Long-term Effects of Hydrogen Sulfide on *Hexagenia limbata* (Ephemeroptera)[1]

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ABSTRACT

Nymphs of the mayfly *Hexagenia limbata* (Serville) were subjected to hydrogen sulfide in test chambers with a mud substrate into which they could burrow. Acute and chronic tests were in flow-through apparatus. At 17.8–18.3°C and O_2 of 4.5–6.6, 96-h LC_{50} was 0.165 mg/liter H_2S and LC_{50} at 12 days was 0.060 mg/liter H_2S. In chronic tests running 138 days with 0.029 mg/liter H_2S, 37% mortality occurred, and at 0.0762 mg/liter H_2S, none survived. No subimagos emerged at concentrations of 0.0348 mg/liter H_2S but below this level 30–70% emerged.

Hydrogen sulfide has been shown to be toxic at relatively low levels to many aquatic organisms. It is evolved from bottom sediments and is produced by heterotrophic sulfate-reducing bacteria or by anaerobic decomposition of organic sediment which contains sulfur. High concentrations (up to 0.08 mg/liter) are generally confined to the first 1 or 2 cm of water above the mud surface (Colby and Smith 1967, Bella et al. 1972, Adelman, unpublished[2]). In the present study the reaction of *Hexagenia limbata* (Serville) to H_2S was investigated. The nymphs inhabit U-shaped burrows dug in fine bottom sediment. Eriksen (1968) demonstrated that the stratum of water available to 20–35 mm nymphs was the 6–7 mm layer above the substrate. Earlier nymphal stages (<1 mm at hatch) have a correspondingly reduced water stratum available. It is therefore apparent that the nymphal stage of the burrowing mayfly is in a situation where exposure to H_2S is likely. Oseid and Smith (1974a) described the acute effects of H_2S on *H. limbata* nymphs under various environmental conditions. The present study was designed to determine the effects of chronic exposure to H_2S under general habitat conditions as close as possible to optimum for the species. A second objective was to compare low levels of H_2S which have adverse effects on nymphs with 96-h LC_{50} concentrations.

Materials and Methods

Nymphs of the same age group were collected from the Crystal Beach area of Rainy Lake near Ranier, Minn., on May 19, 1973, at 2–4 ft. Mud with nymphs still in the burrows was placed in a screen-bottomed box, suspended in lake water, and the mud gently washed out. Nymphs were transferred to the laboratory in lake water. The mean length of the nymphs from the tip of the rostrum to the tip of the abdomen was 18 mm (range 16–20 mm). Prior to testing nymphs were held in glass aquaria (50×25×20 cm) with a water depth of 16 cm. Three cm of mud from the same area in which nymphs were collected was placed on the bottom. The 200 nymphs held in each aquarium formed burrows within a few hours after placement. Water at 18°C, *p*H 7.8, and saturated with O_2 was passed through each aquarium at the rate of 500 ml/min during the pretest holding period. The nymphs were fed a finely ground suspension of lettuce twice daily and recently hatched brine shrimp once each day. At the start of each test nymphs were randomly distributed to test chambers.

The acute test was conducted in flow-through apparatus with 5 toxicant concentrations and one control as described by Colby and Smith (1967) and Adelman and Smith (1970). Toxicant was added as sodium sulfide from stock solution by the dipping bird dispenser. H_2S levels were determined from ionization constant of Pomeroy (1941). Test chambers for acute studies were identical with those used for laboratory maintenance of the nymphs. Water flow through each chamber was 300 ml/min and a light cycle of 12-h light and 12-h darkness was maintained. Nymphs were not fed during the first 96 h of each test but thereafter were fed on the same schedule as the laboratory stock. Analyses were made 3 times each day for H_2S and once each day for *p*H, temperature, dissolved oxygen, and total alkalinity. Water samples for the determination of H_2S, *p*H, temperature, and dissolved oxygen were siphoned from near the mud-water interface of each chamber. H_2S samples were fixed immediately, insuring that the exact concentration bathing the nymphs was determined. Test water (Table 1) from the laboratory well was delivered to test apparatus through PVC pipe.

Quantitative analysis for H_2S was made in accordance with methods outlined by American Public Health Association et al. (1960), except that a colorimeter and a dissolved sulfide standard curve were used. Hydrogen sulfide concentrations in acute tests ranged from 0.0251–0.4723 mg/liter with one control (Table 2). Total alkalinity as $CaCO_3$ was constant at 235 mg/liter. A total of 10 individuals was placed in each test chamber.

Chronic tests employed 2 diluters modified from that of Brungs and Mount (1970) and the same test chambers described for nymph maintenance. Each test consisted of 4 concentrations of H_2S ranging

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[2] Adelman, I. R. 1969. Survival and growth of northern pike (*Esox lucius* L.) in relation to water quality. Ph.D. Thesis, Univ. Minnesota, St. Paul, 195 pp. (Univ. Microfilm, Ann Arbor, Mich. No. 69-20,075).

Table 1.—Analysis of well water used in laboratory tests.[a]

Item	Concentration (mg/liter)
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Iron	0.02
Chloride	<1
Sulfate	<5
Sulfide	0.0
Floride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Copper	0.0004
Manganese	0.0287
Zinc	0.0044
Cobalt, nickel	<0.0005
Cadmium, mercury	<0.0001
Ammonia nitrogen	0.20
Organic nitrogen	0.20

[a] Water taken from well head and before aeration and heating; *p*H 7.5.

from 0.0–0.0762 mg/liter and one control (Table 3). Temperature and *p*H were controlled and water was saturated with O_2 in the head tanks. Chemical analyses were made in the same manner as in the acute tests. H_2S, *p*H, and temperature were determined 3 times per week and dissolved oxygen once weekly. The feeding schedule was that used for laboratory maintenance. Chronic treatment of nymphs was started on July 9, 1973, and terminated 138 days later. Mortality of nymphs and emergence of subimagos were determined daily. LC_{50} values were calculated by graphical interpolation.

Results

The LC_{50} concentration of H_2S in acute tests dropped from 0.312 mg/liter at 48 h to 0.165 mg/liter at 96 h and at 12 days was 0.060 mg/liter (Table 4). Percentage survival dropped in the lowest treatment after 6 days but in all others after the first or second day.

On the basis of nymphal survival and the percentage which emerged as subimagos in the chronic tests, it was shown that concentrations up to 0.0152 mg/liter H_2S in the Diluter 1 test and 0.0129 mg/liter H_2S in the Diluter 2 test are not different from the controls (Table 5). Mortality was low (0–9%) in all concentrations lower than 0.0290 mg/liter H_2S. At this concentration mortality was 37% and at 0.0762 mg/liter H_2S none survived. There was no significant reduction in length of subimagos as the H_2S concentration increased in each experiment. Nymphs exposed to 0.0152 mg/liter H_2S were 6% shorter and at 0.0129 and 0.0290 mg/liter H_2S were 3% shorter than the controls. The percentage of subimagos emerging varied from 30–75% at levels below 0.0348 mg/liter H_2S. At this concentration and higher no emergence occurred.

Discussion

These tests indicate that the no-effect concentrations of H_2S may be exceeded under natural conditions and frequently are exceeded where sludge or other organic deposits result from pollution. Authors cited above found H_2S concentrations at detrimental levels in potential habitats for mayflies. Since Oseid and Smith (1974a) noted avoidance reactions of mayflies well below acute levels of H_2S, the organisms may seek favorable conditions before H_2S rises to a deleterious concentration.

Application of a factor to acute toxic concentration of a material in order to predict no-effect levels for an organism has been widely used to set water

Table 2.—Characteristics of test during exposure of *Hexagenia limbata* in the acute test.

H_2S—mean (mg/liter)	0	.0251	.0466	.1078	.2890	.4723
H_2S—Std. Dev. (mg/liter)	–	.0123	.0159	.0227	.0230	.0151
*p*H—mean	7.99	7.90	7.90	7.84	7.73	7.67
Temperature—mean (C)	18.3	18.2	18.2	18.1	17.8	17.9
Dissolved O_2—mean (mg/liter)	6.63	6.03	6.22	5.81	4.94	4.53

Table 3.—Test conditions during chronic exposure of *Hexagenia limbata*.

	Diluter 1				
H_2S—mean (mg/liter)	0	.0011	.0060	.0152	.0348
H_2S—Std. Dev. (mg/liter)	–	.0009	.0022	.0050	.0105
*p*H	7.9	7.9	8.0	8.0	8.0
Temperature—mean (C)	17.7	17.8	18.0	17.7	17.8
Dissolved O_2—mean (mg/liter)	7.53	7.65	7.37	6.86	5.52
	Diluter 2				
H_2S—mean (mg/liter)	0	.0042	.0129	.0290	.0762
H_2S—Std. Dev. (mg/liter)	–	.0017	.0060	.0096	.0153
*p*H	7.8	7.9	7.9	7.9	8.2
Temperature—mean (C)	17.5	17.8	17.6	17.7	17.9
Dissolved O_2—mean (mg/liter)	7.34	7.11	6.84	6.06	4.75

Table 4.—Percentage survival of nymphs and calculated LC_{50} of H_2S to *Hexagenia limbata* on succeeding days test.[a]

Day	LC_{50} (mg/liter H_2S)	Test concentrations of H_2S (mg/liter)					
		0.0	.0251	.0466	.1078	.2890	.4723
1	–	100	100	100	100	100	100
2	0.312	100	100	90	100	60	0
3	0.185	100	100	90	90	30	–
4	0.165	100	100	80	80	20	–
5	0.135	100	100	80	80	0	–
6	0.134	100	100	60	80	–	–
7	0.120	100	90	50	70	–	–
8	0.110	100	90	50	60	–	–
9	0.090	100	80	50	40	–	–
10	0.072	100	80	50	20	–	–
11	0.072	100	80	50	20	–	–
12	0.060	100	80	50	0	–	–

[a] Survival values have been corrected on the basis of survival in the control tank (Abbott 1925).

Table 5.—Summary of survival of *Hexagenia limbata* nymphs and emergence of subimagos during the chronic test.

	Diluter 1					Diluter 2				
H_2S (mg/liter)	0	.0011	.0060	.0152	.0348	0	.0042	.0129	.0290	.0762
Initial number	12	13	10	11	11	13	13	12	11	11
Deaths (%)	8	8	0	9	36	8	0	0	37	100
Subimagos emerged (%)	50	38	30	36	0	54	62	75	36	0
Emerged or survived in 138 days (%)	92	92	100	91	64	92	100	100	73	0

quality standards. In the present study it was possible to compare such a factor for the mayfly with another arthropod that we reported on elsewhere.

With a 96-h LC_{50} concentration of 0.165 mg/liter H_2S and the highest safe level of 0.0152 mg/liter, the application factor would be 0.09. A ratio based on the 12-day LC_{50} concentration of 0.060 mg/liter H_2S would be 0.25. A comparison of LC_{50}, highest safe concentration, and application factors was made between the *H. limbata* and data from Oseid and Smith (1974b) on the amphipod, *Gammarus pseudolimnaeus* Bousfield (Table 6). It is apparent that although there are wide differences in the 96-h LC_{50}, 12-day LC_{50}, and highest chronic safe level, the differences in application factors are quite small. It is also noted that these factors are in the same general range as those noted for some other toxicants by various authors working on fish.

Table 6.—Relationships between LC_{50}, chronic safe, and application factor values for *Hexagenia limbata* and *Gammarus pseudolimnaeus*.[a]

Item	*Hexagenia*	*Gamarus*
96-h LC_{50} (mg/liter H_2S)	0.165	0.022
12-day LC_{50} (mg/liter H_2S)	0.060	0.011
Highest safe level (mg/liter H_2S)	0.015	0.002
Application factor based on 96-h LC_{50}	0.09	0.10
Application factor based on 12-day LC_{50}	0.25	0.20

[a] *Gammarus* data from Oseid and Smith 1974b.

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