

Swimming Endurance and Resistance to Copper and Malathion of Bluegills Treated by Long-term Exposure to Sublethal Levels of Hydrogen Sulfide¹

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ABSTRACT

After 126 or 148 days of exposure to H₂S concentrations ranging from .0004 to .0146 mg/l, young-of-the-year bluegills were tested for swimming endurance and resistance to copper or malathion.

Swimming tests at low speeds indicated increased endurance for fish exposed to .0004 mg/l H₂S, but fish exposed to higher concentrations had progressively less endurance than the controls. In the tests conducted at higher speeds fish in all test concentrations showed less endurance than the controls. Resistance to copper was increased by exposure to H₂S, but resistance to malathion was not affected except in the lowest test concentration. The chronic exposure to H₂S also reduced growth in the highest concentration, and gill irrigation rate increased progressively with increased concentrations.

INTRODUCTION

Hydrogen sulfide in very low concentrations is detrimental to fish eggs, fry, and juveniles (Colby and Smith, 1967; Adelman and Smith, 1970; and Smith and Oseid, 1972). Because it occurs in many natural and polluted situations as a by-product of decomposition and breakdown of other materials, it has been investigated in a series of studies in University of Minnesota fishery laboratories. Long-term exposure of fish to sublethal levels of H₂S results in slower growth, gradually increasing mortality rate, and in some species reduced fecundity (L. L. Smith, Jr., unpublished data). The study reported herein has attempted to determine the effect of long-term exposure of bluegills (*Lepomis macrochirus*) to sublethal levels of H₂S on their physical endurance and resistance to other toxicants. Specifically, the objectives were to determine swimming capability against various water velocities and resistance to copper sulfate and malathion.

Colby and Smith (1967) showed that 50% of walleye fry (*Stizostedion vitreum*) were killed in 96 hours by total sulfide levels of .05 mg/l. Adelman and Smith (1970), working

on northern pike (*Esox lucius*), showed that eggs, fry, and juveniles were adversely affected by H₂S levels as low as .008 ppm and 96-hr median tolerance limit (TL₅₀) for eggs was .037 ppm and fry was .008 ppm. Smith and Oseid (1972) reported on effects of H₂S on eggs and fry of several freshwater species and found the 96-hr TL₅₀ varied from .028 to .064 ppm and that exposure of 12 days resulted in a TL₅₀ of .018 ppm.

MATERIALS AND METHODS

Experimental Fish

Young-of-the-year bluegills were collected from Medicine Lake, Hennepin County, Minnesota with bag seine on 7 October, 1969 when water temperature was 16.7 C. The mean total length was 3.2 cm (range 2.7–4.8 cm). From the time of collection to the start of chronic exposure tests, fish were held at 21 ± 1 C in laboratory water (Table 1) and were fed three to six times daily with brine shrimp (*Artemia* sp.) and ground pork liver. On 3 successive days immediately following collection they were given a prophylactic treatment with 20 mg/l neomycin sulfate without flushing. Two days after completion of neomycin treatment, fish were subjected to similar treatments for 3 days with a solution of 2.65 mg/l methylene blue.

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Acute and Chronic Tests with Hydrogen Sulfide

A series of 96-hr TL_{50} tests were conducted 28, 42, and 99 days after collection of fish in apparatus described by Adelman and Smith (1970). Test chambers were 6 liter acrylic cylinders 14.5 cm in diameter. Gaseous H_2S was employed and test concentrations were determined by analysis of water in centers of test chambers. Four levels of H_2S and one control were used and water flowed through chambers at a rate of 300 ml/min.

Long-term exposure tests were started on 100 fish 14 days after collection. They were placed in 5 tanks each divided into three sections: 2, $15.5 \times 41.1 \times 21$ cm and 1, $9 \times 30 \times 21$ cm. Ten fish were placed in each of 2 sections with the third and smaller section kept in reserve for holding fish after raceway tests. Dilutions of a stock solution of sodium sulfide were made and dispensed to the test tanks by apparatus modified from that described by Mount and Brungs (1967) and Brungs and Mount (1970). Four test levels and one control were maintained and checked by analysis in each section. Fish in long-term tests were fed six times per day with various combinations of brine shrimp, ground beef liver, fresh ground minnows, and $\frac{1}{8}$ " Oregon moist pellets. Water temperatures were maintained at desired levels by hot water coils of polyethylene controlled with thermostat and solenoids on the hot water source. A constant level of 7.8 pH was maintained with H_2SO_4 dispensed from a diluter similar to that used for the toxicant.

Endurance Test Apparatus

The swimming endurance of treated bluegills was tested in a stainless steel oval raceway with a channel width of 8.5 cm, a depth of 15 cm, and a length of 218 cm. The channel included 2 straight sections each 50 cm long. Untreated laboratory water was maintained at a depth of 9 cm in the channel and the desired current created with a stainless steel paddle wheel with 16 blades. The apparatus was similar to that described by Lemke and Mount (1963). Water velocity was regulated by a rheostat on the $\frac{1}{8}$ hp motor

drive of the paddle wheel. Fish were kept in position until they failed by an electrical shocker placed downstream from their swimming location. One hundred and twenty volt AC current was used with the minimum amperage required to keep the fish away from the poles. One fiberglass screen was placed ahead of the poles to prevent fish from being injured by drifting between the electrodes and a second screen 22 cm upstream to form a swimming area 8.5×22 cm. Temperatures were maintained by immersion of the raceway in a water bath.

Swimming Test Procedure

A single fish was selected at random from one of the long-term exposure tanks, placed in a 400 ml beaker without removal from water, and transferred to the raceway swimming area. It rested in this position without electrical stimulation or water current for 30 minutes. Temperature of water was held the same as that in the long-term experiment. Low speed and high speed tests were conducted with the same procedure until water flow was started. In low speed tests after the 30 minute rest period, current velocity was set at 8 cm/sec. After 5 minutes current was increased to 11.2 cm/sec, and after 7 minutes to 15 cm/sec. At this time the electrical shocker was activated to prevent the fish from lying against the screen or holding its position by resting the caudal fin against the screen. At 9, 11, and 13 minutes the velocity was increased to 17.0, 19.5, and 22.5 cm/sec, respectively. The maximum velocity was maintained until the fish fell back against the screen. The time was then noted, electrical current shut off, and water movement stopped. After 5 minutes, fish were anesthetized in MS 222, weighed, measured, and returned to the unoccupied section of their original test tank. One fish was taken from each tank successively until all fish from one section were tested. They were then returned to their original test section.

In the high speed tests, fish were taken from the second section of each long-term test and placed in the raceway. After a 30-minute rest, water velocity was started at 8 cm/sec. After 5 minutes velocity was in-

TABLE 1.—Analysis of well water used in laboratory test¹

| Item | Value |
|-------------------------------------|-----------|
| pH | 7.5 |
| Total hardness as CaCO ₃ | 220 mg/l |
| Calcium as CaCO ₃ | 140 mg/l |
| Iron | .02 mg/l |
| Manganese | .04 mg/l |
| Chloride | <1.0 mg/l |
| Sulfate | <.5 mg/l |
| Fluoride | .22 mg/l |
| Total phosphates | .03 mg/l |
| Sodium | 6 mg/l |
| Potassium | 2 mg/l |
| Ammonia nitrogen | .20 mg/l |
| Organic nitrogen | .20 mg/l |

¹ Water taken from well head and before aeration and heating.

creased to 10.7 cm/sec and at 7 minutes was further increased to 17.5 cm/sec and the electrical shocker activated. Subsequently at 9, 11, 13, 30, and 45 minutes velocity was increased to 19.7, 22.5, 25.0, 27.0, and 28.0 cm/sec, respectively. After swimming failure of the fish procedure was the same as in low speed tests.

Chemical Stress Tests

Chemical stress tests were carried out with apparatus similar to the proportional diluter used in long-term tests except that it was designed to dispense the same toxicant levels to all test chambers. The toxicant was introduced in a mixing box and then to a distribution box which split the volume into 5 equal portions for distribution to test chambers, each of which was 50 × 26 × 30 cm. Water depth was 22 cm and cycle time was 2 minutes. Dilution water was from common laboratory source (Table 1) which contained no H₂S.

Reaction to Long- and Short-term Exposure

The 96-hr TL₅₀ tests were conducted on untreated fish 28, 42, and 99 days after bluegills were collected. Mean water conditions during the tests were 20 ± 0.1 C, 6.2 ± 0.4 mg/l dissolved oxygen, 7.90 ± .05 pH, and 235 ± mg/l total alkalinity. TL₅₀ was calculated from five test levels and one control. 96-hr

TL₅₀ values for the three tests were .0325, .0320, and .0325 mg/l of H₂S. As noted above, each test tank in long-term tests was divided into 3 sections with fish placed in the first two. Since there was a reduction of H₂S levels between the first (A) and second (B) sections, analyses were run on each section. In series "A" H₂S concentrations ranged from .0004 to .0146 mg/l, and in series "B" from .0004 to .0067 mg/l (Table 2).

After 126 days exposure in series "A," the concentrations had no effect on survival (Table 3), and growth in length appeared to be affected adversely only at the highest concentration (.0146 mg/l) where weight was less than two-thirds that attained in the control. Gill irrigation rate of treated fish was significantly increased over that in controls for all treatments and was 139% greater in the highest concentration.

After 148-day exposure in series "B," survival was not affected, and growth was not adversely affected. Gill irrigation rate determined visually increased in fish from all treatments and those from the highest H₂S concentration (.0067 mg/l) had 42.5% greater irrigation rate than the controls.

SWIMMING ENDURANCE

Swimming endurance was adversely affected by chronic exposure to H₂S (Fig. 1). In series "A" where fish had been exposed to the higher concentrations, the low speed tests indicated that those from the lowest concentration (.0004 mg/l) of the series had slightly increased capability to endure swimming stress. Controls swam for 201 minutes before failure, and fish from the highest treatment (.0146 mg/l) for 31 minutes or 84% shorter time than the controls (Table 3). Fish from series "B," which had been exposed to lower maximum concentrations of H₂S but to greater swimming stress, had less endurance. Those in the lowest concentration had the least resistance (36% less than controls). The controls in the "B" series had 86% less time to failure than controls in the "A" series, strongly suggesting that the series "B" fish were being subjected to a major physical stress by the swimming test.

TABLE 2.—Test conditions in chambers of Series A and B during chronic tests

| Item ¹ | Chamber—Series A | | | | |
|---------------------------------|------------------|-------|-------|-------|-------|
| | 5a | 4a | 1a | 3a | 2a |
| H ₂ S mg/l (mean) | 0 | .0004 | .0015 | .0048 | .0146 |
| Standard deviation (mg/l) | — | .0008 | .0012 | .0020 | .0050 |
| pH | 7.72 | 7.74 | 7.77 | 7.8 | 7.90 |
| Temperature (C) | 24.1 | 24.0 | 24.0 | 24.1 | 24.1 |
| Dissolved O ₂ (mg/l) | 6.20 | 6.49 | 6.58 | 6.66 | 6.49 |
| Total alkalinity (mg/l) | 191 | 191 | 191 | 191 | 191 |

| Item | Chamber—Series B | | | | |
|---------------------------------|------------------|-------|-------|-------|-------|
| | 5a | 4a | 1a | 3a | 2a |
| H ₂ S (mg/l) | 0 | .0004 | .0007 | .0022 | .0067 |
| Standard deviation (mg/l) | — | .0007 | .0006 | .0014 | .0032 |
| pH | 7.72 | 7.73 | 7.77 | 7.79 | 7.91 |
| Temperature (C) | 23.7 | 23.5 | 23.6 | 23.7 | 23.7 |
| Dissolved O ₂ (mg/l) | 5.87 | 6.21 | 6.18 | 6.13 | 6.01 |
| Total alkalinity (mg/l) | 191 | 191 | 191 | 191 | 191 |

¹ All values given are means of weekly or biweekly tests.

TABLE 3.—Effect of chronic exposure of bluegills to various levels of H₂S for 126 days (A) and 148 days (B) on growth, gill irrigation rate,¹ and swimming endurance. (A-series tests at 8.0 cm velocity/sec; B-series at 8.28 cm/sec²)

| Item | Chamber—Series A (Slow) | | | | |
|---|-------------------------|-------|-------|-------|-------|
| | 5a | 4a | 1a | 3a | 2a |
| H ₂ S concentration (mg/l) | 0 | .0004 | .0015 | .0048 | .0146 |
| Fraction of 96-hr LC ₅₀ | — | 1/81 | 1/22 | 1/7 | 1/2 |
| Survival (%) | 90 | 100 | 90 | 90 | 90 |
| Mean total length (cm) ³ | 5.68 | 5.21 | 4.78 | 5.51 | 4.54 |
| Mean weight (g) | 3.69 | 2.88 | 2.03 | 3.58 | 1.91 |
| Mean gill irrigation rate (No./min) | 46 | 57 | 65 | 62 | 110 |
| % of control | 0 | +24 | +41 | +35 | +139 |
| Swimming endurance Time to failure (min) | 201 | 241 | 95 | 110 | 31 |
| % of control | — | +20 | -53 | -41 | -84 |

| Item | Chamber—Series B (Fast) | | | | |
|---|-------------------------|-------|-------|-------|-------|
| | 5b | 4b | 1a | 3a | 2a |
| H ₂ S concentration (mg/l) | 0 | .0004 | .0007 | .0022 | .0067 |
| Fraction of 96-hr LC ₅₀ | — | 1/81 | 1/46 | 1/13 | 1/5 |
| Survival (%) | 100 | 100 | 90 | 100 | 100 |
| Mean total length (cm) ³ | 5.42 | 5.00 | 5.22 | 5.40 | 5.14 |
| Mean weight (g) | 2.86 | 2.16 | 2.59 | 3.18 | 3.42 |
| Mean gill irrigation rate (No./min) | 52 | 71 | 73 | 73 | 74 |
| % of control | 0 | +36.5 | +40.5 | +40.5 | +42.5 |
| Swimming endurance Time to failure (min) | 28 | 18 | 21 | 19 | 22 |
| % of control | — | -36 | -25 | -32 | -21 |

¹ Gill irrigation check after 106 days exposure.

² Text explanation for speed change.

³ Fish in all chambers had a mean length at start of chronic test of 3.23 cm (range 2.70–4.80).

TABLE 4.—Resistance of bluegill with chronic treatment of H_2S to subsequent exposure of copper sulfate (as Cu) (A) and malathion (B)¹

| Item | Chamber—Series A | | | | |
|--|------------------|-------|-------|-------|-------|
| | 5a | 4a | 1a | 3a | 2a |
| H_2S concentration (mg/l) ¹ | 0 | .0004 | .0015 | .0048 | .0146 |
| Copper mg/l (unfiltered) | 5.5 | 7.5 | 9.2 | 8.2 | 7.0 |
| Copper mg/l (filtered) | 3.8 | 3.8 | 4.0 | 3.8 | 3.8 |
| Temperature (C) | 24.3 | 24.1 | 24.3 | 24.5 | 24.0 |
| Dissolved O_2 (mg/l) | 6.65 | 6.61 | 6.63 | 6.63 | 6.58 |
| Survival time (hours) | 13.5 | 13.5 | 12.5 | 24.0 | 52.5 |
| % control | | 0 | -7 | +78 | +288 |

| Item | Chamber—Series B | | | | |
|-----------------------------|------------------|-------|-------|-------|-------|
| | 5a | 4a | 1a | 3a | 2a |
| H_2S concentration (mg/l) | 0 | .0004 | .0007 | .0022 | .0067 |
| Malathion (mg/l) | .075 | .075 | .075 | .075 | .075 |
| Temperature (C) | 24.0 | 24.0 | 24.1 | 23.9 | 23.9 |
| Dissolved O_2 (mg/l) | 7.79 | 8.05 | 8.05 | 7.87 | 8.02 |
| Survival time (hours) | 72.5 | 94.0 | 75.5 | 72.5 | 72.5 |

¹ Total alkalinity in Series A 212 mg/l and in Series B 216 mg/l; pH in Series A 7.5 and in Series B 7.6.

RESISTANCE TO COPPER AND MALATHION

After fish finished swimming tests, they were returned to the treatment tanks and allowed to remain for 18 days before being subjected to tests with copper or malathion. Fish of the "A" series were used in copper tests. While efforts were made to maintain the same copper treatment with all fish tested, the con-

centration of total copper in unfiltered samples varied from 5.5–9.2 mg/l (Table 4). Copper in filtered samples was 3.8 mg/l from all chambers except the one containing fish treated at .0015 mg/l H_2S where concentration was 4.0 mg/l. Survival time varied from 13.5 hours in controls and lowest H_2S group to 52.5 hours in highest H_2S group (+288% of control survival time).

The "B" series fish were tested with malathion at a concentration of .075 mg/l. Controls and fish exposed to the highest concentration survived for 72.5 hours. Fish conditioned at the lowest H_2S treatment (.0004 mg/l) had a significantly longer survival time than controls or those receiving the highest concentration (94.0 hours) (Table 4).

DISCUSSION

The gross effects of long-term exposure to H_2S were reduced growth in the highest concentration and progressively increased gill irrigation rate with increased concentrations of H_2S . This increase in irrigation rate suggests decreased efficiency in the oxygen uptake or transport. In the lower speed swimming stress tests given the series "A" fish, pretreatment at the lowest concentration appeared to increase endurance; but at treatment levels of .0015 mg/l and higher, fish

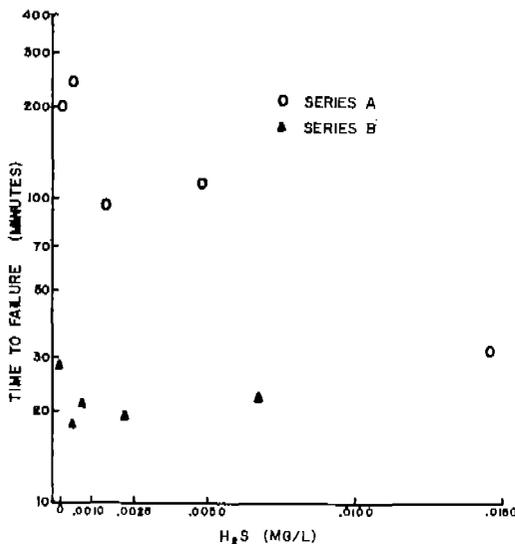


FIGURE 1.—Swimming endurance of fish treated at low levels of H_2S for extended periods (126–148 days) at low speed (A) and high speed (B). Resistance expressed as minutes to swimming failure.

had progressively less endurance. Fish treated at the highest level (.0146 mg/l) were much more easily stunned by electrical shock at the time of failure than those treated at lower levels. In the high speed tests all fish showed much less resistance to the swimming stress, but differences among treatments and controls were less marked than in low speed tests at comparable treatment levels.

Resistance to copper was increased by exposure to H₂S but resistance to malathion was not affected except in the lowest concentration. Since copper affects oxygen uptake by gills and H₂S-oxygen relationships in the blood, the increased irrigation rate induced by exposure to higher levels of H₂S may account for the higher tolerance to copper by treated fish. At the cellular level, H₂S combines with metallic elements (Goodman and Gillman, 1955). Whether this reaction influenced resistance to copper in the present experiments was not determined. Unpublished data by the authors on the fathead minnow (*Pimephales promelas*) show that long-term exposure to H₂S increases tolerance to H₂S in 96-hour acute tests.

From the data developed in this study, it is apparent that slow speed swimming tests will reveal adverse effects of long-term exposure to H₂S on bluegills better than the other gross indicators of changes commonly used. It is also apparent that extended expo-

sure to H₂S levels of .0015 mg/l and greater reduces the physical capability of the fish. The ecological effect of this reduction will be determined by other environmental stress factors which affect the fish.

ACKNOWLEDGMENTS

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LITERATURE CITED

ADELMAN, I. R., AND L. L. SMITH, JR. 1970. Effect of hydrogen sulfide on northern pike eggs and sac fry. *Trans. Amer. Fish. Soc.* 99(3): 501-509.

BRUNGS, W. A., AND D. I. MOUNT. 1970. A water delivery system for small fish holding tanks. *Trans. Amer. Fish. Soc.* 99(4): 799-802.

COLBY, P. J., AND L. L. SMITH, JR. 1967. Survival of walleye eggs and fry on paper fiber sludge deposits in the Rainy River, Minnesota. *Trans. Amer. Fish. Soc.* 96(3): 278-296.

GOODMAN, L., AND A. GILLMAN. 1955. The pharmacological basis of therapeutics. MacMillan Co., 2nd ed. p. xiii + 1-1831.

LEMKE, A. E., AND D. I. MOUNT. 1963. Some effects of alkyl benzene sulfonate on the bluegill, *Lepomis macrochirus*. *Trans. Amer. Fish. Soc.* 92(4): 372-378.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. *Water Res.* 1: 21-29.

SMITH, L. L., JR., AND D. OSEID. 1972. Toxic effects of hydrogen sulfide on juvenile fish and fish eggs. *Proc. 25th Purdue Ind. Waste Conf.*, p. 739-744.