sublethal exposure may be attributed to the initiation of specific protein synthesis or increased to detoxify the toxicant (Connell *et al*., 1999). The increase in the protein synthesis in the above process is expected to be accompanied by an increase in respiration. Thus, the increase in protein synthesis will eventually be reflected in an increase in respiration (oxygen consumption) rate.

Since most fish breathe in water in which they live, changes in the chemical properties thereof may be reflected in the animals ventilatory activity, particularly if the environment factors affect respiratory gas exchanges (Mushigeri, 2003). The fluctuated response in respiration may be attributed to

Table 2. Oxygen consumption (ml of oxygen consumed/g wet wt. of fish/h) of the fish, *L. rohita* following exposure to lethal (9.0 µl/L) and sublethal (0.9 µl/L) concentrations of malathion

Estimations	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Oxygen consumption	0.1662^{H}	0.2832^{D}	0.2938^{B}	0.3000^{A}	0.2836^{C}	0.1691^{G}	0.2017^{E}	0.1898^{F}	0.1588^{I}
±SD	0.0002	0.0003	0.0003	0.0002	0.0004	0.0002	0.0004	0.0003	0.0004
% Change	-------	70.39	76.77	80.50	70.63	1.74	21.35	14.19	-4.45

Values are means ±SD (n=6) for oxygen consumption in a column followed by the same letters are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

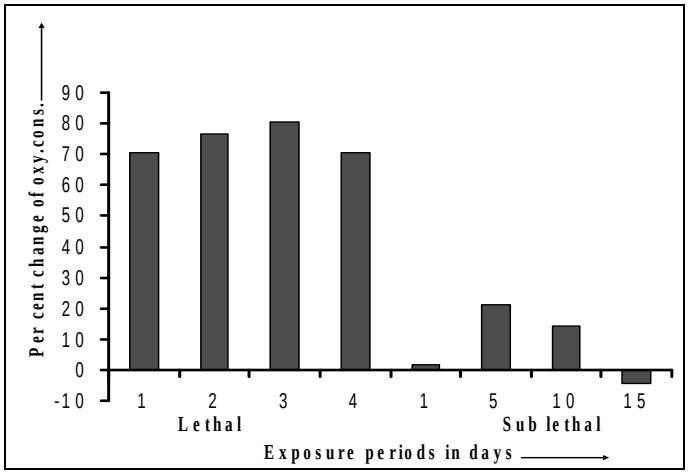

Figure 1. Oxygen consumption of the fish, *Labeo rohita* following exposure to lethal and sublethal concentrations of malathion.

respiratory distress as a consequence of the impairment of oxidative metabolism. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambica* (David *et al*., 2003).

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (Magare and Patil, 2000). Pronounced secretion of mucus layer over the gill lamellae has been observed during malathion stress. Secretion of mucus over the gill curtails the diffusion of oxygen (David *et al.*, 2002), which may ultimately reduce the oxygen uptake by the animal.

If gills would be destroyed due to xenobiotic chemicals (Grinwis *et al.*, 1998) or the membrane functions are disturbed by a changed permeability (Hartl *et al.*, 2001), oxygen uptake rate would even rapidly decreased. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. Kalavathy *et al.* (2001) reported that the dimethoate is efficiently absorbed across the gill and diffuse into the blood stream resulting toxic to fish.

The analysis of data from the present investigation evidenced that malathion is toxic and had profound impact on behaviour and respiration in *L. rohita* in both lethal and sublethal concentrations. Thus it has led to the altered fish respiratory physiology. Variation in the oxygen consumption in malathion treated fish is probably due to impaired oxidative metabolism and pesticide induced respiratory stress. Hence, dysfunction of behaviour and respiration can serve as an index of malathion toxicity.

References

Almazán-Rueda, P., Schrama, J.W. and Verreth, J.A.J. 2004. Behavioural responses under different feeding methods and light regimes of the African catfish *Clarias gariepinus* juveniles. Aquaculture, 231(1-4): 347-359.

APHA. 2005. Standard methods for the examination of water and waste water, American Public Health Association. Washington, DC.

Brack, W., Schirmer, K., Kind, T., Schrader, S. and Schuurmann, G. 2002. Effect-directed fractionation and identification of cytochrome P450A-inducing halogenated aromatic hydrocarbons in contaminated sediment. Environ. Toxicol. Chem., 21: 2654 -2662.

Cohn, J. and MacPhail, R.C. 1996. Ethological and experimental approaches to behavior analysis, implications for ecotoxicology. Environ. Health Perspect, 104: 299-304.

Connell, D., Lam, P., Richardson, B. and Wu, R. 1999. Introduction to ecotoxicology. London, 170 pp.

David, M., Mushigeri, S.B. and Prashanth, M.S. 2002. Toxicity of fenvalerate to the freshwater fish, *Labeo rohita*. Geobios, 29: 25-28.

David, M., Shivakumar, H.B., Shivakumar, R., Mushigeri, S.B. and Ganti, B.H. 2003. Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the freshwater fish, *Tilapia mossambica*. Indian J. of Environ. Toxicol., 13(2): 99-102.

Diez, S., Abalos, M. and Bayona, J.M. 2002. Organotin contamination in sediments from the Western Mediterranean enclosures following ten years of TBT regulation. Water Res., 36: 905-918.

Drummond, R.A. and Russom, C.L. 1990. Behavioural toxicity syndromes, a promising tool for assessing toxicity mechanisms in juvenile fathead minnows, Environ. Toxicol. Chem., 9: 37-46.

Duncan, D.M. 1955. Multiple range and multiple tests: Biometrics.

Finney, D.J. 1971. Probit analysis, 3rd (Ed.), Cambridge University Press, London, 333 pp.

Grinwis, G.C.M., Boonstra, A., Vandenbrandhof, E.J., Dormans, J.A.M.A., Engelsma, M., Kuiper, R.V., Vanloveren, H., Wester, P.W., Vaal, M.A., Vethaak, A.D. and Vos, J.G. 1998. Short-term toxicity of bis (tri-n-butyltin) oxide in flounder, *Platichthys flesus*, pathology and immune funktion. Aquat. Toxicol., 42: 15-36.

Hartl, M.G.J., Hutchinson, S. and Hawkins, L. 2001. Organotin and osmoregulation: quantifying the effects of environmental concentrations of sediment-associated TBT and TPhT on the freshwater adapted European flounder, *Platichthys flesus* L. J. Exp. Mar. Biol. Ecol., 256: 267-278.

Israeli-Weinstein, D., Kimmel, E. 1998. Behavioural response of carp, *Cyprinus carpio* to ammonia stress. Aquaculture, 165(1): 81-93.

Jensen, C.S., Garsdal, L. and Baatrup, E. 1997. Acetylcholinesterase inhibition and altered locomotor behaviour in the carabid beetle *Pterostichus cupreus*. A linkage between biomarkers at two levels of biological complexity. Environ. Toxicol. Chem., 16: 1727-1732.

Kalavathy, K., Sivakumar, A.A. and Chandran, R. 2001. Toxic effects of the pesticide dimethoate on the fish, *Sarotherodon mossambicus*. J. Ecol. Res. Bio., 2: 27-32.

Katja, S., Georg, B.O.S., Stephan, P. and Christian, E.W.S. 2005. Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behaviour, body growth and enzymatic biotransformation activities (GST) of young carp, *Cyprinus carpio*. Aquatic Toxicology, 71: 49-59.

Khan, K. 1996. Integrated pest management and sustainable agriculture. Farmer and Parliament, 30: 15-17.

Little, E.E. and Finger, S.E. 1990. Swimming behaviour as an indicator of sublethal toxicity in fish, Environ. Toxicol. Chem., 9:13-19.

Magare, S.R. and Patil, H.T. 2000. Effect of pesticides on oxygen consumption, red blood cell count and metabolites of a fish, *Puntius ticto*. Environ. Ecology, 18: 891-894.

Mushigeri, S.B. and David, M. 2005. Fenvalerate induced changes in the Ach and associated AChE activity in different tissues of fish, *Cirrhinus mrigala* (Hamilton) under lethal and sub-lethal exposure period. Environmental Toxicology and Pharmacology, 20: 65-72.

Mushigeri, S.B. 2003. Effect of fenvalerate on the metabolism of Indian major carp, *Cirrhinus mrigala*. PhD. thesis, India, Karnataka, Dharwad: Karnatak University.

Parma de Croux, M.J., Loteste, A. and Cazenave, J. 2002. Inhibition of plasma cholinesterase and acute toxicity of monocrotophos in Neotropical fish, *Prochilodus lineatus* (Pisces, Curimatidae). Bull. Environ. Contam. Toxicol., 69: 356-362.

Rao, J.V., Rani, C.H.S., Kavitha, P., Rao, R.N. and Madhavendra, S.S. 2003. Toxicity of chlorpyrifos to the fish, *Oreochromis mossambicus*. Bull. Environ. Contam. Toxicol., 70: 985-992.

Saroja, K. 1959. Oxygen consumption in relation to body size and temperature in the earthworm, *Megascolex marutii* when kept submerged under water. Proc. Indian Acad. of Sci., 49: 183-193.

Shivakumar, R. and David, M. 2004. Toxicity of endosulfan to the freshwater fish, *Cyprinus carpio*. Indian J. of Ecology, 31(1): 27-29.

Tadehl, H. and Häder, D.P. 2001. Automated biomonitoring using real time movement analysis of *Euglena gracilis*. Ecotoxicol. Environ. Saf., 48: 161-169.

Ural, M.S. and Simsek Koprucu, S. 2006. Acute toxicity of dichlorvos on fingerling European catfish, *Silurus glanis*. Bull. Environ. Contam. Toxicol., 76: 871-876.

USEPA. 2005. United states environmental protection agency.

Welsh, J.H. and Smith, R.I. 1953. Laboratory exercises in invertebrate physiology. Burgess Publishing Company, Minneapolis, USA.

Winner, B.J. 1971. Statistical Principles in Experimental design. 2nd Edition, Mc Graw-Hill, New York.