

# The Effect of Phenylmercuric Acetate upon the Frequency of Hatching of Eggs from the Zebrafish

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The rapidly increasing wasting of mercury and mercuric compounds into natural waters (1,2) raised the question, of whether the reproduction of aquatic animals is affected by these substances. The present communication deals with the effect of phenylmercuric acetate upon the frequency of hatching and the time required for development of eggs from zebrafishes.

## Materials and Methods

About 400 sexually mature zebrafishes (*Brachydanio rerio*) with a slight excess of males have been used for the experiments. The animals were distributed amongst thirteen experimental groups. Each group was kept in a funnel-shaped polythene-container having a total volume of about 30 liters. The diameter of the container was 36 cm, and the slope of the walls within the funnel  $54^{\circ}$ . At this inclination the eggs roll along the walls and are collected in the narrow part of the funnel. The bottom part of the funnel opened into a PVC-tube, ending in a conical flask. Within the container there was a net bag, made from nylon, preventing the fishes from reaching the walls and the deep parts of the funnel but permitting the passage of the eggs. Once a day the animals were given deep-frozen cod roe. The water used consisted of 2 parts of tap water from the town of Uppsala (total hardness in German degrees 13-16) and, in order to diminish the hardness, 1 part of distilled water. The water was continuously aerated and renewed once a week. The spawning thus took place in non-polluted water. The temperature of the water was thermostatically regulated at  $+27^{\circ}\text{C}$ . By means of black curtains and electrical lights the animals were subjected to a diurnal cycle with 12 hours of darkness throughout the year.

The contents of the conical flasks below the aquaria were collected once a day. The eggs obtained were counted, and every second egg transferred to a petri dish containing non-polluted, aerated water. The remaining eggs were placed in aerated water containing 10, 20, or 50 ng of phenylmercuric acetate per g (corresponding to  $3.0 \times 10^{-8}$ ,  $6.0 \times 10^{-8}$ , and  $1.5 \times 10^{-7}$  M-solutions). At each of these concentrations all the thirteen groups of fishes were represented by approximately the same

number of eggs. The dishes contained at the most 2 eggs per ml of water and were kept at  $+27^{\circ} \pm 0.5^{\circ}\text{C}$ .

The water in the dishes was renewed, and the dead eggs were removed once a day. The number of dead and hatched eggs were counted once a day until no further hatching occurred. Totally about 2700 eggs were studied. The polluted water was prepared from  $3.0 \times 10^{-5}$  M stock-solution of  $\text{C}_6\text{H}_5\text{HgO}_2\text{C}_2\text{H}_3$  (Hopkins & Williams, Ltd.).

### Results

As seen from Table 1 the frequency of hatching is significantly higher in water containing 10 ng phenylmercuric acetate per g than in the controls. At a concentration of 20 ng per g of water the frequency of hatching is the same as in the controls, and none of the studied eggs survived at a concentration of 50 ng/g (Table 1).

Table 1

The effect of phenylmercuric acetate upon frequency of hatching of eggs from zebrafishes. The eggs have been fertilized in non-polluted water and then transferred into water containing phenylmercuric acetate.

ng phenyl- mercuric acetate per g of water.	Total number of eggs studied.	Per cent of eggs hatched.	Number of eggs hatched.	Number of eggs not hatched.	Analysis  $\chi^2$ p
0	486	39	187	299	5.770 < 0.025
10	486	46	224	262	
0	739	30	224	515	0.012 $\approx$ 0.90
20	739	31	226	513	
0	138	34	47	91	56.646 < 0.0005
50	138	0	0	138	

Most eggs hatch about three days after fertilization. However, the frequency of eggs hatching up to and including the third day after fertilization is significantly higher in water containing 10 and 20 ng phenylmercuric acetate per g than in non-polluted water (Table 2).

Table 2

The effect of phenylmercuric acetate upon the time required for development from fertilization to hatching of eggs from zebrafishes. The eggs were fertilized in non-polluted water and then transferred into water containing phenylmercuric acetate.

ng phenyl- mercuric acetate per g of water	Total number of hatched eggs	Eggs hatched up to and including the third day after fertilization		Eggs hatched from the fourth day on. Number	Analysis	
		Per cent	Number		$\chi^2$	P <
0	187	46	85	102	20.138	0.0005
10	224	67	151	73		
0	224	42	93	131	19.667	0.0005
20	226	62	141	85		

### Discussion

*Brachydanio rerio* was used for the experiments as this species produces eggs throughout the year, is easy to breed, and has a rapid and carefully studied development (3). The mercuric compound studied, used in pulp and paper manufacture, has been chosen for practical reasons, as fishes seem to incorporate mercury more efficiently when the pollution comes from pulp and paper factories than from chlorine factories (4). However, in all species of fishes studied so far almost 100 per cent of the mercury found exist as methyl mercury (5, 6), irrespective of the form of mercury occurring in the environment. The explanation may be found in the fact that, liver homogenates (7) as well as aquatic microorganisms (8, 9, 10, 11) are able to methylate mercury. Moreover, also a non-enzymatic transfer of methyl groups from  $\text{Co}^{+++}$  to  $\text{Hg}^{++}$  may occur in biological systems under reductive conditions (11).

Phenylmercuric acetate is used industrially because of its bactericidal and fungicidal activity. The increased hatchability in water containing 10 ng phenylmercuric acetate per g, therefore, may be due to a decrease in effect of the aquatic microorganisms upon the developing eggs. However, it is also possible that low concentrations of mercuric compounds may increase the hatchability in other ways as well. Thus,

Borg (12, 13) found the hatchability of eggs from pheasants "to be slightly increased after feeding with mercury-treated seed for a few days but impaired after more prolonged mercury feeding."

The studied concentrations of phenylmercuric acetate are much higher than those up to present found in fresh water in nature, 0.01-0.1 ng Hg/g (14), 0.01 ng Hg/g (15), 0.13 ng Hg/g (2), 0.02-0.21 ng Hg/g (16), 0.02-0.12 ng Hg/g (17) and in rainwater, 0.2 ng Hg/g (18). However, recent studies indicate that even much lower concentrations of mercuric compounds will disturb the reproduction of fishes in other ways. These latter results will be published elsewhere.

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