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IMPACT OF CHLORDANE ON BEHAVIOURAL PATTERNS AND HAEMATOLOGICAL INDICES OF *CATLA CATLA*

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ABSTRACT

Freshwater fishes are important source of animal protein to human population. They are adversely affected by aquatic pollutants such as pesticides used in agriculture, which are easily washed off into the water bodies through the skin, gill, intestine, wound, etc. Acute toxicity of an organochlorine insecticide is characterized by their persistence and ability to accumulate in aquatic organisms. Our present study is aimed to elucidate the effect of Chlordane on Haematological indices of carp fish *Catla catla*. 1/5th sub lethal concentration of Chlordane is 0.201 µg/L was used for the toxicity experiment. Blood samples are used to analyze for the haematological total erythrocyte count (TEC), total leucocyte count (TLC), total haemoglobin count (Hb) and Packed cell volume (PCV or Hct), The experimental fish treated with Chlordane over an exposure period of 3, 7, 14, 30 and 45 days. Chlordane caused a significant decrease in total Erythrocyte count (-69.02%), total Haemoglobin content (-66.06%) and Haematocrit (-62.29%) and induced a significant elevation in the total Leucocyte count (91.04%) compared to control group.

KEYWORDS: *Catla catla*, Organochlorine pesticide, Chlordane, LC_{50}, Behavioural patterns, Haematological Indices.

INTRODUCTION

Pesticidal pollution posing a great risk to aquatic fauna especially to aquatic animals, which comprise one of the important sources of protein rich food for human beings (Sharma and Singh, 2007). Injudicious and indiscriminate use of agrochemicals such as fertilizers, pesticides, insecticides and fungicides to boost crop production with the sole aim of getting more yield, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted. Normally these pesticides reach the aquatic environment through surface runoff, sediment transport from treated soil and direct application as a spray to water bodies to control the inhabiting pests (Kumari et al., 2010). The pesticides in aquatic ecosystems affect non target organisms such as fishes and prawns. Pesticide hazard on fish mortality, growth and tissue damage has been amply reported by Wildish et al. (1971).

Chlordane was first produced in 1947 and was used as an insecticide for agricultural crops and livestock, for lawns and gardens, and also for underground treatment around the foundation of homes. In 1978, because of concern over cancer risk, evidence of human exposure and danger to wildlife, EPA cancelled its use on food crops and phased out its other above-ground uses. From 1983 to 1988 its only approved use was as a termiticide around home foundations, and all uses were cancelled after 1988. However, residues still exist in soils and sediments and chlordane bio accumulates in fatty tissue of fish and humans; this bioaccumulation is a source of current concern.

Chlordane is a chlorinated hydrocarbon used as a non-systemic contact insecticide for lawns and crops. Actually a complex mixture of isomers, other chlorinated hydrocarbons, and by-products, chlordane is used in termite and ant control, and as a protective treatment for underground cables. Chlordane may be irritant and toxic by ingestion, inhalation, or skin absorption; toxic effects may be cumulative. When heated to decomposition, chlordane emits toxic fumes of carbon monoxide, hydrogen chloride, chlorine, and phosgene. Chlordane is very persistent in the environment, surviving in soils for more than 20 years.

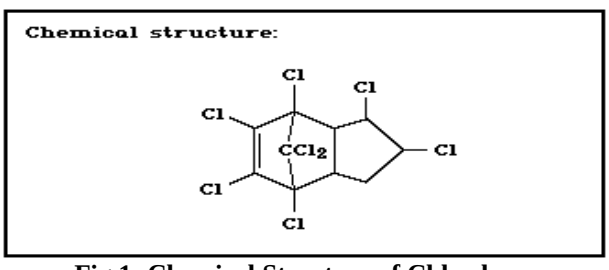

Fig 1: Chemical Structure of Chlordane.

Pure chlordane is a viscous, colourless, odourless liquid. Its solubility in water is approximately 9µg/L at 25°C. It is highly soluble in most organic solvents, including petroleum hydrocarbons (Brooks, 1974a).

Assessment of toxicity on particular organism exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. Toxicity tests will reveal the organism's sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem.

LC_{50} is measured in micrograms (or milligrams) of the material per litre, or parts per million (ppm), of air or water; lower the amount, more toxic the material. Used in the comparison of toxicities, LC_{50} values cannot be directly extrapolated from one species to the other or to humans. Also called median lethal concentration or population critical concentration 50.

Acute toxicity caused by different toxicant on freshwater fish can evaluate by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against pesticides toxicity (Kousar and Javed, 2012, Azmat et al., 2012, Ebrahimpoure et al., 2010). Toxicity testing with a single chemical composition is inadequate identification of pollutant selective toxicity on aquatic biota and does not allow to evaluation pollutant hazard to the environment. Apart from detecting a threshold above which fish are likely to be killed, data obtained on the concentration of selected individual pollutants which are lethal to fish can also provide very necessary information.

Haematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc (Blaxhall et al., 1972; Duthie et al., 1985).The use of haematological technique in fish culture has made it possible for researchers to use it in environmental monitoring and fish health conditions (Mulcahy, 1975).

Fish haematology is one of the most recent branches of physiology to assess the status of general health. Blood is the transport medium, a defence system, and an acid/base buffer system. Circulating blood is the common denominator of health and illness and alteration in its chemical or cellular illness and composition can indicate haematological and non haematological diseases. Erythrocytes occupy the largest fraction of the formed elements of the blood. In the present paper work we discussed about Toxicity evaluation and Haematological indices of *Catla catla* exposed to sub lethal concentration of Organochlorine insecticide Chlordane for a period of 45 days.

MATERIALS AND METHODS

Live specimens of *Catla catla* of (26.0 ± 1.5g) were collected from AP Govt. Fish Breeding and Hatchery Centre, Kalyani dam, near Tirupati, Chittoor district and immediately transferred to transparent polypropylene tank of 500L capacity filled with filtered, well aerated and dechlorinated bore well water. The fish were fed with a commercial pelletized formulated fish feed twice a day. The water quality is maintained constantly throughout the experimental period in control medium.

Toxicity Evaluation

LC_{50} is the concentration at which 50% of the test animals are killed. Lethal concentration of Chlordane was determined by probit analysis method of Finney (1971). The 96hrs LC_{50} tests are conducted to measure the susceptibility, survival and potential of organisms to particular toxic substances such as pesticides. Suggestions made by Duodoroff et al. (1951) were followed, hence constant biomass ratio with water volume was maintained (one gram/1litre). Fishes were acclimated for week days. Aeration facility was provided for fishes. Feeding the fishes was stopped for 2 days prior to the experiments. Fishes were also exposed to Log.2 concentration for the same exposure period to determine LC_{50} value by adapting Dragstedt and Behrens's equation given by Carpenter (1975).

Haematological Analysis

The blood was collected from the fishes by puncturing the heart by using 1ml insulin syringe. For serological analysis the collected blood were centrifuged at 2500 rpm for 14min. Total Erythrocyte Counts (TEC) (10^6 mm^{-3}) were estimated using diluted with Dacie's fluid in the ratio 1:4 (Blaxhall and Daisey, 1973) with haemocytometer under a microscope. Total Leucocyte (TLC) (mm^3x10^3) cells counted using by Neubaur Haemocytometer (Shah and Altindag, 2005). Blood was diluted to 1:20 with Turks diluting fluid and placed in haemocytometer at 640x. The Haemoglobin content (mg/100) of blood was analysed following the cyanmethaemoglobin method using Drabkins fluid and the absorbance was read in spectrophotometer at 546nm. The Hematocrit (PCV) is used to deliberate the volume percentage (%) of red blood cells in blood, and determined by the Microhematocrit tube method.

Statistical analysis

The Probit mortality was found out using SPSS software 16.0 and biochemical data processed using SPSS 13 statistical program. All data were expressed as arithmetic mean ± SD, for the analysis of the experimental parameters Student's -t test was used.

RESULTS AND DISCUSSION

To evaluate the toxicity of chlordane to *Catla catla* (12.50 ± 0.17g) exposed for 96 h showed zero mortality at 0.085 µg/l and 100% mortality was observed at 0.680 µg/l. The mortality rate increased with increase in the concentration of chlordane. When percent mortality was

plotted against log concentration of chlordane, a sigmoid curve was obtained (Fig- 2). The 96 h LC_{50} value was obtained from sigmoid curve was 0.422 µg/1. The percent mortality after transforming to probit mortality was plotted against log concentration of chlordane using probit method (Fig-3). In this a straight line was obtained and the LC_{50} value obtained from the graph was 0.422 µg/l also determined the 96 h LC_{50} value (Fig- 3). The 96 h LC_{50} value was further verified by the method of Dragstcdt-Behren's equation (Carpenter, 1975) and the value calculated by this method was found to be 0.422 µg/1**.** The upper and lower 95% confidence limits were found to be 4.241µg/L and 3.668 µg/L, respectively.

The acute test for a long time has been a major component in toxicity testing (Braunbeck and Lammer 2005). In which acute chemical toxicity is determined as a 96 h LC_{50} value. However the environmental significance of death of individuals after short term exposure to high concentration is questionable (Marigoudar, et. al, 2009). The 96 h LC_{50} value of chlordane to *Catla catla* was found to be 0.422 µg/1 (Fig-2). $1/5^{th}$ sub lethal concentration of chlordane is 0.201 µg/L was used for the toxicity experiment. Viability of animals in the present study is comparable to the few previously published studies that exist, for *rainbow trout* 96 h LC_{50} ranges between 7.5-8.0 µg/1, *Salmo gairdaneri* 8.0µg/1, but that sub lethal concentration (LC_{50}) exceeded this concentration (Shankar et al., 2013). In the present study, the fish maintained in normal freshwater behaved in usual manner and they were very active with their well co-ordinated movements. They were alert at slightest disturbance.

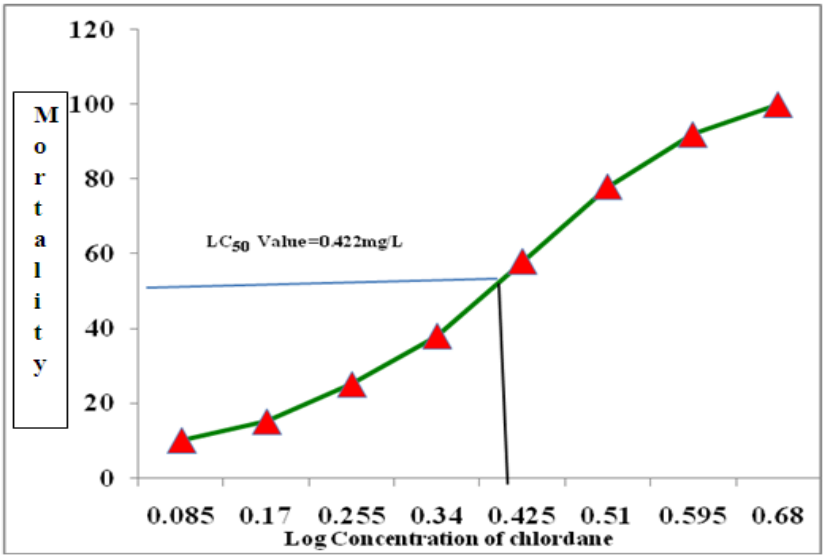

Figure- 2: Representing Percent mortality Vs log concentration of Chlordane.

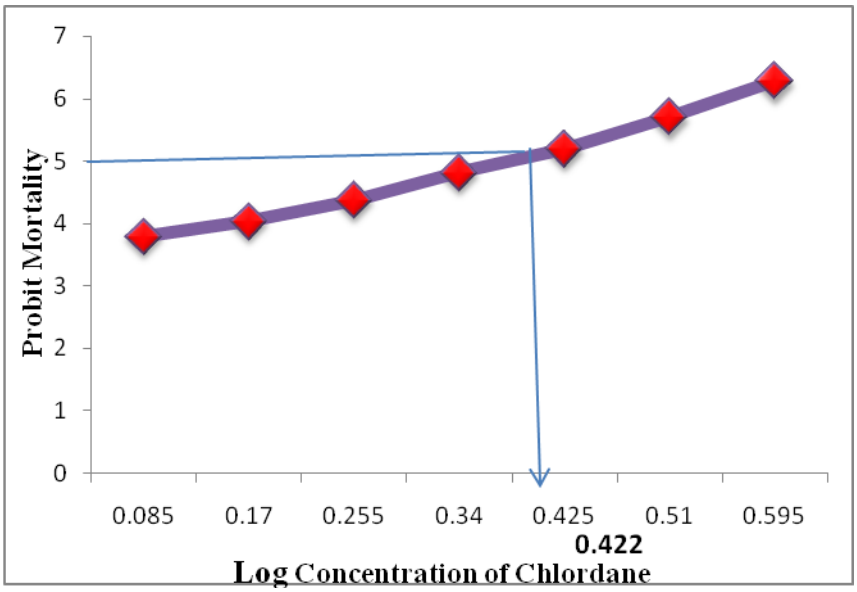

Figure- 3: Representing Probit mortality Vs Log Concentration of Chlordane.

Behavioural manifestations were observed in animals after exposed to sub lethal concentration of chlordane they became irritable and hyper-excited. Jumping movements as well as restlessness were observed and finally the fish turned upside down. Mucus secretion and loss of equilibrium were also observed. They slowly became sluggish with short jerky movements, surfacing and gulping of air and erratic circular movements (Table-1).

Table-1: Behavioural abnormalities monitored in the fish *Catla catla*, treated with sub-lethal concentration of Lihocin at 24h, 48h, 72h and 96h.

S.NO	EXPOSURE PERIODS	MONITORED BEHAVIOURAL CHANGES
1.	**After 24 hrs exposure**	-restlessness, rapid surfacing, -peeling of skin and colour fading
2.	**After 48 hrs exposure**	- slightly reduced activity - gradual increase in colour fading - gill adhesion and a thin film of mucous -nudge and nip
3.	**After 72hrs exposure**	- fish moving towards surface water and gulping of air is increased, - loss of balance and jerky movements during swimming.
4.	**After 96hrs exposure**	- haemorrhages were also identified on fins and trunk of some fish - a thick mucous film was formed on whole body and gills, in all test fishes - fishes lost their natural colouration and become almost reddish black / dark red in colour. S-jerk movements and burst swimming fin flickering

Finally they settled down at the bottom with loss of equilibrium and rolling of the body, convulsions prior to death. The fish very often come to the surface in order to avoid toxic environment. Moreover, the gill of dead fish revealed that the gill lamellae colour was changed from red to brown, swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom, which might be due to inactivation of (AChE) acetyl cholinesterase activity which results in excess accumulation of acetylcholine in the cholinergic synapses leading to hyper stimulation. Alterations in oxygen consumption may be due to respiratory distress as a consequence of impairment in oxidative metabolism (Shankar et al., 2013).

Haematological Indices

Haematological studies play an important role in understanding variations of blood characteristics in relation to factors like phylogenetic position, ecological habitat, pollutants, food selection, etc. The regular monitoring of the fish blood is a diagnostic tool in establishing the health status of the fish in farm. It helps in evaluating the response of different types of blood cells and its components in the conditions of physiological stress due to toxicity, as it quickly reflect the poor conditions of fish than other commonly measured parameters. The blood composition of a fish reflects to some extent to metabolic and other physiological processes. Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alterations in fish caused by pollution of the aquatic environment (Anand kumar, 2001). Fish are subjected to many environmental influences which alter the healthy haemogram, i.e., the baseline data for cellular and plasma components. Haematocrit, haemoglobin and the erythrocytic haemoglobin concentration values indicate the oxygen carrying capacity in teleosts. Such parameters are highly variable among the species interfering in the oxygen-carrying capacity (Affonso, 2001; Tavares-Dias and Moraes, 2004; Wells et al., 2005). The vital functions that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication, makes the hematopoietic system unique as a target organ. Blood is a vehicle for quickly mobilizing defense against trauma and diseases. Since, fishes differ considerably in their activity patterns and respond to the aquatic pollutant. The haematological indices like Red blood corpuscle (RBC), white blood corpuscle (WBC), Haemoglobin (Hb), Packed cell volume (PCV), are commonly studied in fishes to assess the impact of pesticides in aquatic biota (Wells et al., 2005).

Table 2: Variations in Haematological Parameters of *Catla catla* exposed to Sub lethal concentration of Chlordane.

Parameter	Control	Days of Exposure Periods to Chlordane				
		3d	7d	15d	30d	45d
RBC (10^6 /mm^3)	6.15±0.12	5.31±0.15	4.98±0.18	3.48±0.11	2.64±0.16	1.89±0.13
% Change	----------	(-13.65)	(-19.02)	(-43.41)	(-57.07)	(-69.02)
Hb(g/dl)	5.54±0.19	5.16±0.13	4.94±0.12	3.75±0.16	2.49±0.11	1.88±0.06
% Change	----------	(-6.85)	(-10.83)	(-32.31)	(-55.05)	(-66.06)
PCV (%)	31.11±0.11	28.89±0.12	26.35±0.09	24.81±0.05	15.16±0.16	11.73±0.13
% Change	----------	(-7.13)	(-15.30)	(-20.25)	(-51.26)	(-62.29)
WBC (10^4 /mm^3)	17.30±0.08	18.19±0.12	22.02±0.06	25.58±0.04	30.15±0.05	33.05±0.07
% Change	----------	(5.14)	(9.94)	(47.86)	(74.27)	(91.04)

In our Present study animals exposed to sub lethal levels of Chlordane (0.422 µg/L)) showed a significant decrease in RBC count, Hb and PCV content and oxygen carrying capacity of blood, while Leucocytes (WBC) showed the contrasting trend (Table- 2 and Fig-4). The alterations observed in haematological parameters such as TEC, Hb, PCV values were decreased significantly and WBC values increased significantly after 15, 30 and 45 days of exposure periods to chlordane respectively, in comparison with control.

In the present study shows that a similar decreasing trend in all the haematological indices such as RBC, Hb content and PCV suggesting that the Organochlorine pesticides, chlordane also induce changes which give evidence for decrease haematopoiesis followed by anaemia induction in animals (Park et al., 2003). The decreased TEC and Hb content observed in the present study may be due to the disruptive action on the erythropoietic tissue, which in turn affected the cell viability. The increase in WBC count coordinated with an increase in antibody production, which helps in survival and recovery of the fishes exposed to the pesticide chlordane (Seth and Saxena, 2003).

Table -2, shows that WBC significantly increased during exposure periods. WBC plays a major role in the defence mechanism of fish which may be directly proportional to the severity of the causative stress condition and may be attributed to an increase in leucocytes mobilization. Haematological parameters such as erythrocyte count, haemoglobin concentration, haematocrit value vary in fish in response to various toxicants (Chakrabarty and Banerjee, 1988a) Alterations in erythrocytic indices have been reported in fishes subjected to environmental stressors (Gill and Pant, 1987; Khangarot et al., 1988; Areechon and Plumb, 1990) and alterations in erythrocyte morphology (Gill and Pant, 1985). There are reports on the changes on serum protein (Abidi, 1990); blood glucose level (Tilak et al., 2005); total erythrocyte count (Sastry and Siddiqui, 1984; Kumar and Nelson, 1997) and haemoglobin percentage (Sastry et al., 1982) due to various types of pesticides.

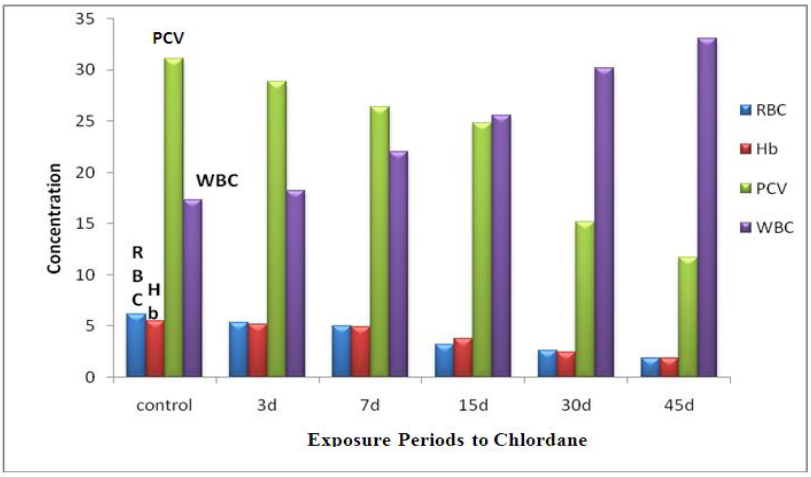

Figure- 4: Variations in Haematological Parameters of *Catla catla* exposed to Sub lethal concentration of Chlordane.

A significant elevation in WBC count (Table-2 and Fig-4) in the present study indicate a hypersensitivity of leucocytes to organochlorine pesticide, chlordane and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by chlordane (Ramesh and Saravanan, 2008) . In toxicity study, it is concluded that exposure to sub lethal concentrations of chlordane results in a significant alterations in different haematological parameters and

this kind of physiological changes may directly affect the survivability of these fishes in their natural habitat.

Leucocytes are involved in the control of immunological function and the changes in WBC counts after exposure to various toxicants may indicate a decrease in nonspecific immunity of the fish. The significant increase in WBC count might be due to generalized immune response and a protective response to chlordane stress (Nussey et al., 2002). In general, increased WBC count in fish exposed to sublethal concentration of pesticide indicates leucocytosis (Dick and Dixon, 1985). Thrall (2004) suggested that stimulation of lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissue under toxic stress may causes to elevate the Leucocyte count..

CONCLUSION

In the present study indicate that chlordane exposure to fishes induce significant changes in the haematological indices of *Catla catla*. The moderate exposure to pesticides may be tolerable but repeated exposure leads to overwhelm health hazards. Sub lethal concentrations of Organochlorine pesticides results in significant alterations in mortality, behavioural and haematological indices. These physiological alterations may directly affect the viability of these fishes in environmental habitat.

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