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EFFECT OF CYPERMETRIN ON THE HAEMATOLOGICAL INDICES OF AFRICAN CATFISH (*Clarias gariepinus*)

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ABSTRACT

Ninety (90) sub adults of *C.gariepinus* were exposed to sub lethal levels of 0.00 (control), 0.0125ppm and 0.025ppm Cypermetrin solution in triplication in rectangular glass aquaria for 36 and 45 days to determine the effect of the exposure on some haematological parameters such as Haemoglobin (Hb), Red Blood Cells (RBC), Packed Cell Volume (PCV), White Blood Cell (WBC),Differential Counts (neutrophils, lymphocyte, eosinophils) and Mean Corpuscular Volume (MCV). The values of the haematological indices were subjected for Analysis of variance (ANOVA) and a positive T-test to further separate the means. The result showed a significant (P>0.05) reduction in Hb, RBC, PCV and Lymphocyte from treatment 0.00 (control) to 0.025ppm, during the exposed period of 36 to 45 days. Whereas WBC and neutrophils increased significantly (P<0.05). In all the exposure periods the alterations were most pronounced in the fish exposed to 0.025m/l of Cypermetrin. The results showed that Cypermetrin could cause high level of stress to *C.gariepinus* by causing changes indicated in the haematological indices of the fish under consideration.

Keywords: Cypermetrin, Haematology, Pyrethroid, anthropogenic.

1. INTRODUCTION

The African catfish, *Clarias gariepinus* of the claridae family is widely spread in the waters of tropical Asia, Syria and Africa. They are the most cultured in Nigeria because of their ability to survive wide variety of environmental extremities. More so, their positive attributes such as fast growth, high fecundity, efficient air breathing organs, disease resistant and ease of larval production in captivity makes it of commercial importance (Safina *et al,* 2013).
Pyrethroid insecticides are widely used for the control and elimination of pests. Most of these insecticides as well as heavy metals can be introduced into the aquatic environment directly or indirectly by anthropogenic processes such as water run-off, industrial effluents or aerosols carried by wind, thereby playing a major role among pollutants of environmental concern *(*Meise *et al, 2006)*.

Cypermetrin, a pyrethroid insecticide present in water proves to be toxic due to the inability of fish to metabolize it. Also, some investigations suggest that these substances can be absorbed through fish gills into the blood stream where they persist and cause alteration in haematology *(*Radu *et al,* 2009*)*. When the poisoned fishes are eaten, it can affect the health of humans. The presence of Cypermetrin and other pesticides have attracted researchers' worldwide to pay attention to their extensive use in agricultural, chemical and industrial processes that are becoming threats to aquatic organisms (Meise *et al,* 2006).

Haematological indices are important parameters in many fields for ichthyological research, fish farming, areas of toxicology and environmental monitoring as possible indicators to evaluate the pathological status of the fish. Studies are carried to either determine the relationship between species or to have knowledge of their physiology (Palidis *et al*, 2007).Of a few, the blood parameters includes; packed cell volume, Haemoglobin, Leucocytes differential count, Red Blood cells and the erythrocyte indices (Mean Corpuscular Haemoglobin- MCH, Mean Corpuscular Haemoglobin Concentration- MCHC and Mean Corpuscular Volume - MCV). Blood is an active component of the fish and its impositions must be kept constant under normal conditions for life processes to be carried out. Changes in haematological parameters depend in the aquatic biotope, sexual maturity, fish species, age and health status (Patriche *et al.,* 2011).

Haematological parameters are closely related to the response of the animal to its environment, indicating that where the fish lives could exert some influence on the blood parameters. Recently, the use of hematological techniques has been useful in assessing the toxic effect of chemicals in aquatic organisms with the help of its intimate relationship with its environment (Gabriel *et al.*, 2006, Akinrotimi *et al.*,

2010).According to Adeyemo *et al*., 2005, these parameters may reveal conditions within the fish's body long before the symptoms manifest, therefore providing diagnostic information once reference values are established under standardized conditions and to forecast the consequences of long term exposure to toxicants. Hence, hematological parameters are defined as an index of a fish's health status (Oshode *et al*., 2008).Irrespective of much studies of toxicant effects on *Clarias gariepinus,* very little is known on the haematological changes of this species when exposed to Cypermetrin.

2. MATERIALS AND METHODS

This research was carried out at the laboratory of Institute of Oceanography and the Endocrinology section of Department of Biochemistry, University of Calabar, Calabar. A total of 90 African Catfish *(Clarias gariepinus)* sub-adults(2 months) were purchased during the early hours from the University of Calabar fish farm with the help of scoop nets and transported in plastic buckets with well aerated water(manual air induction) to the laboratory. Care was taken to prevent agitation which may stress the fish to death. Cypermetrin was also obtained from the Ministry of Agriculture, Barracks Road, Calabar. It was placed in an airtight container and transported to the Laboratory of Institute of Oceanography for subsequent use. The fish was acclimated in plastic aquaria (30 x 30 x 60cm) for 14 days, where the fish was fed with commercial diet (35% crude protein) once daily at 1 % their total body weight. A stock solution of Cypermetrin was prepared by dissolving 20ml in 1000mls of dechlorinated water. Different concentrations of test chemical were prepared using the formula, $N_1 V_1 = N_2 V_2$ (APHA, 2011).

Thus, the concentrations to be used were 0.00, 0.0125 and 0.025ml/L and their introductions were done by the use of Pasteur's pipettes. After acclimation, the sample fish were placed in three (3) treatment levels with two (2) replicates. Five (5) fishes were introduced into each aquarium containing the various concentrations of the toxicant, 30 litres of dechlorinated water was used and the fish were fed as in the acclimation period. The water was filled to 25L before the introduction of the toxicant, after which the water was made up to 30L mark. The solution was renewed daily after cleaning the aquarium. All the physicochemical parameters like dissolved oxygen, PH, alkalinity and other were measured according to the standard methods prescribed in APHA (2011).At the end of the experiment, blood samples were collected from the caudal peduncle of both the control and test fish samples that survived the exposure period of the toxicant with the use of separate heparinised disposable syringes and hypodermic needles. The blood samples were preserved in EDTA bottles as anticoagulant.

Standard hematological methods described by Blaxhall and Daisly (1973) to determine PCV, RBC and Erythrocyte Sedimentation Rate (ESR) were used. The hemoglobin concentrations were determined by the cyanomethmoglobin method (1961). The microhaematocrit methods of Sudova *et al*, (2008) were used to determine the haematocrit. The hematological indices: Means Corpuscular Volume (MCV) was calculated from the equation given by Anderson and Klontz (1965).

Data were collected and subjected to a one way Analysis of Variance (ANOVA) to determine the differences and a positive T-test, student Kneuman Keuls (SNK) test, Duncan Multiple Range Test (DMRT) and Predictive arithmetic software (PAS) were used.

3. RESULTS

The haematological changes produced by the effects of different exposure time of *C.gariepinus* to different concentration of Cypermetrin under static bioassay with renewal are shown in table 1. There was a significant decrease ($P<0.05$) in PVC where the lowest values (23.60±0.10 and 22.75±0.25%) were obtained in fish exposed to 0.025ppm for 36 and 45 days respectively. There was a significant reduction ($P<0.05$) in RBC, Hb and MCV with the lowest values obtained from 0.025ppm concentration compared to 0.00 and 0.0125ppm comparably, the group of fish exposed in 0.025ppm for 45 days noted lower values in Hb (6.65 ±0.15g/dl), RBC ($1.55±0.05×10^6$/mm^3) and MCV (123.50±3.50fl) than those exposed for 36 days, (table 1). On the other hand, WBC count showed a significant increase ($P<0.05$) with the highest value reflected on fish exposed in 0.025ppm treatment for 45 days ($509.00±9.00×10^3$/mm^3) as seen in table. But there was a drop in the WBC count for fish group exposed for 36 days as concentration increased from 0.0125ppm to 0.025ppm (509.00±9.00 to $393.00±2.00×10^3$/mm^3).

Statistically significant reduction was observed in lymphocyte values obtained in 36 and 45 days. The lymphocyte of fish expose in 0.025ppm concentration showed the highest reduction (69.00±1.00 and 75.50±0.50%) compared to 0.00 and 0.0125ppm for 36 and 45 days respectively. (Table 2) No significant difference was observed in the monocyte concentration at 36 days while a decrease was noticed for fish exposed for 45 days from 0.0125 to 0.025ppm (2.5±0.50 to 2.0±0.00%) as shown in table 2. There was a significant reduction ($P<0.05$) in eosinophils from 0.0125ppm to 0.025ppm for both exposure times. Unlike fish exposed for 36

days, there was a significant increase in the neutrophils of fish exposed for 45 days.

4. DISCUSSION

It has been noted that responses in fish to stress are non-specific and enables the fish to cope with the condition and in the maintenance of its homeostatic state. If the stress becomes severe and persistent, the response becomes mal-adaptive and threatens the fish health and well being (Barton, 2002).

The evaluation of haematological parameters of fish has become an important means of understand normal, pathological processes and toxicological impacts *(Sudova et al.,* 2008) which thus provides important information about the internal environment of the organism. The present study showed a decrease in the Hb concentration with increase in Cypermetrin concentration and exposure time. This result is similar to reports in *C.gariepinus* exposed to sunset (Okomoda *et al.,* 2013). The decrease in Hb is usually caused by the effect of toxicants on the gills of fish.

Gaafar *et al.,* (2010), reported that prolonged reduction in Hb content is deleterious to oxygen transport and degeneration of the erythrocyte could be due to pathological condition of the fish exposed to the pesticide. There was a decrease ($p<0.05$) in PCV and RBC counts This is similar to the reports by some authors: *Svobodova et* al., (2001) in *Cyprinus carpio* exposed to diazinon and Gabriel et al., (2007) in C. *gariepinus* exposed to refined crude oil products kerosene. The reduction may be due to the inhibition of erythrocyte synthesis and increase in the rate of erythrocyte destruction.

A gradual reduction was observed in the trend of MCV though with a slight fluctuation (fig.4). Cells released from the spleen, which is an erythropoietin organ would have lower MCV values when compared with the control. This is attributed to direct or feedback responses of structural damage to RBC membrane resulting in haemolysis and impairment in haemoglobin synthesis *(Shah,* 2006). A similar observation was made for *Cyprinus carpio* after cadmium exposure *(Koyama and Ozaki* 1984). Generally a gradual increase was observed in the WBC count in fig.5 with increasing level of toxicant which is likely due to heightened immune mechanism of the fish stimulated to fight against the toxicant. Similarly, *Gabriel et al.,* (2007) attributed these changes to possible excitation of the defense mechanism of the fish to counter the effect of the toxicant. However, a reduction in trend for WBC count was noticed from 0.0125 to0.025ppm fish exposed for 36 days (fig.5). This reduction is as a result of the leucocytes count which shows a weakening of the immune system *(Svoboda,* 2001, *Witeska,* 2003).

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Table 1: Haematological parameters of *C. gariepinus* exposed to 0.00 (control), 0.0125 and 0.025 ppm Cypermetrin concentration

Indices	Days	*Concentration (ppm)		
		0.00 (control)	0.0125	0.025
Pcv (%)	36	27.80± 0.10	27.20±0.20	23.60±0.10
	45	28.15± 0.50	25.75± 0.15	22.75± 0.25
Hb (g/l)	36	8.20± 0.10	7.90 ±0.10	7.15± 0.05
	45	8.05± 0.05	7.10 ± 0.10	6.65±0.15
RBC (10^6mm^{-3})	36	2.08 ± 0.01	1.86± 0.02	1.81 ±0.01
	45	2.15± 0.05	1.80±0.00	1.55± 0.01
WBC (10^3mm^{-3})	36	383.00±1.00	506.00± 9.00	393.00± 2.00
	45	388.00±1.00	48.50± 0.50	509.00±9.00
Mcv (fl)	36	133.00±1.00	149.50±0.50	124.00±1.00
	45	131.50±0.50	130.50± 0.50	123.50± 3.50

*Values are means of 3 determinations

Table 2

Leucocytes differential count parameters exposed to 0.00 (control) 0.0125 and 0.025ppm Cypermetrin concentration.

Indices	Days	Concentration (ppm)		
		0.00 (control)	0.0125	0.025
Lymphocyte (%)	36	90.50± 0.50	88.00 ± 0.00	69.00±1.00
	45	86.00 ± 1.00	79.50±0.50	75.50 ±0.50
Monocyte (%)	36	0.50 ± 0.50	9.50±0.50	0.50± 0.50
	45	1.00 ± 0.00	2.50± 0.50	2.00±1.00
Eosinophil (%)	36	0.50± 0.50	3.00±0.00	1.00 ± 0.00
	45	0.50±0.50	3.00± 0.00	1.50± 0.50
Neutrophil (%)	36	12.00±1.00	25.00±2.00	11.00±1.00
	45	14.00±1.00	15.00±1.00	21.00±1.00

*Values are means of 3 determinations

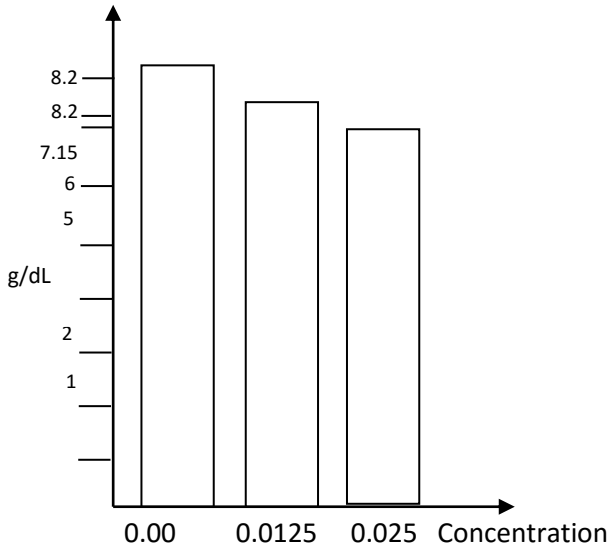
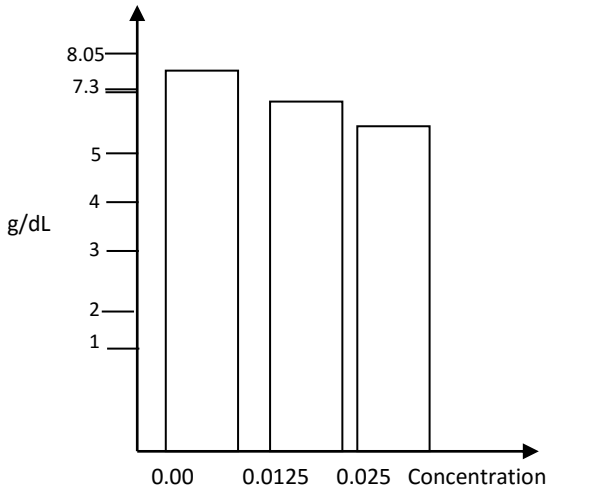


Fig 1. A) Haemoglobin concentration lymphocyte exposed for 36 days



B) Hb Concentration exposed to cypermetrin for 45 days

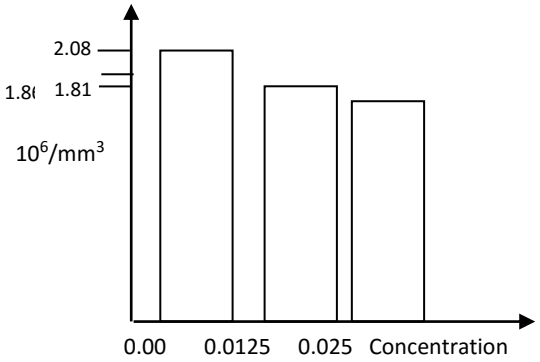
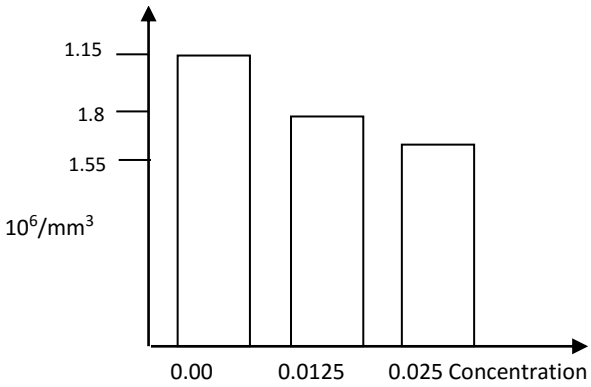


Fig 2: A) RBC exposed for 36 days



B) RBC exposed for 45 days

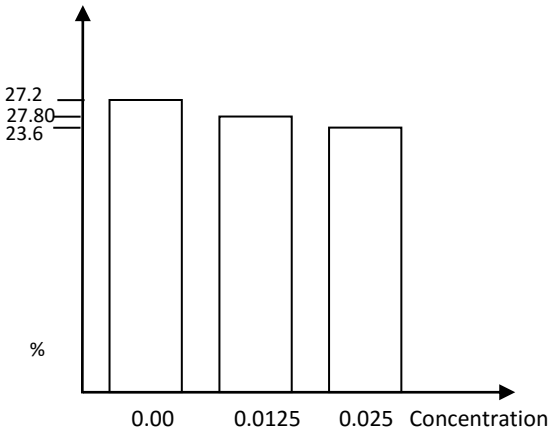
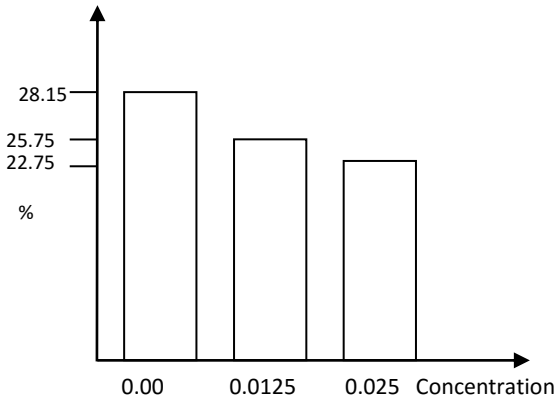
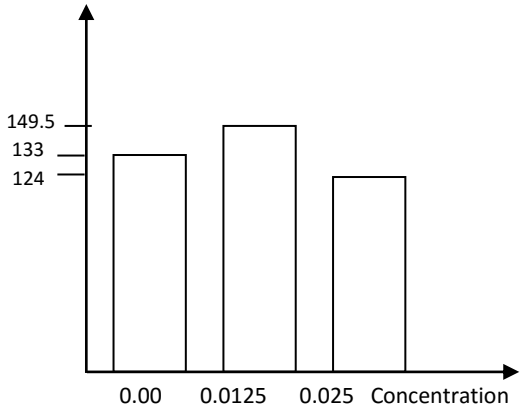


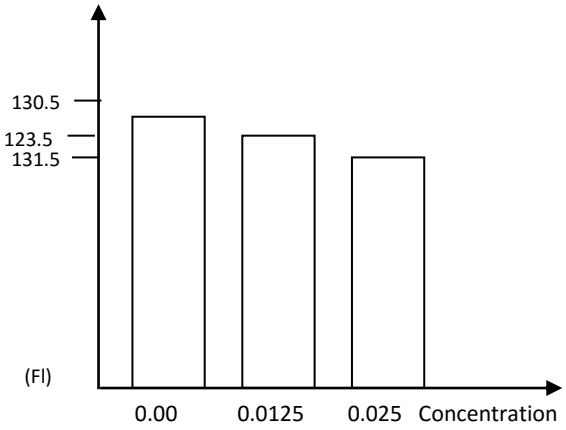
Fig 3. A) PVC exposed for 36 days



B) PVC exposed for 45 days



(Fl) Fig 4. A) MCV exposed for 36 days



B) MCV exposed for 45 days

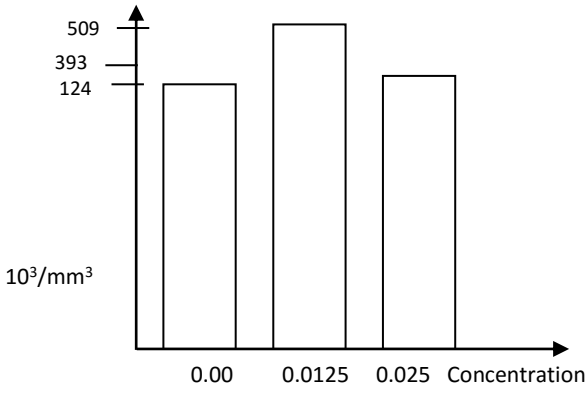
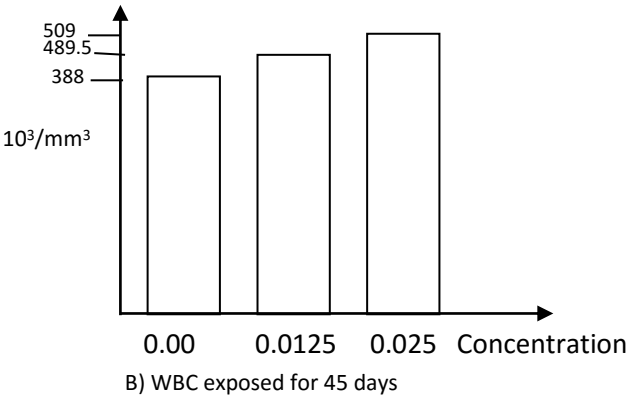
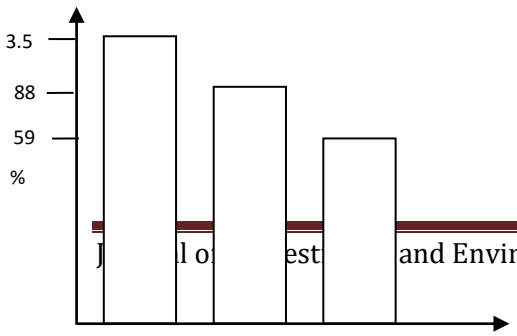
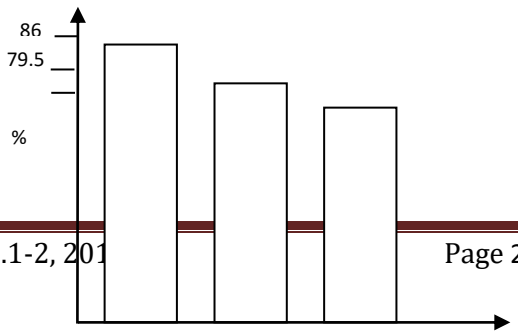


Fig 5: A) WBC exposed for 36 days



B) WBC exposed for 45 days





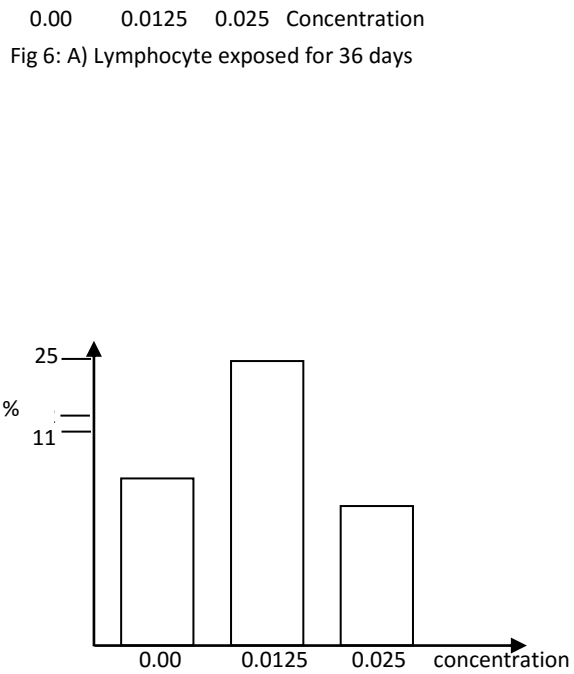
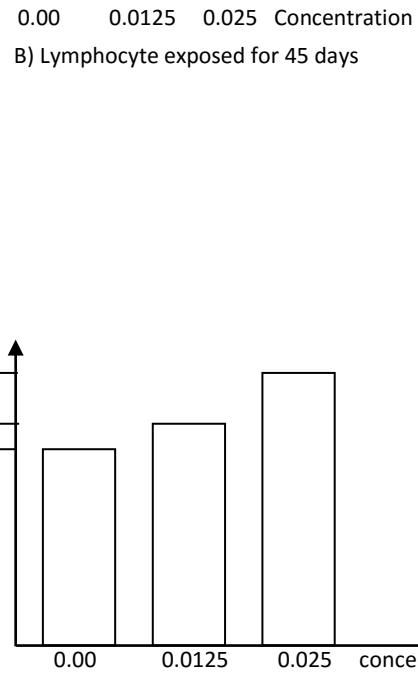


Fig 6: A) Lymphocyte exposed for 36 days



B) Lymphocyte exposed for 45 days

Fig. 7: A) Neutrophil exposed for 36 days

B) Neutrophil exposed for 45 days

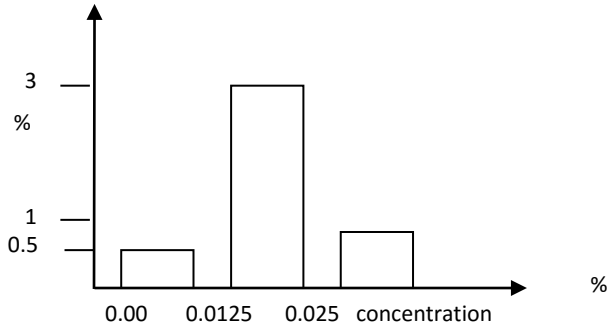
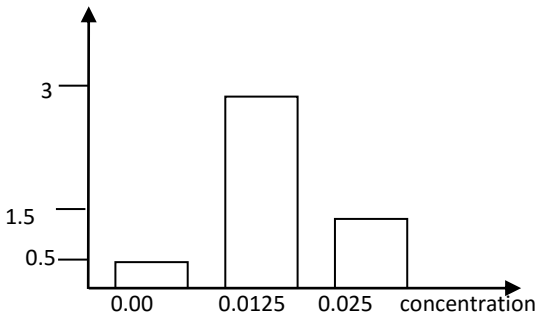


Fig. 8: A) Eosinophils exposed for 36 days

%



B) Eosinophils exposed for 45 days

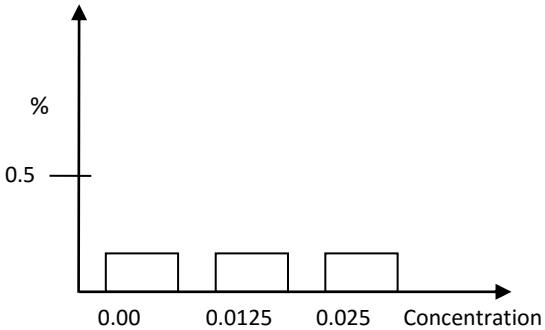
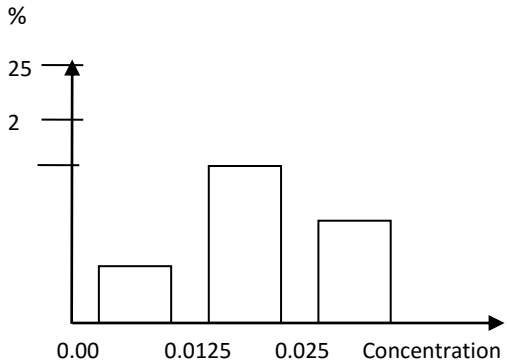


Fig. 8: A) Monocyte exposed for 36



B) Monocyte exposed for 45 days

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