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FACTORS INFLUENCING ACUTE TOXICITY ESTIMATES OF HYDROGEN SULFIDE TO FRESHWATER INVERTEBRATES*

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Abstract—Acute bioassay tests of hydrogen sulfide were run on *Asellus militaris* Hay, *Crangonyx richmondensis laurentianus* Bousfield, *Gammarus pseudolimnaeus* Bousfield, *Baetis vagans* McDonough, *Ephemera simulans* Walker and *Hexagenia limbata* (Serville). Size and type of test chamber, type of substrate for burrowing forms or those seeking shelter in gravel, oxygen concentration, pH, and season of collection influenced the sensitivity of organisms. Hydrogen sulfide exposure at sublethal levels reduced feeding activity of *Gammarus*. Data indicate that test conditions should approximate natural habitat conditions as closely as practical. The most acceptable 96-h LC_{50} hydrogen sulfide concentrations for the various species are: *Asellus* 1.07 mg l^{-1}, *Crangonyx* 0.84 mg l^{-1}, *Gammarus* 0.059 mg l^{-1}, *Baetis* 0.020 mg l^{-1}, *Ephemera* 0.316 mg l^{-1}, and *Hexagenia*, 0.111 mg l^{-1}. Chronic exposure tests now in progress suggest that the no-effect levels are 8–12 per cent of the 96-h LC_{50}.

INTRODUCTION

As a part of a study investigating the toxic effects of hydrogen sulfide on various stages of fish and invertebrates, laboratory studies of one isopod, *Asellus militaris* Hay, two amphipods, *Crangonyx richmondensis laurentianus* Bousfield and *Gammarus pseudolimnaeus* Bousfield and three Ephemeroptera, *Baetis vagans* McDonough, *Ephemera simulans* Walker, and *Hexagenia limbata* (Serville) were conducted. Field studies and surveys have indicated that hydrogen sulfide is often present in both polluted and some natural ecosystems at levels that are detrimental to fish and invertebrates. A series of papers have indicated that concentrations of hydrogen sulfide usually not measured or overlooked as unimportant can be extremely toxic to fish or have more subtle chronic effects which reduce the potential production in fish populations (Colby and Smith, 1967; Smith and Oseid, 1971; Adelman and Smith, 1970, 1972; Smith and Oseid, 1972; Smith and Oseid, 1974). Limited references in the literature to the effect of hydrogen sulfide on various invertebrates suggested that a rigorous evaluation of acute toxicities in a representative series of aquatic organisms associated with fish populations would permit an overall evaluation of the importance of hydrogen sulfide in the various systems. Because preliminary studies suggested that methods of bioassay and the chemical and temperature characteristics under which tests were conducted would have a significant influence on toxicity evaluation, a series of studies was designed to determine the influence of various bioassay procedures and also to define the range of acute toxicity levels of hydrogen sulfide to the organisms.

The specific objectives of this study were: (1) to describe the effect of various oxygen concentrations and temperature levels on sensitivity of various species; (2) to determine the effect of chamber size, substrate, and acclimatization on acute toxicity estimates; (3) to determine seasonal differences in sensitivity of organisms; (4) to determine the effect of size and sex on sensitivity; and (5) to determine the method giving the best estimates of median tolerance limits of the species tested. Since these objectives could not be met with all species, tests were set up to obtain maximum use of the most available species. Final estimates of best LC_{50} levels were based on results of test conditions judged to be most similar to stream conditions. The species used in the tests were selected to include organisms from a wide range of habitat conditions varying from clear, cool water and firm substrate to warmer, turbid

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waters with substrates where insects might burrow in the mud and where hydrogen sulfide levels might normally be relatively high.

MATERIALS AND METHODS

Test organisms

The isopods, *Assellus militaris*, used in the experiments were collected from Jackfish Bay, Rainy Lake, Minnesota and had a mean length of 8 mm (range of 5–13 mm). *Crangonyx richmondensis laurentianus* was also taken from Jackfish Bay and ranged from 6 to 15 mm in length, with a mean of 10 mm. *Gammarus pseudolimnaeus* was collected in Valley Creek, Washington County, Minnesota and varied in size from 8 to 16 mm, with a mean of 11 mm. *Baetis vagans* was also taken from Valley Creek and ranged in size from 4 to 6 mm, with a mean of 5 mm. Nymphs of *Hexagenia limbata* varied from 14 to 35 mm, with a mean of 23 mm, and were taken from Jackfish Bay and Crystal Beach, Rainy Lake, Minnesota. *Ephemera simulans* ranged from 13 to 21 mm, with a mean of 17 mm, and were taken from Crystal Beach, Rainy Lake, Minnesota. The organisms were collected with a Peterson dredge, hardware cloth scoop or drift net. Maximum care was exercised to avoid injury to the animals during separation from the detritus.

Test water

All tests were conducted in water taken from a deep well and transmitted through PVC piping. The water was high in alkalinity (Table 1) with pH stabilized by aeration.

Table 1. Analysis of well water used in laboratory tests*

Item	Value ($mg\,l^{-1}$)
pH 7.5	
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Iron	0.02
Manganese	0.04
Chloride	<1
Sulfate	<5
Fluoride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Ammonia nitrogen	0.20
Organic nitrogen	0.20

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

Table 2. Summary of bioassay tests conducted with hydrogen sulfide on species of invertebrates

Species	Number of tests	Duration (days)	Range H_2S concentration ($mg\,l^{-1}$)
Assellus	4	4	0.044–2.196
Crangonyx	8	4	0.029–2.671
Gammarus	10	4–10	0.008–0.112
Baetis	2	2–4	0.008–0.064
Ephemera	5	4–11	0.106–0.617
Hexagenia	39	2–11	0.005–0.702

Test apparatus

All tests were conducted in flow-through apparatus as described by Colby and Smith (1967) and Adelman and Smith (1970). The former apparatus consisted of a cylindrical chamber 3.8 cm dia by 9.5 cm deep with a Nitex screen at the bottom. The second type was an acrylic box 7.6 × 7.6 × 5 cm with various substrates into which the organisms could burrow. Hydrogen sulfide levels were maintained by dissolving hydrogen sulfide gas in oxygen-free water and mixing this water at the entrance to the test chambers with a proportioned amount of oxygen-saturated water to achieve the desired oxygen and hydrogen sulfide concentrations. After mixing the solution passed through the test chamber in not more than 90 s. Analyses of the hydrogen sulfide content were routinely made at least once each day by determination of total sulfides and calculation of undissociated hydrogen sulfide from pH levels.

Experimental design

Tests set up to determine median tolerance limits consisted of five hydrogen sulfide concentrations and a control (Table 2). Temperature and oxygen were adjusted to meet the requirements of the various tests as subsequently outlined. In some cases substrate, oxygen, and pH were varied while a constant level of hydrogen sulfide was maintained. Experiments on the effect of feeding and non-feeding of *Gammarus* during bioassay and on the effect of hydrogen sulfide on feeding characteristics were conducted.

RESULTS

Effect of test conditions on toxicity estimates

Temperature and oxygen. To determine the influence of ambient oxygen concentration on resistance to hydrogen sulfide, *Gammarus*, *Ephemera* and *Hexagenia* were subjected to various levels of oxygen and hydro-

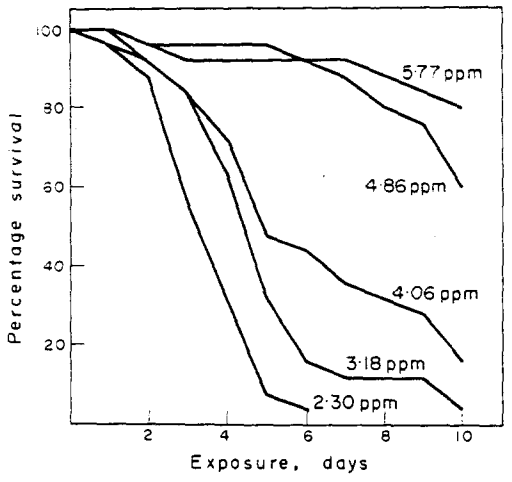

Fig. 1. Percentage survival of *Gammarus* exposed to 0.094 mg l^{-1} H_2S and five levels of oxygen during a 10-day period.

gen sulfide. In the first series of tests *Gammarus* and *Ephemera* were treated with constant levels of hydrogen sulfide and varied concentrations of oxygen. *Gammarus* was subjected to 0.094 mg l^{-1} H_2S and 5.77, 4.86, 4.06, 3.18 and 2.30 mg l^{-1} O_2 at 11°C for 10 days or until all organisms were dead. At the highest oxygen concentration, 80 per cent survived for 10 days and at the lowest, 4 per cent survived through 6 days (Fig. 1). *Ephemera* were exposed to 0.20 mg l^{-1} H_2S and 7.9, 6.1, 3.9 and 1.7 mg l^{-1} O_2 at 15°C. With 7.9 mg l^{-1} O_2 no mortality occurred in 10 days but at 1.7 mg l^{-1} O_2 all died in 3 days (Fig. 2). At intermediate oxygen levels 40 per cent or more survived 10 days. *Hexagenia* were

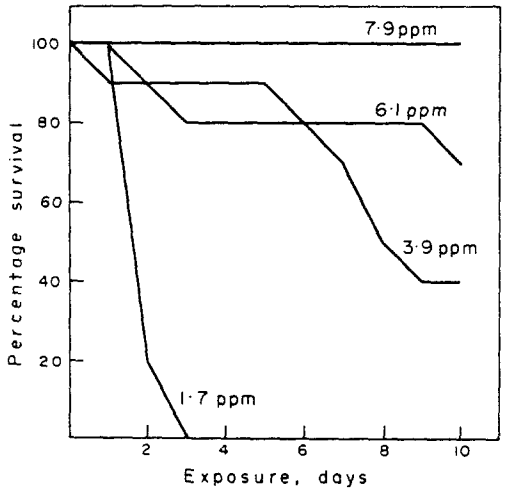

Fig. 2. Percentage survival of *Ephemera* exposed to 0.20 mg l^{-1} H_2S at four levels of dissolved oxygen during a 10-day period.

exposed to 2 and 4 mg l^{-1} O_2 and five levels of hydrogen sulfide from 0.076 to 0.88 mg l^{-1} at 15°C in cylindrical chambers (two replications). At 2 mg l^{-1} O_2, LC_{50} was 0.620 and 0.460 mg l^{-1} H_2S at 24 and 48 h, respectively. In 4 mg l^{-1} O_2, LC_{50} was 0.640 and 0.535 mg l^{-1} H_2S at 24 and 48 h.

A series of four tests on *Gammarus* at 6 and 4 mg l^{-1} O_2 and at 10 and 15°C were run to determine the combined effect of the two variables on LC_{50} of hydrogen sulfide. The tests were run for 10 days at five hydrogen sulfide levels (0.011–0.075 mg l^{-1}) and in the cylindrical chambers. After 10 days the LC_{50} varied only 0.007 mg l^{-1} (Table 3). The lowest was 0.042 mg l^{-1} H_2S at 4 mg l^{-1} O_2 and 15°C.

At 10°C and 6 mg l^{-1} O_2 early resistance was high (0.095 mg l^{-1} H_2S) but decreased rapidly up to 6 days and little thereafter. At 15°C early resistance was less and after 6 days decreased slowly. With 4 mg l^{-1} O_2 resistance was less at the higher temperature. These data indicate that after initial difference in resistance oxygen and temperature within the ranges tested do not have much influence on long-term response.

Hydrogen ion concentration. The effect of pH on hydrogen sulfide toxicity aside from its relationship to dissociation was considered to be substantial by Bonn and Follis (1967) on the basis of 3-h tests with fish. During the present study with *Hexagenia* three 96-h tests 7.4 pH and five 96-h tests at 7.7 pH run in winter had a mean LC_{50} of 0.365 and 0.151 mg l^{-1} H_2S, respectively. Seven summer tests run at 7.7 pH had a mean 96-h LC_{50} of 0.111 mg l^{-1} H_2S. Oxygen concentration and temperature during the tests were 2.0 mg l^{-1} O_2 and 15°C.

Chamber and substrate type. First tests with *Hexagenia* were done in cylinders (Colby and Smith, 1967). Consideration of work by Eriksen (1963a, 1963b) suggested that chamber design and substrate could have a significant effect on the sensitivity of an organism to a toxicant. A box was designed, therefore, which permitted inclusion of a substrate into which the insects could burrow (Adelman and Smith, 1970). Eriksen showed that oxygen consumption was 0.65 cm^3 gr^{-1} dry body weight h^{-1} when nymphs were in burrows

Table 3. LC_{50} of hydrogen sulfide (mg l^{-1}) after various intervals at 4 and 6 mg l^{-1} O_2 and 10 and 15°C for *Gammarus*

	6 mg l^{-1} O_2		4 mg l^{-1} O_2	
Days	10°C	15°C	10°C	15°C
2	0.095	0.071	—	0.062
4	0.059	0.059	0.054	0.058
6	0.053	0.056	0.051	0.052
8	0.050	0.054	0.050	0.045
10	0.049	0.045	0.049	0.042

Table 4. Survival time (days) of *Hexagenia* in different type chambers with hydrogen sulfide* in three paired tests

	Chamber 1		2		3	
	Cylinder	Box	Cylinder	Box	Cylinder	Box
H_2S (mg l^{-1})	0.143	0.178	0.266	0.276	0.355	0.365
Survival time						
50% or more	3.5	8	3	6	2.5	4.5
25% or more	4.5	9.5	3.5	8.5	3.5	5.5

* 2 mg l^{-1} O_2, 15°C.

and 1.1 cc when on bare substrate. During the present study comparative tests with cylindrical chambers and boxes with substrate were made. With an oxygen level of 2 ppm and temperature of 15°C the 48-h LC_{50} for *Hexagenia* in cylinders was 0.460 mg l^{-1} H_2S and in the box with substrate was 0.520 mg l^{-1} H_2S. Oxygen at 2.0 mg l^{-1} was selected for species collected in Rainy Lake because significant hydrogen sulfide concentrations were usually not found where oxygen was higher (Colby and Smith, 1967). The numbers of days to 50 and 25 per cent survival in three pairs of box and cylinder tests under the same conditions were more than twice as great in boxes with mud substrate (Table 4). After 2 days of exposure, controls from nine tests were transferred to Nitex baskets with and without mud substrates for 6 days. When both experimental period and post-treatment periods were with mud substrate the survival was greater (Table 5). In another test, mud substrate was provided in two series of hydrogen sulfide concentrations with box chambers. In one set burrowing was prevented by an overlay of Nitex screen. Where burrowing was permitted the 96-h LC_{50} was 0.120 mg l^{-1} H_2S and where prevented was 0.060 mg l^{-1} H_2S. A 12 day test without hydrogen sulfide in boxes with mud, with mud and screen to prevent burrowing, and with no mud was conducted. Where burrowing was permitted, survival was 62 and 75 per cent; where burrowing was not permitted, survival was 0–12 per cent, and with no mud present was 12 and 38 per cent.

To determine whether the quality of environmental substrate was a factor in resistance, two tests were run with sludge from below a paper mill and mud from an unpolluted habitat of *Hexagenia*. The test chamber was a trough, half of which contained sludge and half mud so that water flowed over both and nymphs had access to both. In the first test after 3 days 78 per cent of nymphs had selected the mud and in the second after 4 days 87 per cent selected the mud in preference to the sludge. In a subsequent 96-h LC_{50} bioassay conducted over mud and sludge there was little difference with 0.320 mg l^{-1} H_2S on mud and 0.310 mg l^{-1} H_2S on sludge. Apparently the type of material into which burrows were made did not alter the reaction to hydrogen sulfide. When *Crangonyx*, a species found where natural hydrogen sulfide may be abundant, was tested in boxes with mud and in cylinders without mud, resistance to hydrogen sulfide was greater when animals could burrow (Table 6).

Gammarus were tested to determine the effect of various substrates on resistance to hydrogen sulfide. Box chambers with mud, fine sand, pebbles (1 cm dia), and no substrate except the box floor were used in a 96-h test at 0.049 and 0.052 mg l^{-1} H_2S with one control. Oxygen was 3.84 ppm and temperature 15.1°C. Percentage survival varied from 4 per cent on mud to 36 per cent on pebbles (Table 7). The course substrate had the highest survival rate and the finest (mud), the lowest. The observed reaction may have been related to reduced activity where suitable cover was available.

Table 5. Effect of chamber type on *Hexagenia limbata* survival without toxicant present—expressed as percentage survival*

Experimental chamber (2 days)	Number of tests	Post-treatment Nitex basket chamber (6 days)	Survival (percentage) Mean	Range
Cylinder without mud	2	Without mud	22.5	0–50
Box with mud†	2	Without mud	40.0	60–100
Box with mud	5	With mud	80.0	60–100

* All tests at 2.0 mg l^{-1} O_2 and 15°C, except as noted.
† One test at 4.0 mg l^{-1} O_2.

Table 6. Effect of box and mud chamber and cylinder without mud on resistance of *Crangonyx* to hydrogen sulfide*

Chamber	LC_{50} of H_2S (mg l^{-1}) 48 h	72 h	96 h
Cylinder without mud	0.540	0.425	0.310
Box with mud†	0.770	0.590	0.510

* 2 mg l^{-1} O_2, 15°C, pH 7.4.
† With mud substrate.

Substrate area and chamber volume. In one test with *Gammarus* at 5.92 mg l^{-1} O_2 and 14.8°C designed to determine the effect of size of chamber on 96-h LC_{50} an increase of tenfold in bottom area and three-fold in volume decreased the 96-h LC_{50} of hydrogen sulfide approximately 25 per cent. Other tests conducted by Siesennop (MS Thesis, 1972) and Smith (unpublished data) where chamber size was 6 and 20 l, respectively, the LC_{50} was depressed still further (Table 8).

Effect of acclimatization

Length of acclimatization period. A series of experiments were run to determine the effect of holding test organisms in the laboratory prior to bioassay tests. *Assellus*, *Ephemera* and *Hexagenia* were held for varied lengths of time prior to the start of each test. Results indicated a marked influence of laboratory acclimatization time one resistance. *Assellus* were held in fresh water in the laboratory for 9, 16, 30 and 44 days prior to testing and then subjected to 96-h LC_{50} tests. The LC_{50}'s were 1.07, 1.21, 1.52 and 1.70 mg l^{-1} H_2S for succeeding acclimatization periods. Temperature was 15°C, O_2 was 2 ppm and pH, 7.3–7.5. *Ephemera* were held for 2 and 17 days in fresh water at 15°C and 2 ppm O_2 prior to testing. After 120-h LC_{50}'s were 0.210 and 0.200 mg l^{-1} H_2S. At 7 days LC_{50}'s were 0.20 and 0.14 mg l^{-1} H_2S, respectively. *Hexagenia* were held in fresh water on mud substrate for 3, 6, 10, 14 and 18 days and then transferred to Nitex baskets without food to determine subsequent survival rates in the absence of toxicants. Holding for up to 6 days resulted in reduced survival after transfer to baskets but longer holding periods did not significantly increase mortality beyond initial losses (Table 9). These results suggest the advisability of a 6 day acclimatization period prior to toxicity tests.

Acclimatization to sublethal hydrogen sulfide. Because bottom-living organisms, which burrow in the substrate, may be exposed continuously to sublethal levels of hydrogen sulfide, two tests were run on *Hexagenia* to determine the effect of sublethal levels on acute response to hydrogen sulfide. Test organisms were exposed to 0.016 mg l^{-1} H_2S and 2 mg l^{-1} O_2 at 15°C and pH 7.7 for 13 days. The 96-h LC_{50} was

Table 7. Effect of substrate type on percentage survival of *Gammarus* at similar hydrogen sulfide levels and varied times

H_2S concentration (mg l^{-1})	Percentage survival				
substrate	Control None	0.049 Mud	0.049 Fine sand	0.051 None	0.052 Pebble*
24 h	100	96	96	96	100
48 h	100	20	24	48	100
72 h	96	4	16	35	100
96 h	96	4	8	26	36

* 1 cm. Dia.

Table 8. Effect of chamber area and volume on 96-h LC_{50} of hydrogen sulfide with *Gammarus*

Number of individuals	Bottom area (cm^2) Total	Per individual	Volume (cm^3) Total	Per individual	96-h LC_{50} (mg l^{-1})
25	11	0.44	107	4.3	0.059
25	116	4.6	325	13.0	0.044
20	400	20.0	6600	330.0	0.035*
40	1288	32.2	20,000	500.0	0.022†

* Siesennop (MS Thesis, 1972).
† L. L. Smith, Jr. (unpublished data).

Table 9. Survival time (days) of *Hexagenia* held on mud in fresh water for varying periods and then transferred to Nitex baskets without food or toxicant

Holding period (days)	Survival rate in baskets 50%	10%	0%
3	10.2	10.8	11
6	5.5	8.5	9
10	7.2	7.8	8
14	4.5	7.5	10
18	5.6	11.5	13

Table 10. LC_{50} values of hydrogen sulfide for *Hexagenia* collected in different months tests at 15 C, 2 ppm O_2, and 7.7 pH

Month	LC_{50} of H_2S (mg l^{-1}) 48 h	72 h	96 h
1967–1968			
August	0.225	0.165	0.115
October	0.400	0.182	0.152
December	0.455	0.260	0.158
February	0.390	0.240	0.102
July	0.250	0.160	0.115

0.108 mg l^{-1} and 0.140 mg l^{-1} H_2S. The 96-h LC_{50} of organisms held in fresh water for the same period and tested simultaneously were 0.103 and 0.098 mg l^{-1} H_2S. Simultaneous tests with the organisms held in fresh water was 0.135 mg l^{-1} H_2S. The data suggest that pretreatment with very low levels may increase resistance to acute levels of hydrogen sulfide but that higher pretreatment levels increase sensitivity. The results are inconclusive but indicate the need for more careful evaluation of effects of sublethal level exposure of bottom invertebrates on subsequent acute lethal tests.

Effect of season, sex and size

It is usually more desirable to catch wild invertebrates shortly before testing rather than to maintain cultures. Because seasonal differences in resistance may occur, acute tests were run at different seasons from 1967–1968 on *Hexagenia* (Table 10). It is evident from the data that organisms taken in summer are more sensitive than those taken in fall and winter. The extent to which this seasonal difference applies to other species was not determined.

In seven hydrogen sulfide bioassays with *Gammarus*, ranging in size from 8.0 to 16.0 mm, mortality rates of males and females were compared and size of mortalities and survivors was noted. No significant difference in sensitivity to hydrogen sulfide of males and females or different sizes was apparent. There was considerable variablitity between tests but no trends.

Behavior under test conditions

Behavior related to hydrogen sulfide concentration. Behavioral effects of hydrogen sulfide on *Ephemera* and *Hexagenia* were noted by observation of emergence from burrows during 10-day tests in chambers with mud substrate at different concentrations of hydrogen sulfide and oxygen. Percentage of emergence was based on the total number of individuals in the test period. At an exposure of 0.20 mg l^{-1} H_2S and four levels of oxygen from 7.9 to 1.7 mg l^{-1}, emergence of *Ephemera* varied from 100 per cent in 3 days at 1.7 mg l^{-1} O_2 to no emergence in 9 days at 7.9 mg l^{-1} O_2 (Table 11). At 2.00 mg l^{-1} O_2 and five levels of hydrogen sulfide from 0.16 to 0.30 mg l^{-1}, emergence was earlier at the higher hydrogen sulfide concentrations. With *Hexagenia* subjected to 2.0 mg l^{-1} O_2 and five hydrogen sulfide concentrations from 0.18 to 0.54 mg l^{-1}, emergence was sooner at higher concentrations (Table 12). In all test series using mud sub-

Table 11. Percentage emergence of *Ephemera* with constant hydrogen sulfide (0.20 mg l^{-1}) and varied oxygen (mg l^{-1}) and with constant oxygen (2.0 mg l^{-1}) and varied hydrogen sulfide (mg l^{-1}) concentration in succeeding days

Days	O_2 mg l^{-1} (with 0.20 mg l^{-1} H_2S) 1.7	3.9	6.1	7.9	H_2S mg l^{-1} (with 2.0 mg l^{-1} O_2) 0.0	0.16	0.19	0.26	0.30
1	0	10	10	0	10	20	40	80	100
2	100	10	10	0	0	20	70	100	100
3	100	10	10	0	0	70	100	100	100
4	—	10	20	0	10	30	100	100	—
5	—	20	20	0	10	50	80	100	—
6	—	20	20	0	10	50	100	100	—
7	—	40	20	0	10	100	100	100	—
8	—	50	20	0	10	100	100	100	—
9	—	60	20	0	10	80	100	100	—
10	—	60	30	0	10	100	100	100	—

Table 12. Percentage emergence of *Hexagenia* with 2.0 mg l^{-1} O_2 and varied concentrations of hydrogen sulfide

Day	H_2S concentration (mg l^{-1}) 0.00	0.18	0.25	0.35	0.43	0.54
1	0	0	0	0	30	90
2	0	11	22	20	70	100
3	10	11	78	80	100	100
4	20	22	78	100	100	—
5	0	33	89	100	100	—
6	—	—	—	—	—	—
7	30	78	100	—	—	—
8	40	100	—	—	—	—
9	20	100	—	—	—	—
10	60	100	—	—	—	—

strates only two nymphs died in burrows rather than emerging before death. The difference in days between 50 per cent emergence and 50 per cent mortality of *Ephemera* varied from less than 1 day at 0.5 mg l^{-1} H_2S to 4 days at 0.15 mg l^{-1} H_2S. With *Hexagenia* the difference was 3 days throughout the same range of sulfide concentrations.

To test the effect of hydrogen sulfide on feeding behavior of *Gammarus* and to determine whether the absence of food affected LC_{50} values with 10 days exposure, a series of tests were run with 3 species of tree leaves, various concentrations of hydrogen sulfide and in pure water. When *Gammarus* was fed *Populus alba pyramidalis* leaves and treated with 0.010–0.047 mg l^{-1} H_2S at 15°C and 5.8–6.0 mg l^{-1} O_2, food consumption varied from 0.431 mg per individual per day in controls to 0.135 mg per individual per day at 0.050 mg l^{-1} H_2S (Table 13).

Table 13. Feeding of *Gammarus* on *Populus alba pyramidalis* leaves at various levels of hydrogen sulfide (mg l^{-1})—intake expressed as mg individual^{-1} day^{-1}

H_2S conc. (mg l^{-1})	Test 1*	Test 2*	Test 3†
0.0	0.677	0.604	0.431
0.010	—	—	0.381
0.013	0.550	—	—
0.016	—	0.680	—
0.020	—	—	0.367
0.028	0.489	—	—
0.031	—	—	0.340
0.033	—	0.428	—
0.039	—	—	0.244
0.047	—	0.196	—
0.050	—	—	0.135

* 6.0 mg l^{-1} O_2, 15°C, and pH 7.51.
† 5.8 mg l^{-1} O_2, 10°C, and pH 7.52.

Upon removal to fresh water after exposure to hydrogen sulfide survivors regained food intake comparable to unexposed individuals.

DISCUSSION

The foregoing data make it apparent that to determine acceptable levels of hydrogen sulfide for aquatic invertebrates, test conditions must approximate natural habitat conditions to the greatest extent possible. The present report indicates that acute toxicity levels vary within species depending on test conditions. While it is recognized that acute levels cannot be used as a basis for setting of stream standards, they are useful as a basis for comparison with fish on which extensive chronic data are available. Chronic tests of three species of invertebrates completed in our laboratory but not yet reported in the literature (Smith and Oseid, unpublished data) indicate that the hydrogen sulfide concentrations which will permit satisfactory completion of all life history phases will be 8–12 per cent of the 96-h LC_{50} concentration when the acute tests are performed under conditions minimizing stresses other than the toxicant.

Test conditions which may cause variations and unrealistic results relate primarily to oxygen concentration, size and shape of test chamber, and type of substrate. The LC_{50} values presented in Table 14 have been selected by the authors as those attained under the test conditions which minimized factors that may induce erroneous results. Ninety-six hour LC_{50} values have been selected as a basis of comparison, although time independent (threshold) acute tests give substantially lower LC_{50} values in some cases.

Acutely toxic concentrations of hydrogen sulfide range from 96-h LC_{50}'s of 1.07 mg l^{-1} in *Asellus* to 0.020 mg l^{-1} in *Baetis vagans* with the cold water

Table 14. LC_{50} values of hydrogen sulfide at 96 h derived from tests most closely approximating habitat conditions for six species of invertebrates

	pH	O_2	°C	96-h LC_{50} (mg l^{-1} H_2S)
Cold water				
Gammarus	7.5	5.9	15.0	0.059
Baetis	7.6	6.2	14.8	0.020*
Warm water				
Asellus	7.5	2.0	15.2	1.07
Crangonyx	7.4	2.0	14.9	0.84
Ephemera	7.4	1.9	15.0	0.316
Hexagenia	7.7	2.0	15.0	0.111†

* Mean of two tests.
† Mean of seven tests.

forms being less tolerant than those found in warm water (Table 14).

Comparison of LC_{50} values for the invertebrates tested in the present study with comparable values for fish found in similar habitats (Adelman and Smith, 1972; Smith and Oseid, 1971, 1972, 1974) indicate that the invertebrate tolerance is equal or greater than that of fish. Since factors applied to acute values to define safe levels for completion of life histories appear to be similar for fish and invertebrates protection of invertebrates will in most cases be achieved by standards set for fish. It should be noted, however, that hydrogen sulfide concentrations in the habitat must be determined in the exact location occupied by the organisms.

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