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Pathological and Biochemical Changes Due to Effect of Organophosphate Pesticide Metasystox in *Channa Punctatus*

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Abstract

Background: Pesticides are a very important group of environmental pollutants used in intensive agriculture for protection against diseases and pests. Pesticides are the some of the deadliest poison produced by human beings, hence present a health hazards in long term exposure even at low levels. Neurotoxicity is major consequence of pesticide effect. *Objectives:* To study the hematological parameters – haematocrit (Hct) and absolute erythrocyte count (AEC) and biochemical parameters - serum glutamate oxaloacetate transaminase (SGOT) in fish Channa punctatus exposed under different concentrations of Metasystox pesticide. *Materials and Methods:* The fishes collected from river Gomti, at Lucknow were brought to the biochemical laboratory in the plastic bags for processing of experimental study. This study was demonstrated in fish Channa punctatus, which were exposed 24 to 96 hours to four different concentrations of Metasystox pesticide. Analysis of haematocrit (Hct) and absolute erythrocyte count had been done as pathological parameters and serum glutamate oxaloacetate transaminase (SGOT) as biochemical parameter to know the adverse effect of the pesticide. *Results and Conclusion:* Significant decrease in absolute erythrocyte count was noticed in fish channa punctatus at all concentrations and the time intervals. The results obtained on hematocrit of the fish channa punctatus were also remarkable; it was fall in level at all concentrations and time intervals. Regarding to biochemical parameter, significant increase in aspartate transaminase enzyme levels were observed in Metasystox pesticide exposed fishes. These results concluded that changes in pathological and biochemical parameters are the adverse effect of the Metasystox pesticide. Therefore it is suggested that pesticide sprayers have to take all the safeguard regarding protection from Metasystox pesticide.

Keywords: Metasystox; Hematocrit; Absolute Erythrocyte Count; Aspartate Transaminase; Toxicity.

Background 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].

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]. There are about 15000 individual compounds and 35000 formulations in use as agricultural pesticides. Though beneficial in their action as pesticide to control agricultural pests, their toxicities account for a significant risk of occupational

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toxicity due to chronic exposure. At cellular level, pesticide has been reported to generate reactive oxygen species, which catalyze increased lipid peroxidation [4].

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 [4]. OPs are the most widely used pesticides worldwide and their metabolites are widespread across different populations [5,6,7]. The adverse short-term effects of exposure to these chemicals have been studied mostly in the nervous system, which is their primary target [8], 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 [9].

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 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 [10].

Metasystox (Dementon- Methyl) is pesticide containing active ingredients o,o- dimethyl, o-ethyl thioethyl phosphorothioate and o,o- dimethyls-ethylthioethyl phosphorothioate widely used throughout country [11]. Metasystox pesticide was selected for this experiment, and effect of this pesticide on hematocrit, absolute erythrocyte count, serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase on fish *Channa punctatus* were seen.

In this research paper we are presenting the results of hematological parameters - hematocrit, absolute erythrocyte count and biochemical parameters - serum glutamate oxaloacetate transaminase of fish *Channa punctatus* which was exposed under Metasystox pesticide. Alterations in parameters are the result of chronic toxicity of metasystox pesticide.

Mode of Action and Neurotoxicity of Organophosphate Pesticides

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 organophosphate 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. The primary mechanism of organophosphates 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 [12]. 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.

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 β-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 – haematocrit (Hct) and absolute erythrocyte count(AEC) and biochemical parameters -serum glutamate oxaloacetate transaminase(SGOT) in fish Channa punctatus exposed under different concentrations of Metasystox 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% $KMnO_4$ 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) [13].

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.

Hematological Analysis

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 [14].

Absolute Erythrocyte Count (AEC)

Absolute erythrocyte were counted by the electrical conductivity method of cell counting. Count were performed on dilution of whole blood in buffered saline diluents which had controlled chemical and electrical characteristics. A 1:62500 dilution of the whole blood was used for erythrocyte count. The transducer was adjusted at the factory such that 0.3125 milliliter of sample was counted. The displayed readings for erythrocyte were in million of cells per cubic millimeter of whole blood [14].

Hematocrit

Percentage of hematocrit (Hct) was analyzed by the electrical conductivity method. In parallel with absolute erythrocyte count, the electrical output from the probe and common electrodes was electronically analyzed as each cell passes through the 100μ aperture. Each cell created an electrical pulse based upon the size of the cell. Since this was done for a given volume (0.3125 ML) of sample solution, with known dilution ratio, the totalized output was accurately calibrated to read hematocrit directly.

Biochemical Investigations

Serum Glutamate Oxaloacetate Transaminase(SGOT)/ Aspartate Transaminase(AST)

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 α-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 [15].

Observations and Results

Absolute Erythrocyte Count

The results obtained on absolute erythrocytes of the fish, *Channa punctatus,* exposed for24 to 96 hours, to four different concentrations of metasystox have been summarized in Table 2.

The fishes became anaemic at all concentrations of pesticide exposure. Erythrocyte counts decreased with gradual increase of pesticide concentration and time exposure, till the end of experiment. At the highest concentration of 1.35% metasystox pesticide, erythrocyte count was lowest within 24 hours and it was 68.38% below control.

Hematocrit

The results obtained on hematocrit of the fish,*Channa punctatus,*exposed for 24 to 96 hours, to four different concentrations of Metasystox have been summarized in Table 3.

The toxicity of pesticide metasystox regarding to hematocrit percentage had been observed as fall in its level at all concentrations and time intervals. Maximum fall in Hct level was observed as 66.08% below control at 1.35mg/l concentration of metasystox within 24 hours of pesticide exposure. While minimum was reported 4.42% at 1.0mg/l concentration of metasystox after 24 hour of pesticide exposure.

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

Water characteristic (Sept month)	
Paramaters	**Values (mean ± S. D) Range in Parenthesis**
Temparature (° C)	23.91 ± 1.45 (22.00 – 25.00)
pH	7.21 ± 0.09 (7.10 – 7.30)
Alkalinity (mg/L)	118.51 ± 2.64 (115.0 – 121.00)
Hardness (mg/L)	114.51 ± 1.29 (113 – 116)
Dissolved oxygen (mg/L)	5.86 ± 0.17 (5.60 – 6.05)

Table 2: Effect of Metasystox on Absolute Erythrocyte Count of fish Channa Punctatus

Pesticide conc. mg/l ,no. of observation 10 in each case	**Absolute Erythrocyte Count (million/cu mm) Mean ±S.D Range in Parenthesis Time of Exposure in hours Control values :4.27 ±0.03 (4.22 – 4.30)**			
	24	**48**	**72**	**96**
1.00	3.08 ± 0.05 (3.03 – 3.13)	2.71 ± 0.09 (2.62 – 2.83)	2.05 ± 0.03 (2.01 – 2.08)	1.61 ± 0.07 (1.52 – 1.71)
1.05	2.85 ± 0.07 (2.74 – 2.94)	2.63 ± 0.07 (2.49 – 2.71)	1.67 ± 0.08 (1.06 – 1.80)	-----
1.25	2.73 ± 0.07 (2.63 – 2.81)	1.76 ± 0.07 (1.71 – 1.86)	-----	-----
1.35	1.35 ± 0.04 (1.30 – 1.40)	-----	-----	-----

Table 3: Effect of Pesticide Metasystox on Hematocrit of fish Channa Punctatus

Pesticide conc. mg/l ,no. of observation 10 in each case	**Hematocrit (%) Mean ±S.D Range in Paranthesis Time of Exposure in hours Control values : 18.31 ± 0.09 (18.20 – 18.43)**			
	24	48	72	96
1.00	17.50 ± 0.09 (17.40 – 17.63)	12.34 ± 0.14 (12.15 – 12.51)	10.31 ± 0.08 (10.19 – 10.40)	9.09 ± 0.04 (9.03 – 9.15)
1.05	11.42 ± 0.11 (11.31 – 11.59)	9.18 ± 0.04 (9.13 – 9.23)	8.66 ± 0.5 (8.60 – 8.73)	-----
1.25	10.71 ± 0.06 (10.63 – 10.79)	7.39 ± 0.09 (7.29 – 7.51)	-----	-----
1.35	6.21 ± 0.05 (6.15 – 6.28)	-----	-----	-----

Table 4: Effect of pesticide Metasystox on SGOT level of fish Clarias Batrachus

Pesticide conc. mg/l no. of observation 10 in each case	S.G.O.T. µmol pyruvate formed /ml/hour Mean ± S.D Range in Paranthesis Time of Exposure in hours Control values 4.15 ± 0.21 (3.15 – 4.72)			
	24	48	72	96
1.00	7.62±0.22 (7.39 – 7.86)	6.74 ± 0.11 (6.63 – 6.89)	6.69 ± 0.12 (6.58 – 6.83)	6.59 ± 0.31 (6.15 – 6.87)
1.05	7.81 ± 0.49 (7.07 – 8.13)	6.80 ± 0.50 (6.07 – 7.18)	6.32 ± 0.14 (6.11 – 6.43)	-----
1.25	7.77 ± 0.18 (7.57 – 7.97)	6.69 ± 0.37 (6.13 – 7.57)	-----	-----
1.35	7.85 ± 0.13 (7.66 – 7.98)	-----	-----	-----

Serum Glutamate Oxaloacetate Transaminase / Aspartate Transaminase (SGOT/AST)

The results obtained on AST of the fish,*Channa punctatus*,exposed for 24 to 96 hours, to four different concentrations of Metasystox have been summarized in Table 4.

The activity of SGOT enzyme was elevated at all concentrations and exposures of the metasystox pesticide for 24 to 96 hours of time intervals.The maximum rise of SGOT enzyme activity was 89.16% above control at the highest concentration of metasystox pesticide (1.35 mg/l), while minimum was 52.88% above control at terminal exposure to the 1.05 mg/l concentration of metasystox pesticide.

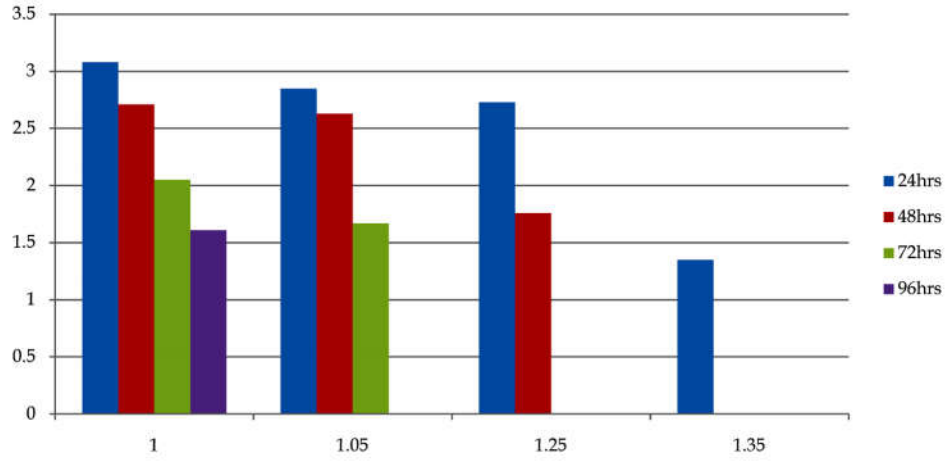

Fig. 1: Effect of Metasystox on Absolute Erythrocyte Count of fish Channa Punctatus

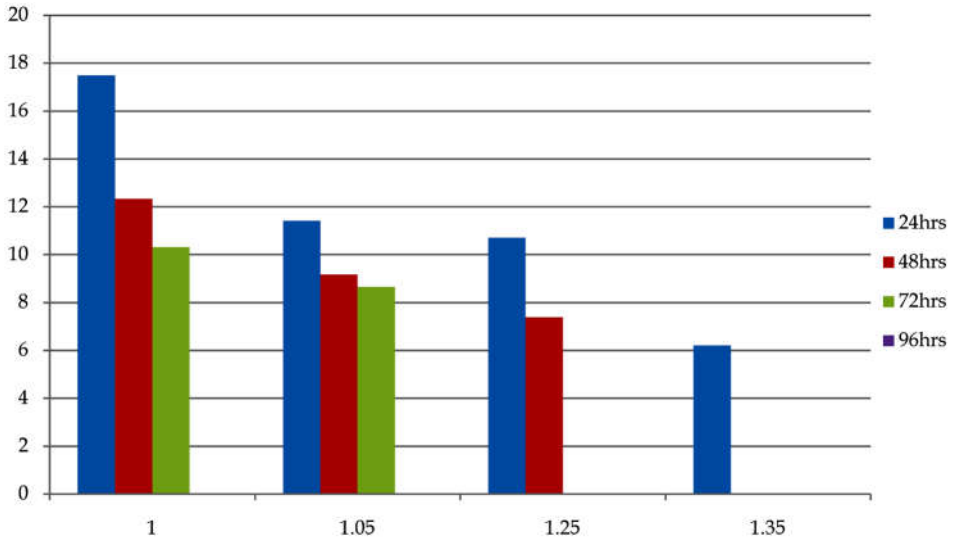

Fig. 2: Effect of Pesticide Metasystox on Hematocrit of fish Channa Punctatus

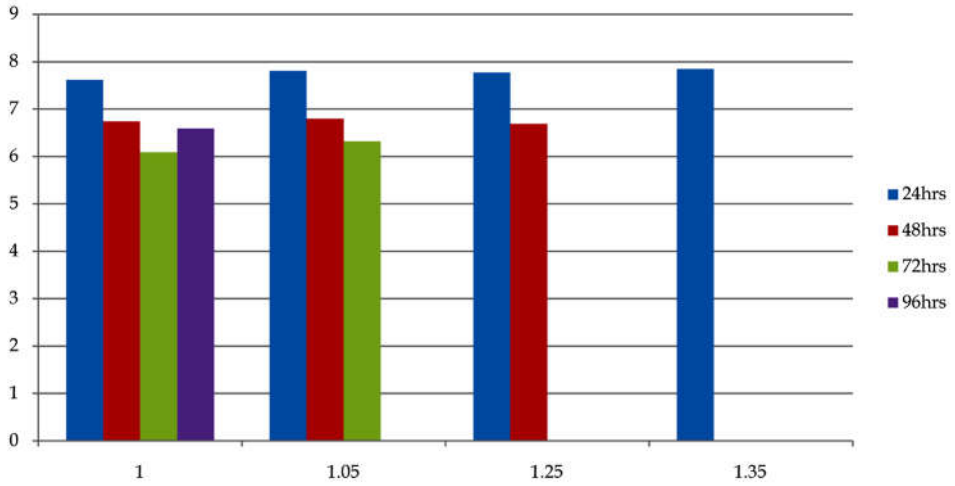

Fig. 3: Effect of pesticide metasystox on SGOT level of fish clarias batrachus

Conclusion and Recommendation

The present study demonstrated a significant decrease in hematocrit and absolute erythrocyte count while increase in aspartate transaminase enzyme in fish *Channa punctatus*, exposed under metasystox pesticide. The above hematological and biochemical parameters were altered because of toxic effect of the metasystox pesticide.The biochemical and hematological parameters can be used as indicator or monitors of prevailing aquatic pollution under natural ecological conditions and in the experimentally created aquatic environment polluted with contaminants. It was also indicated that the metasystox has remarkable effects on hemopoetic system of fishes. These hematological and biochemical parameters no dout plays an important role in diagnosis of diseases even in human beings also. Decrease in hematocrit and erythrocyte count may indicate anaemia, polycythemia, bone marrow disorders, periodontal diseases etc. Same way elevation in aspartate transaminase may indicate mayocardial infarction, hepatocellular diseases,CHD etc. On the basis of above conclusion it is recommended to pesticide workers to take all the precautions regarding protection from pesticides such as to bear aprons , protection shields, long boots , face masks, hand gloves etc.

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