ACETYLCHOLINE ESTERASE ACTIVITY IN BRAIN AND OVARY OF THE TEST ANIMAL

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Abstract: In the present paper, we studied about acetylcholine esterase activity in brain and ovary of the test animal. Maturable oocytes of Heteropneustis fossilis exposed to LH and commercial formulation of four OPI, viz, malathion, cypermethrin, birlane and gardona at various concentrations in vitro to explore the possibility of any direct effect of these insecticides on LH-induced oocytes maturation. In the present study, an attempt has been made to study this in H. fossilis.

Keywords: OPI, Gonadosomatic index, Indian Cat Fish.

1. INTRODUCTION

In an earlier study (Haider and Upadhyaya, 1985), they have observed the loss of stage II and III oocytes accompanied by a significant fall in gonadosomatic index, cessation of vitellogenesis, and inactivation of steriodogenis in gravid catfish, *Clarius batrachus* following 12 weeks chronic exposure to sublethal concentration of commercial formulations of four OPI. Since the maintenance of stage III oocytes, vitellogenesis and steroidogenesis depends on the pituitary gonadotropic stimulation, its absence was though to be the possible reason for these effects induced by OPI.

Mani and Saxena (1985) also opined that low degree of recrudenscence in the ovaries of fenitrothion (an OPI) treated *Channa punctatus* may be attributed to low titers of gonadotropins. However, the possibility of direct action of these OPI on affected organs cannot be ruled out, since it is known that OPI affect several enzymes other than acetylcholinesterase (Corbett, 1974; Eto, 1974; Cremlyn, 1978). Hence, to find out whether there exists any direct effect of these OPI on the ovaries, in the present investigation I have considered the oocyte maturation as the parameter.

In the present paper, maturable oocytes of *Heteropneustis fossilis* exposed to LH and commercial formulation of four OPI, viz, malathion, cypermethrin, birlane and gardona at various concentrations in vitro to explore the possibility of any direct effect of these insecticides on LH-induced oocytes maturation.

Lead is reported to form nuclear inclusion bodies in the oocytes of fish (Katti and Sathyanesan, 1987) but no such report available on organophosphorus insecticides. In the present study, an attempt has been made to study this in *H. fossilis*.

Weiss (2009) worked on the determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation in vivo and reported that the pesticide treated fish showed abnormal behaviour including loss of balance staying motionless in group at bottom, lying laterally at bottom, swimming in spiral fashion with jerks revolving in water opened north and rapid opercular movements.

2. MATERIALS & METHOD

2.1 Test Organisms

Females of catfish (*Heteropneustes fossilisconmunis*) were used as test organisms for several reasons.

- Ecotoxicological studies of cat fish are of potentially great importance, as they have a wide distribution throughout India including Kashmir water bodies.
- They are designated as toxicity test fish by United States Environmental protections agency (U.S. EPA, 1979).
- *Heteropneustes fossilis* is a representative of an ecologically important group.
- It occupies a position within a food chain leading to man.
- It is widely available, amendable to laboratory tests, early maintained and genetically uniform, and

- There is an adequate background data on the organism (*Heteropneustes fossilis*) i.e. physiological, genetics, Taxonomy, Embryology etc.

The fish is voraciously omnivorous; efficiently converting the food ingested, into flesh, grows very fast and is prone to artificial feeds. It naturally breeds in confined waters, spawning occurs in shallow marginal weed infected areas. Breeding season is mid January to March and again July to August.

Fish for the present work were procured from the local market throughout the year 2012, for the experimentation of different parameters related to reproductive activities of the fish. Such as effect on GSI, ova diameter, ovary weight in ovary.

2.2 Toxicants (Test Substances)

For the present study, Dimethoate (Rogor) and Dichlorvos (DDVP) were chosen as toxicants based partially on the probability of their having reproductive effects.These are employed routinely in the integrated farming practice to protect crops and animals from insects, weeds and diseases. The liberal use of these organophosphate pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce is posing great danger to aquatic environment.

These organophosphate pesticides are more frequently used because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment.

IUPAC Name = dimethyl S- (Nmethyl. Carbanoyl methyl) phosphordithioate

Trade Name = Rogor, cygon, perfekthion etc.

Dimethoate

Molecular formula: C5H12NO3PS2

Molecular weight: 229.3 g/mol

Solubility in water: 90% (Mangle. G. 1987)

Vapour pressure: 98.0% (Tecter, D. 1988)

Dimethoate formulations are used to control a wide range of Acari, Aphididae, Aleyrodidae, Cleoptera, Diptera, Collembola, Lepidoptera and thysanoptera in cereals, citrus, coffee, cotton, fruit, grapes, pastures, potatoes, pulses and vegetables.

They are also used for control of flies in animal houses. Dimethoate is a systemic insecticide and a acaricide with contact and stomach action. It acts as a cholinesterase inhibitor (Tomlin 1997)

Dichlorvos: (DDVP)

IUPAC Name = DDVP (0,-o-dimethyl-0-2, 2-dichlorovinyl phosphate

(USEPA, 2007)

Trade Name = Neuon

Structural Formula =

Molecular formula: C4H7Cl2 O4P

Molecular weight: 220.98 g/mol

Solubility in water: 10 g/l at 200C

Vapour pressure: 200C 0.012 mmHg

Dichlorvos an organophosphate insecticide is used as an agricultural insecticide on crops, stored products, and animals. It is used as an insecticide for slow release on pest-strips for pest control in homes. It is also used as an antihelminthic (de-worming agent) for dogs, swine and horses as a botacide; agent that kills fly larvae (USEPA, 1994).

Detailed risk characterization of dichlorovos has been well documented in CEPA (1996), its toxicological profile in ATSDR (1997) and environmental assessment in APVMA (2008).

Dichlorvos specifically inhibits cholinesterase enzymes. It is poisonous if swallowed, inhaled or absorbed through the skin.

Atropine and pralidoxime are specific antidotes and artificial respiration may be needed (WHO, 1999, Gupta, 2006).

2.3 Principle of the Test Method

The fish were exposed to the test substance added to water at a range of concentrations for a period of 96 hours. Mortalities were recorded at least at 24 hour intervals, and the concentration killing 50% of the fish (LC50) at each observation time were calculated where possible.

Information on the test substance

It is necessary to know the water solubility of the substance under the conditions of the test. A reliable analytical method for the quantification of the substance in the test solution must also be available.

2.4 Validity of the Test

For a test to be valid the following conditions were fulfilled. The mortality in the control (s) was not allowed to exceed 10% (or one fish if less than ten are used) at the end of the test.Constant conditions were maintained as far as possible throughout the test. The dissolved oxygen concentration was at least 60 percent of the air saturation value through out the test. The concentration of the test substance was satisfactorily maintained and preferably it was at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentrations was greater than 20% results were mainly based on the measured concentration.

2.5 Description of the Method

Apparatus

Normal laboratory equipment and especially the following were employed:

a) Oxygen meter/equipment for determination of oxygen of H2O

b) Equipment for determination of hardness of water.

c) Adequate apparatus for temperature control.

d) Tanks made of chemicals insert material and of a suitable capacity in relation to the recommended loading.

Holding of fish

All fish (test organism) were obtained and held in the laboratory for at least 20 days before they are used for testing. They were held in water of the quality used in the test for at least seven days immediately before testing and under the following condition.

Light: 12 to 16 hours photo period daily.

Temperature: Appropriate to the species.

For *Heteropneustes fossilis*, recommended test temperature range is 20-24oC (OECD, Paris 1981)

Oxygen concentration: At least 80 percent of air saturation value

Feeding: Three times per week or daily until 24 hours before the test is started.

Following a 48-hour settling in period, mortalities were recorded and the following criteria applied.

Mortalities of greater than 10% of population in seven days: regulation of entire batch.

Mortalities between 5 and 10% of population: acclimatization continued for seven additional days.

Mortalities of less than 5% of population: acceptance of batch.

2.6 Water (Test Medium)

Good quality natural water or reconstituted water was preferred, although drinking water (dechlorinated if necessary) may also be used.

Waters with total hardness of between 10 and 25mg CaCO3 per liter, and with a pH 6.0 to 8.5 was preferable.

2.7 Test Solution

Test solutions of the chosen concentrations were prepared by dilutions of stock solution.

Test solution of Dimethoate and Dicholorvos were prepared in water into the three different concentration of each as:

Dimethoate (Rogor)

LC50 = 1.70mg/l

50% of LC50 = 0.85 mg/l

70% of LC50 = 1.20mg/l

90% of LC50 = 1.53 mg/l

Dichlorvos (DDVP)

LC50 = 1.30mg/l

50% of LC50 = 0.65mg/l

70% of LC50 = 0.90mg/l

90% of LC50 = 1.17mg/l

The test was carried out without adjustment of pH. If there was evidence of marked change in the pH of the tank water after dilution of the test substance, it is advisable that the test be repeated, adjusting the pH of the stock solution to that of the tank water before dilution of the test substance.

This pH adjustment was made in such a way that the stock solution concentration was not changed to any significant extent and that no chemical reaction or precipitation of the test substance was caused HCl and NaOH are preferred.

2.8 Procedure

Conditions of exposure

Duration: preferably 96 hours
Loading: Maximum loading of 1.0g fish/liter for static test was used.
Light: 12 to 16 hours photo period daily.
Temperature: 20-240C
Oxygen concentration: Not less 60% of the air saturation value. Aeration can be used provided that it does not lead to a significant loss of test substance.
Feeding: None
Distribution: Disturbances that would change the behavior of the fish were avoided

2.9 Administrations of Pesticides

The common organophosphate pesticides used for this study were dimethoate (Rogor) and 'Neon' pesticides were administered to glass aquaria containing the experimental fish (*Heteropneustes fossilis*). These pesticides were mixed thoroughly with test medium by glass rod without disturbing the test animal.

Thus, the pesticides were imbibed via the gastrointestinal tract and the surface of gills and skin of the experimental fish.

2.10 Determination of Lc50

Toxicity was determined by renewal of static bioassay. All experiments were conducted in 5 rectangular glass jar containing 10 liters of dechlorinated water to which volumes of dimethoate was added (into two separate experimental designs) to achieve different concentrations of the toxicant. Thirty minutes after preparation of test solution, 10 experimental fishes were carefully placed into each replicate tanks of 5 different concentrations of each pesticide as (0.5mg/l, 0.8mg/l, 1.5mg/l, 1.8mg/l and control 0.00mg/l) for dimethoate and 0.2mg/l, 0.5mg, 0.8mg/l, 1.00mg/l and control 0.00,g/l in case of dichlorvos (DDVP) was used. All experiments were conducted at room temperature and the tanks aerated.

Fish were not fed during the experiment (Reish and Oshida 1986). Observations were recorded every 12 hours, number of dead fishes were removed. Experiment lasted for 96 hours for the different concentrations of dimethoate and dichlorovos (DDVP). The susceptibility of fish to pesticide was determined using probit log method of Finney and Stevens (1948) for LC50 at 96 hours. From the results of acute toxicity, sublethal concentration as 50%, 70% and 90% of the LC50 value were prepared for both pesticides.

Dimethoate (Rogor)

LC50 = 1.70mg/l
5o% of LC50 = 0.85 mg/l
70% of LC50 = 1.20mg/l
90% of LC50 = 1.53 mg/l

Dichlorvos (DDVP)

LC50 = 1.30mg/l
50% of LC50 = 0.65mg/l
70% of LC50 = 0.90mg/l
90% of LC50 = 1.17mg/l

Ten glass jar were used with 3 replicates per treatment and with same conditions as in acute toxicity. Percentage morality of *Heteropneustes fossilis* exposed for several hours of exposure to different sublethal concentrations of dimethoate and dicholorvos were recorded.

3. RESULTS & DISCUSSION

3.1. Results of Dimethoate Treated Testes

3.1. (a) Control Group

The parenchyma cells like hepatocytes, billiary epithelial tissues, nuclei and non parenchyma tissues like bile ducts, hepatopancreas, arteries and veins of the testes in control groups were normal and systematically arranged.

3.1. (b) Experimental Groups

After 10 days of interval, histopathological examination of the testes of *Heteropneustes fossilis* clearly shows that the parenchymal architecture of the testes is disturbed and hepatocyte show dissociation, the hepatocyte appears swollen and cytoplasm appears granular. The hepatocyte nuclei become pycnotic. During these 10 days of exposure, patchy degeneration and isolated degenerated elements

around the parenchyma cells were observed with progressive increase of fibro connective tissue. As a result, signs of congestion were noticed at the sinusoid.

In long-term (20 days) treatment, the effect became more prominent with appearance of apoptotic cells. Blood capillary endothelium ruptured and blood was spilled into the testes tissues. Acute and extensive necrosis of testes cells was observed particularly focal necrosis a common feature in catfish. The density of the connective tissue increased markedly leading to more congestion. The size was variable with concentration and was usually located in the vicinity of hepatic arteries and bile ducts.

Shukla et al. (2005), noticed in his observation that when the catfish Clarias batrachus is exposed to the increased concentration (0.16/mL) of the organophosphate pesticide Nuvan, the hepatocytes exhibited reduction in their size and peripheral accumulation of cytoplasm. The nuclei of the hepatocytes lost their rounded appearance and the cell boundaries became obliterated at places after 20 days of pesticide exposure. The hemorrhage in testes was evident by increased volume of sinusoidal space.

In our present study we have recorded histopathological changes due to Malathion toxicity in the testes which mainly included architectural changes in the testes, hepatocytes swelling, dissociation of hepatocytes, hepatocytes showing pycnotic nuclei, broken sinusoidal endothelium, ruptured blood vessels with haemorrhage and vacuoles in the haepatocytes. At the dose of 0.2 ppm, severe necrotic hepatocytes, pyknosis, hypertrophy, haemorrhage and vacuolation were observed for the fishes in the experimental group. Fishes injected with experimental dose (0.2 ppm) showed areas with disrupted parenchymal architecture and necrosis. The hepatocyte nuclei began to condense and cytoplasm of these cells was highly vacuolated.

Thus, the effects resulting from various combinations of pesticides are often as complicated as they are unpredictable and are commonly referred to as interactions.

4. ACETYLCHOLINE ESTERASE ACTIVITY IN BRAIN AND OVARY OF THE TEST ANIMAL

Organophosphate pesticides are competitive inhibitors of acetylecholineesterase (AChE), the key enzyme in the transmission of nerve impulse. AChE is readily phosphorylated by the organophosphate pesticides at the active site serine (Aldrige and Reiner, 1972; Taylor, 1990) the selectivity of action of organophosphates is that it causes inhibition of AChE and accumulation of acetylecholine at the synapse (Loskowsky and Dettbam, 1975) over stimulating the postsynaptic cells (Pope *et al*., 1995). Reports also demonstrated that the organophosphate pesticide agents can bind to the acetylecholine receptors and this direct interaction is responsible for the manifestation of stress (Pope *et al*., 1995).

Therefore, the AChE activity in different tissues (Brain and Ovary) in experimental animal *Heteropneustes fossilis* has been used in the present investigation as the neurophysiological marker or early signs of organophosphate neurotoxicity. Results of previous studies have suggested that even the undetectable quantity of organophosphate pesticides will affect the enzymatic activity. Several authors have reported that enzymes of the same tissue of different species show difference in the sensitive to various organophosphate insecticides (Pan and Dutta, 1998; Monserret and Bianchini, 1998). In the present work all the groups treated with Dimethoate and dichlorvos organophosphate pesticides revealed significant ($P>0.01$) inhibition of AChE activity in the brain as well as ovary of exposed fish. The inhibition of enzyme was more significant at higher doses of pesticides to fish in both the cases. The time, dose and species related differences in enzyme susceptibility to organophosphate pesticides can primarily be attributed to dissimilar enzyme amount and inhibitor affinity degree to cholinesterase receptor. Although 50% or more depletion is supposed to be life threatening, available investigatrion shows that some fish are capable to tolerate over 90% inhibition in AChE activity (Day and Scott, 1990). More than 90% depletion was also reported by Balint *et al.* (1995); Pan and Dutta, (1998) in fish exposed to various insecticides. The highest reduction in the present study 72% which is considerably low. Oruce and Usta, (2007) reported that *Heteropneustes fossilis* showed to be more resistant to diazinon, this may be because of its low rate of bioactivation and relatively high activity of detoxicating enzymes (Keizer *et al*., 1991).

Rath and Misra, (1981) and Ansari and Kumar, (1984) reported that inhibition of acetylecholine activity has relation with age of fish, concentration of pesticide and time of exposure. Their findings extended a considerable support to our observations.

Therefore, the present study demonstrates that both the organophosphate pesticides (dimethoate and dichlorvos) are potent inhibitors of brain and ovarian AChE activity, but under identical dose the rate of enzyme inhibition was different for different pesticides.

5. SUMMARY & CONCLUSION

The general conclusion which we can draw is the obvious one that pesticides have an inhibitory effect on reproduction. Pesticide exposure of both dimethoate (Rogor) and dichlorovos (Neuon) leads to impairment in reproductive functions of female carp, *Heteropneustes fossilis*. The quality of the natural surroundings of fish has an important role in their development and reproduction. Even slight changes in concentration of certain chemical compounds can negatively affect the reproductive properties of fish. Hence, on the basis of this study we can compare toxicity of these selected pesticides to other pesticides and can also use common carp as a model for other fish species. The reported results would be useful contribution in ecotoxicity risk assessment studies of these organophosphate pesticides on fish species.

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