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Peripheral Effects of Cadmium on the Blood and Head Kidney in the Brown Bullhead (*Ictalurus nebulosus*)

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The disposal of an ever-increasing number of cadmium-containproducts at dump sites is causing concern for the health of individuals using dump sites for various purposes (BARBOUR 1978). Chemical leaking of the cadmium-containing refuse can lead to contamination of drinking water (KNEIP 1978). Thus, there is a need for a standard method for biologically monitoring for the presence of cadmium in water. Our experiments indicate that fish are useful as a monitor.

The following study with fish was undertaken with the following objectives: 1) to determine the amount and rate of uptake of cadmium via atomic absorption measurements; 2) to determine whether acute cadmium exposure (61 ppm) provides alterations in the blood and head kidney. The results indicate that the peripheral blood and head kidney of indigenous fish species feeding in the area of the dump provide the best immediate monitor for water quality. The head kidney and peripheral blood coupled with chemical analysis for cadmium indicate that the head kidney may be one of the initial target organs of cadmium toxicity. After an initial 2-h exposure of 61 ppm cadmium, an immediate and 24-h intervals were used to examine the effect uptake, storage and clearance of cadmium from the peripheral blood and head kidney.

The studies were done on fish maintained in environmentally controlled aquaria. The catfish (Ictalurus nebulosus) was chosen since it is a local sedimental feeder.

MATERIALS AND METHODS

Brown bullhead catfish (I. nebulosus) were used at the New York University Lanza Laboratory. The subadult bullheads (average length = 15 cm, average weight = 50 g) were obtained from Northeastern Biologists, Inc., Rhinebeck, NY and maintained in a 20-L aquaria. Each tank housed four fish using aged filtered tap water. The constant environment was maintained at an average temperature 19.5 $\pm$ 0.8°C. Dissolved oxygen and pH averaged 11 ppm and 6.6, respectively. The fish were fed a diet of Agway Strike commercial fish food daily and fasted 24-h prior to bleeding. The bullheads

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were exposed to 61.3 ppm $CdCl_2$ for 2-h. Fish were sacrificed immediately after exposure or after one day. The remaining fish were maintained in uncontaminated water with weekly changes of water for two months.

Blood Collection and Analysis. Unanesthetized bullheads were bled via a cardiac puncture pre- and post-cadmium exposure at each time-clearing interval. Blood-letting was done with a 20-gauge needle premoistened with a 3.8% solution of sodium citrate anticoagulant in saline and did not exceed 0.1 cc to avoid anemia. Erythrocyte and leukocyte counts were made simultaneously using the Shaw's "Avian" solution technique (1930). Peripheral blood smears and differential counts were done according to standard technique (KLONTZ & SMITH 1968). Two hundred cells per slide were counted. Peripheral blood smears were stained with Wright's and Giemsa stain (WEINBERG et al. 1972).

Head Kidney Myelogram. The head kidney located anterior to the trunk kidney was removed and sliced transfersely. Serial imprints of the cut surface were made on alcohol-cleaned slides and stained with Wright's and Giemsa stains according to ASHLEY & SMITH (1963). A differential myelogram was done counting 300 cells per slide.

Uptake Analysis. Cadmium concentrations indicating uptake, storage and retention were determined with a single beam atomic absorption spectrophotometer. Methods for sample preparation and corrections for background have been previously described (ANDERSON et al. 1978). Measurements of cadmium concentrations in the blood and head kidney were determined by single linear regression analyses. The t-test was used for comparison of cadmium concentrations in control and experimental groups. The confidence levels were 95% for cadmium concentration in the control and experimental groups.

RESULTS AND DISCUSSION

The results of this study indicate that cadmium chloride at a high concentration has an immediate effect on the peripheral circulatory system and head kidney of the brown bullhead (I. nebulosus).

The peripheral response can be seen in Table 1 as loss in red blood cells (RBC) only after 24-h concurrently with an increase of total white blood cells (WBC) both immediately and after 24-h. No apparent effect on the hematocrit was observed. However, there was a slight increase in the hemoglobin level. If it is significant, it may be due to a response to respiratory distress.

In Table 1 a differential analysis of selected WBC immediately and after 24-h indicated a significant decrease in lymphocytes (lymphopenia) accompanied by significant increase in thrombocytes and neutrophils (thrombocytosis and neutrophilia). The lymphopenia thrombocytosis and neutrophilia can be directly attributed to the internal hemorrhaging and toxic effects of the cadmium chloride. Other blood elements were largely unaffected and hence are not

included in the table.

This study indicates in Tables 2 and 3 the toxicity of cadmium on the head kidney elements. As indicated by the fluctuation in the types of read and white cell response, particularly the hemoblasts, mature and immature RBC's, fine and coarse progranulocytes culminating in the destruction or elimination of all formed hemopoietic elements except the mature RBC's within 24-h. The results can be due to the direct effects of cadmium on the head kidney hemopoietic cell lines as indicated by a significant increase in the amount of cadmium present in the head kidney in Table 3.

Atomic absorption analysis of the peripheral blood and head kidney showed a significant uptake of cadmium which remained unchanged in the peripheral blood but continued to increase in the head kidney indicating a potential capacity to store cadmium (Table 3).

The toxic effects of cadmium on the head kidney have previously gone unnoticed in toxicity studies on fish. This study on I. nebulosus presents a new and promising method of monitoring the presence of cadmium in fish. Other investigations on the Hudson River blue crab Callinectes sapidus have documented the distribution of cadmium in the muscle and hepatopancreas (KNEIP & O'CONNOR 1980). Further parallel studies are planned utilizing PCB as a toxic agent on their effects on peripheral blood and primarily the head kidney of other fish species from the Hudson River.

Table 1. Effect of Cadmium on the Total RBC, WBC and Differential Counts

Treatment Group (4) fish	Hours Exposure	Pre-RBC / Post-RBC (10^6 cells/mm^3)	Pre-WBC / Post-WBC (10^3 cells/mm^3)
I Immediate	0	1.36±0.1	*43.58±2.4
Sacrifice	2	1.20±0.1	133.87±1.4
II 24-h	0	*2.00±0.1	*63.25±0.9
Uptake	2	1.63±0.1	125.37±3.5

Treatment Group	Lymphocytes (%)	Thrombocytes (%)	Neutrophils (%)
I Immediate	*80.2±2.9	*10.0±2.7	*3.5±0.2
Sacrifice	68.2±2.4	17.0±1.5	22.7±0.9
II 24-h	*82.0±2.2	* 9.7±3.4	* 1.5±0.6
Uptake	43.0±2.4	26.2±4.3	28.2±2.1

*($P<0.05$) values are reported as pre-exposure over post-exposure
± S.E. for 200 cells counted per smear preparation

Table 2. Effect of Cadmium on Head Kidney Elements

	Treatment Group			
	I Immediate Sacrifice		II 24-h Uptake	
	Control	Treated	Control	Treated
Hours Exposure	0	2	0	2
Hemoblast (%)	2.8±0.7	*10.6±1.1	*3.2±0.8	0
Immature RBC (%)	*10.6±1.4	0	*9.4±0.7	0
Mature RBC (%)	*40.5±2.4	23.1±1.7	*40.4±2.2	22.2±1.4
Fine Progranulocyte (%)	*14.7±0.9	22.4±1.3	*15.4±0.9	0
Coarse Progranulocyte (%)	1.8±0.5	2.3±0.6	*2.5±0.4	0
Neutrophil (%)	1.4±0.4	0.7±0.3	*1.8±0.2	0
Eosinophil (%)	0.3±0.5	0	0	0
Immature Lymphocyte (%)	*3.7±0.5	0	*4.3±0.7	0
Mature Lymphocyte (%)	*18.3±0.9	24.5±1.2	*17.2±1.1	0
Round thrombocyte (%)	*4.6±0.8	16.0±1.2	*5.1±0.7	0

The values are reported as the mean ±S.E. for 300 cells counted per imprint preparation.
* ($P<0.05$)

Table 3. Cadmium Residue in Peripheral Blood and Head Kidney Atomic Absorption Analysis

Treatment Groups	No. Fish/ Group 4	Hours Exposure	Blood ppm Cd (±S.E.m) (ug/Cd/ml)	Head Kidney ppm Cd (±S.E.) (ug Cd/g Dry Wt)
I Immediate Sacrifice	Control	0	0.29 ± 0.1	0.91 ± 0.2
	Treated	2	**1.97 ± 0.8	**3.69 ± 0.6
II 24 h Uptake	Control	0	0.16 ± 0.1	1.37 ± 0.4
	Treated	2	*1.03 ± 0.1	**6.12 ± 1.5

* ($P<0.05$)
** ($P<0.01$)

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