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STUDY ON THE ACUTE TOXICITY OF CHLOROCID 20 EC TO *Tubifex* sp. Lamarck, 1816

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ABSTRACT

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Static bioassay tests were conducted to assess the acute toxicity of chlorocid 20 Emulsified Concentration (EC) to *Tubifex* sp. Lamarck, 1816 during March to August 2006 in the Zoological Garden at Carzon hall campas of the Dhaka University. *Tubifex* sp. was exposed to the toxicant for different duration (24-96 hours) with and without debris in the exposure media. In the Bioassay test 1 (without debris), Lethal Concentration$_{50}$ (LC_{50}) values of chlorocid 20 EC at 24, 48, 72 and 96 hours of exposure time were 7.144 mg/l, 4.116 mg/l, 2.212 mg/l and 1.607 mg/l respectively and 9.690 mg/l, 6.943 mg/l, 5.708 mg/l and 4.084 mg/l respectively in Bioassay test 2 (with debris). The acute toxicity of chlorocid 20 EC gradually decreased with time, which was indicated by toxicity curve becoming asymptotic with the time. These asymptotes mark the approximate values of the threshold concentration (=incipient LC_{50}). The results show that higher levels of toxicant are needed to induce mortality under debris condition. The pre-exposure acclimation to 0.50 mg/l of chlorocid 20 EC afforded slight resistance to the toxicant as evident by delayed and reduction in mortality, when challenged with higher lethal doses, compared to un-acclimated *Tubifex* sp.. Acclimation to 1.0 mg/l chlorocid 20 EC for a period of four days did not afford any protection to *Tubifex* sp. when challenged with lethal doses. The result of the experiment reveals that debris as well as pre-exposure acclimation with chlorocid 20 EC influences the mortality rate of *Tubifex* sp.

Key Words: Toxicity, Bioassay test, Chlorocid 20 EC, *Tubifex* sp.

INTRODUCTION

Tubifex sp., the common benthic organisms occupy the important spatial and trophic niche in the aquatic ecosystem. Benthic organisms, commonly known as benthos are attached or resting on the bottom or living in the bottom sediments. They vary widely with different bottom condition and with depth. They play an important role in the release of nutrients from sediment (Pelegri and Blackburn, 1996). They are also used as indicators of water quality and trophic conditions (Lindegraad, 1995) and reflect characteristics of both the sediments and water column (Cook and Johnson, 1974). Most of them are of micrscopic forms; consist of mainly oligocheats, insects and molluscs. *Tubifex* sp. belongs to the order Plesiopora under the class Oligocheata of the phylum Annelida. They are essential for production of fish food and proper growth of fish that ultimately increase fish production in the water body. *Tubifex* sp. can be an index to compare the relative fertility and fishery potential of different water. Fish health directly depends on the plankton and benthos those can be easily digested by fishes and those contain high calories of energy. Now it is universally expected that the richest fisheries of the world are closely related to the plankton and benthos production. Benthos constitutes important food items of bottom dwelling fish, also the carnivore, omnivore and scavenger fish. On the other hand, organic fertilizers are especially efficient in increasing the abundance of benthos particularly *Tubifex* in the water body. So benthos is important community in aquatic food chain which connected with the terminal biological production.

Bangladesh is mainly agricultural country and large amount of agrochemical and pesticides are used to enhance production. Few hundreds of these pesticides are marketed and a large number of them are also illegally smuggled into country. But indiscriminate use of these pesticides is contributing most to the pollution which has led to wide spread development of pesticide resistant strains, destruction of non-target organism e.g. *Helistis* sp. in the cotton bee and several disruptions in aquatic and terrestrial animals. Such as affects not only to fish, fish eggs, larvae and fry but also other aquatic lives. Chlorocid 20 EC, an organophosphrous pesticide, is a recently marketed pesticide in Bangladesh. But no investigation has yet been done on the toxic effects of chlorocid 20 EC to fish or any other organism. So it is felt necessary to determine the toxic effects of this pesticide to benthic organism especially *Tubifex* in aquatic environment.

A large number of works have been carried out in Bangladesh concerning the toxic effects of pesticide to animals. However, most of the studies were concentrated on fish as they are economic commodities (Rita and Nair 1979; Mount, 1962; Tripathi, 1992; Sultana, 1994; Barman, 1996). Unfortunately, few studies investigated the toxic effects of pollutants to benthic organisms (Mani and Konar, 1984; Pal and Konar, 1986). Considering the importance of benthos especially *Tubifex* sp. the present investigation was performed to determine the LC_{50} values for the Chlorocid 20 EC at different exposure times, to assess the acute toxicity to *Tubifex* sp., whether the pre-

exposure acclimation to low level of Chlorocid 20 EC has any effects on modifying the effects of lethal toxicity and to assess the effects of organic debris in modifying the lethal toxicity.

MATERIALS AND METHODS

Study and duration of the research work

The experiment was carried out in the Aquarium House of the Department of Zoology, University of Dhaka located at the animal garden of the department. Analytical and other laboratory works were done in the Laboratory of the Department. The present experiment was performed during March to August 2006.

Collection and acclimation of Tubifex sp.

Tubifex sp. were obtained from the drainage system of Curzon Hall area in live condition and kept in glass beaker with water containing debris. The collected *Tubifex* sp. were further acclimated in glass aquaria (8×8×8 cubic inch) containing debris, supplied with tap water for a period of 72 hours in static condition. However, water exchanges were done at every two days. Only healthy organisms of approximately similar size were transferred to the experimental system. The major water quality of the parameter of the acclimation tank was monitored at times and found within the normal ranges.

Preparation of Toxicant Concentration

Test stock solution of the toxicant was made by adding requisite amount of the chemical, calculated in terms of active ingredient, to tap water. Initially stock a solution of 100 mg/l water of chlorocid 20 EC was prepared. Calculated amount of stock solution was then added to the experimental tanks to obtain the desired concentrations of the chemical in the experimental tanks. The test chemical was measured by pipette.

The Experimental System

The experimental trials were conducted in a laboratory bioassay system. The system consisted of 14 glass aquaria (8×8×8 cubic inch), each had water holding capacity of 4 litres. Before and after each bioassay trial, the aquaria were washed with commercial detergent and then washed thoroughly with tap water. The aquaria were dried in the sun before the organisms were exposed to the toxicant and placed on a steel platform serially. Draining of water from the aquaria was done by siphoning. Air stones connected to air compressors were used for oxygenation.

Experimental Procedure and Protocol

Bioassay Tests: Prior to the actual study, two preliminary trials were carried out using arbitrary concentrations of the toxicants. On the basis of the observed mortality in the preliminary trials (0, 5, 10, 15 and 20 mg/l of chlorocid 20 EC used), a series of closely spaced concentrations (0, 1, 2, 4, 6, 8 and 10 mg/l of chlorocid 20 EC used in bioassay test 1 and 0, 1, 2, 4, 8, 12 and 14 mg/l in bioassay test 2) were selected to be used in the actual or bioassay tests. Each concentration was tested in duplicate. Two bioassay tests– Bioassay test 1 (without debris) and bioassay test 2 (with debris) were done.

Acclimation Tests*:* A batch of 100 *Tubifex* was brought to the laboratory and acclimated to the laboratory conditions for 3 days. During this time debris were given as their feed. The water medium was changed at 24 hours interval.

After acclimation of benthos to laboratory conditions, 60 individual of *Tubifex* sp. were randomly selected and divided into three groups. First group (Group-A) received no toxicant treatment. Second group received doses of 0.5 mg/l and the third group received a dose of 1.0 mg/l of chlorocid 20 EC continuously. It was observed during the previous experiments (bioassay tests) that these doses were not lethal to the test animal during a four days exposure period and it was also observed that this particular dose did not exert much stress to the test animal. Therefore, it was hoped that the use of these concentrations might induce acclimation process in benthos. During this time debris were given as their feed. The water medium was changed daily. The acclimation of benthos under the mentioned condition was continued for a period of 3 days. A concentration of 8, 12 and 14 mg/l of the toxicant were used during pre-exposure acclimation and subsequent challenge exposures.

Twenty individuals of *Tubifex* sp. was used in each experimental tank, containing 4 liters of test water. It was hoped the loading of organisms would not exceed the loading capacity of the test water for the test animal. Each experimental trials whether preliminary or actual was conducted for a period of 96 hours to determine the median lethal concentrations (LC_{50}) as recommended by Sprague (1969, 1973); Alabaster and Lloyd (1980) and American Public Health Association (APHA, 1980). According to Sprague (1963) 96 hours LC_{50} is most reproducible.

Mortality and Criteria for Death

Records of mortality were made at logarithmic time intervals (Sprague 1973). However, in addition to these fixed time observations, several inspection were made in between these periods and benthos were removed from the aquaria as soon as they were found dead to prevent water quality deterioration. If the organism did not shown any sensitiveness to prodding the animal was regarded as dead.

Data Analysis

The median lethal concentration (LC_{50}) value for different exposure times were estimated by using computerized EPA probit analysis program (version-1.5). The facility was available in Safeway Pest Control, Dhaka.

RESULTS AND DISCUSSION

The toxicity of Chlorocid 20 EC was determined using *Tubifex* sp. as a test organism. The result of the bioassay test 1 and bioassay test 2 (Figure 1 and 2, Table 1 and 2) revealed that the mortality was increased with the increased concentration of doses. However, there were some differences between the two conditions which showed in Figure 1. These tests showed that mortality was delayed in lower concentrations of chlorocid 20 EC in debris condition compared to without debris's condition. It seems that debris probably absorb or bind with organic matters of the chlorocid 20 EC and thereby decrease toxicity level of it. Rand and Petrocelli (1985) suggest that many toxicants bind with organic debris and its toxic effects are reduced.

Table 1. Cumulative mortality (%) of *Tubifex* sp. at different concentrations of chlorocid 20 EC during exposure period of 96-hr (bioassay test 1)

Treatment No.	Conc. of chlorocid 20 EC (mg/l)	No. of *Tubifex* sp. released	Exposure time					
			Cumulative mortality (%) with times (hours)					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	1	20	0	0	0	10	25	30
2	2	20	0	0	10	20	40	60
3	4	20	0	0	20	40	60	80
4	6	20	0	0	30	50	90	100
5	8	20	0	30	50	80	100	100
6	10	20	30	60	80	100	100	100

However, Mani and Konar (1984) reported much lower value of LC_{50} for malathion to *Diaptomus forbesi* which ranged from 0.001-0.54 ppm and in case of *Branchiura sowerbyi* it was 0.05-0.66 ppm. Pal and Konar (1986) estimated LC_{50} values for *Diaptomus forbesi* and *Branchiura sowerbyi* which ranged from <1-1.01 ppm, <0.05-0.93 ppm, <1-6.5 ppm and 7.5-14 ppm, <0.005-0.425 ppm, 0.34-2.92 ppm, respectively in case of phosphamidon, methyl parathion and mixture of DDVP, phosphamidon, methyl parathion. Compared to the present investigation, Sahib and Rao (1980) reported that LC_{50} value of 48-hrs of malathion to *Tilapia mossambica* as 5.5 mg/l. Similarly, higher LC_{50} value (15.05 mg/l) was reported by Sultana (1994) for the fingerlings of *Labeo rohita*. The differences in LC_{50} values observed in the present experiment with that of above mentioned studies might be due to differences in the species, chemicals and other experimental conditions.

Table 2. Cumulative mortality (%) of *Tubifex* sp. at different concentrations of chlorocid 20 EC during an exposure period of 96-hrs (bioassay test 2)

Treatment no.	Conc. of chlorocid 20 EC (mg/l)	No. of *Tubifex* sp. released	Exposure time					
			Cumulative mortality (%) with times (hours)					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	1	20	0	0	0	0	0	0
2	2	20	0	0	0	0	0	20
3	4	20	0	0	0	10	20	40
4	8	20	0	0	30	50	75	80
5	12	20	10	30	70	100	100	100
6	14	20	30	60	90	100	100	100

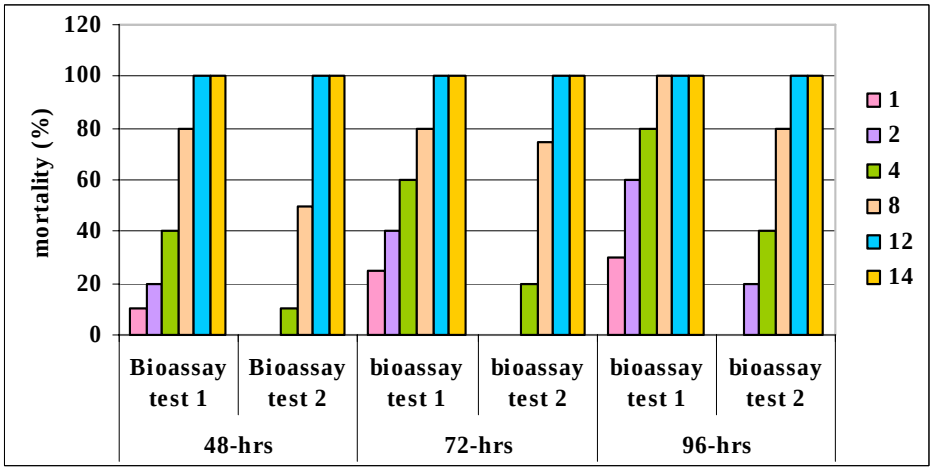

Figure 1. Toxicity curve of chlorocid 20 EC for *Tubifex* sp. during 48-, and 96-hrs experimental period (bioassay test 1 and 2)

As it was evident from the results the acute toxicity of chlorocid 20 EC of its respective 95% CL gradually decreased with time. This is similar to the observation made by Korn and Earnest (1974), Sahib and Rao (1980), Pal and Konar (1986), Sultana (1994). It was evident from the result of authors that asymptotic mark of the toxicity curve was reached and a clear asymptotic mark was established by 96-hrs exposure. However, a clear asymptotic mark was established by 96-hrs exposure during the present study.

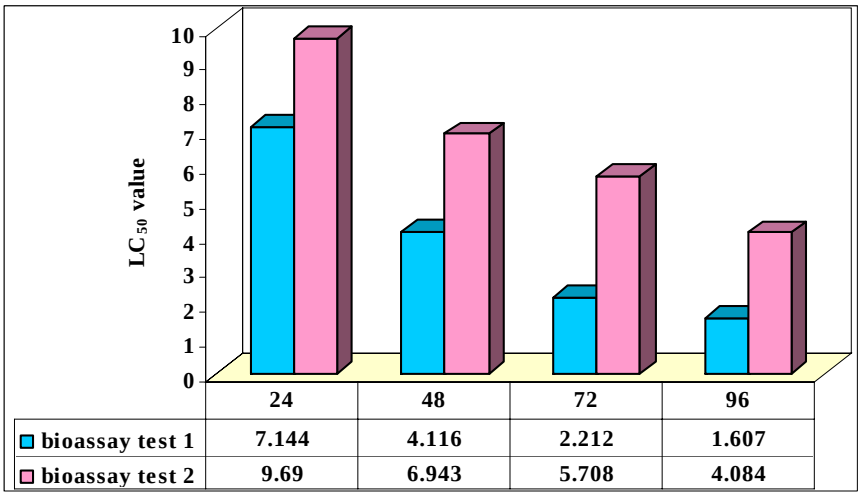

Figure 2. Toxicity curve of LC_{50} for *Tubifex* sp. at different hours-experimental periods of bioassay test 1 and 2

Mortality of acclimation tests

From the result of the present study it can be concluded that previous acclimation to 0.5 mg/l of chlorocid 20 EC for 3 day afforded slight protection against mortality when challenged with higher concentrations of the toxicant, but animal acclimatized to1.0 mg/l did not afford any protection. And this result was showed in Figure 3 and Table 3. However, both cases, mortality were delayed compared to un-acclimatized *Tubifex* sp. The delayed mortality in previously acclimated *Tubifex* sp. might be due to modulation of adaptive mechanism during long term low level of exposure. On the other hand, Barman (1996) observed that the acclimated fish become more sensitive to the toxicant compound than the fish groups, which were not acclimated previously. In conformity with the present observation, Vison *et al*. (1963) reported increased tolerance in mosquito fish (*Gambusia affinis*) previously exposure to sub-lethal level of DDT. Dixon and Sprague (1981b) observed that sensitivity increased by one third of the lethal level to cyanide following acclimation to low level of the toxicant.

During study it was found that the water quality parameters did not fluctuate greatly not only among the different treatment aquaria but also between different experimental trials. In the first experiment fluctuations in temperature was very little (range 26^0C-30^0C). Aeration of water ensured adequate supply of oxygen. During the experimental trials the *Tubifex* sp. were not fed which helped to avoid large fluctuations in their metabolic wastes and fouling of test solution.

Table 3. Cumulative mortality (%) of three grouped *Tubifex* sp. challenged with different concentration of chlorocid 20 EC for a period of 96-hrs after continuous pre-exposure acclimation.

Acclimation conc. (mg/l)	Group of *Tubifex* sp.	Challenge conc. (mg/l)	Exposure time (hours)					
			Cumulative mortality (%) with times (hours)					
			6	12	24	48	72	96
0.0	A_1	0	0	0	0	0	0	0
	A_2	8	0	25	50	80	100	100
	A_3	12	20	40	75	100	100	100
	A_4	14	50	80	100	100	100	100
0.5	B_1	0	0	0	0	0	0	0
	B_2	8	0	0	10	30	40	60
	B_3	12	0	20	50	80	100	100
	B_4	14	20	40	80	100	100	100
1.0	C_1	0	0	0	0	0	0	0
	C_2	8	0	0	30	40	80	100
	C_3	12	0	40	65	95	100	100
	C_4	14	40	70	100	100	100	100

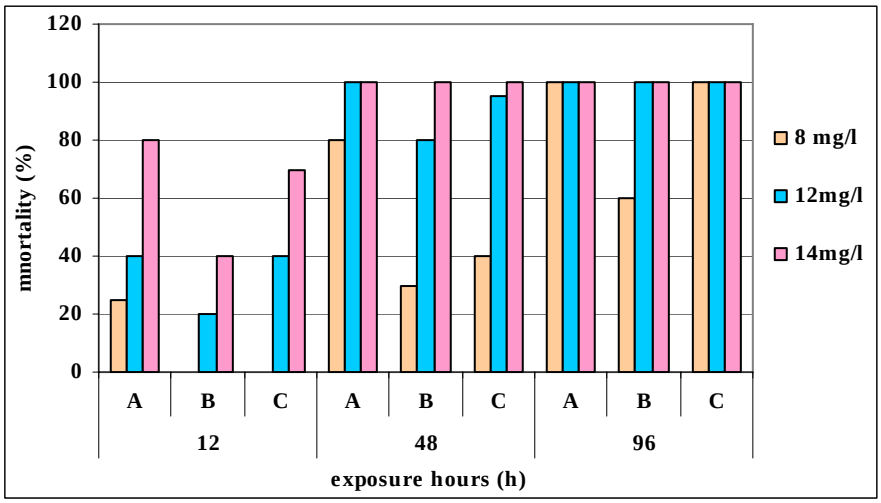

Figure 3. Graphical presentation on mortality of *Tubifex* sp. a different exposure time during acclimation tests

It can be concluded from the present study that chlorocid 20 EC not only decline the *Tubifex* sp. in the aquatic environment, but also produce resistance variety of *Tubifex* which are ultimately consumed by fish and affect our fishery resources adversely. Therefore, further extensive research should be undertaken to prepare a guideline to use chlorocid 20 EC in our aquatic environment.

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