The effect of chlorpyrifos exposure on carp fish at twin lakes of West Sumatra Indonesia

T Ihsan*, T Edwin¹, D Paramita¹, N Frimeli¹

¹Department of Environmental Engineering, Faculty of Engineering, Universitas Andalas, Kampus Unand Limau Manis, Padang, West Sumatra 25163, Indonesia
taufiqihsan@eng.unand.ac.id

Abstract. This study was conducted to analyze the sublethal effect of chlorpyrifos on the Feed Conversion Ratio (FCR), Sustainable Growth Rate (SGR), and carp behaviors. The concentration used 1/7 and 1/14 of the LC50 carp. The study was conducted with the number of fish per aquarium was five fishes, and the testing exposure time was 14 days. The results of the FCR and the SGR at a concentration of 1/7 and 1/14 LC50 was 0.2907 and 0.3031, and 1.551% and 1.9581%, respectively. Changes in behavior during exposure, a decline in appearance such as being alone in the corner of the aquarium, moving irregularly and stress (r = 0.75 - 1), lots of mucus (r = 0.800 - 1), anal excretion (r = 0.6 - 0.799), and breathe to the surface (r = 0.800 - 1). This situation happened because chlorpyrifos begins to affect the nervous system of carp to reduce other body functions. The FCR and SGR had a significant effect through the statistical test due to the chlorpyrifos concentration. Whereas in carp's physiological behavior changes, there was no significant effect due to the level of concentration provided.

1. Introduction
Chlorpyrifos, as one type of insecticide that was widely used in Indonesia. Chlorpyrifos was an insecticide shaped like a crystal of white to brownish color and has a sharp odor [1]. If chlorpyrifos enters the water body, it will potentially pollute surface water to affect water biota, one of which is fish. Insecticide contamination in the fish body will damage the respiratory and nervous system of fish and affect fish's metabolic network that needs oxygen as an energy source. Therefore, damage to the respiratory, nervous system results in disruption of the body's metabolic activities, including the amount of oxygen to be consumed by fish, fish's ability to digest food, and the growth rate of fish will also be disrupted [2]. Carp fish (Cyprinus carpio L) was used as a biological test animal because organisms that were sensitive to toxic materials and changes in the environment spread widely and are easily obtained in large quantities, had economic value, were easily maintained [3]. Research into the potential sublethal effect of chlorpyrifos exposure on carp has been conducted frequently [4-6]. We conducted similar research with cases in the Twin Lakes area, West Sumatra Province. There was a runoff of the agricultural regions in this area that used chlorpyrifos to go to the carp cultivation area. In this carp cultivation area, it had a chlorpyrifos concentration of 0.007 mg/l and chlorpyrifos LC50 of 0.03 mg/l [7]. The location of Twin Lakes in the West Sumatra province is shown in figure 1. This study aimed to analyze the effect of sublethal toxicity of chlorpyrifos in carp on oxygen consumption parameters, Feed Conversion Ratio (FCR), Sustainable Growth Rate (SGR), and carp changes behavior. The results of this study can be used as consideration in the use of insecticides in agriculture.
2. Methodology
The tool used was a glass-based aquarium with a size of 35 x 30 x 30 cm as many as nine pieces each of 3 aquariums for control, for concentrations of 1/7 of LC50 and 1/14 of LC50 chlorpyrifos insecticides. The aquarium was equipped with an aerator. Clean water without chlorine-filled to aquariums, the volume of which was adjusted to the amount of fish, which is one liter of water for 0.8 grams of fish weight [3]. The test animals used in the study were carp with an average carp of 4-5 cm, weighing 2-3 grams each [8]. The number of fish was 45, of which one aquarium consists of five fishes [9].

Acclimatization test animals were carried out for 14 days in order to test animals' adaptability to the environment (laboratory) by gradually being transferred from 100% maintenance water to 100% test water. At the time of acclimatization, the test animal is given food in the form of fish pellets and given oxygen through an aerator. During acclimatization, deaths of <3% of the test animal population [9] occurred, so that test animal was considered to be eligible for sublethal testing.

Feed Conversion Ratio (FCR) and Sustainable Growth Rate (SGR) were observed by measuring carp and feed load weight using the Metler Toledo brand's analytical balance sheet. Behavior observation was done visually, using a video camera to record. Recordings were played on the media player at speeds of 0.05 slower, making it easier to document carp's behavior. The observation parameters consisted of being alone in the aquarium corner, moving irregularly and experiencing stress, lots of mucus, anal excretion, and breathing to the surface. During the 14 days of testing, the measurement and recording data of behavior for each parameter used in statistical analysis, namely data on day 1st, day 4th, day 7th, day 10th, and day 14th. Water quality parameters observed were dissolved oxygen saw at the beginning and end of the replacement test media, temperature, and pH found every day.

2.1. Feed Conversion Ratio (FCR)
Feed Conversion Ratio (FCR) was calculated using data on the amount of feed given to carp compared to carp's weight at the beginning of the observation and the carp's mass at the end of the view (day 1). Work steps for retrieving parameter data for feed conversion ratio.

After getting the FCR value of each aquarium, the three experiments' average was calculated at each concentration. The relationship between the FCR and observation time and chlorpyrifos insecticide concentration was obtained from regression and correlation analysis.
2.2. **Sustainable Growth Rate (SGR)**

Carp growth rates were calculated using carp weight data at the beginning of the observation, carp weight at the end of the view (day t), and time of view.

After obtaining the SGR value of each aquarium, it was averaged for three experiments at each concentration. The relationship between SGR carp with the time of observation and the concentration level of chlorpyrifos insecticides was obtained from the statistical analysis.

3. **Results and discussions**

3.1. **FCR and SGR**

Figure 2 shows an increase in the FCR of carp exposed to chlorpyrifos insecticides, starting from the 1\textsuperscript{st} to 14\textsuperscript{th} day. The increase of FCR on day 1\textsuperscript{st} to day 14\textsuperscript{th} at concentration of 0.002 mg/l was 0.2907 (from 0.7280 to 1.0187) and at a level of 0.004 mg/l was 0.3031 (from 0.8444 to 1.1475). The increase occurred in carp exposed to chlorpyrifos insecticides. Fish is exposed to chlorpyrifos, which inhibits metabolic processes in the fish body so that fish experience stress and cause a high feed conversion ratio in fish. Under normal circumstances, the lower the FCR value, the better because the amount of feed spent to produce a certain weight will be less \cite{10 - 14}.

The ANOVA test results, the significance value on the relationship of the FCR to exposure time, and variation in concentration were 0.001 (p <0.05). This value shows the influence of the FCR on carp due to differences in concentration exposure. The higher the level presented, the higher the FCR in fish.

In figure 3, there was a decrease in the growth rate of carp exposed to chlorpyrifos insecticides. Based on the coefficient of determination, the correlation coefficient ranges from 0.800-1.000. This value means that the relationship between observation time and the growth rate of carp is stable. Decreasing the growth rate on day 1\textsuperscript{st} to day 14\textsuperscript{th} at a concentration of 0.002 mg/L was 1.551, and at a level of 0.004 mg/l was 1.9581.

![Figure 2. Graph of Relationship between the FCR and observation time.](image-url)
If the metabolic activity goes well under normal circumstances, there would be increased fish growth [10,12,13,15-17]. Figure 3 shows the relationship between the SGR of carp and the time of observation.

Decreasing the SGR occurred due to the disruption of the fish's body's metabolic processes due to damage to the respiratory and nervous systems. This situation was due to the function of the gills and organs that were directly related to the gills in carp, which begin to experience damage due to insecticide contamination so that the gills cannot supply oxygen adequately into the body. Biota water required oxygen in the combustion of food to carry out activities, such as swimming, growth, reproduction, etc. Therefore, the fish's body's lack of oxygen could disrupt the fish's life, including perishing in its growth [18-20].

The results of the ANOVA test, the significance value obtained was 0.0001 (p <0.05). This value shows an influence on carp's growth rate due to differences in exposure to the concentration given. So the higher the level of chlorpyrifos, the lower the growth rate of feed-in carp.

3.2. The general appearance of carp body movements

Frequent sightings in healthy fish one of them were to move actively. Changes in carp presence were seen from the reasonable condition of fish every day 1st, day 7th, and day 14th in control, concentrations of 0.002 ppm, and 0.004 ppm. Observations produce the average value of the percentage of fish with regular body movements from the total number of fish in an aquarium.

Based on the relationship between observation time and carp gestures' general appearance, a correlation coefficient $r = 0.75-1.000$. This value means having a stable relationship. The percentage of stressed carp with inactive and aloof fish characteristics will decrease further along with the length of time of observation and concentration of exposure.

In the Kruskal-Wallis test, the significance value was 0.384 (p> 0.05), which means no significant difference in changes in carp movements' general appearance to variations in exposure concentration. After being exposed to chlorpyrifos insecticides, the general decrease in carp's presence on the first day, namely some fish, began to stress, but swimming movements were still agile with an unusual swimming position with irregular movements or classified as panic by moving in all directions. The next day the number of fish that experienced a decline in general appearance increased. The symptoms obtained were fish that were not actively moving, dwelling, and alone at the base or on the surface and getting weaker. So it can be seen that the longer the carp during exposure to chlorpyrifos insecticides, the lower the body movements in carp.

The high concentration of chlorpyrifos given also affects the regular general appearance of carp changes. Fish that infected with chlorpyrifos insecticides had a general decline in presence. This might be because chlorpyrifos has affected the nervous system and disrupted muscle movement. So, the fish
begin to weaken and move irregularly. Chlorpyrifos, which acted as a neurotoxin, so the higher the concentration was given to carp, the more nervous the fish's system is disturbed, making the fish's body move abnormally. Strange or abnormal fish movements were caused by a lack of coordination of the nervous and muscle systems created by the accumulation of acetylcholine in synaptic and neuromuscular junctions [21-23].

3.3. Mucus production
Mucus functions to reduce body friction with water that could make fish swim faster and prevent the entry and exit of water through the skin as a protector produced more so that there was a mucus [24]. There was a small amount of mucus in the fish's body, but it would be a problem if the mucus produced was a lot because it could inhibit gas exchange through the gills. The body's surface coated with mucus was a way for fish to protect themselves from toxic substances.

In the Kruskal-Wallis test, the significance value was 0.191 (p>0.05), so that there was no significant difference in fish mucus production to differences in exposure concentration. Fish had a lot of mucus seen in their non-agile movements because mucus influences carp's actions, more solitude at the bottom of the aquarium, and pectoral fins, which sometimes attach to the body due to a large amount of mucus. Increased mucus secretion after exposure to toxic substances and poor water conditions is the fish's response to counter the effects of poisonous substances on the body [8]. Excess mucus secretion was a specific reaction to toxins to reduce direct poison contact with the skin. Simultaneously, fish alone by leaning in an aquarium is a fish response that indicates toxic media. If the amount of mucus was significant, it could cause death in fish because fish produced excessive mucus, which results in disruption of the oxygen exchange system because of the gill lamella walls were filled with mucus.

3.4. Anal secretion
The body of living things had tolerance abilities that were not needed by the body (poisons) through the excretion process [25]. In this study, fish excretion was observed visually. Anal secretion was related to the amount of metabolic waste released by fish through feces and urine into the water, which was toxic to fish.

In the Kruskal-Wallis test, the significance value was 0.061 (p> 0.05), so that there were no significant differences regarding the appearance of fish excretion on the variation in exposure performed. However, fish that got chlorpyrifos exposure had more anal secretion (this refers to the number of feces accumulated on the aquarium wall in the form of dark solids or some scattered in aquarium water that caused water to experience turbidity) compared to control-test fish. This condition showed that exposure to chlorpyrifos insecticides influences the excretion of carp. Exposed body conditions make the body's immune system weakened, so it was easily infected with bacteria. Infected fish showed symptoms such as lack of appetite, lethargy, swimming erratically, dark feces, and slimy white, sometimes left behind on the anus to float. The amount of dirt excreted by fish was more, not because of the increased appetite of fish but because fish tried to remove toxic substances in the body through excessive excretion. The animal's body could tolerate specific amounts of poisonous substances.

4. Conclusion
The length of the exposure time and the higher the concentration of exposure to chlorpyrifos insecticides, the higher the feed conversion ratio. At the highest concentration of 0.004 mg/l, the ratio increases to 0.3031. The carp's growth rate is also getting lower to 1.9581 at the highest concentration of 0.004 mg/l. Time of exposure and variation in concentration affect changes in physiological behavior of carp such as general appearance with a correlation coefficient (r) = 0.75-1, mucus r = 0.800-1 anal excretion r = 0.6-0.799 and breathing to the surface r = 0.800-1. The length of the exposure time and the higher the concentration, then the more changes will occur in carp's physiological behavior.
Acknowledgments
The Engineering Faculty of Universitas Andalas support the publication of this article.

References
[1] Agency for Toxic Substances and Disease Registry Toxicological profile for chlorpyrifos (USA: ATSDR) p 217
[2] Lu F C and Kacew S 2002 Lu’s Basic Toxicology, Fundamentals, Target Organs, and Risk Assessment 4th ed (New York: Taylor and Francis) p 647
[3] Rice E W, Baird R B, Eaton A D and Clesceri L S 2012 Standard Methods for the Examination of Water and Wastewater (USA: APHA) p 1546
[4] Sharbidre A A, Metkari V and Patode P 2011 Pestic Biochem Physiol 101 (2) 132-41
[5] Marigoudar S R, Nagarjuna A, Karthikeyan P, Mohan P and Sharma K V 2018 Chemosphere 211 89-101
[6] Raibeemol K P and Chitra K C 2020 Fish Shellfish Immuno. 102 1-12
[7] Ihsan T, Edwin T, Husni N and Rukmana W D 2018 J. Ilmu Lingkungan 16(1) 98-103
[8] Halappa R and David M 2009 Turk J Fish Aquat Sc. 9 233-38
[9] United States Environmental Protection Agency (USEPA) 2002 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms 5th ed (Washington, DC: EPA) p 275
[10] Besson M, Aubin J, Komen H, Poelman M, Quillet E, Vandeputte M, van Arendonk J A M andde Boer I J M 2016 J. Clean. Prod. 116 100-09
[11] Cresson P, Travers-Trolet M, Rouquette M, Timmerman C, Giraldo C, Lefebvre S anErmande B 2017 Mar. Pollut. Bull 123 (1-2) 279-85
[12] Omasaki S K, Janssen K, Besson M and Komen H 2017 Aquaculture 481 124-32
[13] Hoseini S M, Yousefi M, Hoseinifar S H and Doan H V 2019 Aquaculture 503 452-59
[14] Zhou Y, Luo W, Yu X, Liu, Q and Tong J 2019 Comp. Biochem. Physiol. Part D Genomics Proteomics 29 221-27
[15] Zeng L Q, Fu C and Fu S J 2018 Comp. Biochem. Physiol. Part A Mol. Integr. Physiol 217 26-34
[16] Ai F, Wang L, Li J and Xu Q 2019 Aquaculture 507 393-401
[17] Gjerde B, Mahapatra K D, Reddy P V D K, Saha J N, Jana R K, Meher P K, Sahoo M, Khaw H L, Gjedrem T and Rye M 2019 Aquaculture 503 381-88
[18] Marcon L, Lopes D S, Mounteer A H, Goulart A M A, Leandro M V and Benjamin L A 2016 Ecotoxicol. Environ. Saf 131 135-42
[19] Narra M R, Rajender K, Reddy R R, Murty US and Begum G 2017 Chemosphere 168 350-57
[20] Jiao W, Han Q, Xu Y, Jiang H, Xing H and Teng X 2019 Fish Shellfish Immunol 86 239-45
[21] Khan A K, Aldosari F, Hussein S M 2018 J. Saudi Soc. Agric. Sci 17(2) 195-99
[22] Ihsan T, Edwin T and Angraeni W 2018 Environ. Health Eng. Manag 5(4) 205-10
[23] Ihsan T, Edwin T and Yanti R D 2019 Nat Environ Pollut Technol. 18(4) 1399-403
[24] Botte E S, Jerry D R, Codi-King S, Smith-Keune C and Negri A P 2012 Mar Pollut Bull 65(4-9) 384-93
[25] Toledo-Ibarra G A, Diaz-Resendiz K J, Pavon-Romero L, Rojas-Garcia A E, Medina-Diaz I M and Giron-Perez M I 2016 Vet Immunol Immunopathol 176 58-63