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Acute Toxicity (LC_{50} 96 Hours) of Organophosphate Pesticide With Poksim Active Compound and Haematology and Histopathology Review Goldfish (*Cyprinus carpio L*)

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Abstract

This research has the objectives to determine LC_{50} 96 hours value and observation toward hematology, gills and kidney histology of goldfish (*Cyprinus carpio*) exposed to organophosphate pesticide with Poksim active compound (trade brand FOKKER 500 EC). Method used in this paper is experiment to determine LC50 96 hour value with probit analysis. Descriptive method was used for gills and kidney tissues microanatomy observation with hematoxilin eosin (HE) coloring and hematology. Toxicity result of Fokker 500 EC pesticide toward goldfish obtained LC50 96 hours with 41,7 ppm. Histological result showed that increasing exposure doses in real test had caused increase gills and kidney tissues damage. Hematology observation result during research obtained that along with the increasing exposure doses of pesticide in the real test, acute toxicity test would caused reduction in total erythrocyte, leukocyte and hemoglobin of goldfish. Result also showed that Fokker pesticide usage was allowed until 1,8 ppm dose based on histology and hematology evaluation.

Keywords: Acute toxicity, goldfish, histology, hematology, pesticide

INTRODUCTION

Inefficient pesticide usage could raise a threat for ecosystem [1] such as water pollution which disturb organism life system as a result of pesticide residual content [2] thus it could poisoning organism and even could trigger death for water organism [3]. Water pollution by pesticide mainly occurs through water flow from places using pesticides (Subiyakto, 2003). Pesticide poisoning mechanism through gills or skin also through its food would eventually accumulate in gastric cavity and thus would damage digestion organs [4]. Dangerous pesticides mostly used were from organophosphate group. One brand of organophosphate pesticide is Fokker 500 EC with poksim 500 g/l as its active compound.

Goldfish (*Cyprinus carpio*) is a fish that highly sensitive environmental change. Goldfish is a species which had narrow tolerance toward water quality and toxic compound in water body thus this organism mostly used as subject for pollution toxic test mostly known as bioassay [5]. Toxic characteristic of pesticide from organophosphate group would cause morphological abnormality in interior organ of goldfish. Organophosphate group works mode of action by paralyzing nerve with affecting nerve function by inhibiting cholinesterase [5]. Pesticide from organophosphate group had the characteristic of contact poison which damaged nerve tissues and gastric cavity [7].

This research carry the objective to determine LC_{50} 96 hours of pesticide from organophosphate group with Poksim active compound (trade brand FOKKER 500 EC) toward goldfish (*Cyprinus carpio*) and to discovered the effect of pesticide toward gills and kidney tissue damage (histology) of goldfish (Cyprinus carpio) and haematology observation includes total erythrocyte, leukocyte and haemoglobin. Observation result toward tissues micro anatomy (histology) and blood haematology would be important to support toxicity test result. Toxicity result integrated with histology and haematology observation was expected to be more detail in determining safe benchmark related with this pesticide effect toward water environment.

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MATERIALS AND METHODS

Study material is lethal benchmark value of LC_{50} 96 hours for organophosphate pesticide with poksim active compound (trade brand FOKKER 500 EC) toward goldfish (*Cyprinus carpio*) and its review toward gills, kidney histology and haematology of goldfish (*Cyprinus carpio*). Histology preparate was done in anatomy laboratory of Medical Faculty Brawijaya University. Technique used is *hematoxilin eosin* (HE) coloring. Preparates would then scanned and observed using dot slide microscope (Olympus SN 3K19322). Result obtained would then being identified concerning its type of damages in gills and kidney tissues.

Method used in this paper is experimental. Study was done for 96 hours to obtain lethal value LC_{50} 96 hours for organophosphate pesticide to goldfish (*Cyprinus carpio*). To observe tissue damage, histology analysis was done for gill, kidney. This observation was conducted by haematology analysis to review total erythrocyte, leukocyte and haemoglobin in goldfish blood. Data collection technique in this study was done by direct observation toward studied object under examination either in real situation or in artificial situation [8]. Data analysis for toxicity test in determining LC_{50} 96 hours was using probit analysis [9].

Data Analysis

Toxicity Test

To determine lethal value LC_{50} 96 hours of organophosphate pesticide with poksim active compound (trade brand FOKKER 500 EC) toward goldfish (*Cyprinus carpio*) we use preliminary test to determine upper and lower lethal concentration values. Subsequent phase would be real test to determine LC50 96 hours values. Data in real test would be analyzed in probit to determine LC_{50} 96 hour value.

Gills and Kidney Histopathology

Histology analysis was done to observe micro-anatomy of fish's gills and kidney after pesticide exposure.

Blood Gold Fish Haematology

Hematology observed in this study concern with haemoglobin content, total erythrocyte, and total leukocyte. Haemoglobin measurement was done by using Sahlimeter by observing the color of fluid surface and compared it with scale color for sahli tube.

Calculation for erythrocyte and leukocyte would refer to Svobodova & Vyukusova (1991). Total erythrocyte calculation was done by counting total erythrocyte within 5 fields of view in haematocytometer and it is enumerated using formula:

Amount of erythrocyte: $\frac{A}{N} \times \frac{1}{V} \times F_p$

Information:
A= amount of calculated cells
V= volume of haemacytometer chamber
N= amount of observed haemacytometer chamber
Fp= dilution factor

Amount of total leukocyte was calculated for 5 fields of view and its amount was calculated using formula: total leukocyte = amount of calculated cells x 50 cell/mm^3.

RESULT AND DISCUSSION

Toxicity Test of Fokker 500 EC Pesticide toward Goldfish (*Cyprinus carpio*)

Result of preliminary test showed that the lower concentration is 1 ppm and the upper concentration is 10 ppm. This result would become basic for lethal concentration used to determine *median lethal concentration* value (Guthrie dan Perry, 1980). Result of real test showed that in FOKKER 500 EC pesticide exposures of 0; 1,35; 1,8 ppm concentration, there were no mortality occur in goldfish. In exposure dose of 24 ppm, there were 10% mortality, in exposure dose of 4,2 ppm there were 50% and 60% mortality in total 96 hours, in exposure dose of 6,5 ppm, there were 70% and 90% mortality, while in exposure dose of 8,7 ppm there were 100% mortality. Calculation result to determine probit value was showed in Table 1.

During study there were no mortality occur in control thus $M_{control}$=0. Next phase would be making probit graphic using Microsoft Excel where x=log concentration and y=probit value, result showed in Figure 1.

Based on probit graphic (Figure 1) it is obtained line equation Y=9.021x-0.743 if assumed that value of LC_{50} 96 with amount of mortality is y=5 (50%) from test animal thus value x=0.6366 and probit value of antilog 0.6366=41.7. This value showed that exposure in Fokker 500 EC pesticide dose with poksim active compound for 41.7 ppm (~42 ppm) would cause 50% population of test subject would experience death in exposed time period of 96 hours.

Table 1. Calculation Table for Probit Values to Determine the Value of LC_{50} 96 hours Pesticides Fokker 500 EC on Goldfish

Concentration (ppm)	Log^{10} concentration	Amount of subject	Average mortality	% mortality	Correction of % mortality	Probit value
0,0	-	10,0				
1,35	0,13	10,0				
1,8	0,2	10,0				
2,4	0,3	10,0	1	10		
3,2	0,5	10,0	2,5	25	25	4,32
4,2	0,62	10,0	5,5	55	55	5,13
6,5	0,81	10,0	8	80	80	5,84
8,7	0,93	10,0	10	100	100	8,71

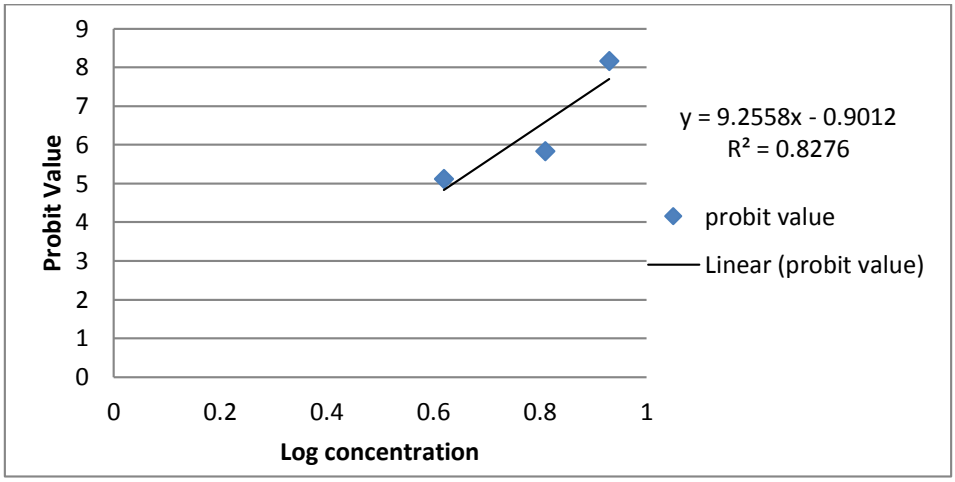

Figure 1. Probit Graphic to Determine the Value of LC_{50} 96 hours Pesticides Fokker 500 EC on Goldfish

Result of Histology Analysis of Goldfish Gills (*Cyprinus carpio*)

Structure of goldfish gills tissues (*Cyprinus carpio*) which being observed using dot slide microscope (Olympus SN 3K19322) in 100µm scale with 400x magnification using hematoxilin eosin (HE) coloring method was showed in Figure 2.

Histology of gills tissues in dose 1,35; 1,8; 2,4 ppm would experience light damage with total damage about 5-17%. Doses 4,2 and 6,5 ppm would experience medium damage with total damage about 33-66%, while in highest dose of 8,7 ppm there were heavy damage with total damage about 71%. Heavy damage was showed with missing part of cells over tissues which could lead toward mortality. This was commonly called as necrosis (Takashima dan Hibiya, 1995).

Result of Histology Analysis of Goldfish Kidney (*Cyprinus carpio*)

Kidney tissue structure of goldfish (*Cyprinus carpio*) observed using dot slide microscope (Olympus SN 3K19322) in 100µm scale with 400x magnification was using coloring method with HE (hematoxilin eosin) showed in Figure 3.

Histology of kidney tissue in dose 1,35 experiencing light damage with total damage about 4%. Doses 1,8; 2,4; 3,2; 4,2 ppm experiencing medium damage with total damage about 32,1-69% while in highest doses 6,5; 8,7 ppm it experience heavy damage with total damage about 76-85%.

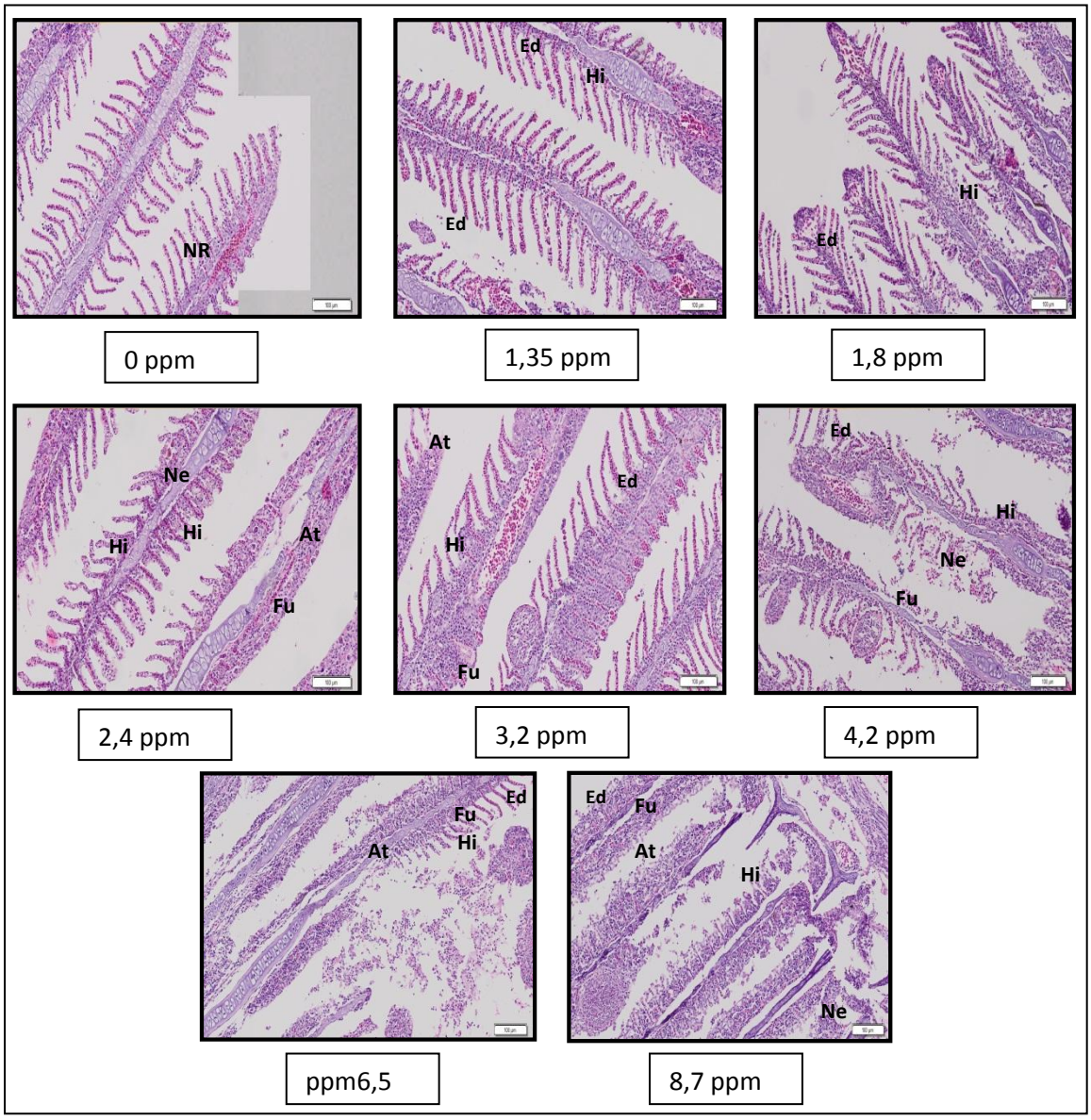

Figure 2. Microanatomy of Goldfish Gills Tissues Exposed toward Fokker 500 EC Pesticide in Different Concentration, NR=Normal, Ed=Oedema, Hi=Hyperplasia, Fu=Fusion, At=Athrophy, NE=Necrosis. (Using application Scan OlyVia in scale magnification of 100µm)

Poisoning by a pollutant could occur due to pollutant transformation process becoming more active metabolite due to interaction with other physical-chemical factors in waters. During pollutant conversion it is commonly form a reactive product. Due to its reactivity, product would bond with tissue substance in the body thus would create lesion (injured) toward cell/tissue inside target organ [10].

Haematology

Haematology observation would include observation toward total leukocyte, erythrocyte and haemoglobin. Observation result toward total leukocyte was shown in Figure 4.

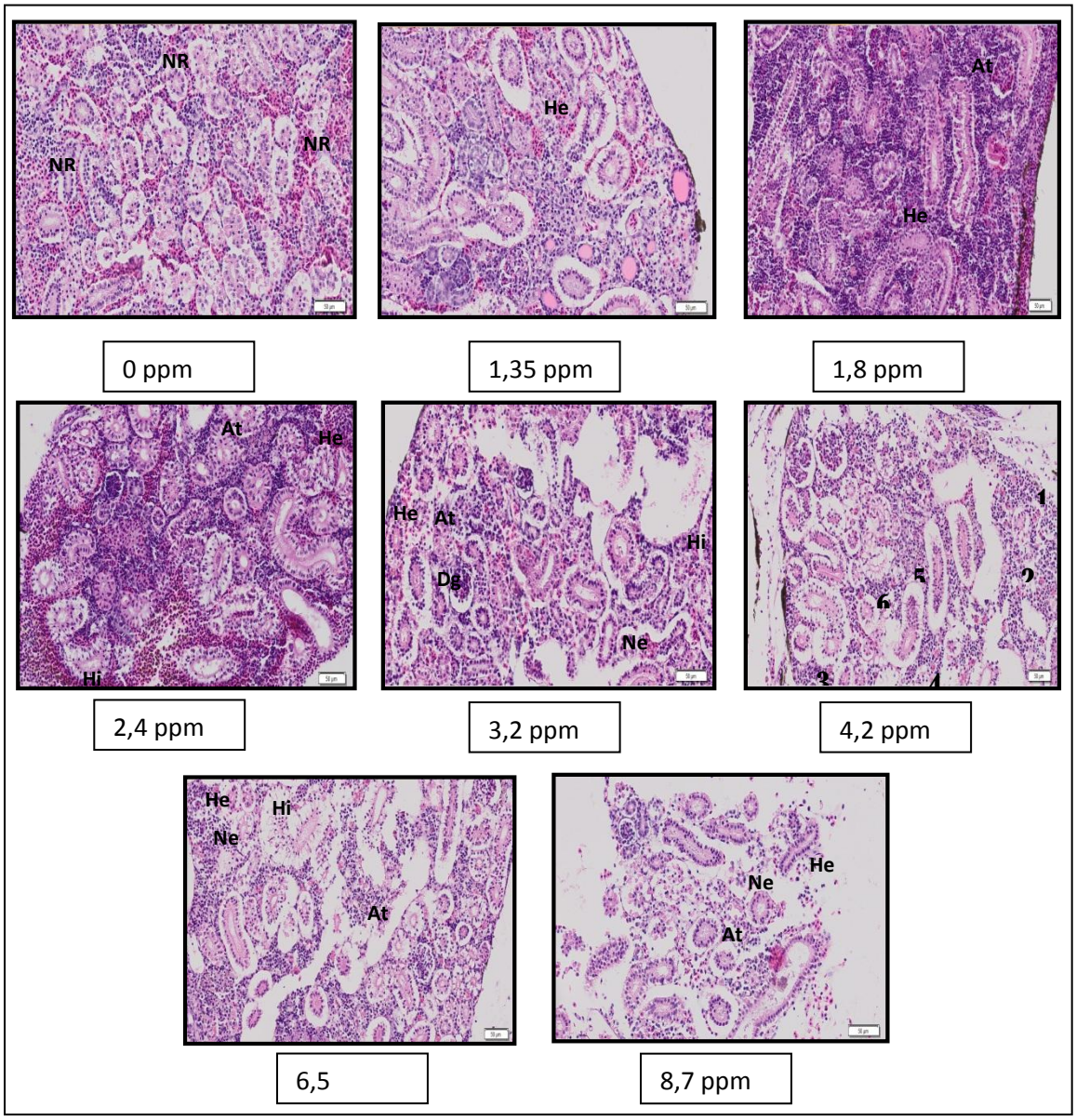

Figure 3. Microanatomy of Gill Tissue of Goldfish Exposed toward FOKKER 500 EC Pesticide in Different Concentration. Information HE=Hemorrhage, At=Atrophy, Hi=Hypertrophy, Ne=Necrosis, Dg=Degradation from tubular epithelium tissues, Ne=necrosis 18% (Using application ScanOlyVia in scale magnification 50µm)

Figure 4 showed the highest leukocyte was obtain in goldfish with dose 0 ppm (control), while lowest leukocyte was obtain in goldfish with dose 4,2 ppm. Total leukocyte in blood was showing fish health condition. Fish experiencing stress caused by environmental change or by foreign object would show increasing response of leukocyte cells [11]. Decreasing response would cause by leukocyte inside blood vessel would very low (decreasing) due to most of leukocyte would move toward infected tissues. This is in accord with Nuryati et al. (2010) that reduction in leukocyte amount was due to active leukocyte and it is gone out from blood vessel toward infected tissues [12]. This is fish response to identify and remembering type of pathogen entering its body. Next immunity role was taken over by humoral immunity that is antibody.

In general, reduction of leukocyte in treated fish showed that the leukocyte was presumed to be active and out of blood vessel toward infected tissues. This study only count leukocyte in blood vessel. Increasing amount of leukocyte functioned in cellular immunity occur

immediately until several days after infection, and a week later it would decrease. The next immunity was taken over by humoral immunity that is by antibody. Increasing leukocyte amount in exposure dose of 6,5 and 8,7 was caused since in high dose, fish would experience acute poisoning which cause fish mortality. Response of fish immune system which was characterized by lowering leukocyte amount haven't been done maximum but fish had already poisoned.

Total examination of erythrocyte was done to observe fish health condition by observing total erythrocyte in the blood. Average result of total erythrocyte in tested fish could be seen in Figure 5.

The result showed that erythrocyte content decreased as dose of pesticide increasing means of red blood cell (erythrocyte) of goldfish blood in each treatment showed that highest erythrocyte was found in 0 ppm dose (control) while lowest erythrocyte was in 8,7 ppm dose. Lukistyowati (2011) suggest that erythrocyte cell status could give important information concerning physiology and showed fish health condition [13]. Lowering amount of erythrocyte cell caused by discripted to red blood cell producing organs in kidney and spleen in producing red blood cell if being infected by bacteria, thus erythrocyte amount would decrease [14]. Erythrocyte is one of the most important components of blood cell, due to haemoglobin content which has the role in binding oxygen from environment and taken to all part of the body that needed. Lower erythrocytes lead fish unable in taking oxygen in large amount although oxygen availability in environment is sufficient. As a result, fish would experiencee anoxia [15].

Observation toward haemoglobin content is highly related with gills' histopathology condition. High histological damage would lowered fish haemoglobin content.

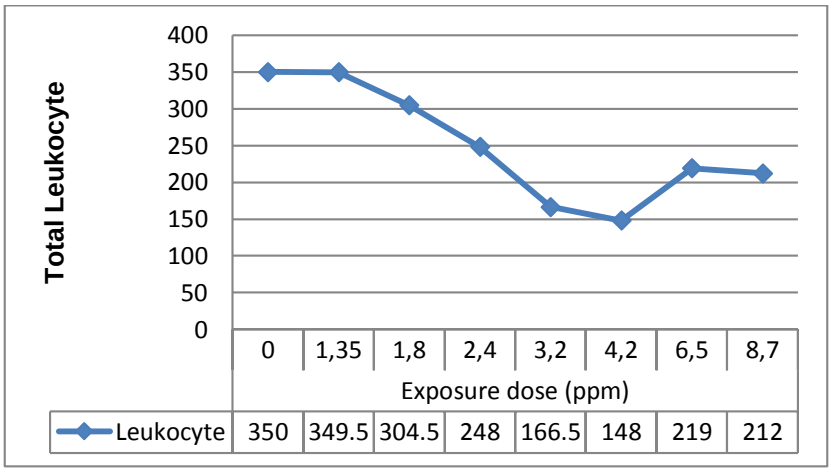

Figure 4. Total Leukocyte of Goldfish Exposed FOKKER 500 EC Pesticides

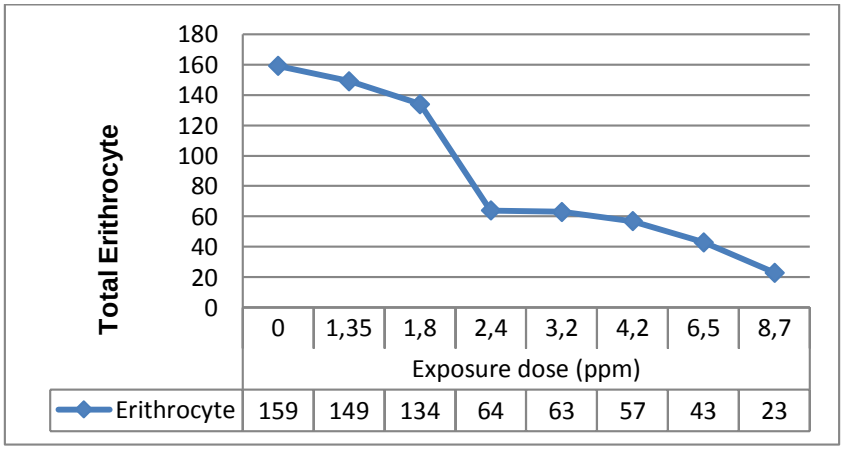

Figure 5. Total Erithrocyte of Goldfish Exposed FOKKER 500 EC Pesticide

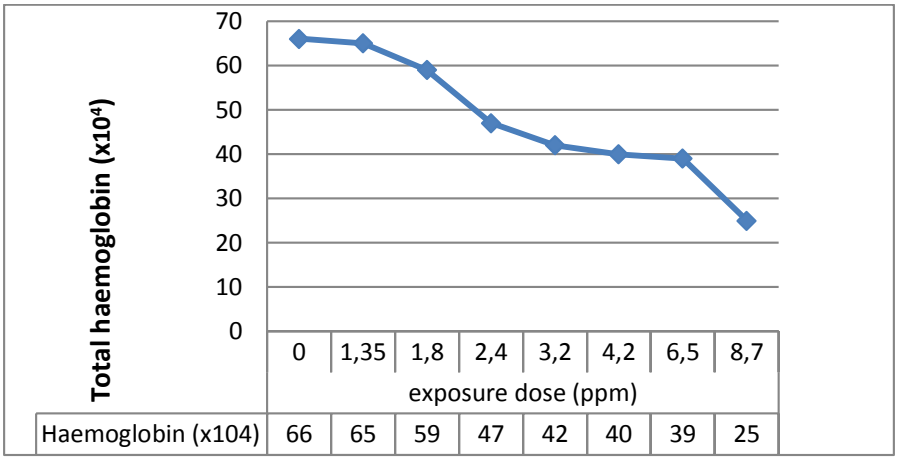

Figure 6. Haemoglobin Content of Goldfish Exposed FOKKER 500 EC Pesticides

Lower amount of oxygen molecules in the water plus lower oxygen absorption by gills would disrupt fish metabolism process. Thus, fish would unable to synthesize compounds or substance needed such as in normal erythrocyte synthesis. Though normal erythrocyte synthesis was still ongoing, but erythrocyte being produced would be abnormal or premature which cause in lower ability to fixate. Haemoglobin content was shown in Figure 6.

Figure 6 showed that in control (0 ppm dose) it is gained that haemoglobin content was highest as 66 x 10^4 and lowest with dose as 8,7 ppm with 25 x 10^4. There was hemoglobin decrease along with higher exposure dose. This showed that gill had disruption in absorbing oxygen as a result of toxic response from pesticide. According to Vinodhini (2009) and Ersa (2008) lower hemoglobin content in fish was due to several factors such as pollution and lack of nutrition [16]. Toxic substance would enter the blood and disturbing haemoglobin and red blood cell balance. Lower haemoglobin content would decrease metabolism rate and lowered energy resulted. This would make fish becoming weak and did not have any appetite to eat and was seen motionless in the bottom or hung below water surface [17].

CONCLUSION

Conclusion of this study shown toxicity result of FOKKER 500 EC pesticide toward goldfish is 41,7 ppm as LC_{50} 96 hours value. Result of histology analysis showed that increasing exposure dose in real test would cause increase damage percentage. Result of hematology analysis obtain that more exposure would results decreasing number of total erythrocyte, leukocyte and hemoglobin of goldfish. Study result obtain LC_{50} 96 hours value and this study had been evaluating histology, hematology and community perception concerning pesticide usage of Fokker which was allowed until 1,8 ppm dose based on histology and hematology evaluation.

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