Bull. Environ. Contam. Toxicol. (1984) 32:693–697

Bulletin of Environmental Contamination and Toxicology

Time Course Study of Occurrence of Anomalies in Medaka's Centrum by Cadmium or Fenitrothion Emulsion

Yukio Hiraoka and Hisanori Okuda

Department of Hygiene, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan

In the present study, developing medaka eggs were exposed to cadmium (Cd) or a fenitrothion emulsion (MEP), and the transition of incidence of centrum-damaged fish was examined.

Exposure of fish eggs to chemical substances results in inhibition of development (Hisaoka 1958) and the occurrence of various anomalies (Anderson and Battle 1967, Hisaoka 1958) can sometimes be observed depending on the kind and concentration of chemicals involved. Delayed anomalies sometimes occur in fish after hatching normally from eggs exposed to chemicals. Hiraoka (1980a, b, c, d) reported that the exposure of medaka eggs to cadmium, zinc, oxadiazon emulsion (herbicide), or fenitrothion emulsion (organophosphorous pesticide) during development of the eggs caused frequent vertebral abnormalities during adulthood.

Recently Bengtsson (1975) and Kanazawa (1976) presented a summary of the skeletal abnormalities of fish, in which a few cases of skeletal abnormalities in medaka are cited. But there is no mention of the time at which skeletal abnormalities become apparent as a result of exposure to chemical substances during egg development, and the frequency of such occurrence with medaka growth.

MATERIALS AND METHODS

Adult medaka (the red variety of Oryzias latipes) were purchased from the Ito Fish Farm and reared in our tank. Later, eggs were spawned by these fish. The harvested fertilized eggs were divided into 3 groups. The first group was maintained in dechlorinated water containing 0.01 ppm Cd (Cd group), the second in dechlorinated water containing 0.4 ppm MEP (MEP group), and the third, in dechlorinated water (control group) for one week up to the retinal pigment stage. Following this, each group was reared in dechlorinated water for 4 months to adulthood. The temperature of the water was 25 ± 2°C.

The medaka were collected from each group once a week, starting from the commencement of hatching for a period of six weeks. Following this, they were collected in control and MEP groups during the 8th, 10th, 12th, and 16th weeks. The number of collected medaka is shown in Tab. 1. To study the bone and cartilage, freshly hatched fry and larvae were stained, using a modification of the method of Dingerkus and Uhler (1977), and young and adult fish, by a modification

of the method of Inouye (1976). These specimens were observed under a phase contrast or stereoscopic microscope.

RESULTS AND DISCUSSION

None of the newly hatched fry stained with alizarin red S (for bone staining), but most of maxillary, suspensorium, and gill arches of the medaka fry, immediately after hatching stained light blue with alcian blue (for cartilage staining). Possibly these regions changed to cartilage initially in order to take food and respire to stay alive. From the 2nd through the 4th week, the hypural bone and pterygiophore stained with alcian blue and apparently changed to cartilage. It may be inferred that the capacity of medaka for locomotion and balance develops gradually. At about the 5th week, most of the anterior portion of vertebral column of the medaka stained deep red with alizarin red S. Etoh (1981) reported that the vertebrae stained with alizarin red S approximately a month after hatching, which is in close agreement with our results. The cartilage in the skull stained blue initially and then red. Ossification gradually took place and was completed by the 10th – 12th week. During the 10th – 12th week, the skeleton size nearly approximated that of the adult. The ossification process in all three experimental groups was essentially the same.

In the control group, centrum-damaged fish were observed in the 3rd week and continued to increase in number (Tab. 1 and Fig. 1). The cumulative frequency of centrum-damaged fish was 8% in the 10th week but remained constant after this. The incidence of centrum-damaged fish in the control group in the present study was neither 0% nor near 0%. The reason for this may be that since vertebral abnormalities in fish arise from many factors (Bengtsson 1975, Kanazawa 1976), it was not always possible in our particular rearing system to account for the occurrence of several percent of vertebral abnormalities arising in medaka.

In the Cd group, centrum damage was not observed in newly hatched fry; however, centrum-damaged fish were found in the 1st week, and increased rapidly up to the 4th week. The cumulative frequency of centrum-damaged fish was 13% and 14% in the 5th and 6th weeks respectively and seemed to remain constant after this. There was a significant difference in the incidence of centrum-damaged fish in the control and the Cd groups during the 4th, 5th, and 6th week ($p < 0.01$, χ^2-test).

In the MEP group, there was no centrum damage in newly hatched fry but centrum-damaged fish were observed in the 1st week, and increased rapidly up to the 6th during which the cumulative frequency of centrum-damaged fish was 21% and remained as such. There was a significant difference in the incidence of centrum-damaged fish in the control and MEP groups, which persisted throughout the experimented period following the 2nd week ($p < 0.01$, χ^2-test).

From these results, it is apparent that the cumulative frequency of centrum-damaged fish increased rapidly before the period of 1 – 2 weeks during which the vertebrae stained with alizarin red S. Following this, the frequency value remained constant. Thus, it may be inferred that the centrum damage observed in the medaka exposed to Cd or MEP took place in most cases before ossification of the centrum had been completed.

Table 1. The number and body lengths of examined fish and frequency of centrum-damaged fish

		Rearing period (weeks)										
		0	1	2	3	4	5	6	8	10	12	16
Control group	A	52	37	42	43	39	32	42	33	37	35	33
	B	4.3±0.3	5.1±0.4	5.8±0.7	5.5±0.8	6.4±1.0	7.9±1.0	8.9±1.8	8.1±1.3	11±1.4	12±1.7	20±1.5
	C	0	0(0)	0(0)	9(2)	8(3)	9(4)	17(6)	15(7)	16(8)	9(8)	9(8)
Cadmium group	A	29	31	30	29	30	30	14	—	—	—	—
	B	4.1±0.2	5.1±0.4	5.6±0.5	5.9±0.7	7.0±1.3	6.6±1.6	9.4±1.2	—	—	—	—
	C	0	3(2)	13(6)	14(8)	20(10*)	30(13*)	14(14*)	—	—	—	—
Fenitrothion group	A	39	20	41	40	42	36	41	30	35	34	35
	B	4.2±0.2	4.6±0.4	5.2±0.4	5.5±0.5	6.9±0.9	6.2±0.7	7.4±1.2	10±1.0	12±1.5	13±2.0	15±1.6
	C	0	10(3)	22(11*)	13(11*)	19(13*)	36(20*)	42(21*)	23(21*)	23(21*)	15(21*)	26(21*)

A = the number of examined fish, B = body length (mm, Mean ± S.D.), C = frequency of centrum-damaged fish(%)
Figures in parenthesis indicate the cumulative frequency of centrum-damaged fish.
* : $p < 0.01$, compared to the control, as evaluated by the χ^2 -test.

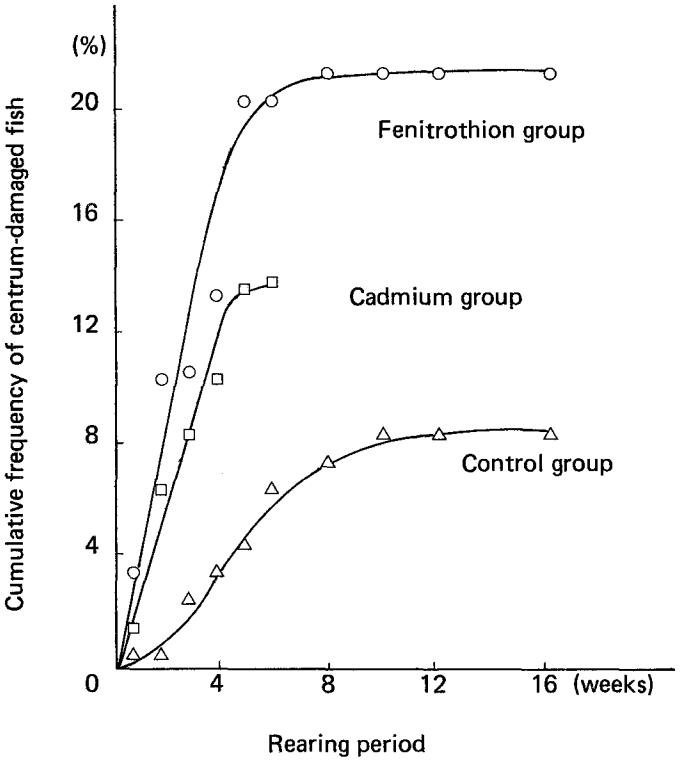

Fig. 1 Increase in cumulative frequency of centrum-damaged fish with the passage of time.

Though agricultural control chemicals differ qualitatively from heavy metals, the occurrence of centrum-damaged fish in the MEP group was similar to that in the Cd group. Thus, it may be concluded that even if medaka eggs are exposed to various other chemical substances, the occurrence of centrum-damaged fish would be basically similar to that found in the present study.

Acknowledgement. This study was supported in part by Grant-in Aid for Scientific Research (57770384) from the Ministry of Education.

REFERENCES

Anderson PD, Battle HI (1967) Effects of chloramfenicol on the development of the zebra-fish, Brachydanio rerio. C J Zool 45: 191–204

Bengtsson BE (1975) Vertebral damage in fish induced by pollutants. In: Koeman JH, Strik JJTWA (ed) Sublethal effects of toxic chemicals on aquatic animals. Amsterdam, p23–30

Dingerkus G, Uhler LD (1977) Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology 52: 229–232

Etoh H (1981) Teratogenicity experimental methods. In: Egami N (ed) In fish, as laboratory animals. Tokyo, p452

Hiraoka Y (1980a) Studies on the vertebral abnormalities of medaka as the biological index of water pollution. III. Experimental investigation concerning cadmium exposure. Hiroshima MJ 28: 625–632

Hiraoka Y (1980b) Studies on the vertebral abnormalities of medaka as the biological index of water pollution. IV. Experimental investigation concerning zinc exposure. Hiroshima MJ 28: 633–642

Hiraoka Y (1980c) Studies on the vertebral abnormalities of medaka as the biological index of water pollution. V. Experimental investigation concerning oxadiazon exposure. Hiroshima MJ 28: 643–650

Hiraoka Y (1980d) Studies on the vertebral abnormalities of medaka as the biological index of water pollution. VI. Experimental investigation concerning MEP exposure. Hiroshima MJ 28: 651–660

Hisaoka KK (1958) The effects of 2-acetylaminofluorene on the embryonic development of the zebra-fish. I. Morphological studies. Cancer Res 18: 527–535

Inouye M (1976) Differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red S. Cong Anom 16: 171–173

Kanazawa J (1976) Vertebral abnormalities in fish and pesticides. Kagaku 46: 108–115

Received October 20, 1983; accepted January 30, 1984