Biochemical changes in certain tissues of *Cirrhina mrigala* (Hamilton) (Cyprinidae: Cypriniformes) exposed to fenthion

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ABSTRACT

Lebaycid (Fenthion), a common organochloride pesticide shows non-target effect on the Indian carp, *Cirrhina mrigala* Hamilton. This study presents a brief report on the impact of incipient lethal levels (1/4th and 1/10th of fenthion on the concentration of substrates (carbohydrates, lipids and proteins) in the four important tissues - brain, gill, intestine and liver. Maximum decrease in glycogen level was observed in liver tissue (-54.4/30 days) and minimum in Intestine tissue (-0.86/60 days). Maximum decrease in protein levels were observed in brain tissue (-40.9/30 days) and minimum in Liver tissue (1.6/60 days). Maximum and minimum decrease in lipid levels were observed in the liver tissue (-51.8/30 days and -0.5/60 days).

Key words: Fenthion, *C.mrigala*, glycogen, protein, lipids, brain, gill, intestine, liver. 96h \( LC_{50} \), probit

1. Introduction

Water pollution is a major problem of this century and addition of pollutants changes the natural qualities of water (Voltz et al; 2005). Pesticides are known to contaminate a number of inland water bodies closer to areas of pesticide applications. Although pesticides are needed for the management of pests, their harmful effects on non-target organisms cannot be ignored. Pesticides leave residues in water and mud even several days after being sprayed in the adjacent crop fields. Pesticides affect growth and nutritional value of fish, when their concentration in water exceeds the critical maximum limit (Arunachalam et al; 1980). The reproductive potential of fish is affected, when reared in water containing pesticide residues (Moore and Waring, 2001). Abhilash and Prakasam (2005) reported alterations in the cellular morphology of pesticide treated fish. The physiological functions of fish get altered upon exposure to different pesticide concentrations (Gupta and Saxena, 2006). Marigoudar et al. (2009) reported changes in the behavioural responses of fishes when exposed to pesticides beyond the maximum tolerance level.

In the present investigation, the mobilization of three substrates, carbohydrates, lipids and proteins in four different tissues - brain, gill, intestine and liver of fenthion treated *Cirrhina mrigala* Hamilton is analysed.

Tissue carbohydrates are affected by pesticide toxicity. Sharma and Mahajan (1983) showed that aldrin affected biochemical factors like blood glucose and liver and muscle glycogen. Vasanthi and Ramaswamy (1987) found a shift in the metabolic pathway and subsequent changes in glycogen mobilization in the tissues of *Sarotherodon mossambicus* Peters exposed...
to thiodon. The tissue proteins are mobilized during stress due to pesticides. (Ahmed, 1976; Rita and Milton, 2006) recorded gluconeogenesis from proteins in S. mossambicus Peters exposed to malathion. (Singh and Gupta, 1985; Sawney, 1997) estimated the protein and glycogen content in the liver, brain and testes of Channa punctatus Bloch exposed to cythion. Palanichamy et al; (1989), reported sublethal effects of malathion, thiodon and carbaryl on the total protein content of muscle, liver, gill and intestine in the fresh water catfish, Mystus vittatus Bloch. Sherekar and Kulkarni (1989) analysed the protein content in the liver, muscle and gills of Channa orientalis Bloch and Schneider exposed to methyl parathion. Tripathi and Verma (2004) treated the fresh water fish, Clarias batrachus L with endosulfan and studied the changes in protein, glycogen and lipid in the liver and muscle tissues. Shivakumar (2005) reported changes in protein and carbohydrate metabolic activity under severe physiological and pathological stress due to endosulfan on the fresh water fish, Catla catla L. Prashanth and Neelagund (2008) studied the effects of cypermethrin treatment on aspartate amino transferase, Alanine amino transferase and glutamate dehydrogenase in the gill, liver and muscles of fresh water fish, Cirrhinus mrigala Hamilton.

Singh and Singh (2006) reported changes in tissue lipids, phospholipids and protein profile in the male Heteropneustes fossilis Bloch exposed to sublethal concentrations of endosulfan. Gad (2009) studied the liver glutathione reductase and glutathione S-transferase, brain acetyl cholin esterase and total protein and lipid contents in the muscle of Oreochromis niloticus and Clarias gariepinus exposed to pesticides. Ghosh et al. (1999) reported an increase in the cholesterol and total lipid content in the liver of Heteropneustes fossilis Bloch exposed to carbofuran, which remained unchanged in the ovary.

In this study, mobilization of carbohydrates, proteins and lipids in the brain, gill, intestine and liver of Cirrhina mrigala Hamilton fingerlings, exposed to incipient lethal levels (one – fourth and one – tenth 96h LC50) of an organophosphate pesticide, Lebaycid (fenthion) (4-methyl mercapto-3 methyl phenyl thiophosphate) is analysed.

2. Materials and methods

2.1 Procurement of the test species

Fingerlings (5 ± 1 cm and 7 ± 1g) of Cirrhina mrigala (Hamilton) - a common herbivorous Indian fresh water fish that feeds on algae and diatoms and much favoured for rural pisciculture operations - cultured under ideal conditions were procured from a local hatchery near Nagercoil (N 8°10’17.2” E 77°.26’21.2”) Kanyakumari district, India.

2.2 Toxicity Studies

Static bioassay tests were conducted to assess the toxicity of fenthion to C.mrigala fingerlings exposed to 17 different concentrations of the toxicant (2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6 mg/l) based on the active ingredient of the pesticide in 100 litre fibre glass tanks by allowing 10 fishes in each tank. Feeding was discontinued a day prior to the experiment and simultaneous controls were run. The test water was renewed daily (Sprague, 1973), to maintain the toxicant concentration in the test water constant, to keep the dissolved oxygen in the optimum level and to remove the debris. The fish were observed for behavioural abnormalities and the number of fish that died after 3, 6, 12, 18, 24, 48, 60, 72, 84 and 96h of exposure was recorded. The results of the static bioassay were analysed using linear regression probit analysis (Finney, 1971) using the statistical package, POLO-PC, (LeOra Software).
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The test fish were continuously exposed to one fourth and one tenth 96h LC$_{50}$ of fenthion for a period of 60 days. The biochemical changes in tissue substrate concentrations were assessed after 30 and 60 days of exposure.

2.3 Estimation of tissue substrates

Glycogen was determined by Anthrone method (Roe, 1954). Freshly weighed (200 mg) tissues were homogenized in distilled water (20ml) and 5% trichloroacetic acid in a homogenizer. The homogenate was centrifuged at 2500rpm for 5 minutes. To every 1ml of the supernatant 4ml of Anthrone reagent was added. A standard glucose solution was also run along with the samples. The samples were boiled in a water bath for 10 minutes for colour development. The optical density was measured in a spectronic 20 spectrophotometer at 620nm. The percentage glycogen content of the tissue was calculated using the OD of the unknown sample and that of standard glucose solution.

Protein was estimated following the method of Lowry et al. (1951). Freshly weighed (100 mg) tissues were homogenised with 5% trichloroacetic acid in a homogenizer. The homogenate was centrifuged at 3000rpm for 10 minutes and the residue was dissolved in 0.1N NaOH. Exactly 0.2ml of this solution was made up to 1ml using 0.1N NaOH. To this 3.5ml of Folín’s reagent was added and thoroughly mixed. After 30 minutes the optical density was measured at 670nm in a spectronic 20 spectrophotometer.

Lipids were extracted as described by Floch et al. (1957), and estimated by the method of Barnes and Blackstock (1973). 50 mg of tissues were homogenised (5% w/v) in a waring blender in chloroform-methanol mixture (2:1). The homogenates were filtered through Whatman No.1 filter paper, and the residue was rehomogenised as before and then filtered. The non-lipid matter from pooled filtrate was removed by shaking vigorously with 0.88% KCL (added as one fourth of the volume). 1 mL of filtrate was taken in a test tube and evaporated under nitrogen and 1 mL of concentrated H$_2$SO$_4$ was added and boiled for 10 min. For estimation of total lipid, 0.2 mL of solution was taken and 5 mL of vanillin reagent was added. The developed colour was read in spectrophotometer at 520 nm against reagent blank.

3. Results

The toxicity curve (fig.1) indicated the time based toxicity response of C.mrigala to fenthion. The LC$_{50}$ values range from 3.05 (96h) to 3.69 (12h) (Table 1). The 96h LC$_{50}$ value (3.05 mg/l) obtained using probit analysis (Table 2) is used for fixing the two incipient lethal level exposure concentrations of 0.76 mg/l (1/4$^{th}$ 96h LC$_{50}$) and 0.305 mg/l (1/10$^{th}$ 96h LC$_{50}$). Maximum decrease in glycogen content (54.4 percent) was observed in the liver tissue after exposure for 30 days to 1/4$^{th}$ 96h LC$_{50}$ (Table 3) and maximum decrease in protein content (40.9 percent) was in the brain tissue after exposure for 30 days to 1/10$^{th}$ 96h LC$_{50}$ (Table 4). The lipid content decreased by 51.8 percent in the liver tissue (maximum decrease) after exposure for 30 days to 1/4$^{th}$ 96h LC$_{50}$ concentration.

4. Discussion

The 96 h LC$_{50}$ of fenthion to C.mrigala is 3.05 mg/l. Since the non-target action of fenthion is limited, the LC$_{50}$ is slightly higher compared to other pesticides. Singh and Sahai (1985) reported 0.17ppm of BHC, 0.2 of endosulphan and 0.19ppm of lindane as the sub lethal concentrations to Puntius ticto Hamilton. Sharma et al (2007), were determined the basis of LC$_{50}$ value in three test concentrations of endosulfan on Mystus vittatus Bloch in of sublethal
1(1/4 \times \text{LC}_{50} = \sim 0.5 \text{ mg/L}),\ sublethal \ 2 (1/8 \times \text{LC}_{50} = \sim 0.25 \text{ mg/L}),\ and \ non \ lethal \ (1/10 \times \text{LC}_{50} = \sim 0.2 \text{ mg/L}).\n
The glycogen content in the brain, gill, intestine and liver decreased significantly on exposures to 1/4^{th}\ 96h \ \text{LC}_{50} \ for \ 30 \ days \ while \ a \ recovery \ in \ the \ glycogen \ content \ was \ be \ observed \ in \ almost \ all \ the \ tissues \ after \ 60 \ days \ of \ exposure \ to \ 1/10^{th} \ 96h \ \text{LC}_{50}.\ In \ chronic \ exposures, \ the \ test \ fish \ gradually \ regained \ normalcy \ due \ to \ systemic \ acclimatization \ which \ can \ be \ clearly \ observed \ at \ the \ macro \ level \ involving \ major \ substrates. \ Jee \ et \ al. \ (2005), \ found \ an \ increase \ in \ levels \ of \ serum \ glutamic \ oxaloacetic \ acid \ transaminase, \ glutamic \ pyruvic \ acid \ transaminase, \ glucose \ and \ alkaline \ phosphatase \ and \ a \ decrease \ in \ the \ concentration \ of \ plasma \ total \ protein, \ albumin, \ cholesterol \ and \ lysozyme \ in \ Korean \ rock \ fish \ \textit{Sebastes schlegeli} \ Hilgendorf \ exposed \ to \ cypermethrin. \ Velisek \ et \ al. \ (2009), \ showed \ that \ exposure \ of \ rainbow \ trout \ to \ 14.7 \text{mg/L} \ of \ pesticide \ Talstar \ 10 \ EC \ caused \ alterations \ in \ haematological \ and \ biochemical \ indices \ as \ well \ as \ in \ tissue \ enzyme, \ all \ of \ which \ resulted \ in \ stress \ to \ the \ organism. \ The \ bifenthrin-based \ pesticide \ Talstar \ 10 \ EC \ therefore \ contained \ substrates \ strongly \ toxic \ to \ fish.

Glycogen mobilization is maximum in the liver (54.4 percent decrease for \textit{Cirrhina mrigala} Hamilton exposed to 1/4^{th} \ 96h \ \text{LC}_{50} \ for \ 30 \ days). \ Liver, \ being \ the \ seat \ of \ glycogen \ metabolism \ supplies \ glycogen \ for \ producing \ more \ energy \ to \ combat \ pesticide \ stress. \ The \ lipid \ level \ decreased \ by \ more \ than \ 50 \ percent \ after \ 30 \ days \ of \ exposure \ to \ 1/4^{th} \ 96h \ \text{LC}_{50} \ of \ fenthion. \ Lipids \ reaching \ the \ liver \ are \ mobilized \ for \ producing \ sufficient \ energy \ for \ handling \ pesticide \ stress \ in \ the \ environment. \ Mobilization \ of \ proteins \ is \ maximum \ in \ the \ intestine \ (38 \ percent \ decrease) \ in \ 1/4^{th} \ 96h \ \text{LC}_{50} \ exposure. \ Proteins \ are \ the \ last \ substrates \ to \ be \ mobilized \ for \ catabolic \ activities. \ In \ chronic \ exposures, \ such \ substrates \ are \ left \ undisturbed. \ Kamalaveni \ et \ al. \ (2001) \ studied \ the \ pesticide \ toxicity \ impacts \ of \ glycogen \ activity \ levels \ in \ succinate \ dehydrogenase \ and \ glucose - 6 – phosphate \ dehydrogenase \ were \ assessed \ in \ various \ tissues \ of \ \textit{Cyprinus carpio} L. \ exposed \ to \ lethal \ concentrations \ of \ different \ pyrethroids \ including \ fenvalerarte \ for \ a \ period \ of \ 72 \ h. \ The \ results \ indicated \ a \ steady \ decrease \ in \ SDH \ activity \ with \ a \ concomitant \ increase \ G6PD \ activity. \ The \ decreased \ SDH \ activity \ indicated \ inhibition \ of \ SDH \ at \ mitochondrial \ level \ and \ the \ increased \ G6PD \ activity \ an \ enhancement \ of \ an \ alternative \ pathway \ of \ carbohydrate \ metabolism, \ viz. \ the \ hexose \ monophosphate \ shunt \ pathway \ as \ a \ biochemical \ adaptation \ to \ overcome \ the \ toxic \ stress. \ Sancho \ et \ al. \ (1998) \ reported \ that \ total \ properties \ had \ decreased \ in \ liver \ of \ the \ European \ eel, \ \textit{Anguilla anguilla} L \ after \ fenitrothion \ exposure \ for \ different \ time \ intervals \ up \ to \ 96 \ hrs. \ Reduction \ in \ protein \ content \ in \ liver \ of \ fenvalerate \ exposed \ fish \ might \ be \ due \ to \ either \ arrested \ metabolism \ in \ the \ liveror \ the \ use \ of \ proteins \ to \ build \ up \ new \ cells \ or \ enzymes \ to \ reduce \ the \ stress. \ Oguegi \ and \ Auta \ (2007), \ analysed \ the \ impact \ of \ short \ term \ exposure \ of \ 96h \ to \ 0.008, \ 0.009, \ 0.010, \ 0.011 \ and \ 0.12mg \ L^{-1} \ of \ water \ borne \ lamda-cyhalothrin \ on \ \textit{Clarias gariepinus} \ Burchell \ through \ changes \ in \ selected \ biochemical \ parameters \ in \ serum \ glucose, \ protein, \ cholesterol, \ triglycerol, \ glutamic \ pyruvic \ acid \ transaminase \ (GPT), \ glutamic \ oxaloacetic \ acid \ transaminase \ (GOT) \ and \ Alkaline \ phosphatase \ (ALP) \ there \ were \ significant \ (P < 0.05) \ dose-dependent \ alternations \ in \ all \ the \ exposed \ groups. \ Begum \ and \ Vijayaragavan \ (2001) \ reported \ that \ the \ free \ fatty \ acids \ (FFA) \ content \ decreased \ in \ the \ liver \ after \ 24 \ and \ 72 \ hr \ and \ increased \ after \ 144 \ hr \ of \ experiment \ in \ carbofuran \ insecticide \ in \ \textit{Clarias batrachus} L., \ whereas \ the \ muscle \ free \ fatty \ acids \ increased \ after \ 24 \ hr \ followed \ by \ decrement \ after \ 72 \ and \ 144 \ hr. \ These \ results \ clearly \ indicated \ that \ after \ 24 \ hr \ of \ carbofuran \ intoxication, \ only \ liver \ free \ fatty \ acids \ were \ oxidised \ and \ utilized \ as \ a \ source \ of \ metabolic \ energy. \ But, \ after \ 72 \ hr \ both \ liver \ and \ muscle \ free \ fatty \ acid \ would \ have \ been \ utilized \ to \ yield \ energy \ to \ mitigate \ the \ toxic \ stress \ due \ to \ sub \ lethal \ concentration \ of \ commercial \ carbofuran \ stress. \ At \ the \ end \ of \ 144 \ hr \ muscle \ FFA \ would
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have been sufficient to meet the energy requirements, therefore liver FFA increased after 144 hr of exposure. Somneuk et al. (2007) analysed the AChE activity in the brain, liver, muscle and gill tissues of hybrid catfish, Clarias macrocephalus × Clarias gariepinus exposed to a sublethal concentration of an organophosphate, chlorpyrifos and a carbamate, carbaryl for 4 days. AChE inhibition increased rapidly with insecticide concentration. Relative inhibition of AChE was higher in larger fish but did not differ significantly with sex. Relative inhibitions of AChE accompanying insecticide exposure were highest in the brain tissues and progressively less in the liver, muscle and gill tissues. Insecticide concentrations and AChE inhibition in the brain increased over 4 days of sublethal exposure. After transfer to insecticide free water, AChE inhibitions and insecticide residue in the brain decreased but remained above control values over the 4 days recovery period.

Most of the pesticides and heavy metals affected fresh water organisms even at very low concentrations. Sometimes the effects of these deleterious chemicals go unnoticed because most of the changes occurring in the body are subtle and can be noticed only when the conditions have become irreparable. The present investigation has thrown light in to biochemical alterations in the vital organs of C. mrigala brought about by incipient lethal doses of an organophosphorus insecticide, fenthion. In concentrations which do not produce immediate death, statistically significant alterations in biochemical factors could be noticed, mainly associated with acclimation to the toxic environment, an in-built adaptation to combat chemical stress with a significant norm of reaction.

5. Acknowledgements

The authors wish to thank and Dr. M. Jezer Jebanesan, Principal, Scott Christian College (Autonomous), Nagercoil, for the facilities provided.

6. References


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**Figure 1:** LC$_{50}$ values of (mg/l) of fenthion to *Cirrhina mrigala* fingerlings

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<th>UCL</th>
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<tr>
<td>24</td>
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**Table 2:** Probit analysis showing the response of *Cirrhina mrigala* to fenthion after 96h of exposure

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Statistics

$SW = 34.830$  $SWX = 51.546$  $X Bar = 1.480$  $SWY = 169.511$

$Y Bar = 4.867$  $SWX*Y = 76.308$  $SWY*Y = 251.533$

$b value = 28.912$

Regression equation $y = 28.912 \times - 37.92$

If $Y = 5.0$ then $x = 1.485$. This corresponds to dose of 3.052
Biochemical changes in certain tissues of Cirrhina mrigala (Hamilton) (Cyprinidae: Cypriniformes) exposed to fenthion

Variance 0.0000 Chi-square 1.02 (with 5 degrees of freedom p)
Lower limit 1.4727 Log Dose 1.4845 Upper limit 1.4963

Table 3: Glycogen content (in mg/g wet weight of tissue) in C. mrigala fingerlings exposed to fenthion

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatments</th>
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<th>Gill</th>
<th>Intestine</th>
<th>Liver</th>
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<td>Control</td>
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<td>¼th 96h</td>
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<td>31.8 ± 4.0</td>
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<td>¼th 96h</td>
<td>40.2 ± 3.7</td>
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Table 4: Protein content (in mg/g wet weight of tissue) in C. mrigala fingerlings exposed to fenthion

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<td>64.5 ± 3.1</td>
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<td>59.2 ± 4.8</td>
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<td>62.4 ± 5.2</td>
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<td>54.9 ± 3.8</td>
<td>88.2 ± 7.1</td>
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Table 5: Lipid content (in mg/g wet weight of tissue) in C. mrigala fingerlings exposed to fenthion

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<td>25.2 ± 3.4</td>
<td>33.2 ± 1.6</td>
<td>46.6 ± 1.1</td>
</tr>
<tr>
<td>60</td>
<td>¼th 96h</td>
<td>41.8 ± 2.1</td>
<td>19.8 ± 1.1</td>
<td>30.6 ± 1.8</td>
<td>51.8 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>¼th 96h</td>
<td>47.2 ± 1.8</td>
<td>23.4 ± 2.8</td>
<td>37.4 ± 2.8</td>
<td>58.7 ± 4.1</td>
</tr>
</tbody>
</table>

Note for tables 2, 3 and 4: NS – Not significant; all other deviations significant at p≤0.05 (Student’s t-test)
Biochemical changes in certain tissues of Cirrhina mrigala (Hamilton) (Cyprinidae: Cypriniformes) exposed to fenthion

Figure 1: Toxicity curve showing the response of *C.mrigala* fingerlings to fenthion (mg/l)

LCL – Lower confidence limit
UCL – Upper confidence limit
LC$_{50}$ – Lethal concentration for 50 percent of the exposed fish