Bull. Environ. Contam. Toxicol. (1986) 36:181–185

Environmental Contamination and Toxicology

Methylmercuric Chloride and Serum Cholesterol Level in the Bluegill (*Lepomis macrochirus*)

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Cholesterol is an important physiological substance because it is the precursor of certain hormones and the bile acids. It also forms one-third of a cell membrane (as steroid cholesterol) (Vander, et al. 1970). The bimolecular lipid layer (cholesterol is one of them) is the main barrier to the diffusion of molecules through the cell membrane. Therefore, any amount of disruption in the cholesterol layer of the membrane is expected to result in a marked increase in cell membrane permeability as well as the normal function of hormones and the bile acid.

External factors affect cholesterol levels in several species. Styryl-hexahydroindolinol can cause hypocholesterolemia in rats, dogs, and monkeys (Bagdon et al. 1983). High doses of Niridazole can increase the cholesterol level in male rats (Ikegwuonu et al. 1983), while high doses of Aflatoxin B can increase the cholesterol levels in chicks (Maurice et al. 1983). There are, however, no reports on the effect of heavy metals, such as mercury, on the cholesterol levels in fish. The purpose of this study was to investigate the effect of methyl mercuric chloride on the cholesterol level in fish. Several researchers have found that extensive damage can be caused in fish as a result of exposure to methyl mercuric chloride (Choi et al. 1978; Hildebrand et al. 1980; Lock et al. 1981; Dutta et al. 1983). The damages imparted by methyl mercuric chloride to the different organs and tissues in fish are mainly due to the destruction of the cell membrane in the organs and tissues. A previous study by the authors has shown that there is an increase in the serum protein in fish after exposure to mercury for 48 hrs. which seems to occur with the release of membrane protein into the serum as the result of its destruction (Dutta et al. 1983). The study of the effect of this heavy metal on the cholesterol levels might give a better understanding of the mechanism which is responsible for the inverse relationship between the level of protein and the cholesterol in blood serum.

MATERIALS AND METHODS

Adult bluegills (5-8 cm in total lengths) were collected from a local pond. Twenty-four fish were kept in aquaria water (400 L

Table 1 Data base cholesterol levels in control and mercury exposed bluegill fish

			Experimental (mercury exposed)					
		#1		#2		#3		#4
Set #1	cont 1	cont 2	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3
mg/ml(chol)	0.22	0.23	0.58	0.68	0.041	0.047	0.22	0.18
Set #2	cont 1	cont 2	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3
mg/ml(chol)	0.03	0.03	0.34	0.40	0.12	0.12	0.22	0.18
Set #3	cont 1	cont 2	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3
mg/ml(chol)	0.40	0.34	0.40	0.58	0.09	0.05	0.22	0.27
Mean		0.30		0.50		0.08		0.20

cont = control chol = cholesterol

pH=7.25-7.35, temperature=22-25°C, regular tap water) for three days and fed tetra Doromin, fish chow ad libitum. Three sets were used in this experiment, with eight fish in each set (using 22-L aquarium for each set) (Table 1). In each set two fish were used as control and the remaining six were treated with methyl mercuric chloride. All eight fish in each set were kept under similar conditions for the duration of the experiment (3 days). The six treated fish in each set were kept continuously in a 8.728×10^{-4} ppb(w/v) of methyl mercuric chloride containing aquarium water (6.9% of the lethal dose) for three days. Blood samples were taken at 24 (day one), 48 (day two), and 72 hours (day three) from both the control and experimental fish of each set. In order to separate the serum samples from the collected blood samples, the blood samples were kept at room temperature for 30 min and then centrifuged at 700xg for 10 min and the supernatants (serum) were separated and analyzed. The serum samples collected from both the experimental and control groups of each set were assayed for total cholesterol concentration using the method described by Wynbenga (1970).

A statistical analysis was made by running t-tests between control and day one, control and day two, control and day three, day one and day two, day one and day three, and day two and day three variables. It was found from the 2-tail probability scores that all the means of the above combination of variables were highly significant (Table 2). This indicates that the cholesterol levels in each variable are distinctly different from each other.

Table 2 t-Test between variable groups: Control, day one, day two, day three cholesterol levels of mercury exposed bluegill fish

Variable Group	Mean	Standard Deviation	t Value	2 - Tail Probability
Control	0.30	0.07		
			-3.22	0.009
Day 1	0.50	0.14		
Control	0.30	0.07		
			7.01	0.000
Day 2	0.08	0.04		
Control	0.30	0.07		
			2.71	0.022
Day 3	0.22	0.03		
Day 1	0.50	0.14		
			7.34	0.000
Day 2	0.08	0.04		
Day 1	0.50	0.14		
			4.97	0.001
Day 3	0.22	0.03		
Day 2	0.08	0.04		
			-6.76	0.000
Day 3	0.22	0.03		

Cases - 6 for each variable
Degree of Freedom - 10 for each variable group

RESULTS AND DISCUSSION

The means of the cholesterol levels in the serum have been shown in Table 1. The total level of cholesterol increased after 24 hr of treatment with methyl mercuric chloride, and decreased after 48 hr of exposure. After 72 hr the level increased again, but did not reach the level detected at 24 hr.

The effect of methyl mercuric chloride at the same concentration on the total protein level in serum of bluegill was examined by Dutta et al. (1983). They found that there was an increase in the total protein level after 48 hr of treatment. These results combined with those from the present study suggest that there might be a relationship between protein and cholesterol levels in serum of bluegill exposed to methyl mercuric chloride. It is possible that the rise in the total cholesterol level came about as a result of the destruction of the cell membrane (as it was explained in the introduction). The damage to the cell membrane can be done by denaturation of the protein moiety in the membrane.

This denaturation of proteins can also explain the decrease in the total protein in serum after 24 hr of treatment with methyl mercuric chloride, while the increase in the total cholesterol can be the result of the cholesterol release from the cell into the blood.

Brinster et al. (1982) have detected that heavy metals like Zn and Cd can stimulate protein synthesis in liver of mice. Consequently, another possible explanation for the decrease in cholesterol is that mercury might be able to stimulate protein synthesis in the bluegill liver, which increases the level of apo-HDL and as a result increases the level of HDL (high density lipoprotein) in serum. It has been shown by Miller and Miller (1975) that HDL, in mammals, functions as a carrier of cholesterol, from tissues and serum to liver. It is possible that the increase in the HDL level, synthesized in liver (as a result of protein induction by mercury), can account for the decrease in the cholesterol level after 48 hr of treatment. This corresponds with the increase in protein level at the same period in serum (Dutta et al. 1983).

Our results suggests that there is an inverse relationship between the serum cholesterol level and serum protein level in bluegill exposed to methyl mercuric chloride.

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Received June 17, 1985; Accepted August 17, 1985