

## Lethal Concentration 50 (LC<sub>50</sub> - 96 hours) Nile Tilapia (*Oreochromis niloticus*) exposed Cypermethrin-based Pesticide

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### Abstract

In aquaculture of an irrigation, the use of cypermethrin-based pesticide can harm Nile tilapia which cultured within and its impact on humans who consume the fish. Thus it is necessary to study to determine the threshold of this cypermethrin-based pesticide. Determining the thresholds was using the test of LC<sub>50</sub> - 96 hours (Lethal Concentration 50-96 hour) which tested tilapia that dead by 50%, which can be used as a benchmark threshold for cypermethrin-based pesticide. Test of LC<sub>50</sub> - 96 hours consist of preliminary test and advanced test. Lethal Concentration 50 (LC<sub>50</sub> - 96 hours) of cypermethrin-based pesticide towards Nile tilapia (*Oreochromis niloticus*) is 0.082 ppm. At these doses, tilapia death reached 50%. This concentration is expressed as a threshold usage of cypermethrin-based pesticide on the environment around the aquaculture of tilapia.

**Keywords:** Cypermethrin-based pesticide, LC<sub>50</sub>96 hour, Nile tilapia.

### INTRODUCTION

Pesticides are substances used to control population of certain species which are considered as pests that directly or indirectly harm the interests of human beings. The addition of pesticides also generates environmental impacts. The environmental impact will be carried along the food chain. In agriculture, the most widely used pesticide is Cypermethrin. Cypermethrin widely used in farming activities and household pest control of the world [1]. Despite its low toxicity to mammals, Cypermethrin is highly toxic to aquatic organisms and honeybees. Farmers use Cypermethrin-based pesticides at a concentration of 500 ppm per 1 liter of water.

Toxicity tests used to study the effect of a chemical toxic to certain organisms. Toxicity tests normally used are LC<sub>50</sub> -96 hour, i.e. the levels of toxic materials that can cause the death of 50% of the population or the test organism within 96 hours [2]. Acute lethal toxicity is a toxic process or the entry process of toxic substances into the body causing the interference of working mechanism and the target organ. Acute toxicity test or toxicity of lethal acute also means a trial

designed to evaluate the relative toxicity of a chemical to aquatic organisms in a limited and specified period of time. The criteria effects commonly used in lethal acute toxicity tests are the death percentage (in fish), the absence of movement (immobility) and balance, and growth [3].

This study used Nile tilapia (*Oreochromis niloticus*) as the test organism; types of fish that would be suitable for use in similar aquaculture research as this study [4]. Nile tilapia is one of the biota of water recommended by the USEPA (US Environmental Protection Agency), as the test animals for toxicology. This is because the distribution is quite extensive, widely cultivated has high capability in tolerating a bad neighborhood and is easily maintained in the laboratory. Additionally, Nile tilapia fish is also an organism which categorized as important in aquaculture. Nile tilapia is used as bio-indicators because fish have a high resistance to a wide range of changes that occur around the neighborhood of his life, fast growth, resistance to disease and classified as sensitive fish [5]. This study was aimed to determine the threshold on cypermethrin based pesticide dose which allowed in the waters with referring to the LC<sub>50</sub> 96 hour.

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## MATERIALS AND METHODS

### Acute Toxicity Test Procedure (LC<sub>50</sub>-96 hours)

Research procedures of acute toxicity test was performed on two stages, a preliminary test and continued with the real test. The range for testing is a multiple of 24 hours. Doses that used is based on a logarithmic scale which is read by progressive bisection [6].

#### Preliminary test

Preliminary test is intended to determine the range of appropriate levels of cypermethrin-based pesticide that took place during the short period. The range of concentration is expressed as the threshold lethal consisting of bottom threshold lethal (LC<sub>0</sub> 24 hour) and the above threshold lethal (LC<sub>100</sub> 12 hour) with the range of the closest with LC<sub>50</sub> 96 hour. Lethal threshold levels are then used to determine the median lethal concentration (LC<sub>50</sub> 96 hour). The procedure is as follows.

Basin test capacity of 16 liters was prepared for 8 units for each concentration. Then a solution of cypermethrin-based pesticide made with a concentration of 0 ppm; 0.0001 ppm; 0.001 ppm; 0.01 ppm; 0.1 ppm; 1 ppm; 10 ppm and 100 ppm according to base figures on a logarithmic scale column 1. Then, Nile tilapia (*Oreochromis niloticus*) is in medium-sized 7 cm - 9 cm as many as 10 individuals included in each treatment. During toxicity testing, we used continuous aeration. Observations were made every 12 hours for 96 hours to determine the mortality. The observed parameter is the number of dead on Nile tilapia once every 12 hours, and calculated on a cumulative 96 hours. The mortality percentage is calculated from the number of dead fish divided by the total number originally on each treatment level.

#### Advanced test

Stages on advanced tests (acute toxicity test) is the first LC<sub>0</sub> -24 hour (bottom lethal threshold) and LC<sub>100</sub> 12h (above lethal threshold) which obtained from a preliminary test. Then the results are use to determined variations in the levels of advanced test is based on a logarithmic scale [6] precise targeting of the to test advanced by way of progressive bisection. Then, the process of acute toxicity testing (advanced test) where the procedure is the same as a preliminary test procedure. Parameter observed is the number of dead Nile tilapia every 12 hours for 96 hours calculated cumulatively [7].

#### Probit analysis

Data analysis in this study using probit analysis. Probit analysis is generally used to determine the relative toxicity of chemicals on living organisms. It is used to test the response of test organisms in a variety of chemical concentrations and compared them. The probit analysis was calculated through statistical data with Microsoft Excel.

## RESULTS AND DISCUSSION

### Preliminary test

Preliminary test carried out to obtain a concentration above the threshold (LC<sub>100</sub> -24 hour) as the lowest concentration in which all test fish is tilapia die within 24 hours of exposure. While the lower threshold (LC<sub>0</sub> -48 hour), which is the highest concentrations where all the fish are still alive in the time of exposure 48 hour [8]. Data from acute toxicity test pesticide with active ingredient cypermethrin towards the Nile tilapia (*Oreochromis niloticus*) in the preliminary test can be seen in Figure 1.

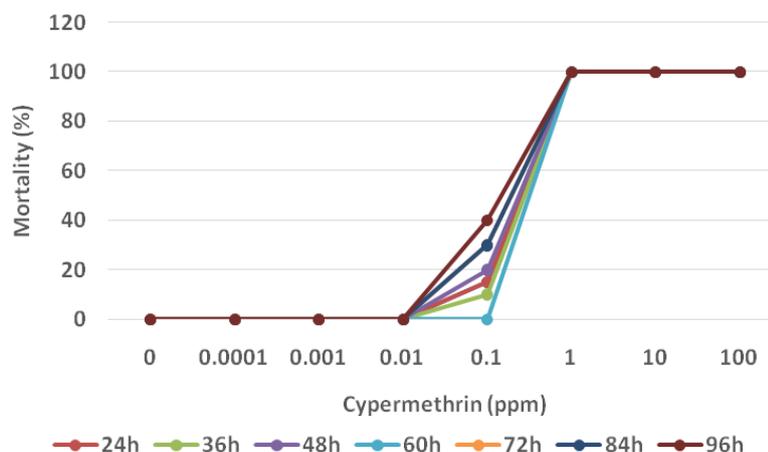


Figure 1. Mortality of Tilapia on Preliminary Test

Based on preliminary test results showed that Cypermethrin pesticide have lower threshold value 0.01 ppm to percentage mortality of 0%. The concentration is the highest concentration of the test, in which all fish is still alive (100%) within 48 hours. A threshold value above 0.1 ppm with a percentage of 70%, but the concentration is not representative or not the lowest concentration of a test in which all fish (100%) died within 24 hours. This concentration is used as the upper threshold because within 96 hours the percentage of mortality of the largest fish approaching 50%. According to Husni and Esmiralda [9] a preliminary test carried out to determine the limits of the range of critical (critical range test), which became the basis of the determination of the concentrations used in advanced test or toxicity tests indeed. That concentration can cause most of the deaths was close to 50% and lowest mortality approach 50%.

**Advanced test**

Advanced test is performed to determine the concentration where 50% fish dead during the period of exposure of 96 hours (LC<sub>50</sub> - 96 hours). The dose used in this advanced test based on the logarithmic scale bisection [10]. This dose is guided by values above the threshold and the lower threshold limit value on a preliminary test carried out previously. The range is based on a logarithmic scale Random concentration, i.e. 0 ppm; 0.0135 ppm; 0.018 ppm; 0.024 ppm; 0.032 ppm; 0.042 ppm; 0.065 ppm; 0.087 ppm of Cypermethrin pesticides. Data from acute toxicity test pesticide with active ingredient Cyper-

methrin towards tilapia (*Oreochomis niloticus*) on advanced test can be seen in Figure 2.

The mortality rate of fish vary in accordance with the concentrations of pesticides are given. At the low concentration of 0.0135 ppm, 0.018 ppm, 0.024 ppm, 0.032 ppm there are no dead fish. The accumulation of contaminants in organisms differ depending on the concentration of pollutants in the water/environment, temperature, state of the test animals and physiological activity [11]. Therefore, at these concentrations, the fish can tolerate the levels of given pesticides, thus the fish survived.

At a concentration of 0.042 ppm; 0.065 ppm and 0.087 ppm, death of the fish is start happening and the highest mortality at a concentration of 0.087 ppm up to 70% mortality occurred. The fish mortality has increased with the growing number of pesticide concentrations given. A decrease in the survival test fish caused by the inability of the adaptation of fish to pollutants given in the media [12]. As a result, the fish are not able to neutralize effect that pollutants contained in the test medium. In the water, Cypermethrin is in the form of metane ethyl carboxylate, alkylation can react with Fe in hemoglobin and may replace oxygen in erythrocytes [13]. Biochemical reactions that show this biological imbalance that causes the proliferation of blood cells does not occur, as a result of blood volume remained. However, erythrocytes decreased, resulting in death due Fe Cypermethrin bound by the active ingredient.

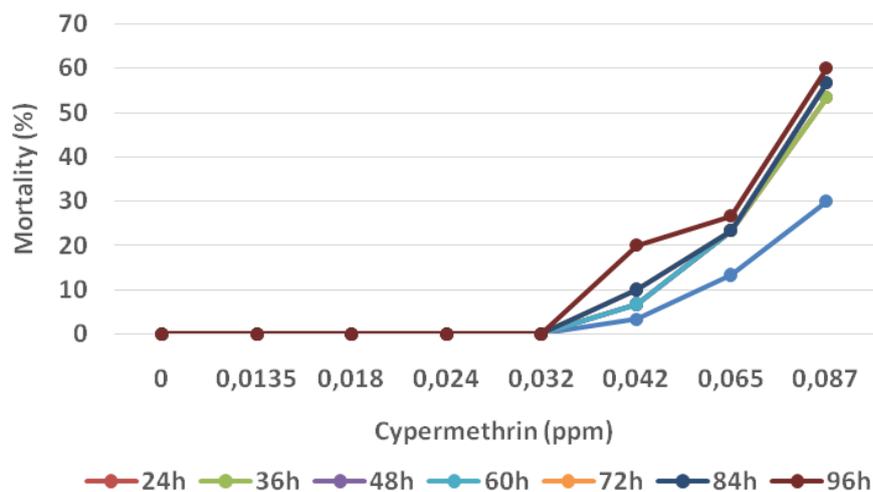


Figure 2. Results Mortality Data Tilapia on Advanced Test

Table 1. Table of Probit Value Calculation

| Cons. (ppm) | log. Cons (x) | Σ organism test | Repeat |   |   | the average number of deaths | % mortality | Probit value * |
|-------------|---------------|-----------------|--------|---|---|------------------------------|-------------|----------------|
|             |               |                 | 1      | 2 | 3 |                              |             |                |
| 0           | -             | 10              | 0      | 0 | 0 |                              |             |                |
| 0.0135      | -1.8696       | 10              | 0      | 0 | 0 |                              |             |                |
| 0.018       | -1.7447       | 10              | 0      | 0 | 0 |                              |             |                |
| 0.024       | -1.6197       | 10              | 0      | 0 | 0 |                              |             |                |
| 0.032       | -1.4948       | 10              | 0      | 0 | 0 |                              |             |                |
| 0.042       | -1.3767       | 10              | 3      | 2 | 1 | 2                            | 20          | 4.1584         |
| 0.065       | -1.1871       | 10              | 2      | 3 | 3 | 2.67                         | 26.67       | 4.375          |
| 0.087       | -1.0605       | 10              | 5      | 6 | 7 | 6                            | 60          | 5.2533         |

Note: \* Value is determined by probit transformation table

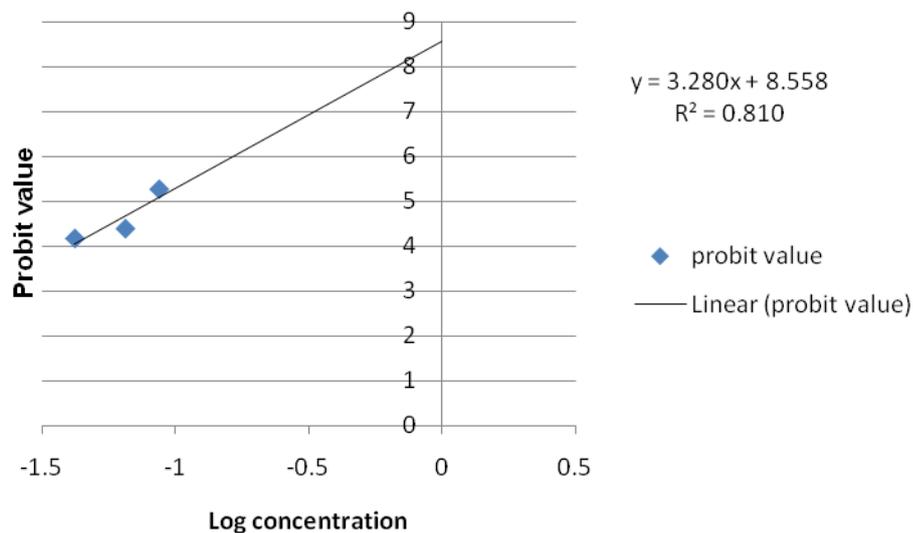


Figure 3. Probit Graph

The observation of advanced test showed that the higher the concentration of pesticides given the higher fish mortality. It conformed to the previous study that mentioned the percentage of survival of tilapia fish (*Oreochromis niloticus*) decreases with increasing concentrations of the pollutants [14]. Basic calculations with observations on mortality data of tilapia (*Oreochromis niloticus*) for 96 hours at the advanced test, the results of calculations to determine the probit values are presented in Table 1.

The next step probit analysis is to create charts using Microsoft Excel where  $x = \log$  concentration, and  $Y = \text{probit value}$ , the results are presented in Figure 3. Based on the graph probit (Fig. 3), we obtained line equation  $Y = 3280 + 8558$  if it is assumed that the value of LC<sub>50</sub> 96 hour with the number of deaths is  $y = 5$  (50%)

of the test animals, the value  $x = -1,084$  so that the value probit is the antilog of  $-1,084 = 0.082$ . The value shows that the exposure dose of cypermethrin-based pesticide is 0082 ppm causes 50% of the population of test animals dying within 96 hours.

### CONCLUSION

Lethal Concentration 50 (LC<sub>50</sub> - 96 hours) of cypermethrin-based pesticide towards tilapia (*Oreochromis niloticus*) is 0.082 ppm. At these doses, tilapia will die 50%. This concentration is expressed as a threshold cypermethrin-based pesticide used on the environment around the cultivation of Nile tilapia.

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