

Toxic effects of endosulfan on cholesterol levels of liver, brain and gills of stinging catfish, *Heteropneustes fossilis*

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Abstract: Stinging catfish *Heteropneustes fossilis* exposed to different concentrations of an organochlorine pesticide endosulfan under static conditions, revealed statistically significant increase in cholesterol contents of liver, brain and gill tissues even at the lowest concentration (0.0010 mg/l). Fish also elicited behavioral changes due to pesticide toxicity. Hypercholesterolemia appeared to be due to stress induced metabolic alterations caused by intoxication.

Keywords: Endosulfan, Toxicity, Hypercholesterolemia, Behavioral alterations

INTRODUCTION

Pollutants such as pesticides are known to alter the behavioral pattern, growth, reproduction and resistance to diseases of aquatic organism, affecting on a variety of biochemical and physiological mechanism (Geraldine *et al.*, 1999). Organochlorine pesticides are still used for crop protection in several countries without considering their toxic effects on aquatic life. These pesticides reach in water bodies mainly through run-off from agricultural fields and affect the life of non-target organisms like fishes, which are more susceptible than invertebrates (Schoettiger, 1970; and Tandon and Dubey, 1983). Endosulfan is a non-systemic insecticide and is commonly used for agricultural purposes, forestry and gardening. Cholesterol is an important component of great significance in living systems. It holds a key and central position in the metabolism of many closely related biologically important compounds (Shell, 1961), precursor of steroid hormones (Lehninger, 1975) and an important constituent of cell membrane where it modulates their fluidity, fragility and consequently the membrane function (Suhail *et al.*, 1988). Present paper embodies observations on toxic effects of endosulfan on cholesterol contents of liver, brain and gill tissues of freshwater stinging catfish *Heteropneustes fossilis*, an active and hardy fish of economic importance and has a great demand because of its medicinal value (Talwar and Jhingran, 1991; and Daniels, 2000).

MATERIALS AND METHODS

Live *Heteropneustes fossilis* obtained from river Gomti at Lucknow were transported and acclimatized to the laboratory environment, as described earlier (Chandra, 1988). Only healthy looking and active fishes of weight

range 100-150 grams were selected for the experiment. They were properly fed and then starved for 24 hours before the experiment. Fish from the same group kept under normal conditions, were used as control. The recommendations made by Doudoroff *et al.* (1951) and APHA (1992) were followed for the static bioassay tests. The pesticide concentrations, selected on the basis of 80-100% survival of fishes (Table I). Fish were exposed to different concentrations of pesticide from 24 to 96 hours depending upon their survival at the particular concentration. Fish were taken out regularly after every 24 hours interval from the experimental as well as from control group and washed with distilled water. Their liver, brain and gill were dissected out, washed and kept in 0.7% cold saline solution. 2.5% homogenates were prepared in glacial acetic acid and cholesterol level was estimated following method of Rosenthol *et al.* (1957), using Bausch and Lomb Spectronic - 20 spectrophotometer at 560 nm. The physicochemical characteristics of dechlorinated tap water during the experiment were- temperature 19-21 °C; pH 7.0-7.4; dissolved oxygen 7.2-7.6 mg/l; total alkalinity 108-115 mg/l; and total hardness 118-128 mg/l.

RESULTS

The effects of endosulfan toxicity on cholesterol levels in liver, brain and gill tissues are summarized in Table 2. At the lowest concentration of 0.0010 mg/l the cholesterol levels in liver, brain and gill increased continuously upto 72 hours of pesticide exposure and then decreased after 96 hours but remained still above their respective control levels. At 0.0015 mg/l, 0.0020 mg/l and 0.0025 mg/l pesticide concentration the cholesterol levels in liver and brain rose continuously until the terminal hours of exposure.

Table 1. Percent survival of fishes* in endosulfan activity.

Pesticide conc. mg/l	Time of exposure in hours				
	24	48	72	96	120
Control	100%	100%	100%	100%	100%
0.0010	100%	100%	90%	80%	45%
0.0015	100%	90%	80%	40%	20%
0.0020	100%	80%	50%	10%	00%
0.0025	80%	30%	10%	00%	00%

* No. of observations-20 for determining % survival.

In gills the cholesterol contents increased upto 48 hours of toxicant exposure at 0.0015 mg/l concentration and then declined. However, with increasing concentration of pesticide, cholesterol levels of gills increased during initial exposure period but later decreased. Interestingly the maximal rise in cholesterol level during the experiment was observed in gills (36.09%), followed by brain (29.65%) and liver (27.07%) at 0.0015mg/l concentration. Elevated cholesterol contents in liver, brain and gills due to toxic effect of endosulfan were statistically significant ($P < 0.01$) as compared to their respective control levels. Endosulfan also produced behavioral changes in *H.fossilis* such as convulsions which were more frequent during the terminal hours of experiment, rapid air gulping, copious mucus secretion, loss of balance and sensitivity to touch and sound.

DISCUSSION

The toxicants on entering into fish body cause deleterious effects on functional activity of endocrine system and metabolism leading to physiological, pathological and biochemical disorders (Bais and Arasta, 1995; Arasta *et al.*, 1999, Pandey *et al.*, 2000;

Karuppasamy 2002; David *et al.*, 2003). Certain pathological informations along with physiological and biochemical data may provide accurate and early indication of toxicity of organochlorine pesticide (Krishnagopal *et al.*, 1988). Endosulfan had significantly increased cholesterol levels of liver, brain and gill tissues of *H.fossilis* at all concentrations in these experiments. It was further observed that the rate of increase in cholesterol was higher in the initial period of pesticide exposure to fishes but following prolonged exposure the same was much lower. However, the level remained above the control level. Lipids provide an essential, readily available energy source for fish, of which cholesterol is of major importance because of its relationship to many physiologically active steroids and hormones (Tiez, 1970; Evans, 1998). Alterations in cholesterol level may be an indirect result of toxicant effect on metabolic enzymes (Mayer *et al.*, 1992; Padmini *et al.*, 2004). Marked biochemical alterations in blood and tissues of freshwater cat fish *H.fossilis* have been reported by Singh and Srivastava (1998) and Chandra (2008) following formothion and malathion exposure. Biochemical changes are primarily due to shift in the respiratory metabolism caused by pesticide in the ambient environment and utilization of organic reserves yielding excess energy to compensate the stress. Toxic stress to fish *H.fossilis* was quite evident in these experiments showing abnormal behavioral changes. Rao *et al.* (1981) reported reduced oxygen consumption in fish *M.aculeatum* treated with endosulfan. Exposure to sublethal concentration of thiodon lead to 40% decline in oxygen consumption rate in *Mystus vittatus* (Reddy and Gonathy, 1977). Varied respiratory response may occur in fishes (Thosar and

Table 2. Cholesterol levels of different tissues of *H.fossilis* following endosulfan toxicity.

Pesticide conc. mg/l	Cholesterol mg/100mg. Fresh weight			
	Mean \pm Std. deviation			
	Time of exposure in hours			
	24	48	72	96
	Liver – Control -2.29 \pm 0.12			
0.0010	2.62 \pm 0.13	2.80 \pm 0.17	2.85 \pm 0.30	2.60 \pm 0.30
0.0015	2.80 \pm 0.16	2.98 \pm 0.15	3.14 \pm 0.32	
0.0020	3.04 \pm 0.42	3.10 \pm 0.24		
0.0025	3.00 \pm 0.25			
	Brain –Control- 2.80 \pm 0.08			
0.0010	3.27 \pm 0.17	3.46 \pm 0.24	3.70 \pm 0.23	3.40 \pm 0.42
0.0015	3.40 \pm 0.38	3.66 \pm 0.30	3.98 \pm 0.31	
0.0020	3.60 \pm 0.28	3.84 \pm 0.42		
0.0025	3.37 \pm 0.38			
	Gills-Control-1.08 \pm 0.10			
0.0010	1.28 \pm 0.13	1.35 \pm 0.08	1.40 \pm 0.06	1.20 \pm 0.12
0.0015	1.39 \pm 0.08	1.69 \pm 0.12	1.46 \pm 0.28	
0.0020	1.48 \pm 0.19	1.60 \pm 0.21		
0.0025	1.22 \pm 0.25			

Lonker, 2004) depending upon exposure period of toxicant, stress and the intensity of damage caused to gills. Further dose dependent behavioral changes in fish *H.fossilis* clearly indicated stages of stress response, the alarm reaction, the state of resistance and the stage of exhaustion in present experiment. It appeared that fishes exposed to lowest concentration of pesticide did not reach upto the state of exhaustion, rather they were able to accommodate and adapt the stress. However, fishes exposed to higher concentration of endosulfan could not resist the stress for longer duration and died. Nath (2003) also reported abnormal behavior in *Clarias batrachus*, *Heteropneustes fossilis* and *Labeo rohita* of wetland pond of Bihar, contaminated with endosulfan. Matsumura (1980) reported that susceptibility and survival time in fishes are directly related to body size, weight and age in ambient environment. Deshmukh and Sonawane (2007) reported significant changes in blood corpuscles number and attributed as an adaptation to meet stressful condition in the fish *Channa gachua* following long term exposure to endosulfan. Chandra *et al.* (2007) reported significant increase in Glutamic oxalacetic and pyruvic transaminase in brain and liver of *C.batrachus* due to toxicity of malathion and dimicron pesticides which indicated impaired carbohydrate and protein metabolism. Aldolase, an important gluconeogenic enzyme which plays an essential role in mobilization of energy to tolerate additional stress, was also reported to escalate significantly in liver, brain and gill of *C. batrachus*, following pesticide exposure (Chandra, 2010). Such biochemical changes clearly indicated utilization of organic reserves yielding excess energy to cope up the toxic stress of environment at their level best.

Since cholesterol plays an important role in body metabolism and being associated with almost every organ of body, was the foremost biochemical component of tissues revealing significant alterations in present observations. The percentage elevation of cholesterol were noted to be higher during early period of exposure to toxicant than prolonged one, which suggested that the fish tried its best to accommodate with the situation and doing so body metabolism became rapid to fulfil energy requirement, utilizing organic reserves, yielding excess energy to compensate the toxic stress. The mobilization of energy substances as secondary response and reduced capacity to tolerate additional stress have been described as a part of integrated response in fish (Bonga, 1997). Increased glucose contents in brain of *H.fossilis* (Shrivastava *et al.*, 2002) and changes in liver cholesterol have been observed following exposure of fish to various pollutants (Kasthuri and Chandran, 1997; and Kulkarni and Dharmadhker, 1998). Gills being the primary sites of absorption but having poor drug metabolizing capacity, indicated

maximum rise (36.09%) in cholesterol level. Endosulfan toxicity have also been reported to cause degeneration, necrosis, hyperplasia and hypertrophy in gills, liver and muscle tissues (Kumar *et al.*, 2000; and Sharma *et al.*, 2001). These observations further support the decreasing cholesterol levels in liver, brain and gill tissues of *H.fossilis* following prolonged exposure and increasing doses to pesticide when fish became exhausted. Thus it can be concluded that even very low concentration of endosulfan pesticide significantly altered cholesterol level of fish *H.fossilis* and behavioral changes are the early symptoms to gauge the potential toxicity of a toxicant.

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