Indian Journal of Medical & Health Sciences
Volume 2 Number 1, January - June 2015
DOI: https://dx.doi.org/10.21088/ijmhs.2347.9981.2115.3

Original Article

Bio-Chemical and Hematological Alterations Due to Toxicity of Dimicron Organophosphate Pesticide in Fish *Clarias Batrachus*

Mishra B. P.*, Bhupinder Kaur Anand, Lingidi Jhansi Lakshmi***, Sapna Jaiswal******

IJMHS (Jan-Jun 2015) 02 (01): 15-22 / ©Red Flower Publication Pvt. Ltd.

Abstract

Objective: To study the biochemical parameters of serum glutamate oxaloacetate transminase (SGOT), serum cholesterol and hematological parameters hemoglobin, total leucocyte count (TLC) in fish *clarias batrachus,* exposed under pesticide dimicron. *Materials and Methods:* This study was concluded in fish *clarias batrachus* exposed 24 to 96 hours to four different concentration of dimicron pesticide. Serum cholesterol, SGOT was estimated as bio-chemical biomarkers while hemoglobin and total leucocyte count as hematological biomarker. *Result:* There were significant increase in serum cholesterol, SGOT, TLC and altered hemoglobin observed in dimicron pesticide exposed to fish *clarias batrachus. Conclusion:* Hypercholesterolemia, elevated SGOT, leucocytosis and altered hemoglobin is result of toxicity of dimicron pesticide in fish *clarias batrachus.* The elevated cholesterol and SGOT are the biochemical biomarker for the disorder like myocardial infarction, heart attack, biliary obstruction, alcoholic cirrhosis etc in human beings.

Keywords: OPP (Organophosphate Pesticide) SGOT (Serum Glutamate Oxaloacetate Transminase) Dimicron, TLC (Total Leucocyte Count).

Author's Affiliation: *Associate Professor ***Senior Resident, Department of Biochemistry, Mayo Institute of Medical Sciences, Barabanki, U. P, India. ** Professor, Department of Community Medicine,****Senior Lecturer, Department of Biochemistry, Career Institute of Medical Sciences, Lucknow, U.P, India.

Reprint request: Brijendra Pratap Mishra, Associate Professor, Department of Biochemistry, Mayo Institute of Medical Sciences, Faizabad Road, Gadia, Barabanki, Dharsania, Uttar Pradesh -225003

E-mail: bpmishra_72@yahoo.com, bpmishra_72@rediffmail. com

Introduction

Organophosphate and carbamates are the pesticides most commonly used worldwide in the pest control of crops. With green revolution and industrialization, they have become household items of the agriculturists. Unfortunately, because of their easy availability and accessibility [1].

The acute toxicity and clinical manifestations of organophosphate pesticide are caused by excessive synaptic accumulation of acetylcholine (AChE) **[2].** Organophosphorus compound irreversibly inhibit the enzyme acetyl cholinesterase resulting in excessive accumulation of ACh (acetylcholine), leading to the paralysis of cholinergic transmission in the C.N.S. auto ganglia, parasympathetic nerve endings, some sympathetic nerve endings and neuromuscular junctions [3].

Dimicron is a pesticide with broad spectrum of activity. It is a stomach poison and continued exposures of these highly toxic pesticides have proved hazardous to the health of human and others. It is used against aphids, beetles, bugs and trips etc [4].

Hematology covers many aspects of controlled synthesis and function from the development of many specialized cell and they support peripheral circulation for the maintenance of the circulatory environment [5].

In this research paper, the results obtained due to the effect of Dimicron on hematological parameters– Hemoglobin (Hb), Total leucocytes count (TLC), and bio-chemical parameters–serum cholesterol, SGOT (Serum glutamate oxaloacetate transminase) of fish *clarias batrachus*, is given.

Materials and Methods

The fishes collected from river Gomti, at Lucknow were brought to the biochemical laboratory in the plastic bags in natural water, washed three times in tap water and treated with 2% $KMnO_4$ to remove external parasitic infections, normal and healthy fishes were selected for the biochemical experiment. The fishes of uniform rate (80–95 gms) and length (14.1–17.5 cms) were taken for the experiment. They were transferred to large glass aquaria and acclimatized for 96 hours. Water characteristics–temperature (^{0}C), pH, alkalinity (mg/l), hardness (mg/l) and dissolved oxygen (mg/l) were analyzed by using standard method (APHA et al; 1991) [**6].**

A. Collection of Sample

Blood was collected from caudal vessels, either by serving off the caudal end or directly from heart and ventral aorta. Anticoagulants, like EDTA, Potassium citrate, Potassium oxalate, and ammonium oxalate were used. The collected blood was transferred to clean dry test tube and allowed to clot, at 10 ^{0}C. Soon after contents of the test tube were centrifuged at 2000 rpm and serum transferred to another clean dry test tube and stored in refrigeration at 2–8 ^{0}C.

B. Serum Cholesterol Estimation

Sample was placed for the estimation of serum cholesterol by the modified method of Zlatkis, A et al (1953). 0.1 ml serum was taken in large glass stoppered test tube having 5 ml glacial acetic acid contents were filtered and 0.5 ml filtrate was taken in another glass stoppered test tube and the volume was made up to 8.0 ml with glacial acetic acid. To this 2.0 ml colour reagent (1.0 ml-10 % $FeCl_3$ + 99.0 ml concentrated sulfuric acid) was thoroughly mixed by brisk circular motion of the test tube. Simultaneously, a blank was prepared by using glacial acetic acid in place of filtrate .The test tube were kept in dark for colour development and heat loss. Optical density was determined at 540 nm. Standard curve was plotted for gradually increasing volume of standard cholesterol solution (25 mg/dl). Cholesterol level was calculated as cholesterol mg/dl of serum [7].

C. Serum Glutamate Oxaloacetate Transminase Estimation (SGOT)

SGOT was estimated according to method of Reitman and frankel (1957). As given by Wootton (1964). 0.9 ml DL-asparatic acid solution (222 mm) and 0.1 ml á-ketogluteric acid solution (20mm) were mixed to make the substrate. The substrate was taken into two separate clean dry test tube one for test and other for control. 0.2 ml serum was added in the test and incubated at 30 ^{0}C for 60 minutes. 1.0 ml of 2, 4-dinitrophenyl hydrazine solution (1mm) was added in each test tube, 0.2 ml serum was then added to 'control' and mixed thoroughly, then 10.0 ml 0.4 N NaOH was added and mixed. Optical density was determined at 505 nm against water blank and standard were also prepared as given in the method. Sodium pyruvate was used in standard and volume of serum was replaced by water. SGOT level was calculated as micro mole pyruvate formed /hour /ml serum[8].

D. Hematological Estimation

(a) Preparation of Blood Film: Blood films were prepared for confirming the protozoan and other infections. Standard glass slides washed with 90 % ethyl alcohol were taken and then uniform, film was immediately prepared, slides were air dried, stained in Giemsa's leishman's and Wright's stain according to methods of Grandwhol (1943) and Wintrobe (1957) and then properly labeled [9].

(b) Hemoglobin: Hemoglobin was determined by the cynomethaemoglobin method and it was expressed as gms%. 40 micro liter capillary having sample (whole blood) was diluted in 9.960 milliliters of dilute 1:250 dilution plus lysing reagent. A vial of the properly diluted sample was placed on the lowered haemoglobin carries block. The light shield door was closed and the test was automatically completed. Hemoglobin displaced readings were in grams per deciliter of whole blood.

(c) Total leucocytes count (TLC): TLC leucocytes were counted by the electrical conductivity method of cell counting. Count was performed on dilution of whole blood in buffered saline dilutants which had controlled chemical and electrical characteristics. A 1:250 dilution of the whole blood and lysing hemoglobin reagent was used for leucocytes count. The transducer was adjusted at the factory should that 0.3125 milliliter of sample was counted. The displayed readings for leucocyte count were in thousand of cell per cubic millimeter of whole blood.

Observations and Results

Serum Cholesterol: The result obtained on serum cholesterol level of the fish *clarias batrachus* exposed for 24 to 96 hours, to four different concentrations of Dimicron, have been summarized in table no. 2

Maximum rise of 16.75% in cholesterol level was observed after exposure of 48 hours at 2.70 mg/l Dimicron. The minimum rise of 0.65 % was at the same concentration. At 2.40 mg/l concentration, the levels elevated 9.99%, 2.55 %, and 5.27 % after 24, 48, 72, hours of exposure respectively, above control. The level had however fallen 1.24 % below control in 96 hours.

At 2.70 mg/l concentration, slight increase of 0.65% above control was observed in 24 hours. The increase level seen after 48 hours and 72 hours of exposure were 16.75% and 15.68% respectively above control. At 2.90 mg/l concentration, 50% fishes died after 48 hours, when the peak cholesterol level of 20.84% above control observed. At 3.05 mg/l concentration 1.11 % increase was observed, after 24 hours of exposure above control.

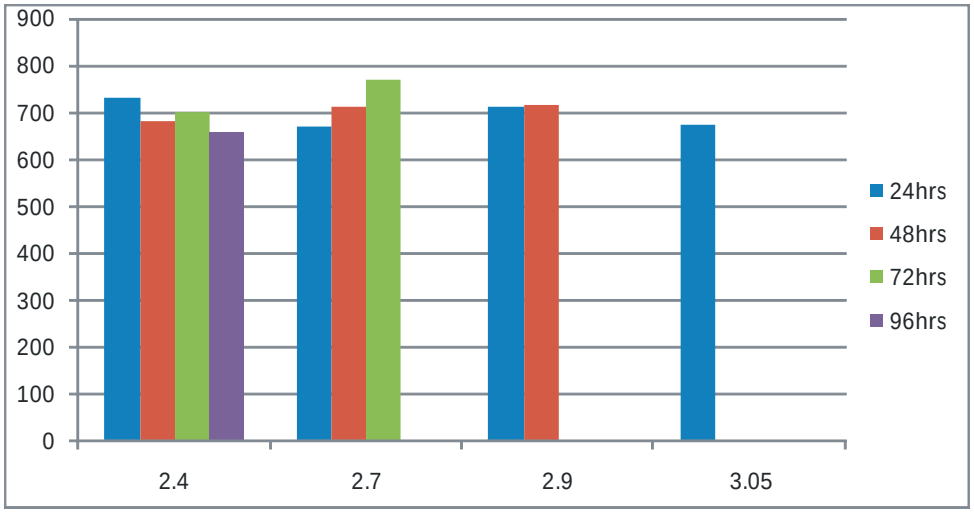
Table 1: Analyzed water characteristics in month July at the beginning of the experiment

Paramaters	Water characteristic (July month) Values (mean ± S. D) Range in Parenthesis
Temparature (° C)	28.92 ± 1.53 (27.00 – 30.30)
pH	7.22 ± 0.09 (7.10 – 7.30)
Alkalinity (mg/L)	119.50 ± 2.66 (115.0 – 121.00)
Hardness (mg/L)	115.50 ± 1.29 (113 – 117)
Dissolved oxygen (mg/L)	5.87 ± 0.17 (5.70 – 6.10)

Table 2: Effect of pesticides Dimicron on Serum Cholesterol levels of Fish *clarias batrachus.*

Pesticide conc. mg/l no. of observation 10 in each case	Serum cholesterol mg/100ml Mean ± S.D Range in Paranthesis Time of Exposure in hours			
	Control values (598.75 ± 660.00)			
	24	48	72	96
2.40	730.95 ± 92.08 (605.55– 810.73)	681.55 ± 27.09 (550.32–902.39)	699.59 ± 59.94 (629.75–775.69)	656.36 ± 66.32 (581.71– 730.33)
2.70	668.99 ± 74.33 (581.75–752.81)	713.09 ± 52.92 (660.17–785.23)	769.95 ± 51.40 (700.81– 815.32)	
2.90	712.99 ± 97.93 (600.75– 811.23)	714.73 ± 58.67 (652.75– 785.32)		
3.05	671.98 ± 80.93 (591.38– 749.24)			

Fig.1: Effect of pesticides Dimicron on Serum Cholesterol levels of Fish *clarias batrachus*



SGOT

The result obtained on serum glutamate oxaloacetate transminase levels of fish, *clarias batrachus*, exposed for 24 to 96 hours , to four different concentration of Dimicron have been summarized in table no - 3

SGOT level increased gradually with increasing intervals of exposed to all concentrations of the pesticides. Maximum rise of 31.01% above control was found at lowest concentration of 2.40 mg/L after longest exposure of 96 hrs.

At 2.40 mg / L concentration, SGOT level had risen 8.16%, 16.78%, 19.72% and 31.06% above control, after 24, 48, 72 and 96 hrs of exposure respectively.

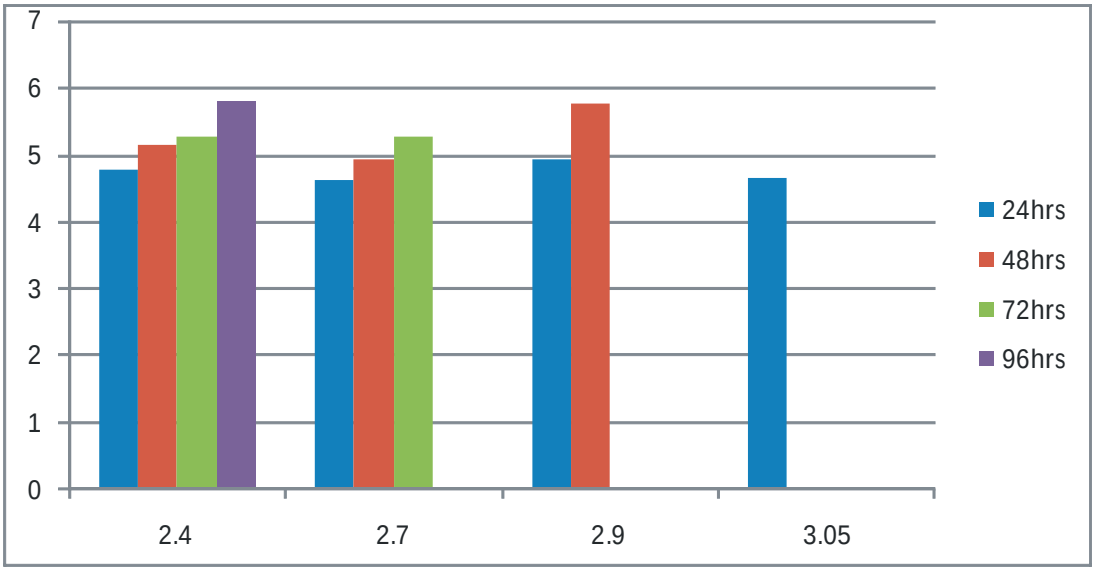
At 2.70mg / L concentration after 24, 48 and 72 hrs of exposure levels increased 4.53%, 11.33% and 19.72% respectively above control.

At 2.90mg / L concentration, after intervals of 24, 48 hrs, and enzyme level increased 11.30% and 30.61% respectively above control showing the peak at the terminal exposure, which proved lethal to 50% fishes.

Table 3: Effect of pesticide dimicron on SGOT level of fish *clarias batrachus*

Pesticide conc. mg/l no. of observation 10 in each case	S.G.O.T. micro mole pyruvate formed /ml/hour Mean ± S.D Range in Paranthesis Time of Exposure in hours			
	Control values 4.41 ± 0.34 (3.99 – 4.83)			
	24	48	72	96
2.40	4.77 ± 0.16 (4.63 – 4.91)	5.15 ± 0.23 (4.99 – 5.49)	5.28 ± 0.24 (4.99–5.49)	5.78 ± 0.15 (5.66–5.99)
2.70	4.61 ± 0.16 (4.49–4.83)	4.91± 0.09 (4.83–4.99)	5.28 ± 0.08 (5.16–5.33)	
2.90	4.91 ±0.34 (4.33–4.99)	5.76 ± 0.24 (5.49–5.99)		
3.05	4.66 ± 0.13 (4.49–4.83)			

Fig. 2: Effect of pesticide Dimicron on SGOT level of fish *clarias batrachus*



At the highest concentration of 3.05mg / L enzyme level was 5.6% above control within 24 hrs.

Hemoglobin

The result obtained on hemoglobin level of fresh *clarius buttrachus*, exposed for 24 to 96 hrs to 4 different concentrations of dimicron have been summarized in table 4.

A lower concentration of this pesticide, within short interval, was not toxic to fish, but high concentrations caused lowering in hemoglobin's levels at all time intervals.

At 2.40 mg / L concentration, interestingly, there were no change in hemoglobin levels after 24 hrs but after 48 hrs intervals an increase of 13.57%above control was observed with increasing time intervals of 72 and 96 hrs decrease of 3.90% and 11.71% respectively below control had occurred.

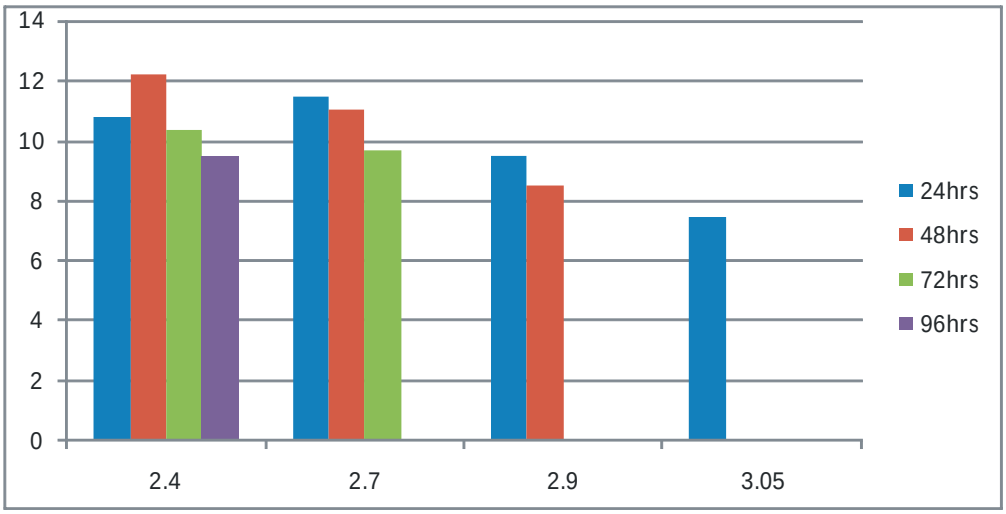
At 2.70mg / L concentration, after 24 and 48 hrs of exposure hemoglobin levels increased 6.69% and 2.60% respectively above control at the end of 72 hrs a decrease of 10.41% below control was seen.

At 2.90mg / L concentration after 24 and 48 hrs of exposure, hemoglobin levels decreased 11.71%, 21.38% respectively below control.

Table 4: Effect of pesticide Dimicron on Hemoglobin in fish *clarias batrachus*

Pesticide conc. mg/l no. of observation 10 m each case	Mean ±S.D Range in Paranthesis Time of Exposure in hours			
	Control values : 10.76 ± 0.36 (10.30 – 11.20)			
	24	48	72	96
2.40	10.76 ± 0.64 (10.20 – 11.20)	12.22 ± 0.31 (11.80 – 12.50)	10.34 ± 0.27 (10.00 – 10.70)	9.50 ± 0.25 ()
2.70	11.48 ± 0.47 (10.80 – 12.00)	11.04 ± 0.38 (10.50 – 11.50)	9.64 ± 0.24 (9.30 – 9.90)	
2.90	9.50 ± 0.25 (9.20 – 9.80)	8.46 ± 0.32 (8.00 – 8.80)		
3.05	7.44 ± 0.36 (7.00 – 7.90)			

Fig. 3: Effect of pesticide Dimicron on Hemoglobin in fish *clarias batrachus*



At the highest concentration of 3.05mg/L decrease of 30.86% below control was seen within 24 hrs and 50% died after 24 hrs.

Total Leucocytes Count (TLC)

The results obtained on total leucocytes count of *clarias batrachus* exposed for 24 to 96 hours in four different concentration of pesticides dimicron have been summarized in table No. 5

The maximum rise of 46.35% leucocytes above control was observed after 24 hours of exposure at the highest concentration of 3.05 mg/l while minimum was 5.90 % above control at the lowest concentration of 2.40 mg/L within 24 hours.

Table 5: Toxic effect of pesticides Dimicron on total leucocytes count of fish *clarias batrachus*

Pesticide conc. mg/l no. of observation 10 in each case	Total leucocytes count per mm Mean ± S.D Range in Paranthesis Time of Exposure in hours			
	Control values 15,600 ± 127 (15,450 – 15,750)			
	24	48	72	96
2.40	16,520 ± 335 (15,300– 15,750)	18,560 ± 396 (18,000 – 18,950)	17,450 ± 233 (17,100– 17,700)	18'410 ±284 (18,100 – 18,750)
2.70	17,810 ± 270 (17,500– 18,000)	18,080 ± 750 (17,100 – 18,800)	19,330 ± 295 (19,100– 19,700)	
2.90	21,660 ± 207 (21,400– 21,800)	22,420 ± 277 (22,000 – 22,700)		
3.05	22,830 + 228 (22,500– 23,050)			

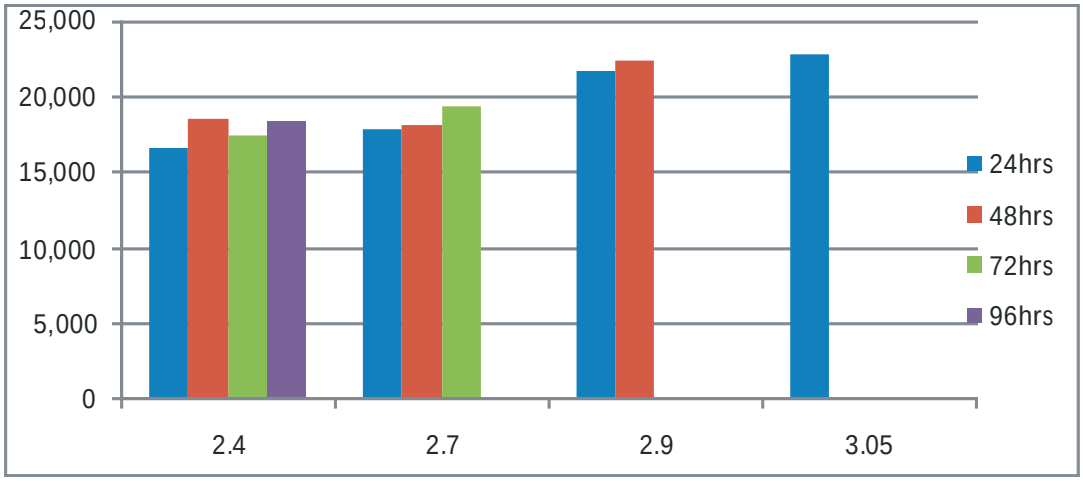
At 2.40 mg/l concentration after 24, 48, 72 and 96 hours of exposures, leucocytes increased 5.90%, 18.97%, 11.73%, and 8.01% above control respectively.

At 2.70 mg/l concentration, increase of 14.17%, 15.90%, and 23.91% above control were seen after 24, 48, and 72 hours of exposure respectively.

At 2.90 mg/l concentration 38.85%, and 43.72%increase had occurred after 24, and 48 hours of exposure respectively.

At the highest concentration of 3.05 mg/ l, 50% of fishes died after 24 hour and leucocytes were 46.35% above control.

Fig. 4: Effect of pesticides Dimicron on total leucocytes count of fish *clarias batrachus*



Discussion

Organophosphates are widely used in agriculture worldwide and are common cause of poisoning that continued to result in significant fatalities. This is because of free availability of pesticides and it's over the counter sale [10]. Organophosphorus pesticides degrade the environment frivolously hematological and biochemical parameter can be used as indicators or monitors of prevailing aquatic pollution under natural ecological conditions and in the experimentally creates aquatic environment polluted with contaminants[11].

Several studies on volunteers reported that repeated long term exposure of organophosphorus pesticides and carbamates decrease the blood cholinesterase activities without clinical manifestations[12].

Significant changes in hematological and bio-chemical parameters of fresh water fishes were observed due to effects of pesticides (Pandey et.al.1976; Sethi and Singh, 1987; Tandon etal.1986; Mishra B.Petal2000) [13].organophosphate pesticides are known to cause death due to asphyxiation. Study of biochemical and hematological parameters no doubt play an important role in diagnosis of disease. In our study significant decrease in hemoglobin content was observes due to effect of dimicron at higher concentration , with increasing time interval similar study was observed due to effect of pesticide malathion on hemoglobin concentration by Mishra B.P et al 2000.

A fall in hemoglobin content is indicative of an impaired hemoglobin bio-synthesis .A fall in hemoglobin content due to variety of pollutants has been recorded by several fishes. Thus our observation is similar with their findings. (Singh 1992; Trivedi et al; 1990,Mishra B.P. et al 2000) [14].

The organophosphate pesticide rogar resulted in increase of leucocytes in *channa punctatus* from 22.55 thousand per (mm to 32.00) thousand per cm in 96 hours at sub lethal dose of 10ppm (Goel and Mya; 1986, Mishra B.P et al 2000) Leucocythemia, a fatal disease in human beings in the cases of leucocytosis. It can be acute or substrate excess exposures of pesticides are cause for cancer in human beings also [14, 15]. Similar study has been observed in this research communication with Dimicron OP pesticides at all concentrations and time intervals.

Arise in serum cholesterol in the fresh water fish *channa punchatus* occurred an exposure to a medium contaminated with 2, 3, 4, triaminobenzene, Malathione, Sumithione and cadmium (Goel and Garg 1980; Awasthi 1982; Dubala and Shah 1981, Mishra et al 2000) [16]. Similar finding observed in

this study with Dimicron pesticides in fish, *clarias batrachus* .Hypercholesterolemia is one of the major cause for coronary heart disease in human beings.

Transamination is reversible reactions of great importance in body metabolism since all α -amino acid take part in formation of different α-ketoaminoacids, Aminotransferase (SGPT and SGOT) levels of blood and tissues of fishes under different solution of life have been studied by several workers. Several studies were reported that the activities of transaminase were significantly increases in experimental rates when fed with OP pesticide and Endrin [17]. In this study have given changes in SGPT levels of fish *clarias batrachus* exposure to 4 concentrations of OP Dimicron for 24 to 96 hours. Normally it was observed in elevation of SGPT at all concentration of OP Dimicron for 24 to 96 hours. Normally it was observed in elevation of SGPT at all concentrations. Alterations in transminase (SGPT) activities have been proved as sensitive indicators for hepatocellular as well as myocardial damage several studies were reported.

In conclusion, workers engaged to pesticides spread in agriculture may show toxic response, hence it is compulsory to use protective gear such as gloves, Face shield, aprons, shoes etc. and use of protective measure to avoid the aquatic toxicity.

Acknowledgement

This study was supported by Chairman, Research and Development unit of Mayo Institute of Medical Sciences, Barabanki, UttarPradesh, India and Central Drug Research Institute (CDRI), Lucknow, India.

References

1. Technical report series .Toxic hazards of pesticides to Mon. No.227; WHO ; Geneva : 1962 The acute toxicity and clinical manifestations of organophosphate pesticides are caused by excessive synaptic accumulation of acetylcholine (Ache).
2. Siwach SB; organophosphate poisoning New challengers. API medicine updates 1998.
3. Vishwanathan R, Srinivasan V. Treatment of OP compound poisoning. J.Indian Med Assoc 1964; 43:494.7.
4. Goel S. and Agrawal V.P.1996 influence of vitamin B12 on Methylparatheon toxicity in an airbreathing fish, channa *punctatus*: Aa hematological approach / Ad Bios 14 (1):85–86.
5. Baltantyre B;Marrs,T And Tumer P.(1993) General and Applied Toxicology .Vol.1,Stockton Press. McMillan Publishers Limited, England.
6. A.P. H.A. (American Public Health Association) 1991: " standard methods for the examination of water and wastewater" 14th Ed. American Public Health Association New York.
7. Zlatkis A; Zak B. and Boyle A.J. 1953: J.Lab clin.Med. 41:486–92.
8. Reitman.S., Frankel.S.,1957: A colorimetric method for the determination of serum glutamic oxaloacetic and pyruvic transaminase. Amer.j.clin.Path.28:56–63.
9. Wintrobe, M.M. Clinical Haematology, Lea and Febiger, USA. 1957.
10. Nadia A Ather; Jamal Ara et al; (2008): Acute organophosphate insecticide poisoning: J of surgerg Pakistan (international) 13:7174.
11. Valcazar A.A.etal : 1990 Arch pharmacology Toxcol.6,750
12. Aldrige W.N. (1971) the nature of the reaction of O.P. compounds and carbamates with esterases Bull World Health Organization 44, 25.
13. Pandey, B. N., Chanchal, A. K. and singh, M. P. 1976. Effect of Malathion on oxygen consumption and blood of Channa Punctatus. Bloch. Ind. J. Zootomy, 16: 95–100.
14. Singh R.K.; Tripathi S.N; Mishra B.P.(2000): hematotoic effect of commonly used pesticides malatheion in Teleost Fish clarias battrachus; J.Environ Res;2000;10(1):17–19.
15. Goel and Meya 1986 hematological Anamalies in *clarias battraches* under the stress Rogers .Ad.Bios; 5: 187–192.
16. Mishra B.P.; S.N.Tripathi; Singh R.K. hemato toxic effect of pesticide Rogor in fish channa punctatus J. Envionment Res,2000;10(1) :3–4
17. Luckens, M. M. and phelps KI (1969): Serum enzyme pattern in acute poisoning with organochlorine insecticide .J.pharam.Sci.58: 569–572.
18. Gomez. S.G. Faircloth G; Lopez-Lazaro,L;JimenoJ; Bueren J.A; Albella B;2001 invitrohemato toxicity of aplidine on human bone marrow and cord blood progeneita cells. Toxicity in vitro 15: 347–350.
19. Jyotsna A.Patil. arun j.Patil etal,2003; biochemical effects of various pesticide a sprayers of Grape gardens; I.J.of clinical biochemistry,18(2): 16–22.

20. Meena K.,Gupta P.K. and Bewa, S.R.(1978) Endrin-induced toxicity in normal and irradiated rats.

21. Kagan J.(1971) Topical questions about the toxicology of phosphoorganic insecticides Ernahrungs for schung 14: 503–514 (in German).