IJPSR (2020), Volume 11, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 November 2019; received in revised form, 25 March 2020; accepted, 30 March 2020; published 01 November 2020

TOXICITY EVALUATION AND BEHAVIOURAL CHANGES OF *LABEO ROHITA* EXPOSED TO ETHION 50% EC

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Keywords:

Ethion, Organophosphate, *Labeo rohita*, $LC_{50,}$ and behavioral studies

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ABSTRACT: Modernization and intensification of agricultural production is generally accompanied by a rapid increase in the use of chemical fertilizers and pesticides. Ethion 50% EC is a broad-spectrum organophosphate insecticide used to control insects, mites, and nematodes by soil, foliar, and seed treatment applications, mainly on potatoes, sugar beet, rice, and maize, citrus. The aim of the present study was to assess the effect of Ethion 50% EC pesticide on common carp *Labeo rohita* for the 96 h LC_{50} value was found 1.20 µg/l and observed the behavioural studies at the of exposed to *Labeo rohita*.

INTRODUCTION: Modernization and intensification of agricultural production are generally accompanied by a rapid increase in the use of chemical fertilizers and pesticides. Pesticides are useful tools in agriculture but the gradual degrading of the aquatic ecosystem. Pesticide contamination of surface water use is a problem worldwide. When pesticide reaches the aquatic environment, it presents there for several days or weeks, depending upon its solubility, producing mass mortality, morphological, physiological, and behavioral changes in the organisms. However, excessive use of pesticides has caused an alarming level of resistance in many types of pests and disease organisms as well as serious contamination of mankind and the environment. Fish have long been valued as an excellent indicator of water.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(11).5579-86

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(11).5579-86

The oxygen consumption is a very sensitive physiological process, and the change in respiratory activity has been used as an indicator of stress in animals exposed to toxicants. Water pollution has focused the attention of the scientific community and the public on environmental problems. Pesticides are one of them used for agricultural purposes and due to their chemical property remain for a longer time in the air, water, and soil. Toxicity testing is an essential component of water pollution evaluation, and the study of changes in physicochemical parameters only does not help much in the assessment effect of pollution in aquatic biota. The toxicologist has demonstrated and advocated the utility of fish or predicting potential damage to the aquatic fauna of water bodies.

The toxicity tests thus throw light on the sensitivity of the organisms to the respective toxicants or chemicals. The lethal concentrations of chemicals to aquatic organisms are measured by exposing them to the variable concentrations. The selection of the toxicity tests is the most important aspect of the toxicology.

Toxicity Tests are Classified: Duration short term exposures, intermediate or long term, exposures 2. Method of adding testes solutions static, re-circulation, renewal, or flow-through methods 3. Purpose effluent quality monitoring, relative toxicity, relative sensitivity, test, order, growth rate, etc. of the organism [1]. These tests determine LC_{50} and are a quick estimate of toxicity, assessment of relative toxicity of different toxicants, and assessment of a toxicant to different species.

These tests are also useful for estimating toxicant concentration used in intermediate and long term tests. The intermediate test is conducted when a toxicity test is dealt with a long life cycle organism or longer life cycle stage, which requires additional time for the determination of LC_{50}. The aquatic environment, with its water quality, is considered as the main factor that affects the state of health and disease in both men and animals. Depending on the exposure of chemicals, single-dose test and multiple-dose tests come into the practice, and on the basis of duration of exposure, toxicity tests are divided into two main types: acute toxicity test and chronic toxicity test.

Acute Toxicity Test: It is a short term exposure to toxicants to determine the concentrations of a chemical or toxicant producing effect on the test organisms under controlled laboratory conditions. The death of the organisms is the most detectable response to find out the lethality tests or LC_{50} concentrations of test animals.

Chronic Toxicity Test: The effect of the chemical on the test organisms exposed to prolonged duration either whole life cycle or a particular stage and sensitive stage is carried out in a chronic toxicity test. The inherent capacity of a compound to affect adversely any biological activity of an organism. The best method for evaluating the toxicity of a toxicant is by the determination of median lethal concentration (LC) or lethal dose (LD), which represents the amount of poison for killing 50% of the particular population of the experimental animals [2]. Toxicity tests are used to evaluate the adverse effects of toxicants on living systems under standardized, reproducible conditions which permit a comparison with other toxicants tested [3, 4]. Different toxicants tests serve different purposes, and the short term acute toxicities conducted for a period of 24, 48, 72, and 96 h. This test is one of the most used tests in the evaluation of toxicities. The above test acute tests also conducted for a long duration, over a period of 30 days to several months,' *i.e.*, chronic toxicities.

Reference Toxicant: Reference toxicants help in comparing the results obtained from time to time with in the same laboratory (or) between different laboratories by providing a baseline set data [5]. Inappropriate behavioral changes to environmental and physiological stimuli due to the toxic effects of aquatic contaminates can have serve implications for survival [6].

Static Renewal Technique: It was accepted as a standard method by the committee on methods for (toxicity test with aquatic organisms [7]. This is an improved static and attempt has been made to maintain water quality and concentration of the toxicant. The test organisms were periodically transferred to a fresh test solution of the same composition, usually once in 24 h, either by transferring the test organisms from one chamber to another or by replacing test solutions.

Duration of the Test: The concentration of pesticides, which might normally in sub-lethal concentration during short-term exposures (24 h or 48 h), might prove to lethal concentration, if the exposure time is extended (up to 96 to 120 h). Since the toxicity of the poison is a function of time, it is customary to expose the test organisms over a fixed period of time to the toxicant, usually for a period of 96 h.

MATERIALS AND METHODS:

Animal Selected: *Labeo rohita* is an edible freshwater fish occurring abundantly in the freshwater bodies, rivers, lakes, and ponds in India [8]. A large population of fish consumers prefers this fish because of a rich source of animal protein, tasty flesh, and fewer bones. Besides its adaptability to the laboratory conditions and suitability to toxicity studies. Hence, this fish was selected as the experimental animal for this investigation. *Labeo rohita* is one of the three major carps produced and consumed in large quantities in India, with global production of 1.35 million tons per year.

Procurement and Maintenance of Fish: Healthy freshwater fish, *Labeo rohita* (Hamilton) size 6 ± 7 cm total length (TL) and 6.5 ± 7.5 g body weight were collected from the Kuchipudi fish farm; Guntur District of Andhra Pradesh, India; fish were immediately transferred in large plastic tanks with required aeration and brought to the laboratory.

Then the fish acclimatized to the ANU laboratory conditions in large cement (200 L) tanks with sufficient dechlorinated ground water for 15 days at room temperature 28 ± 20 ºC. During the acclimation period and subsequent periods of pesticides exposure, fish were held under a photoperiod of 12 h light: 12 h dark. The fish were fed with fish meal, rice and commercial fish pellets once in two days, at the same time water was renewed every day rich in oxygen (aeration) and feeding was stopped one day prior to the experimentation.

Then the fish were separated into the batch of having the length of the fish 6 ± 7 cm and body size 6.5 to 7.5 g were maintained in static water without any flow [7] (Doudoroff *et al*., 1951). All the precautions were laid by [9, 10] APHA *et al*., 1998; 2005 were followed.

As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, the hardness of water, exposure period, the density of the animals, size, and sex, *etc.*, precautions were taken throughout this investigation to control all these factors as far as possible. As a part of it, water from the same source has been used for maintenance of the fish. The size of the animals selected was also maintained strictly throughout the investigation.

Selection of Pesticide: The test compound in this study belongs to an Organophosphate pesticide Ethion 50% EC which is widely used in agriculture field of Krishna and Guntur districts of Andhra Pradesh. Ethion 50% EC is a broad-spectrum organophosphate insecticide used to control insects, mites, and nematodes by soil, foliar, and seed treatment applications, mainly on potatoes, sugar beet, rice, and maize, citrus. Ethion 50% EC was manufactured by PI industries.lim India. The toxicant was purchase from the local pesticide market in Guntur of Andhra Pradesh, India.

Preparation of Stock Solution: The stock solution of the toxicant was prepared in 100% pure acetone. Control group was maintained for each experiment and they were added with the acetone equal to the highest concentration used in the test. Precaution is taken to minimize the acetone as a solvent.

Selection of Lethal Concentrations: Toxicants exist in the aquatic system at concentrations too low to cause rapid death directly, but they might impair the functioning of organisms.

Through pesticide might not presently in lethal concentration, accidental spoilages might result in toxic concentrations. Hence, in the present investigation, 96 h LC_{50} 1.2 µg and $1/10^{th}$ of 96 h LC_{50} 0.12 µg was selected as lethal concentration to study the behavioral responses in the experimental animal.

RESULTS AND DISCUSSION: Aquatic organisms are continually being exposed to various pollutants in the environment. Toxicity of pollutants to plants, animals, fish, or wildlife can be evaluated simply by exposing a group of organisms under controlled conditions such as evaluation that can be performed and is the indices of action. Pesticides can produce adverse effects in a biological system, seriously damaging its structure and function of the living system finally leads to the death of an organism. Those adverse responses might be defined in terms of measurement as acute toxicity. Pesticides are entering into the aquatic ecosystem by agriculture runoff from land, impairing the quality of the water and making it unfavorable for aquatic life [11, 12, 13, 14, 15].

Toxicity is a relative property of a chemical which refers to its potential to have harmful effects on living organisms. It is a function of the concentration of the toxicant and duration of exposure. The toxicity tests provide a measure of the toxicity of compounds to a given species under specific environmental conditions (water quality, pH and temperature *etc.*). The determination of LC_{50} for any period offers more reliable information about the toxicity of a chemical in aquatic hazard evolution [12, 13, 14, 15]. The behavior of the animal can serve as the link between physiological and ecological processes; it might be used for studying environmental pollutant effects [12,

[16]. Sublethal and lethal effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction, and survival [17] that median lethal concentration of chlorpyrifos based pesticide Termifos to African catfish *Clarias gariepinus* were found to be 0.861 mg l^{-1}. Toxicity of pesticides to aquatic organisms also has been being affected by dissolved oxygen (DO), size, age, water quality, and temperature.

Short-term bioassay data are an 'early warning' in predicting acute poisoning in the field; they can be used to predict the toxicities of mixtures, and they can also serve to prognoses effects in various physicochemical conditions.

The information generated from various toxicity tests can be used in the management of pollution for different purposes like a prediction of environmental damage of waste, comparison of various toxicants, animals or test conditions, and regulation of waste discharge. Fish are adapted for aquatic respiration, during which they take water in, through the mouth and pass through gill chambers covered by the operculum. The flow of water is continuous for almost the whole of the respiratory cycle. In its passage, the water gives up oxygen to the blood and takes away the carbon dioxide through diffusion.

The process of oxygen is transported in the circulating fluid by hemoglobin present in the blood corpuscles. Oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental factors because it reflects energy expenditure and, ultimately, the food requirements.

The metabolic rate of fish is usually measured by their rate of oxygen uptake from water (Mo2); Mo2 is a criterion that has been suggested as an index of sublethal for fish and one that, if altered, might directly limit a fish's aerobic performance.

In the present study, the observed percent mortality along with exposure concentration of Ethion 50% EC for 96hr to the fish, *L. rohita* (Hamilton) in static renewal bioassay are given in **Table 3** and **Fig. 2,** respectively. The LC_{50} values are given in **Table 2** and **Fig. 1**.

The results of the LC_{50} (Lethal Concentration) of the present study at 96 h were found to 1.2 $\mu g l^{-1}$ for Ethion 50% EC, results according to Finney Probit analysis, the lower bound and upper bound 95% lethal confidence limits for Ethion 50% EC (1.3503-1.4497) respectively. The percent mortality and probit mortality increased with the increasing concentration of the toxicant. The percent mortality plotted against the log concentration of Ethion 50% EC gave a dose response curve. The 96 h LC_{50} of Ethion 50% EC was obtained by taking the mean LC_{50} derived from the percent and probit mortality curves.

The LC_{50} values of Ethion 50% EC to fish in, the present study shows that 96hr acute toxicity to freshwater fish *L.rohita* is 1.2 µg l^{-1}, which is less toxic as compared to, Bluegill *Lepomis macrochirus* (25.8 µg l-1) [18] (Kumar and Chapman, 1998); *Oreochromis niloticus* (0.046 mg l^{-1}), *Crucian carp Carassius carassius (*90.0 µg l^{-1}); present study Ethion 50% EC is more toxic to fish *L. rohita* as compared with controls.

The median lethal concentration of Neem-On to the freshwater fish *Labeo rohita* was 42.66 ppm. The variation in the LC_{50} values is due to its depending upon various factors *viz.*, sensitivity to the pesticide concentration and duration of exposure, [19]. The present study also supported by many authors [20]. The 96 h LC_{50} value of λ-cyhalothrin to fish *L. rohita* was 2.72 µg l^{-1} [21, 22] Investigated LC_{50} values of organ phosphorus pesticide metasystox to freshwater fish *Niemacheilus botia* was 10.3, 9.131, 7.884, &7.018 ppm for 24, 48, 72, 96 h [13, 14, 15].

In the present study observed that the fish *L. rohita* is less resistant to Organophosphate pesticide Ethion 50% EC to Compare with the different pesticides it was found that diazinon an organophosphate 1530 µg l-1 to the larvae of common carp [22] and cypermethrin a synthetic pyrethroid, has an acute toxic LC_{50} of 0.4 µg l^{-1} to fish *L. rohita* [23, 24.]

The median concentration of methyl parathion to guppy fish, *Poecilia reticulate* was 8.48 ppm. Alterations in the tissues of freshwater fish *L. rohita* exposed to fenvalerate [25, 26] were estimated LC_{50} value as 4 ppm for organophosphate quinolphos exposed to *Oreochromis mossambicus.*

TABLE 1: LC_{50} VALUES WITH 95% CONFIDENCE LIMITS FOR ETHION 50% EC BASED ON DISSOLVED CONCENTRATIONS ESTIMATED ACCORDING TO (FINNEY PROBIT METHOD, 1971

S. no.	Concentration	Exposed Fish	% Mortality	Log of Toxicant Concentration	Lower Bond/Upper Bond
1	1.2 µg/L	10	50	0.1261 ± 0.098	1.3503-1.4497

TABLE 2: THE 96 H ACUTE TOXICITY OF ETHION 50% EC ON FRESHWATER FISH, *LABEO ROHITA* PERCENT MORTALITY

S. no.	Concentration of Ethion (μgL^{-1})	Log Concentration	No of Fish Exposed	No of Fish Alive	No of Fish Dead	Percent Mortality
1	1.05	-0.3010	10	10	0	0
2	1.10	-0.1549	10	9	1	10
3	1.15	-0.0457	10	7	3	30
4	1.20	0.0791	10	5	5	50
5	1.25	0.1461	10	2	7	70
6	1.30	0.2041	10	1	9	90
7	1.35	0.2552	10	0	10	100

TABLE 3: THE 96 h ACUTE TOXICITY OF ETHION 50% EC ON FRESHWATER FISH, *LABEO ROHITA* PROBIT MORTALITY

S. no.	Concentration of Ethion 50% EC	Log Concentration	No of Fish Exposed	No of Fish Alive	No of Fish Dead	Probit Mortality
1	1.05	-0.3010	10	10	0	-
2	1.10	-0.1549	10	9	10	3.72
3.	1.15	-0.0457	10	7	30	4.48
4.	1.20	0.0791	10	5	50	5.00
5.	1.25	0.1461	10	2	70	5.84
6.	1.30	0.2041	10	1	90	6.28
7.	1.35	0.2552	10	0	100	8.09

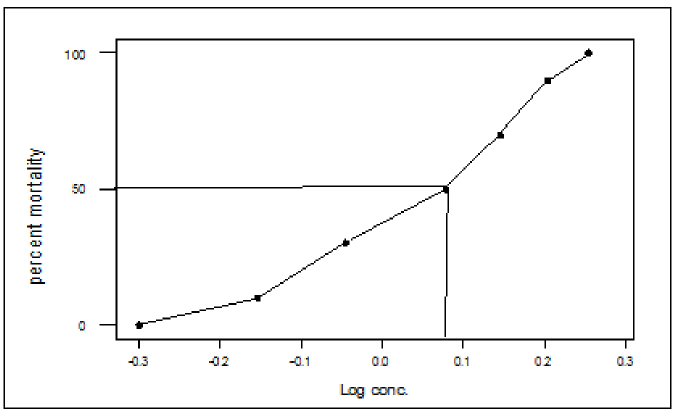

FIG. 1: THE GRAPH SHOWING DOSE RESPONSE CURVE BETWEEN PERCENT MORTALITY AGAINST LOG CONCENTRATION IN FRESHWATER FISH, *LABEO ROHITA* EXPOSED TO ETHION 50% EC

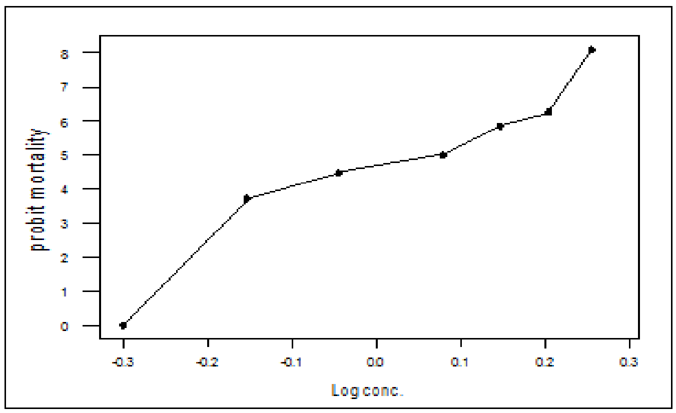

FIG. 2: THE GRAPH SHOWING PROBIT MORTALITY AGAINST LOG CONCENTRATION IN FRESHWATER FISH, *LABEO ROHITA* EXPOSED TO ETHION 50% EC FOR 96 H

Behavioral Changes: Morphological and behavioral changes exhibited by the test fish can be taken as useful parameters in assessing the toxicity caused by pesticides to some extent [27, 28]. Thus, studies on symptomology need much emphasis on understanding the changes in animals [29]. The behavioral changes were seen in the present study, over the duration of 96 h of exposure to Ethion 50% EC, overall statistically significant changes in almost all behavioral patterns were observed in fish *L. rohita* the surfacing phenomenon of fish observed under toxicant exposure.

The control group showed normal behavior during the whole experiment and also normal responses were observed at the low concentration of the toxicant (*i.e.*, 1.2 μgl^{-1}) (Ethion 50% EC) was applied there is no variation takes place at control group.

The present investigation of my research work after 24 h of exposure to Ethion 50% EC significantly increased hyperactivity in terms of surfacing and scraping moments, and schooling behavior was observed in comparison to controls.

At 48 h of exposure, the surfacing as well as scraping moments decreased in fish *L. rohita*, other behaviors increased hypersecretion of mucus significantly, opening mouth for gasping, losing scales, hypersensitivity was observed. After 72 h of exposure, the fish *L. rohita* showed decreased surfacing and jerky movements and increased grasping movements, sank to the bottom of the test chamber, and independency in swimming. Subsequently, fish moved to the corners of the test chambers, which can be viewed as avoidance behavior of the toxicant.

During the 96 h exposure, all body activities were nearly ceased. Complete loss of body balance exhibits irregular, erratic, darting swimming movements and loss of equilibrium followed by hanging vertically in the water. Hyper excitation, loss of equilibrium, increased cough rate, flaring of gills, increasing production of mucus from the gills, darting movements, and hitting against the walls of test tanks were noticed in all the species tested. A film of mucus was also observed all over the body and also on the gills. The increased variation rate by rapid and repeated opening-closing mouth and opercula covering accompanied by partially extended fins (coughing) was observed. This could be due to the clearance of the accumulated mucus debris in the gill region for proper breathing.

Nerve agents act on acetylcholinesterase (AChE), especially dealing with neurotoxic compounds. Their toxicity is not limited to the acute phase; however, chronic has long been noted. Neurotransmitters are acetylcholinesterase; acetylcholine level increased resulting in the failure of transmission of stimuli to the nerves or organs. My study (or) research work *Labeo rohita* exposed to Ethion 50% EC is coinciding with those other researchers. Decreased swimming behavior and increased respiration rate were other effects of insecticide in the present study, exposed to *Labeo rohita* to Ethion 50% EC found that contaminates such as pesticides disturb normal fish behavior after exposure [6]. Pesticides are known to effect fundamental physiological systems such that they affected salmonid olfactory-mediated behaviors in study [30]. Fish kills occur when pesticides are improperly applied to or otherwise end up in bodies of water through either misapplication or drift [31]. The response was initiated at the threshold dose increase intensity of dose and exposure time is increased. This is the basic concept of the dose relationship [32, 13, 14, 15]. This leads to the abnormal functioning of the body, including loss of balance, moving in a circular form (convulsions), and at higher concentrations of insecticides resulting in the death of the organism. Fish in the experimental group applied with the highest concentration of pesticides were cloying laterally at the bottom with the loss of balance, swimming down in a spiral movement with jerks. Thus, the behavioral changes of the fish under insecticidal habitat and might affect the stability of the population reported [12].

It is clear those earlier studies that LC_{50} of insecticides for a freshwater fish varies from species under the influence of a number of factors, including size and time of exposure. Disruption of schooling behavior of the fish, due to the lethal and sublethal stress of pesticides, results in increased swimming activity and entails increased expenditure of energy. The erradic swimming of the treated fish indicates a loss of equilibrium.

In the present study of test organism *Labeo rohita* exposed to Ethion 50% EC showed jerky movements, hypersecretion of mucus, opening mouth for gasping, losing scales, hyperactivity was observed in increase the toxicant concentration. Behavioral characteristics are obviously sensitive indicators of toxicant effects. It is necessary to select behavioral indices a more accurate assessment of the hazards that a contaminant may pose in natural systems. After observing experiments and all author reports, the present study showed that Ethion 50% EC is highly toxic to the common edible fish *Labeo rohita*.

CONCLUSION: In the present investigation of the test species, *Labeo rohita* has shown differential toxicity levels with the function of the period. This shows that the more is the duration period, the less is the concentration required. The observed percentage mortality and probit mortality of *Labeo rohita* for Ethion 50% EC in static tests continuous for different hours and different concentrations were shown in **Table 2**. Control and experimental groups in response to insecticide Ethion 50% EC in presently studied fish *Labeo rohita* (Table no. II) Confirm that Toxicity evaluation (LC_{50} values) and behavioral changes in fishes are very sensitive indicators under toxicity of chemicals. The behavioral changes are affecting the general health status of the fish.

ACKNOWLEDGEMENT: Authors express the deep sense of gratitude to all the professors and Head of the department, Acharya Nagarjuna University, Department of Environmental Sciences, for providing necessary laboratory facilities to carry out this work. IAEC approval number: IJPSR/RA-13511/11-19

CONFLICTS OF INTEREST: I declare that there are no conflicts in research.

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How to cite this article:
Prasanna C, Anithasmruthi C and Rathnamma VV: Toxicity evaluation and behavioural changes of *Labeo rohita* exposed to ethion 50% EC. Int J Pharm Sci & Res 2020; 11(11): 5579-86. doi: 10.13040/IJPSR.0975-8232.11(11).5579-86.