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Time-related and Dose-dependent Biochemical Effects of the Insecticide Chlordecone (Kepone) in the Freshwater Indian Catfish *Heteropneustes fossilis*

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Arun K. Srivastava and Anil K. Srivastava (1995) Time-related and dose-dependent biochemical effects of the insecticide chlordecone (Kepone) in the freshwater Indian catfish *Heteropneustes fossilis*. *Zoological Studies* **34**(2): 106-110. Exposure of freshwater Indian catfish *Heteropneustes fossilis* to acute levels (0.048 mg/l) of chlordecone (Kepone) for 96 hours and to subacute levels (0.024 and 0.012 mg/l) for short-term (10 and 20 days) and long-term (30 and 60 days) periods resulted in markedly decreased total serum protein concentrations and hypercholesterolemia in the fish at all time intervals. However, fish exposed to sublethal concentration (0.008 mg/l) of chlordecone exhibited no significant changes in these parameters during either short- or long-term treatments.

Key words: Toxicity, Fish, Protein, Cholesterol.

Chlordecone (Kepone: decachlorooctahydro-1,3,4 methano-2h-cyclo-buta (cd) pentalen 2-one), hereafter referred to as CD is a polycyclic chlorinated pesticide with a molecular structure similar to mirex. It causes signs of neurotoxicity in man (Taylor et al. 1978). The adverse effects of CD on the central nervous system has also been demonstrated in rodents (Chang-Tsui and Ho 1980). The majority of these symptoms have also been reported in birds and mammals with CD toxicosis (McFarland and Lacy 1969, Desaiah 1984). Because CD is a stable chemical with high resistance to physical and biological degradation, it remains in the environment indefinitely (Eroschenko and Osman 1986). Chlordecone can also be detected in areas where mirex has been applied to control pests because 5 to 10% of mirex is photodegraded in the environment to CD (Carlson et al. 1976). Thus, studies of the biological effects of CD on fish should have a high priority.

Srivastava and Srivastava (1987) determined the LC50 value (Litchfield and Wilcoxon 1949) of CD in catfish as 0.24 mg CD/l for 96 hr by using the static acute toxicity bioassay (APHA et al. 1975). In fish, chlordecone has been reported to cause scoliosis (Buckler et al. 1981, Stehlik and Merriner 1983) and a number of behavioural changes (Roberts and Bendl 1982). Srivastava and Srivastava (1994a,b) have observed that CD produces histopathological changes in the liver and gonads of *Heteropneustes fossilis*. Further, CD has also been reported to alter such hematological parameters as total RBC and WBC counts, percentage of hemoglobin, hematocrit and clotting time (Srivastava and Srivastava 1994) as well as inducing changes in blood glucose, and liver and muscle glycogen levels in fish (Srivastava and Srivastava 1995).

The aim of the present study is to quantify such parameters as total serum protein and total blood cholesterol levels as criteria for evaluation of time-related and dose-dependent effects of CD toxicity on the freshwater Indian catfish *H. fossilis*. Protein and cholesterol levels of the

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blood are considered appropriate parameters in fish in a study of the influence of stressors or toxic stimuli (Grant and Mehrle 1973, Hattingh et al. 1978, Perrier et al. 1979, Wedemeyer and McLeay 1981, Gluth and Hanke 1984).

MATERIALS AND METHODS

The fish *H. fossilis* (weight 35.5 ± 2.20 g; length 15.50 ± 0.75 cm) were collected from a freshwater lake and were acclimated in tap water for fifteen days under natural photoperiod and ambient temperatures (25.40 ± 1.50°C). They were fed daily with wheat flour pellets and ground dried shrimp and maintained at a loading density of 0.495 fish cm/l or a biomass density of 1.71 kg/m^3 (Srivastava and Sahai 1987). Food was discontinued 24 hr prior to experimentation. The following physicochemical characteristics of the water used in the experiments were measured: hardness 114.75 ± 5.25 mg/l as $CaCO_3$; dissolved oxygen content 8.2 ± 2.5 mg/l; chloride 7.9 ± 0.55 meq/l; conductivity 308.60 ± 20.95 μmhos/cm; and pH 7.6 ± 0.02.

A stock solution of CD (1 mg/l) was prepared in acetone. In order to study the effects of CD on total serum protein and total blood cholesterol, groups of thirty to sixty fish (six fish per 20-liter glass jar) were subjected to 0.048 mg CD/l for 96 hr (acute exposure), and concentrations of 0.024 and 0.012 mg CD/l (subacute exposure) and 0.008 mg CD/l (sub-lethal exposure) for periods of 10, 20 (short term), 30 and 60 days (long term). Water with fresh CD in appropriate amounts was renewed daily to maintain water quality and proper CD concentrations. Six fish from each group were selected randomly for analysis of selected variables. Control groups of six fish each were maintained in tap water under parallel conditions, received equal aliquots of CD-free acetone as the treated fish and were sampled at the specified time intervals.

For the autopsy, the fish were anesthetized with 1g/3l MS 222. Blood from the fish was collected from the served caudal penducle with citrated tuberculin syringes for analysis of total serum protein (Lowry et al. 1951) and total blood cholesterol (Zlatkis et al. 1953). Statistical significance between each experimental and control group was calculated by Student's *t*-test.

RESULTS

Tables 1 and 2 indicate the changes in serum protein and blood cholesterol after exposure of catfish to acute concentrations of CD, as well as subacute and sublethal concentrations of CD over both short- and long-term periods. The total serum protein values in the control fish ranged between 6.14 ± 0.18 and 6.73 ± 0.20 g/100 ml. Acute exposure of the fish to 0.048 mg CD/l evoked a significant reduction in total serum protein (Table 1). Both short-term (10 and 20 days) and long-term (30 and 60 days) exposure to 0.024 and 0.012 mg CD/l were sufficient to produce significant serum protein decreases in the catfish (Table 2). However, the total serum protein levels in fish exposed to a sublethal concentration of CD (0.008 mg/l) did not differ significantly from those of the control fish (Table 2).

Total blood cholesterol values in the control fish ranged from 309.00 ± 2.02 to 317.97 ± 5.60 mg/100 ml (Tables 1, 2). Acute exposure to 0.048 mg CD/l caused significantly increased blood cholesterol levels (Table 1). The hypercholesterolemia followed both short- and long-term exposures to subacute concentrations (0.024 and 0.012 mg CD/l) (Table 2). But, as shown in Table 2, the sublethal level (0.008 mg CD/l) evoked hypercholesterolemia only followed ten days of treatment, whereas exposure to the pesticide for twenty to sixty days caused no marked changes in total blood cholesterol level.

Table 1. Total serum protein and total blood cholesterol values in catfish *Heteropneustes fossilis* exposed to acute concentration of 0.048 mg CD/l for 96 hours

Parameters	Control	Treated
Total serum protein (g/100 ml)	6.54 ± 0.24	5.33 ± 0.19**
Total blood cholesterol (mg/100 ml)	317.97 ± 5.60	363.49 ± 4.02***

Values are expressed as mean ± SE (N = 6).
Significant differences from the untreated control groups denoted by *$p < 0.05$, **$p < 0.02$-0.01, ***$p < 0.001$.

Table 2. Total serum protein and total blood cholesterol values in catfish *Heteropneustes fossilis* exposed to subacute (0.024 and 0.012 mg/l) and sublethal (0.008 mg/l) concentrations of chlordecone for short and long terms

Parameters	Short term (Days)				Long term (Days)			
	10		20		30		40	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
			0.024 mg CD/l					
Total serum protein (g/100 ml)	6.14 ± 0.18	5.50 ± 0.12*	6.50 ± 0.17	5.71 ± 0.19*	6.31 ± 0.15	4.82 ± 0.15***	6.67 ± 0.15	4.45 ± 0.21***
Total blood cholesterol (mg/100 ml)	311.93 ± 2.35	322.96 ± 1.99**	312.61 ± 2.97	330.78 ± 2.59***	312.28 ± 0.43	358.69 ± 1.11***	312.85 ± 2.52	384.40 ± 1.25***
			0.012 mg CD/l					
Total serum protein (g/100 ml)	6.73 ± 0.20	5.58 ± 0.15***	6.62 ± 0.15	5.85 ± 0.12**	6.33 ± 0.12	5.52 ± 0.13***	6.20 ± 0.12	5.49 ± 0.15**
Total blood cholesterol (mg/100 ml)	309.00 ± 2.20	319.34 ± 1.49***	312.90 ± 0.90	324.66 ± 1.28***	313.35 ± 1.64	335.51 ± 2.66***	316.68 ± 2.08	354.18 ± 1.29***
			0.008 mg CD/l					
Total serum protein (g/100 ml)	6.30 ± 0.15	6.45 ± 0.18	6.60 ± 0.20	6.62 ± 0.13	6.50 ± 0.20	6.88 ± 0.17	6.60 ± 0.22	6.84 ± 0.29
Total blood cholesterol (mg/100 ml)	311.54 ± 0.21	310.26 ± 0.35*	312.82 ± 0.89	314.73 ± 1.40	313.98 ± 1.42	317.02 ± 1.71	314.12 ± 1.58	316.97 ± 1.72

Values are expressed as mean ± SE (N = 6).
Significant differences from untreated control groups denoted by $^{*}p < 0.05$, $^{**}p < 0.02\text{-}0.01$, $^{***}p < 0.001$.

DISCUSSION

The catfish *H. fossilis* exposed to higher levels of CD in the present study exhibited hypoproteinemia. Mehrle et al. (1971), Grant and Mehrle (1973), and Gluth and Hanke (1984) found significant decreases in serum protein levels in rainbow trout and carp exposed to organochlorine insecticides. They reported a generalized hypoproteinemic effect in fish after exposure to the toxicant and correlated this change with disturbances in osmoregulation. Exposure to acute, subacute and sub-lethal concentrations of the dye, malachite green caused hypoproteinemia in *H. fossilis* after acute, short- and long-term treatment (Srivastava et al. 1995). Srivastava and Narain (1985) have ascribed the hypoproteinemic effect observed in catfish exposed to BHC or endrin to kidney disorders like nephrosis. Toxicants which affect the liver also result in reductions of total serum proteins in fish (Racicot et al. 1975, Pfeifer et al. 1977, Gingerich et al. 1978, Pfeifer and Weber 1979). In a previous study, we found the liver to be severely damaged following CD toxicosis (Srivastava and Srivastava 1994a). Therefore, the hypoproteinemia observed in the present study may be due to liver and kidney disorders.

Hypercholesterolemia was observed in CD-exposed catfish at acute, and sub-acute levels for acute, short- and long-terms. This may be due to the damage to the liver of treated fish (Srivastava and Srivastava 1994a). A review of literature shows that organochlorine pesticides cause hypercholesterolemia in fish (Narain 1981, Gluth and Hanke 1984, Srivastava and Narain 1985). Fish treated with dyes showed increased cholesterol levels (Bano 1982, Sharma et al. 1982, Srivastava et al. 1995). Hypercholesterolemia reported in the present study is, therefore, in accordance with the above observations. The elevated blood cholesterol levels may be due to mobilization of stored cholesterol from tissue.

Hypoproteinemic and hypercholesterolemic effects have also been reported in teleosts during stress (Cordier et al. 1959, Wedmeyer and McLeasy 1981), which also induced a rise in epinephrine (Nakano and Tomlinson 1968). Hypoproteinemic effects of catecholamines are well documented in stressed and epinephrine-injected fish (Haider 1969, Perrier et al. 1973 1979, Grant and Mehrle 1973, Hattingh et al. 1978). Perrier et al. (1973) reported increased levels of serum cholesterol due to increases in the circulating level of epinephrine in stressed, as well as epinephrine-injected rainbow trout (*Salmo gairdneri*). It is possible, therefore, that the changes observed in *H. fossilis* in this study may be due to increased output and circulating levels of catecholamines during the stress of pesticide toxicosis.

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殺蟲劑 Chlordecone 對淡水印度鯰魚之生化效應

Arun K. Srivastava[1] and Anil K. Srivastava[2]

將淡水印度鯰魚分別暴露於0.048, 0.024, 0.012 mg/l chlordecone 96小時、10、20、30、或60天，其結果造成血清蛋白質濃度降低及高血膽固醇現象。但是0.008 mg/l chlordecone短期或長期處理並不引起任何顯著之變化。

關鍵詞：毒性, 魚, 蛋白質, 膽固醇。

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