*Original Article*

Indian Journal of Forensic Medicine and Pathology
Volume 8 Number 4, October December 2015

# Hematological and Biochemical Toxic Effect of Commonly Used Organophosphate Pesticide Malathion in Teleost Fish Clarias Batrachus

**Mishra B.P.*, Rai Pramod Kumar**, M.P.S. Marwaha***, Bhupinder Kaur Anand****, Lingidi Jhansi Lakshmi*******

**Abstract**

*Objective:* To study the hematological parameter - hemoglobin, total leucocyte count and biochemical parameter - serum cholesterol, serum glutamate pyruvate transaminase in fish Clarias Batrachus exposed under different concentrations of Malathion pesticide. *Methodology:* This experimental research study has been done in teleost fish Clarias Batrachus, exposed 24 to 96 hours, to four different concentrations of Malathion pesticide. Estimation of serum cholesterol and SGPT had been done as biochemical parameter and Hemoglobin as well as T.L.C had been analyzed as hematological parameter. *Results:* There were no significant changes in hemoglobin level at lower concentration of Malathion pesticide within short intervals but at high concentrations, lowering in hemoglobin levels had been observed at all time intervals though elevated total leucocyte count had been observed at all concentrations and time intervals. Regarding to biochemical parameters significant increase in serum cholesterol and SGPT enzyme levels observed in Malathion pesticide exposed fishes.

**Keywords:** Cholesterol; Hemoglobin; Malathion; OP; SGPT; TLC.

## Introduction and Conceptual Framework

In this rapidly developing, capitalist world, people are continually exposed to numerous environmental pollutants such as industrial waste, polluted air and pesticides. These invariably comprise complex mixtures of chemicals. The effects of the mixtures and their mode of action in humans are insufficiently well studied. The majority of pollutants are potentially toxic for organisms, some being connected to disease development. In this context, the increase of chronic degenerative disease including cancer in humans is of considerable concern [1].

**Authors Affiliation:** *Associate Professor *****Assistant Professor, Department of Biochemistry, **Assistant Professor, Department of Pathology, Mayo Institute of Medical Sciences, Barabanki, U. P, India. ***Wg Cdr, Aerospace Medicine Specialist, Airfare station, Ambala, Haryana, India. ****Professor, Department of Community Medicine, Career Institute of Medical Sciences, Lucknow, U.P, India.

**Reprints Requests: Brijendra Pratap Mishra,** Associate Professor, Department of Biochemistry, Mayo Institute of Medical Sciences, Barabanki, U. P, India.
E-Mail: bpmishra_72@yahoo.com,
bpmishra_72@rediffmail.com



Pesticides are a very important group of environmental pollutants used in intensive agriculture for protection against diseases and pests [2]. Function wise they are divided into herbicides (protection against weeds), insecticides (against insects), fungicides (against fungi), and others. While their use improves the quantity of agricultural products, it potentially affects their quality, as pesticides may enter human diet [3].

Organophosphorus compounds or organophosphates (OPs) form a large group of chemicals used over the past 60 years for protecting crops, livestock, human health and as warfare agents. On the basis of structural characteristics they are divided into at least 13 types, including phosphates, phosphonates, phosphinates, phosphorothioates (S=), phosphonothioates (S=), phosphorothioates (S substituted), phosphonothioates (S substituted), phosphorodithioates, phosphorotrithioates, phosphoramidothioates [1]. OPs are the most widely used pesticides worldwide and their metabolites are widespread across different populations [4, 5, 6]. The adverse short-term effects of exposure to these chemicals have been studied mostly in the nervous system, which is their primary target [7], but there is a growing concern about their possible toxic effects

in non-target tissues and (long-term) chronic effects that have not been studied in such detail. The majority of people are continually exposed to low OP concentrations, and long-term epidemiologic studies reveal linkage to higher risk of cancer development [8].

The primary mechanism of OPs toxicity is well studied – they function as inhibitors of the enzyme acetylcholinesterase (AchE). Human exposure to OPs is most frequently assessed by measurement of decrease in AchE activity. This method is relevant for professional exposure, where OP concentrations entering to body are relatively high. However, low OP concentrations, which are present continuously, do not cause significantly decreased AchE activity. Exposure of wider populations must lean on assessment of OP metabolites, such as alkylphosphate in urine ( Gupta, 2006).

*Metabolism*

Metabolism of xenobiotics takes place mostly in the liver and to a lesser extent also in the lung and intestine. It comprises two phases; the metabolic enzymes in phase I activate the chemical with the introduction of functional groups, on which phase II reactions can take place. The phase II enzymes attach various hydrophilic groups, e.g. glucuronic acid, sulphate, glycine, glutamic acid, enabling excretion of the metabolite from the organism [9].

*Mechanism of Toxicity*

The toxicity of OPs depends on their chemical structure, metabolism in target organism, concentration (i.e. dose), mode of application, degree of decomposition, mode of entering organisms, etc. [3]. The best described OP toxic effects are the neurological symptoms following acute poisoning as a consequence of the primary target (AchE). Potential secondary targets and toxic effects outside the nerve system have not been well studied, but are nevertheless very important for risk assessment.

*Neurotoxicity of Organophosphate Pesticides*

The primary mechanism of OPs toxicity involves inhibition of the enzyme AchE. AchE is found in synaptic membranes, where it degrades, through its hydrolytic activity, the neurotransmitter acetylcholine, producing choline and acetate, a reaction important for the regulation of synaptic activity in the central and peripheral neural system. OP cholinesterase inhibitors block the function of acetylcholinesterase, causing the accumulation of excessive acetylcholine in the synaptic cleft. This causes neurotoxic effects such as neuromuscular paralysis (i.e. continuous muscle contraction) throughout the entire body [1]. Symptoms of acute OP poisoning can be divided according to the site of acetylcholine accumulation in the organism . In addition to acute symptoms, some OPs can cause other symptoms that arise a few days after exposure or poisoning with OP. Weakness in muscles and breathing difficulties usually appear 1 – 4 days after poisoning while, after 7- 21 days, weakness in peripheral muscles also occurs. The cause of these delayed symptoms is inhibition of the neuropathy target esterase (NTE) located in the neural system, rather than AchE inhibition. NTE belongs to the same group of serine esterases as AchE, however its primary role in the organism is not well known [10]. Several other neurotoxic symptoms that cannot be ascribed to AchE inhibition, but act on different secondary targets inside the neural system, have been also proposed.

Malathion is an organophosphate parasympathominetic pesticide which binds irreversibly to cholinesterase. It is widely used in agriculture, residential landscaping, public recreation areas and in public health pest control programmes such as mosquito eradication.

Organophosphates are basis of many insecticides, pesticides, herbicides and nerve agents. Impaired memory, lack of concentration, disorientation, severe depression, confusion, irritability, nightmares, delayed reaction time, drowsiness, insomnia etc are the symptoms of chronic toxicity of pesticides. A biocide is a chemical or microorganism which can exert a controlling effect on any harmful organism by chemical or biological means.

Malathion has also been used in public health mosquito control and fruit fly eradication programmes (National pesticide information center). Malathion kills insects and pests by preventing their nervous system from working properly. Malathion binds to the enzyme, acetylcholinesterase and prevents the nervous system from stopping.

Newer evidence suggests that organophosphate pesticide may cause developmental neurotoxicity at much lower doses and without depression of plasma cholinesterase levels. Some of the most common naturally occurring brain toxins that lead to neurotoxicity as a result of excessive dosage of $\alpha$ - amyloid, glutamate and oxygen free radicals. Higher concentration of brain toxins can lead to neurotoxicity and death (apoptosis). It is also a major cause for neurodegenerative diseases such as Alzheimer's disease.

Major action of pesticides on parasympathetic nervous system may cause bradycardia, hypotension, hypersecretion, bronchoconstriction, GI tract hypermotility, decrease intraocular pressure. Action on neuromuscular junction may lead prolonged muscle contraction.

*Objective*

To study the hematological parameters of hemoglobin, total leucocyte count and biochemical parameters of serum cholesterol, serum glutamate pyruvate transaminase (SGPT) in fish Clarias Batrachus exposed under different concentrations of Malathion pesticide.

## 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% KMnO4 to remove external parasitic infections, normal and healthy fishes were selected for the biochemical experiment. The fishes of uniform rate (85-95 gms) and length (14.1-17.8 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 etal; 1991) [11].

*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 refrigerator at 2-8 $^0$C.

*Hematological Analysis*

*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 diluent (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 [12].

*Total Leucocytes Count (TLC)*

Total 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 [12].

*Biochemical Investigations*

*Serum Cholesterol Estimation*

Sample was placed for the estimation of serum cholesterol by the modified method of Zlatkis, A; etal (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 % FeCl3 + 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 [13].

*Serum Glutamate Pyruvate Transaminase / Alanine Transaminase (SGPT/ALT) Estimation*

SGPT was estimated according to the method of Reitman and Frankel (1957) as given by wotton (1964). 0.9ml DL-alanine solution (222mm), 0.1 ml $\alpha$ -ketoglutaric acid solution (20mm) was mixed to take the substrate. The substrate was taken in two separate, clean dry test tube, one for the test other for control. 0.2ml serum was added in the test tube and incubated at 37°C for 30 minutes. 1.0ml of 2,4 – dinitrophenylhydrazine solution (1mm) was

added in each test tube, 0.2ml serum was added to control and mixed thoroughly. Then 10.0ml of 0.4N NaOH was added and mixed. Optical density was determined at 505nm against water blank. SGPT level were calculated as micro mole pyruvate formed / hour / ml serum [14].

## Observations & Results

*Hemoglobin*

The result obtained on hemoglobin level of fish *clarias batrachus*, exposed for 24 to 96 hrs to four different concentrations of Malathion have been summarized in Table 2. At lower concentration of Malathion pesticide, within short interval, was not toxic to fish, but at high concentrations caused lowering in hemoglobin levels at all time intervals.

At 2.40 mg/L Malathion 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, reported below control range.

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

At the highest concentration of 3.05mg/L decrease of 30.86% below control was seen within 24 hrs and 50% fishes were 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 concentrations of pesticide Malathion have been summarized in Table 3.

The maximum rise of 46.35% leucocytes above control was observed after 24 hours of pesticide 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 had been observed within 24 hours.

At 2.40 mg/L Malathion concentration, after 24, 48, 72 and 96 hours of exposures, leucocytes increased 5.90%, 18.97%, 11.73%, and 8.01% above control respectively. Though at 2.70mg/L concentration, increase of 14.17%, 15.90%, and 23.91% above control were seen after 24, 48, and 72 hours of exposure respectively.

Elevated T.L.C. percentage of 38.85, and 43.72 above control had been observed after 24 and 48 hours of exposure respectively at the Malathion concentration of 2.90mg/L. At the highest concentration of 3.05 mg/ L, 50% of fishes died after 24 hour and leucocytes were 46.35% above control.

*Serum Cholesterol*

The results obtained on serum cholesterol level of the fish *clarias batrachus* exposed for 24 to 96 hours, to four different concentrations of Malathion, have been summarized in Table 4.

Maximum rise of 16.75% in cholesterol level was observed after exposure of 48 hours at 2.70 mg/L Malathion. The minimum rise of 0.65 % was there at the same concentration after 24 hours.

At 2.40 mg/L malathion concentration, the levels were increased 9.99%, 2.55 %, and 5.27 % after 24, 48, 72, hours of exposure respectively, above control. The serum cholesterol 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 levels seen after 48 and 72 hours of exposure were 16.75% and 15.86% respectively above control.

At 2.90 mg/L concentration, 50% fishes were died after 48 hours, when the peak cholesterol level of 20.81% above control was observed. Though at 3.05 mg/L concentration 1.11 % increase was observed, after 24 hours of exposure above control.

*Serum Glutamate Pyruvate Transaminase / Alanine Transaminase (SGPT/ALT)*

The results obtained on SGPT levels of fish, *clarias batrachus*, exposed for 24 to 96 hours , to four different concentration of Malathion have been summarized in Table 5. The toxic effect of Malathion pesticide also marked with regard to biochemical parameter SGPT levels. At lower concentration with increasing time intervals increase in SGPT level were more pronounced as compared to higher concentration and shorter time intervals.

At 2.40 mg/L Malathion concentration, after 24, 48, 72 and 96 hrs of exposure SGPT enzyme levels were increased 5.79%, 10.67%, 5.18%, and 15.85% respectively above control range. While at 2.70mg/L concentration the levels were increased 11.58%, 24.08% and 27.43% above control, after 24, 48 and 72 hrs of exposure respectively.

At 2.90mg/L Malathion concentration, after exposures for 24 and 48 hrs, SGPT levels increased 7.31% and 4.25% respectively above control. Though at 3.05mg/L concentration, after exposure of 24 hours SGPT levels increased 3.04% above control, after which 50% fishes did not survived.

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

| Paramaters | Water characteristic (Sept month) Values (mean ± S. D) Range in Parenthesis |
|---|---|
| **Temparature (° C)** | **23.92 ± 1.45 (22.00-25.00)** |
| pH | 7.22 ± 0.09 (7.10-7.30) |
| Alkalinity (mg/L) | 118.50 ± 2.64 (115.0-121.00) |
| Hardness (mg/L) | 114.50 ± 1.29 (113-116) |
| Dissolved oxygen (mg/L) | 5.89 ± 0.17 (5.60 - 6.05) |

**Table 2**: Effect of Malathion Pesticide on Hemoglobin of fish Clarias Batrachus

| Pesticide conc. mg/l, no. of observation 10 in each case | Mean±S.D Range in Paranthesis Time of Exposure in hours Control values : 10.76 ± 0.36 (10.4 - 11.12) | | | |
|---|---|---|---|---|
| | **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 (9.70 - 9.20) |
| 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) | | | |

**Table 3:** Effect of Malathion Pesticide on Total Leukocyte 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) | | | |

**Table 4:** Effect of Malathion Pesticide on Serum Cholesterol levels of Fish *Clarias Batrachus*

| Pesticide conc. mg/l, no. of observation 10 in each case | Serum cholesterol mg/dL Mean ± S.D Range in Paranthesis Time of Exposure in hours Control values (644.54 ± 56.67) | | | |
|---|---|---|---|---|
| | 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) | 769.55 ± 51.40 (700.81 – 815.32) | |
| 2.90 | 712.99 ± 97.93 (615.06 – 810.92) | 714.73 ± 58.67 (652.75 – 785.32) | | |
| 3.05 | 671.98 ± 80.93 (591.38 – 749.24) | | | |

**Table 5:** Effect of Malathion Pesticide on SGPT levels of fish Clarias Batrachus

| Pesticide conc. mg/l , no. of observation 10 in each case | S.G.P.T. µmoles formed / ml / hour Mean ± S.D Range in Paranthesis Time of Exposure in hours Control values 3.28 ± 0.38 (2.90 – 3.66) | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| 2.40 | 3.47 ± 0.26 (3.28 - 3.66) | 3.63 ± 0.12 (3.54 – 3.72) | 3.45 ± 0.29 (2.57 – 4.09) | 3.80 ± 0.38 (3.42 – 4.18) |
| 2.70 | 3.66 ± 0.53 (3.28 - 4.04) | 4.07 ± 0.58 (3.57 – 4.71) | 4.18 ± 0.54 (3.80 – 4.57) | |
| 2.90 | 3.52 ± 0.53 (3.14 - 3.90) | 3.42 ± 0.38 (3.04 – 3.80) | | |
| 3.05 | 3.38 ± 0.55 (3.40 – 3.93) | | | |

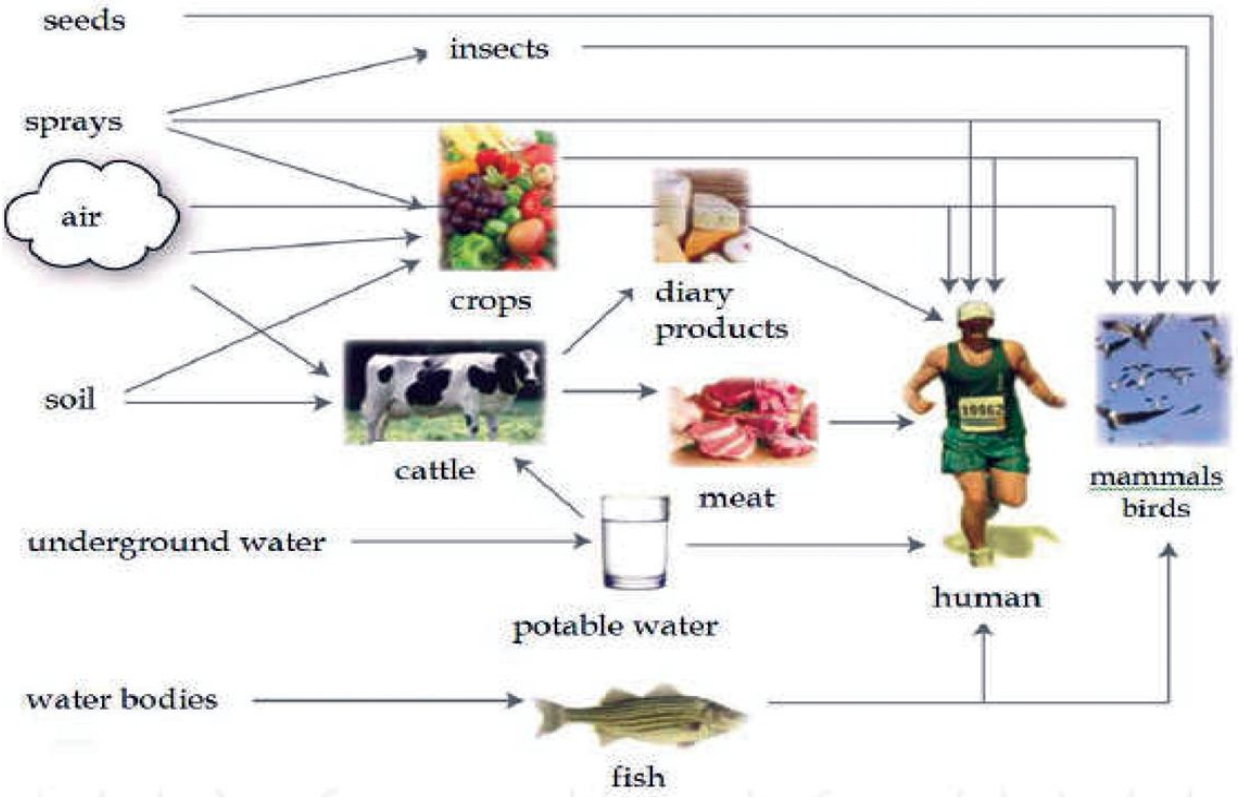


**Fig. 1:** Routes of Exposure to Organophosphates (adapted from WHO, 2001)

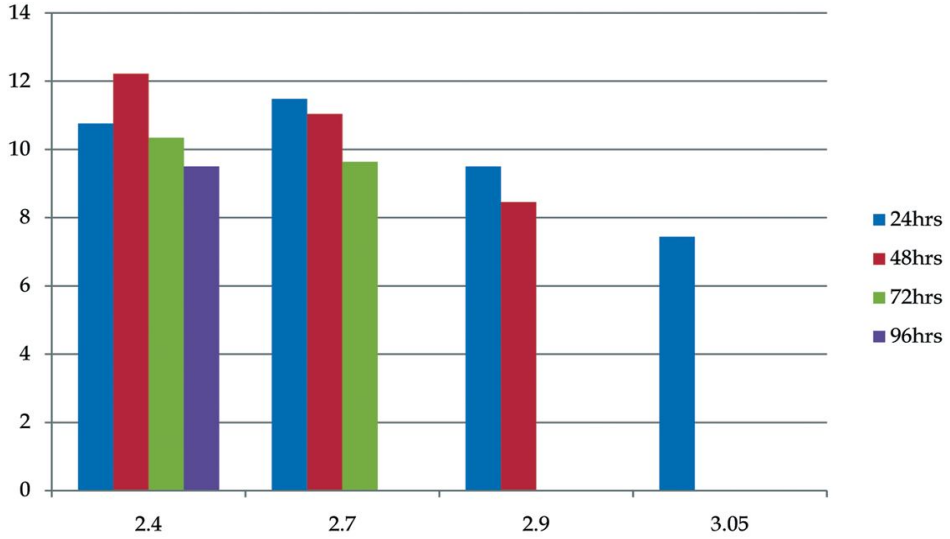


**Fig. 2:** Effect of Malathion Pesticide on Hemoglobin in fish Clarias Batrachus

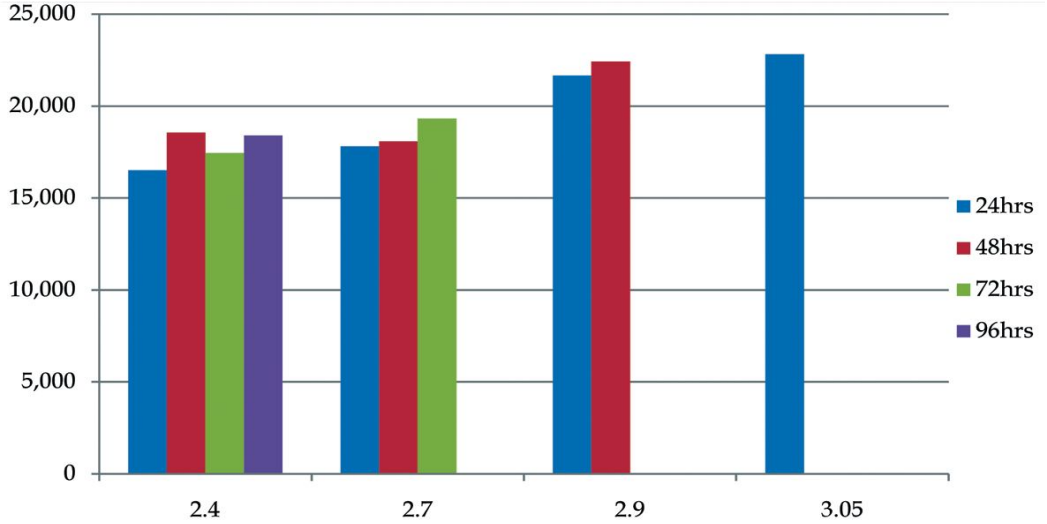


**Fig. 3:** Effect of Malathion Pesticide on Total Leukocyte Count of fish Clarias Batrachus

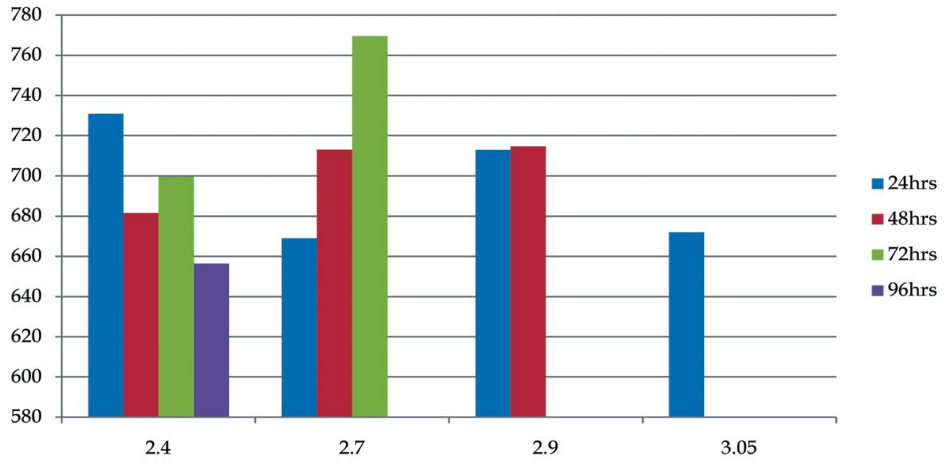


**Fig. 4:** Effect of Malathion Pesticide on Serum Cholesterol levels of Fish Clarias Batrachus

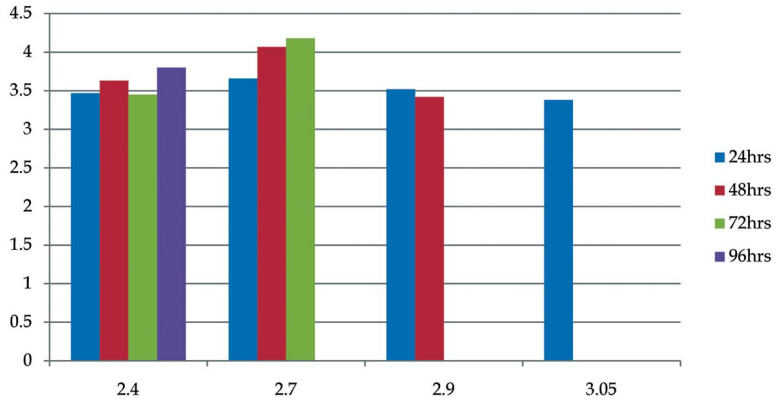


**Fig. 5:** Effect of Malathion Pesticide on SGPT levels of fish Clarias Batrachus

## Conclusion and Recommendation

Leucocytosis, altered hemoglobin, hyper-cholesterolemia and elevated SGPT are result of toxicity of Malathion pesticide in fish Clarias Batrachus. The elevated cholesterol and SGPT are the biochemical markers for bioclinical stress such as Myocardial infarction (MI), Atherosclerosis, CAD, Heart attack, biliary Obstruction, Liver Cirrhosis etc. Leukemia and Myloproliferative disorders are indicator of leucocytosis in human beings. Low hemoglobin levels are predictor of increased risk of death among heart failure patients (Journal of the

American Heart Association). High hemoglobin levels may be indicators of bone marrow dysfunction, kidney cancer, liver cancer, polycythemia vera etc in human beings. On the basis of this conclusion it is advised to pesticide sprayers to take all the precautions regarding protection from pesticide exposure and suitable use of prophylactic supplements for healthy life style.

**Acknowledgement**

The authors are thankful to Dr. K.N. Singh, Chairman, Mayo Institute of Medical Sciences, Barabanki, U.P and Director, CDRI, Lucknow for providing support and assistance.

**References**

1. Gupta, R.C. Toxicology of Organophosphate & Carbamate Compound. Elsevier Academic Press. 2006.
2. Gavrilescu, M. Fate of pesticides in the environment and its bioremediation. Eng Life Sci. 2005; 5(6): 497-526.
3. Grliæ, L. Mali kemijski leksikon Napried, Zagreb 1988.
4. Aprea, C.; Strambi, M.; Novelli, M.T.; Lunghini, L. & Bozzi, N. Biologic monitoring of exposure to organophosphorus pesticides in 195 Italian children. Environ Health Perspect. 2000; 108(6): 521-5.
5. Barr, D.B.; Bravo, R.; Weerasekera, G.; Caltabiano, L.M.; Whitehead, R.D. Jr.; Olsson et al. Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population. Environ Health Perspect. 2004; 112 (2): 186-200.
6. Curl, C.L.; Fenske, R.A. & Elgethun, K. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Environ Health Perspect. 2003; 111(3): 377-82.
7. Gupta, S.; Stravitz, R.T.; Dent, P. & Hylemon, P.B. Down-regulation of cholesterol 7alpha-hydroxylase (CYP7A1) gene expression by bile acids in primary rat hepatocytes is mediated by the c-Jun N-terminal kinase pathway. J Biol Chem. 2001; 276(19): 15816-22.
8. Brown, L.M.; Blair, A.; Gibson, R.; Everett, G.D.; Cantor, K.P.; Schuman, L.M. et al. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res. 1990; 50(20): 6585-91.
9. Josephy, P.D. & Mannervik, B. Molecular Toxicology. New York: Oxford University Press. 2006.
10. Kamanyire, R. & Karalliedde, L. Organophosphate toxicity and occupational exposure. Occupational Medicine-Oxford. 2004; 54(2): 69-75.
11. A. P. H. A. (American Public Health Association). standard methods for the examination of water and waste water, 14th Ed. American Public Health Association New York. 1991.
12. Wintrobe, M.M. Clinical Haematology, Lea and Febiger, USA. 1957.
13. Zlatkis A; Zak B. and Boyle A.J. J.Lab clin.Med. . 1953; 41: 486–92.
14. Reitman.S., Frankel.S. A colorimetric method for the determination of serum glutamic oxaloacetic and pyruvic transaminase. Amer.j.clin.Path. 1957; 28: 56–63.