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Toxic and Teratogenic Effects of Hydrazine on Fathead Minnow (*Pimephales promelas*) Embryos

V. Henderson[1], J. W. Fisher[2], and R. D'Allessandris[2]

[1]*Biology Department, Grambling State University, Grambling, LA 71245;* [2]*Air Force Aerospace Medical Research Laboratory, Toxic Hazards Division, Environmental Quality Branch, Wright-Patterson Air Force, Base, OH 45433*

Hydrazine is widely utilized in commercial industries and is employed as a missile fuel in Air Force operations (BACK et al. 1978). Hydrazine is toxic to aquatic organisms (SLONIM 1977; FISHER et al. 1980) and is capable of producing teratogenic effects (GREENHOUSE 1977; HENDERSON et al.1981). The widespread use of hydrazine suggests a need for assessing its potential impact upon the aquatic environment. Accidential spills during production, storage, fueling operations and transportation are inevitable.

We designed this investigation to simulate conditions of an accidential spill which might occur during the spawning season of the fathead minnow, Pimephales promelas. This study was undertaken to determine the sensitivity of cleaving eggs to hydrazine, in particular morphological abnormalities and how they affect hatching, growth rate and certain physiological functions which may possess survival value.

MATERIALS AND METHODS

Fathead minnow eggs in early cleavage were collected from ponds at the Environmental Protection Agency, Newtown Toxicology Station, OH. Eggs had been laid on the inside of tile pipes or underside of boards. Eggs were transported 85 km in a styrofoam. chest containing pond water at 21°C. Eggs were tempered in the laboratory at 21°C for 2 h prior to experimentation and had reached mid-cleavage when initially exposed to hydrazine.

EXPERIMENTAL DESIGN

Four recirculating units, dosed with 0.01, 0.1, 1.0 and 5.0 mg/L of hydrazine, respectively, and a control unit were held in a 573-L Living Stream containing water at 21°C for 48 h. Each unit consisted of the following: (1) a 40-L glass aquarium containing 28 L of toxicant solution or water for control; (2) a plastic funnel (26 cm in diameter) with an overflow port 2 cm below the lip and a one mm wire mesh platform (15 cm in diameter) placed 7 cm below the lip; (3) a Silent Giant® pump attached to the underside of the funnel by Tygon® tubing; and (4) a plastic container (10.5 cm in diameter and 7.5 cm in depth) with a fine mesh nylon bottom to hold the eggs. The pump provided a recirculating flow rate of 440 mL/min and the platform supported the egg holder. At least 200 eggs were exposed to each concentration

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of hydrazine, except for the 5.0 mg/L concentration which contained 147 eggs. Liquid anhydrous hydrazine (Eastman Chemical Company) was monitored colorimetrically using p-dimethylaminobenzaldehyde (REYNOLDS & THOMAS 1964). Twenty-five percent of the toxicant solution was removed from each aquarium twice per 24 h, then replenished to its original concentration and volume by the addition of a fresh toxicant solution.

Post-exposure incubation of the eggs was carried out by placing each funnel on a plexiglass support (165 cm X 8 mm with holes 21 cm in diameter) positioned above a Living Stream® containing 573 L of water at 21°C. Two submerged pumps provided a recirculating flow rate of about 300 mL/min to each funnel. Each group of treated and control eggs was equally divided between two egg chambers.

All water used in this study was treated by passing it through a one µm rope filter, a charcoal bed, then mixing water 1:1 with house-distilled water. The pH of the water ranged from 7.0 to 7.5, dissolved oxygen 9.0 to 9.8, and the $CaCO_3$ hardness was about 150 mg/L.

BIOLOGICAL ENDPOINTS

Hatching success, time of hatch (in day-degrees) and growth rates at two weeks post-hatching were determined. Physiological anomalies were examined at 24 h during the 48 h exposure of hydrazine, at hatching and two weeks post-hatching. The presence of body movement and hemoglobin were determined at the two later intervals. Heart beat intensity and the presence of pigment in the eye and on the body were determined at 48 h. Heart deformities, scoliosis and gaped mouth were noted at hatching and two weeks post-hatching. Additionally, several other morphological and physiological deformities existed at hatching. The deformities are defined thusly: (1) enlarged pericardial cavity is a condition where this structure is at least 30% greater in volume than normal; (2) tubular heart is a structural defect where the heart remains cylindrical and does not fold or twist upon itself; (3) absence of hemoglobin is a blood condition where there is a lack of color when observing the blood coursing through the capillary beds; (4) scoliosis is a lateral curvature of the spine; (5) body movement refers to the rhythmic contractions of the body parts or locomotion involving the entire body; (6) mouth deformities, primarily gaped mouth, occur when there is a misfit of the mandible and maxillary bones. Developmental arrest refers to a aneuralae-microcephalic condition in which the organism is alive but growth and differentiation are halted.

RESULTS AND DISCUSSION

Embryos exposed to 0.1, 1.0 and 5.0 mg/L of hydrazine for 24 h showed a number of physiological defects (TABLE 1). Morphological appearances of the control and 0.01 groups were identical (TABLE 1).

TABLE 1. Fathead minnow embryo responses at 24 h during exposure to hydrazine.

Hydrazine Conc (mg/L)	Heart Beat	Hemoglobin	Body Movement	Pigment in eye	Pigment on body	Development	
						Normal	Arrest
Control[1]	N	P	N	P	P	X	
0.01	N	P	N	P	P	X	
0.1	SN (SA)	SN (SA)	SN (M)	SN (SA)	P	X	
1.0	SN (M to W)	A	ND	SN (SA)	A		X
5.0	SN (W)	A	ND	SN (SA)	A		X

1. Twenty to thirty embryos were examined at each concentration and control.

2. The letters are identified as follows: N-Normal; P-Present; A-Absent; SN-Subnormal; ND-Non Detectable; SA-Small Amount; M to W- Moderate to Weak; and W-Weak.

The control and 0.01 mg/L groups had rhythmic heart beats and periodic body movement. Blood was a bright red color indicating the presence of hemoglobin and pigment was present in the eyes and on the body. Embryos exposed to 1.0 and 5.0 mg/L of hydrazine were severely affected. The heart rate of the 1.0 mg/L group was moderate to weak, while the 5.0 mg/L group was barely observable. Hemoglobin was absent in both groups blood cells when observed circulating through the blood vessels. There was an absence of muscular contractions and pigment in the eyes of both groups. A small amount of pigment was distributed over the body and the yolk sac regions were greatly enlarged in both groups. The embryo's conditions ranged from aneurulae to microcephalic indicating developmental arrest.

Embryos exposed to 0.1 mg/L of hydrazine for 24 h displayed responses intermediate between the control group and 0.01 and 1.0 mg/L groups. The heart beat was moderate and the blood was pink, indicating that the red blood cells did not possess a full complement of hemoglobin. A small amount of pigment was present in the eyes and a normal amount was distributed over the body surface. Muscular activity was subnormal. No sign of developmental arrest was observed. The reduction or lack of hemoglobin in the embryos exposed to 0.1, 1.0 and 5.0 mg/L did not cause death during the period of treatment. The ability of the embryos to survive may be related to: (1) hemoglobin not being converted to methemoglobin, a form that does not transport oxygen; (2) reduced muscular activity of the embryos thereby requiring less oxygen; (3) oxygen being transported by blood plasma and/or diffusing directly into the body cells. CHAPMAN (1980) suggests that a change in extracellular-intracellular calcium ratio results in reduced hemoglobin synthesis. If hydrazine combines with calcium, as suggested by SLONIM (1972), then the calcium ratio of fathead minnow eggs could have been altered by hydrazine, thereby affecting hemoglobin synthesis. Loss of pigment in the eyes or on the body of embryos exposed to 0.1 and 5.0 mg/L of hydrazine may be due to lack of melanin synthesis or a failure of melanophores to function.

The hatching success of control embryos was 96%. Embryos exposed to 0.01 and 0.1 mg/L of hydrazine were scarcely affected. These two groups had hatching rates greater than 90%. Hatching began on day 8 (141 day-degrees) and continued through day 10 (189 day-degrees) for the above groups. The hatching success in the 1.0 and 5.0 mg/L embryos was affected by hydrazine exposure. In the 1.0 mg/L embryos, 82% hatched beginning on day 10 (189 day-degrees) and extended through day 13 (252 day-degrees). Ninety-five percent of these embryos were in an aneurulae-microcephalic condition. There was an absence of well-defined fin folds, paired and unpaired fins. This group of embryos remained motionless on the bottom of the incubation chamber. Hatching rate of the 5.0 mg/L was significantly less than the control. Only 2% of the embryos hatched and these required 12 days (231 day-degrees). One-half of these embryos was normal and the other one half in an aneurulae-microcephalic condition. The developmental features of the 0.01 and 0.1 mg/L embryos appeared to be

similar to those of the control except in a few cases. Two per cent of the 0.01 mg/L embryos developed enlarged pericardial chambers and scoliosis. Gaped mouth was observed in 4% of the 0.01 mg/L embryos and 16% of the 0.1 mg/L embryos. The 1.0 mg/L embryos/larvae were significantly affected by exposure to hydrazine. Observations at hatching and two weeks post-hatching revealed several deformities (TABLE 2). The most common deformities were gaped mouth, tubular heart and scoliosis. Two week old larvae were unable to swim. The incidence of gross morphological defects in developing fathead minnow eggs parallels that found in other species (STRUHSAKER et al. 1974, KUHNHOLD 1972) exposed to various pollutants.

TABLE 2. Frequency of anomalies in fathead minnow embryos treated with 1.0 mg/L of hydrazine at hatching and two weeks post-hatching.

Anomaly	At Hatching[1]	Two Weeks Post-Hatching
Enlarged Pericardial Chamber	95	92
Tubular Heart	70	70
Absence of Hemoglobin	38	38
Scoliosis	100	100
Body Movement	26	44
Enlarged Abdomen	84	84
Gaped Mouth	80	56

[1]Frequencies are expressed as percentages and the sample size at each sampling period is 20 to 30 specimens.

The effect of hydrazine on larval length two weeks post hatching suggests that the 1.0 mg/L concentration is most critical with regard to influencing growth. The average length of these larvae was 3.7 mm (N=50). When compared with larval lengths of the control (6.22 mm), the average length of the 1.0 mg/L larvae was significantly different from the control. The reduced growth rate in the 1.0 mg/L larvae suggests that this feature was sacrificed in order to maintain embryonic metabolism. Embryonic metabolism has been reported (ANDERSON 1974) to increase during exposure to toxicants thereby leaving little substrate material for growth. Our results could be explained on a similar basis.

A 48 h exposure to 5 mg/L of hydrazine was lethal to fathead minnow eggs in mid-cleavage and 1.0 mg/L caused severe deformities. Eggs exposed to 1.0 mg/L appeared to have little chance for surviving. Concentrations of hydrazine below 1.0 mg/L did not ad-

versely affect developing fathead minnow eggs. These data suggest that in spill situations, concentrations $\geq$ 1.0 mg/L would have a significant effect on fathead minnow egg development.

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