Uptake and Effect of Cadmium on Zebrafish

by ROBERT REHWOLDT
Environmental Science Program
Marist College, Poughkeepsie, N.Y.
and DAVOUD KARIMIAN-TEHERANI
Österreichische
Studiengesellschaft für
Atomenergie Ges. m.b.H.
Lenaugasse 10 A-1082 Wien, Austria

INTRODUCTION - Cadmium is known to be a toxic substance toward many organisms. Much work has been performed evaluating toxic limits for many species as well as investigating the effects of chronic exposure (PRODAN, 1932; REHWOLDT et al., 1974; HOLDEN, 1969). Most of the chronic studies, however, deal with mammals or birds and not with fish.

Zebrafish are well suited for chronic studies as they have a well known life cycle, are easy to maintain in the laboratory and lay eggs daily. Eggs usually hatch within three days after they are deposited (HISAOKA & BATTLE, 1958).

METHODS - Approximately 350 mature zebrafish, Brachydanio rerio, were used in these experiments. The fish were divided into two groups and placed in 50 liter aquaria. One group was fed normal fish food (Tetra Min, Tetra Werke, West Germany) and the other group were fed with the same food with 10 ppm Cd as $Cd(CH_3COO)_2$ added.

The fish were fed daily and, immediately after feeding ceased, an activated charcoal filter was turned on for several hours to remove any cadmium that might have been leached out of the food or fish excreta.

Every week ten random pairs of fish from each tank were removed and placed in a net enclosure in second aquaria for the purpose of breeding. The net prevented the adult fish from eating the eggs. After 24 hours the fish were returned to their original tanks and the eggs allowed to hatch. At the end of each two weeks the offspring from each breeding tank were sacrificed as well as four fish from the experimental group and four from the control group. The adult fish were analyzed as single samples, however, due to the small size of the offspring, they had to be grouped as monthly samples for analysis. The offspring were not fed during the two week development.

Samples were freeze dried and then sealed in quartz ampules. The ampules were irradiated for 24 hours in a neutron flux of $4\text{-}7.10^{13}$ n/cm^2 sec (ASTRA-Reactor) and then measured with an Intertechnique pulse height analyzer connected to a 12 cm^3 Ge(Li) detector, as well as a Labine pulse height analyzer with a 40 cm^3 Ge(Li) detector. The isotope measured was ^{115m}In, 337 KeV, which is a decay product of ^{115}Cd.

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 4

The following water parameters were maintained in all of the aquaria:

Temperature 27.0C	Hardness 25mg/l as Ca ion.
Aeration, continuous	Lighting, natural diurnal cycle.

RESULTS AND DISCUSSION - Figure 1 and Table 1 show the uptake of Cd during the 6 months of the study. As can be seen in Figure 1 there is an initial steep rise which is then followed by a period of slow uptake. Finally, a plateau is reached. Uptake curves such as this are quite usual and have been reported for heavy metal studies with mammals (HEMPHILL, FRIBERG, 1965). The plateau corresponds to the time at which the rate of retention equals the rate of excretion.

It is of great interest to note that there are two distinctly different peak concentrations for male and female. The maximum concentration attained in the females, 12.7μg/g dry weight, is approximately double that attained by the males, 5.1μg/g. There is no obvious explanation for this especially in light of the fact that in mammals cadmium tends to accumulate in the male reproductive system (PARIZEK, 1957) in addition to the other internal organs.

FIGURE 1 CONTENT OF Cd IN ZEBRAFISH

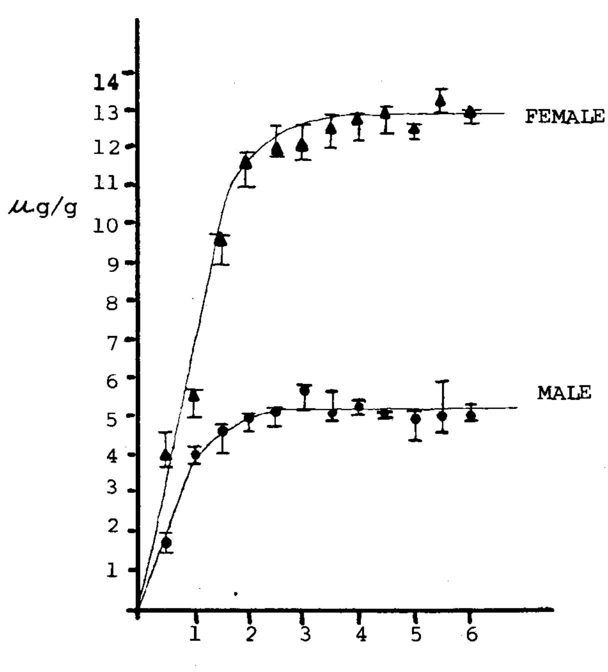

TABLE 1

CONTENT OF CADMIUM $\mu g/g$ DRY WEIGHT

		MONTH											
		0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
MALES	MEDIAN	1.71	4.02	4.61	4.99	5.10	5.61	5.10	5.26	5.06	4.92	5.01	5.19x
	RANGE	1.90	4.12	4.70	5.01	5.21	5.79	5.60	5.29	5.10	5.16	5.92	5.32
		1.68	3.81	4.00	4.77	4.80	5.20	4.99	5.11	5.00	4.41	4.66	5.00
FEMALES	MEDIAN	4.00	5.51	9.57	11.61	12.01	12.00	12.44	12.77	12.91	12.46	13.21	12.95x
	RANGE	4.60	5.61	9.59	11.92	12.61	12.61	12.91	12.92	13.08	12.51	13.64	12.99
		3.70	5.00	9.00	11.01	12.00	11.77	12.06	12.22	12.44	12.33	13.00	12.81
CONTROL	MEDIAN	0.03	ND	ND	0.04	0.04	ND	ND	ND	ND	0.06	ND	ND x
MALES	RANGE	0.09			0.10	0.06					0.08		
		0.08			0.03	0.03					0.04		
CONTROL	MEDIAN	ND	0.10	ND	ND	0.09	0.06	ND	ND	ND	ND	0.09	0.07x
FEMALES	RANGE		0.11			0.10	0.09					0.14	0.09
			0.09			0.06	0.04					0.07	0.06
OFFSPRING			ND		ND		ND		ND		ND		ND

x ANALYSIS BASED ON 40 SAMPLES

ND= NOT DETECTABLE

As can be seen in Table 2 the monthly total of eggs began to decline after the first month. Although a χ^2 test could be applied on to each month's figures to attempt to determine exactly at which point the decrease in eggs becomes statistically significant, perhaps it is better to note that chronic long term ingestion of cadmium caused a marked and real decrease in the number of zebrafish offspring, $P < 0.0005$, at the end of this study.

It must be stated at this point that this work was not designed to isolate and examine the fish eggs so, therefore, we are unable to say at this time whether the decrease in offspring was due to changes in the viability of the eggs or to an actual decrease in the number of eggs deposited.

It is interesting to note, however, that a study by KIHLSTROM & HULTH (1972) showed taht trace amounts of mercury in the water actually increased the hatchability of zebrafish eggs.

Lasko and others have reported that chronic exposure to concentrations of heavy metals can cause an increased incidence of spinal deformities in fish. This was not observed during this study. In the previously cited work, however, the metals were in the water not the food (LASKO)

During these experiments another interesting phenomenon was observed. At the end of the work one could clearly see that the colors and markings on the fish that had been fed the cadmium were less defined. Since this is a somewhat subjective observation it is not possible to say at exactly what point the colors began to change. No explanation is offered at this time, however, it does have interesting implications for fish which rely upon color during their life cycles.

Finally, analysis of the offspring, Table 1, yielded no detectable amounts of cadmium, indicating that the cadmium retained by the parent zebrafish is not transferred to the offspring.

ACKNOWLEDGEMENT - The authors are indebted to Mr. Pinterits and Mr. Graf for their help in the analysis of many of the samples and to Mr. Kehrer for his assistance in the preparation of the manuscript.

TABLE 2 NUMBER OF OFFSPRING- 24 HOUR PERIOD AND 10 PAIRS OF ADULTS

	MONTH											
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
OFFSPRING EXPERIMENTAL	15	16	9	6	7	5	6	2	4	5	2	3
OFFSPRING CONTROL	14	17	12	15	14	9	16	11	11	17	8	14

REFERENCES

FRIBERG, L. Acta Pharmacol. Toxicol. 11, 168 (1965).

HEMPHILL, D.D. Ed., Trace Substances in Environmental Health III, Univ. of Missouri, Columbia, Missouri.

HISAOKA, K.K. & H.I. BATTLE. J. Morph., 102, 311 (1958).

HOLDEN, H. Lancet, 2, 57 (1969).

LASKO, L. Personal Communication.

KIHLSTROM, J. & L. HULTH. Bull. Environ. Contam. & Toxicol. 7, 111 (1972).

LASKO, L. Personal communication.
PARIZEK, J. J. Endocrinol. 15, 56 (1957).

PARIZEK, J. J. Endocrinol. 15, 56 (1957).

PRODAN, L. J. Ind. Hyg. Toxicol. 14, 174 (1932).

REHWOLDT, R., L. MENAPACE, B. NERRIE & D. ALESSANDRELLO. Bull. Environ. Contam. & Toxicol. 14, 174 (1932).