Mercuric Chloride Uptake by Eggs of the Ricefish and Resulting Teratogenic Effects

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Inorganic mercurials are frequent and dangerous pollutants of aquatic environments. Apparently many mercurials may be biologically transformed into methylmercury (WOOD et al., 1968; JENSEN and JERNELOV, 1969; LANDNER, 1971). The inorganic mercurials are often the initial mercury compounds to which polluted ecosystems are exposed since methylation requires an indefinite amount of time.

Mercuric chloride is a particularly common industrial and agricultural pollutant. KONRAD (1970) estimated that for every ton of chlorine produced by chlor-alkali chemical firms, as much as 150 to 200 grams of mercuric chloride were discharged into the U. S. waterways. There were 35 chlor-alkali plants operating in the United States in 1965, and they produced a total of three million tons of chlorine. This means that between 495 and 660 tons of inorganic mercury were introduced into the environment through this source in one year.

In agriculture mercuric chloride is commonly used as a disinfectant for seeds, an anti-disease treatment for corn, tubers and bulbs, and a fungicide for numerous vegetables and potatoes (GOLDWATER, 1971).

Mercuric chloride is dangerous to fish. It is rapidly absorbed from water by goldfish (McKONE et al., 1971). It caused reduction in the activity of certain liver enzymes in killfish, Fundulus heteroclitus, which were exposed to 23 ppb Hg^{++} (JACKIM et al., 1970). JONES (1939) found the ten day threshold toxicity for the stickleback, Gasterosteus aculeatus, was 10 ppb (parts per billion).

Mercury compounds are known to be teratogens which are concentrated by the fetus of higher vertebrates (SENTEIN, 1957; KALTER and WARKAND, 1959; GOLDSTEIN et al., 1969), producing gross abnormalities and death at concentrations less than the adult lethal concentration.

There is a paucity of published information concerning the teratogenic effects of inorganic mercury on fish embryos, or the ability of fish embryos to concentrate inorganic mercury directly from the water. It is the purpose of this paper to investigate these phenomena.

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Procedures

Japanese Medaka, Oryzias latipes, were obtained from the Carolina Biological Supply Company. Every morning egg clusters were removed from the vents of the females. Clusters of six eggs were placed in 100 x 15 mm plastic disposable petri dishes containing 30 ml of embryo rearing solution. The eggs were then incubated at 26°C on a photoperiod with a 16 hour light phase.

The embryo rearing solution had the following composition: one part 10 percent NaCl, one part four percent $CaCl_2 \cdot 2H_2O$, one part three percent KCL, 97 parts deionized water and 10 ppb malachite green.

Six eggs from each female served as controls, while the remaining clusters of six eggs each were placed in embryo rearing solutions with mercuric chloride. The experimental embryo rearing solutions contained 10 ppb, 15 ppb, 20 ppb and 30 ppb of the inorganic mercury. The solutions had a pH of 6.9. Desired concentrations of mercury were prepared by serial dilutions from a 1,000,000 ppb mercury stock solution, which was prepared freshly each day.

Both control and experimental eggs were transferred to fresh solutions each morning for 16 consecutive days using inverted 10 ml pipettes equipped with a suction bulb. This was done to keep the oxygen tension from falling too low, and to minimize the effect of any mercury adsorption by the sides of the petri dishes. KIRCHEN and WEST (1969) state that medaka eggs generally hatch within 11 days of fertilization at 25°C; however, this incubation time did not prove adequate, so an incubation period of 16 days was arbitrarily used.

For the first four days, observations were made four times a day at 7:00 a.m., 2:00 p.m., 6:00 p.m., and 10:00 p.m. Thereafter observations were made at 24 hour intervals until the 16th day. At the end of the 16 day incubation period the eggs were blotted to remove excess water, weighed and immediately frozen. Later, they were digested in HNO_3 and H_2SO_4 (2.5 ml of each). Thirty-four ml of five percent KMNO was added to the digest. The acid solution was heated to 55°C for 16 hours. The eggs were then analyzed for total mercury using flameless atomic absorption (HATCH and OTT, 1968).

Results and Discussion

Hatching results are summarized in Table I. The 16 day hatching percentage for control eggs was 46.7. The hatching value for the 10 ppb experimental eggs was 58.3

percent which does not differ significantly from the control eggs (Chi Square = 3.4, $0.10 < P < 0.05$). The hatching percentage for the 15 ppb experimental eggs was 20.8 which is significantly lower than the control value ($x^2=12.8$, $P > .0005$). None of the 20 or 30 ppb experimental eggs hatched.

TABLE 1

Comparison of Hatching Successes for Controls and Experimental Eggs.

Sample designation	Number of eggs	% hatching
Controls	120	46.7
Experimental		
10 ppb	132	58.3
15 ppb	72	20.8
20 ppb	72	00.0
30 ppb	78	00.0

Fry hatched from the 15 ppb experimental eggs appeared to lack the ability to move their caudal fins. Their pectoral fins moved rapidly. These fish remained on their sides on the bottoms of the petri dishes. Their tails were bent in an anterior and ventral direction assuming a "C"-shaped position.

Hemorrhaging, blood vessel deterioration, and loss of circulating blood cells (Figures 1, 2, and 3 respectively) were observed in 79 percent of the 15 ppb experimental eggs. All the fish that hatched from the 15 ppb eggs appeared normal, while all the 15 ppb eggs which did not hatch exhibited all three abnormalities. Neither the control eggs nor the 10 ppb experimental eggs showed any evidence of the anomalies.

TEJNING (1967) reported subcutaneous bleeding in a chicken embryo produced by a hen fed methylmercury dicyanimide. He also observed severe anemia in those chickens whose blood exhibited the highest concentrations of mercury.

In the present study those experimental eggs exhibiting hemorrhaging had clusters of unconfined red blood elements present over the surface of the yolk. Some of the eggs demonstrated additional hemorrhaging in the caudal region. The size and number of these free blood clusters varied, consisting of from one large cluster to several smaller clusters.

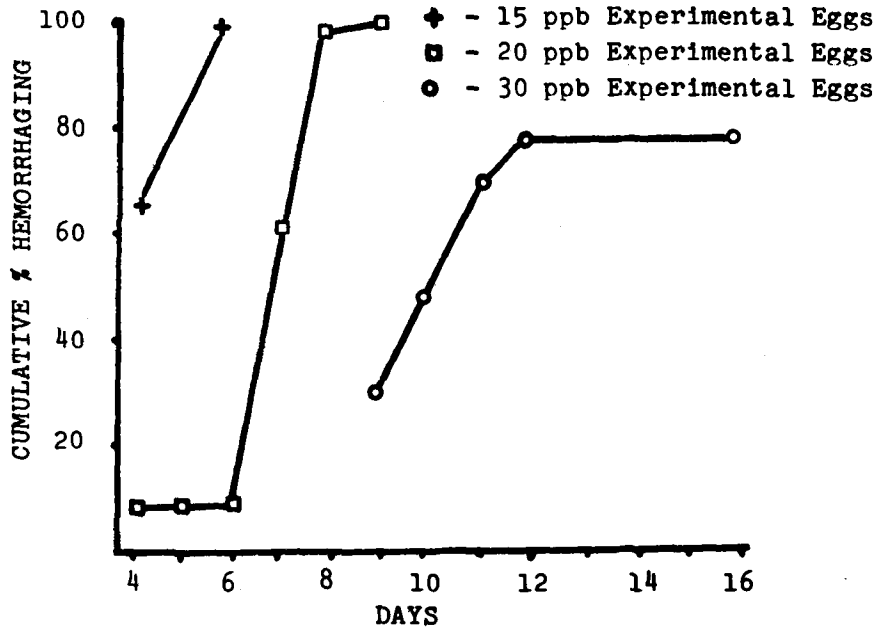

Fig. 1. Cumulative % hemorrhaging for eggs incubated in various concentrations of mercuric chloride.

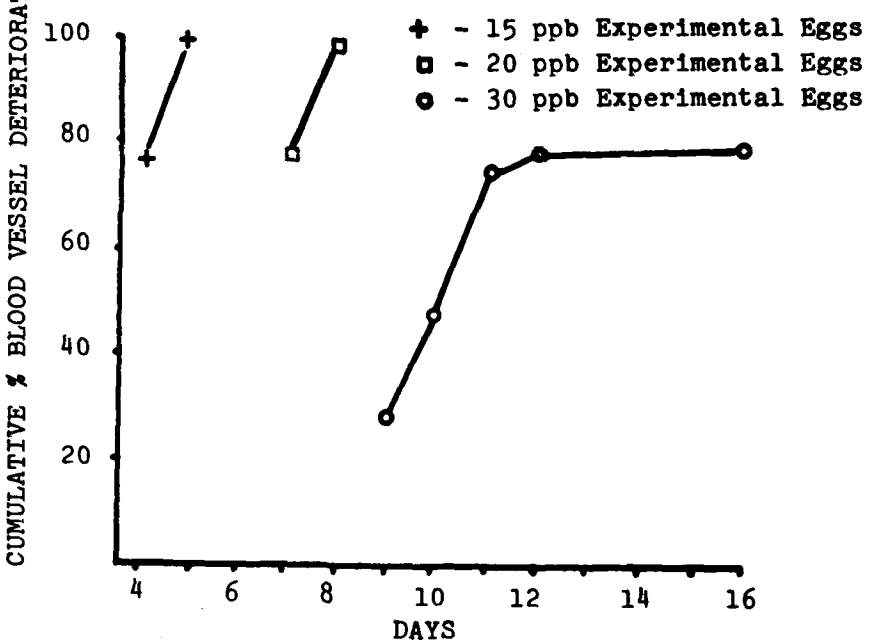

Fig. 2. Cumulative % blood vessel deterioration in eggs incubated in various concentrations of mercuric chloride.

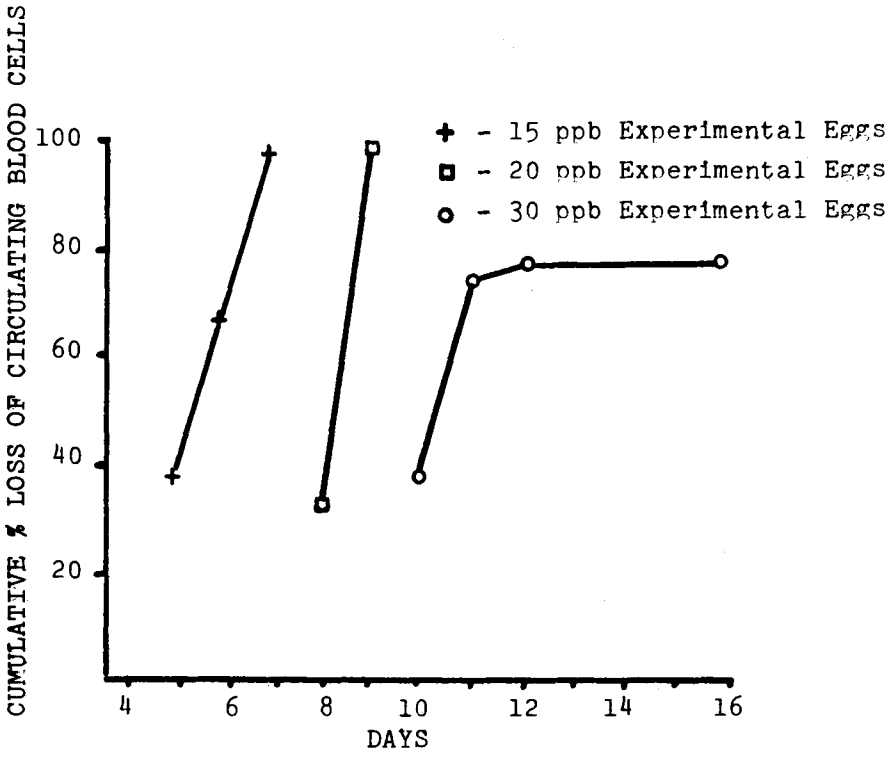

Fig. 3. Cumulative % loss of circulating blood cells in eggs incubated in various concentrations of mercuric chloride.

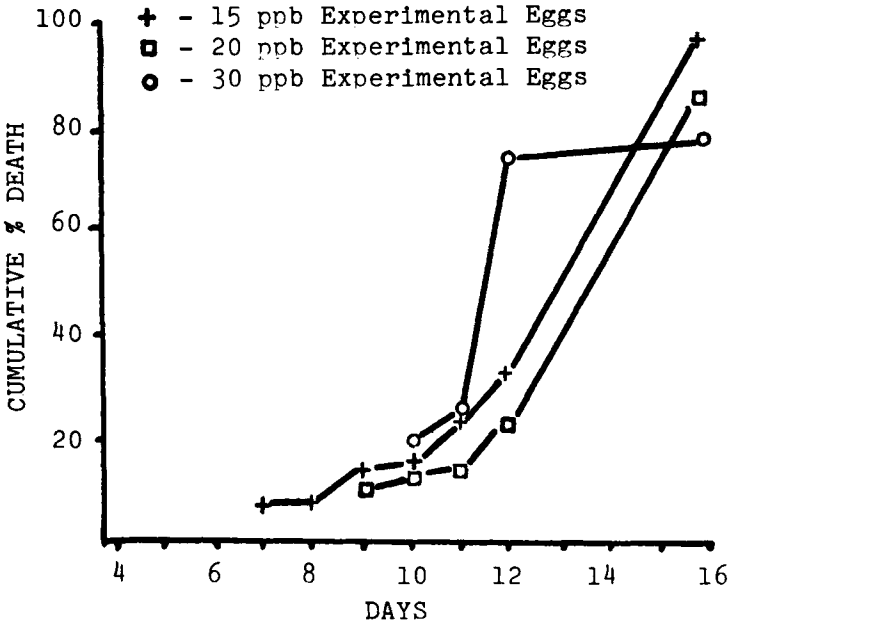

Fig. 4. Cumulative % death of eggs incubated in various concentrations of mercuric chloride.

The red color of the unconfined blood clusters gradually decreased until no pigment was visible. This color decrease took approximately two days. After about three days there was a complete absence of pigment in all portions of the embryo's circulatory system, except where blood elements were trapped within a vessel by vascular adhesion. In the latter case, the erythrocytes retained their red color. At this stage no circulation was visible, although the heart continued to beat. It is possible that the loss of blood color resulted from hemolysis of the erythrocytes and subsequent destruction of the hemoglobin.

Several investigators have recorded hemolysis caused by mercury compounds (BENESCH and BENESCH, 1954; SHEETS et al., 1956 and 1958). JACOB and JANDL (1962) state that hemolysis results primarily from osmotic swelling of the cell caused by the mercurial inhibition of cell membrane sulfhydryl groups. In addition to hemolysis, the hemoglobin, which contains several sulfhydryl groups, is subject to sulfhydryl inhibition by metallic ions (INGRAM, 1955).

Blood vessel deterioration in the 15 ppb, 20 ppb, and 30 ppb experimental eggs started on the ninth, seventh and fourth days respectively. As used here, blood vessel deterioration denotes elongation of the sinus venosis and reduction in diameter of the cardinal and vitelline veins entering the sinus venosis. Approximately 24 hours before death the sinus venosis became extremely elongated and the cardinal and vitelline veins showed severe reduction in diameter. In addition the heart was greatly reduced in size. It is interesting to note that the heart continued to beat for several days despite its reduction in size, total loss of red blood cells and extreme elongation of the sinus venosis.

If we define death as the absence of heart beat, then the 16 day median tolerance limits (TLm) ranged between 10 and 15 ppb mercuric chloride. The TLm is the concentration of a pollutant at which 50 percent of the test animals die (Fig. 4).

The lethal concentration (LC_{100}) in this study was between 20 and 30 ppb. The LC_{100} is the minimum concentration of a pollutant which will kill 100 percent of the test animals. Had the test period been extended past 16 days, the seven living embryos in the 20 ppb experimental group probably would have died, lowering the LC_{100} to between 15 and 20 ppb mercury. These embryos, while still considered to be living, showed no circulation and exhibited extreme blood vessel deterioration.

The final concentration of mercury in the 10, 15, 20, and 30 ppb experimental eggs after the 16 day incubation period was 16,000, 29,000, 54,000, and 56,000 ppb respectively (Table II). The concentration factors were 1,590, 1,907, 2,677 and 1,864 respectively. All control eggs contained less than one ppb mercury.

TABLE II

Total Concentrations of Mercury in Eggs Incubated in Solutions Containing Various Amounts of Mercuric Chloride.

Embryo rearing solution (ppb mercury)	Total mercury concentration (ppb) after 16 days incubation	Concentration factor
Controls	1	
10	16,000	1,600
15	29,000	1,900
20	54,000	2,700
30	56,000	1,900

Several authors have reported high concentrations of mercury in fish taken from mercury contaminated waters (JOHNELS et al., 1967; HASSELROT, 1967; UNDERDAL and HASTEIN, 1971; FIMREITE et al., 1971). Concentration factors of 3,000 times or more occurred as mercury moved from water through an aquatic food chain to pike (JOHNELS et al., 1967). HANNERZ (1967) found a mean concentration factor of 981 with a range of from 87 ppb mercury in the muscle tissue to 2,928 ppb mercury in the gills in juvenile pike exposed to 30 ppb mercuric chloride for eight consecutive days. Hannerz's value represents concentration directly from water to the pike and does not involve a food chain. The experimental eggs in this study also concentrated mercuric chloride directly from an incubating solution without biological magnification due to a food chain.

Summary

Eggs collected from adult Japanese Medaka were incubated for 16 days in solutions containing 10, 15, 20, and 30 ppb mercury as mercuric chloride. The mean hatchability of control eggs was 46.7 percent. Experimental eggs incubated in 10 ppb and 15 ppb mercury had hatching percentages of 58.3 and 20.8 respectively (Chi Square = 12.8, $P < .0005$). None of the 20 ppb or 30 ppb experimental eggs hatched. Hemorrhaging, blood vessel deterioration and loss of blood cells were observed in 79 percent of the 15 ppb experimental eggs and all the 20 and 30 ppb experimental eggs. Neither the control nor the 10 ppb experimental eggs demonstrated any of these abnormalities. The LC_{100} for 384 hours was between 20 ppb and 30 ppb.

The 10, 15, 20 and 30 ppb experimental eggs concentrated mercury directly from the incubating solution 1,600, 1,900, 2,700, and 1,900 times, respectively. The total concentration of mercury in these eggs after 16 consecutive days was 16,000, 29,000, 54,000, and 56,000 respectively.

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