Bull. Environ. Contam. Toxicol. (1996) 57:450-457

Bulletin of Environmental Contamination and Toxicology

Sulfide Tolerance of Four Marine Species Used to Evaluate Sediment and Pore-Water Toxicity

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Received: 31 August 1995/Accepted: 20 March 1996

Hydrogen sulfide, which occurs naturally in marine and estuarine sediments, may be present at levels that are toxic to organisms used to evaluate sediment toxicity. It is necessary, therefore, to evaluate the possible contribution of sulfides to sediment toxicity before effects attributed to xenobiotic contaminants can be assessed. This is especially important because sediments in bays and estuaries often contain relatively high levels of sulfides (Bagarinao 1992).

We investigated the sulfide tolerances of four marine species that are commonly used to evaluate amphipod survival in bulk sediment (*Rhepoxynius abronius* and *Eohaustorius estuarius*) and embryo development in pore water (*Mytilus edulis* and *Strongylocentrotus purpuratus*). Because sulfide is volatile and subject to oxidation under static exposure conditions, we used sealed static and flow-through exposure systems to control the concentrations of sulfide to which organisms were exposed. Because the sensitivities of these organisms to sulfide have not been reported previously, this study provides benchmark data for evaluating the potential contribution of sulfides as a confounding factor in sediment and pore-water toxicity tests.

MATERIALS AND METHODS

Adult bay mussels (*M. edulis*) were acquired from Carlsbad Aquafarms, (Carlsbad, California) and the Tomales Bay Oyster Company (Tomales Bay, California). *M. edulis* embryos were subsequently obtained via spawning in accordance with standard methods (ASTM 1989). Adult purple sea urchins (*S. purpuratus*) were acquired from the Bodega Marine Laboratory (Bodega Bay, California). *S. purpuratus* embryos were obtained in compliance with standard methods (Dinnel and Strober 1985). Adult amphipods (*E. estuarius* and *R. abronius*) were obtained from North West Aquatic Sciences (Newport, Oregon). All adult organisms were held in filtered, refrigerated (15°C) seawater

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salinity = 32‰) under static conditions prior to use in experiments. All experiments were conducted with the same source of seawater.

Because pore-water toxicity tests are likely to be conducted under conditions that are conducive to the loss of sulfides via volatilization (i.e., in open containers), the initial period of exposure is likely to be the most important. Initial experiments were performed, therefore, to identify early periods of exposure that are critical to the development of bivalve embryos. *M. edulis* embryos were exposed to sulfides for 2, 4, 6, 8 and 10 hr in triplicate, in sealed glass scintillation vials that did not contain head space. Nominal test concentrations of 96-, 192- and 288-µg total sulfide/L (i.e., 3-, 6- and 9-µ*M*/L) were prepared by adding appropriate volumes of a stock solution of sodium sulfide ($Na_2S.9H_2O$ dissolved in N_2-deaerated distilled water) to 0.45-µm filtered seawater. Embryos were placed in plastic cylinders fitted with 20-µm Nitex® screens at both ends so that the embryos were immersed in the test solution. When each exposure period was attained (e.g., 2 hr, 4 hr), the cylinders were transferred to vials that contained filtered seawater but not sulfides. Embryos were then allowed to develop until the termination of the test at 48 hr. Dissolved oxygen and pH were measured at the beginning and end of each test period with the appropriate meters.

Flow-through exposures were conducted to maintain constant concentrations of sulfide over a 48-hr test period. Solutions of hydrogen sulfide were prepared as described above and were held in Tedlar® gas-sampling bags with no headspace. This stock solution was mixed with 0.45-µm filtered seawater via a peristaltic pumping system, which transported the solutions into sealed 1-L exposure jars that contained the test organisms. Sulfide concentrations were maintained over a range of exposure concentrations by varying the flow rates of the stock solution and the seawater. Initial tests of the flow-through system indicated that nominal concentrations of total sulfide were difficult to attain, probably as a result of sulfide oxidation in the stock solution and exposure water (Morse et al. 1987). Therefore, the mean values of sulfide concentrations measured at 0, 24 and 48 hr were used to assess sulfide exposures and toxicity.

M. edulis and *S. purpuratus* embryos were exposed in the screened cylinders that were described above. Four cylinders were placed in each 1-L exposure jar. After 48 hr, embryos were transferred from the plastic vials into glass scintillation vials and fixed with 1 mL of 5% gluteraldehyde. Percent normal development was determined by microscopic examination for both bivalves and echinoderms following the exposure period. Amphipods were exposed in groups of 20 and survival was assessed at the end of the 48-hr exposure period.

Dissolved oxygen and pH were determined at the beginning and end of each exposure.

For all flow-through experiments, sulfide concentrations were determined using a modification of the methylene-blue method (APHA 1985). Ten-mL samples and standards were preserved in scintillation vials using 1 mL of 5-*M* NaOH. One mL of mixed diamine reagent was added to each sample and standard, and color was allowed to develop for 20 min. The absorbance of each sample and standard was measured at 664 nm with a Hitachi spectrophotometer in the order that they were collected. Total sulfide concentrations were calculated using a regression analysis derived from a standard curve. Using this method, the detection limit for total sulfides was approximately 3 µg/L.

Statistical analyses were performed according to USEPA methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (USEPA 1991). Effective concentration (EC50) estimates were calculated using the probit method. No observed- (NOEC) and lowest observed- (LOEC) effect concentrations were determined using the William's test.

RESULTS AND DISCUSSION

The influence of exposure time and total sulfide concentration on *M. edulis* development are presented in Fig. 1. A reduction in normal development occurred within 2 hr for all exposure concentrations, and there was a direct relationship between the length of exposure and increased abnormal development for all sulfide concentrations. Levels of dissolved oxygen remained constant (8.5 mg/L) during all exposures.

Experiments conducted with the flow-through system resulted in concentrations of total sulfides that remained relatively constant during the course of the 48-hr exposures (see Table 1). The concentration of hydrogen sulfide was approximately 9-10% of the total sulfide concentration at the pH of the test solutions (8.0 ± 0.1; APHA 1985). Oxygen concentrations remained relatively constant (8.9 ± 0.9 mg/L) during the course of all experiments.

Results of the 48-hr flow-through exposures indicate that abnormal development of *M. edulis* and *S. purpuratus* embryos was positively associated with increasing sulfide concentrations (Fig. 2). *M. edulis* was more sensitive than *S. purpuratus* as evidenced by its greater response over the range of exposure concentrations, its lower threshold for inhibition of normal development, and its lower EC50 (Table 2). In addition, the normal development of *M. edulis* was completely inhibited at 256 µg total sulfide/L whereas total inhibition for *S. purpuratus* development did not occur until 640 µg total sulfide/L.

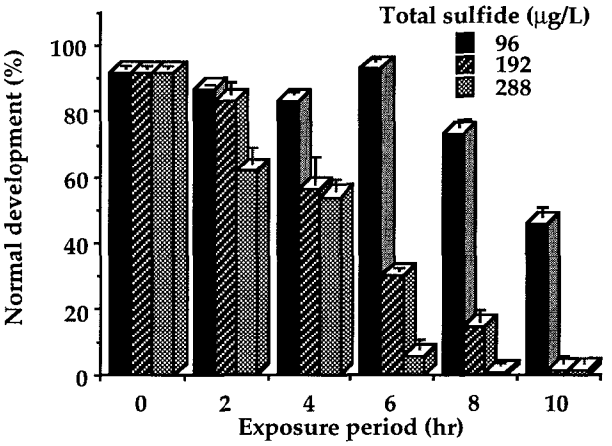

Figure 1. Results of the static-renewal experiments conducted with *M. edulis* embryos (error bars represent standard errors). Exposure concentrations are reported as nominal values.

Table 1. Concentrations of total sulfides measured during flow-through experiments.[a]

Total sulfides		
µg/L	mg/L	
M. edulis /S. purpuratus	*R. abronius*	*E. estuarius*
10 (3)[b]	0.00 (0.00)	0.01 (0.01)
29 (13)	0.32 (0.05)	0.35 (0.05)
83 (6)	0.61 (0.04)	0.90 (0.09)
115 (13)	0.80 (0.07)	1.22 (0.13)
205 (19)	0.99 (0.11)	1.92 (0.15)
256 (42)	1.47 (0.15)	2.72 (0.07)
448 (10)	2.50 (0.18)	3.71 (0.51)
640 (29)		4.35 (0.15)

a Values are means of measurements taken at 0, 24 and 48 hr.
b (Standard error).

Results of the 48-hr flow-through exposures conducted with *R. abronius* indicate that mortality was only observed at the two highest exposure concentrations (Fig. 3). Significant mortality first occurred at 1.47 mg total sulfide/L, which was approximately equal to the LC50 (Table 2). Complete mortality occurred at the highest exposure concentration of 2.50 mg total sulfide/L.

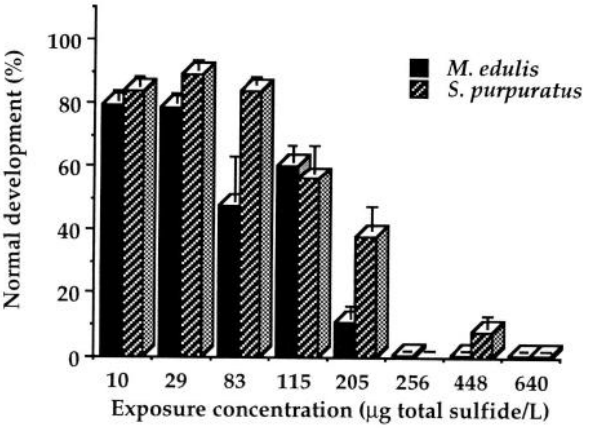

Figure 2. Results of the 48-hr flow-through experiments conducted with *M. edulis* and *S. purpuratus* embryos (error bars represent standard errors). Exposure concentrations are means of measured values.

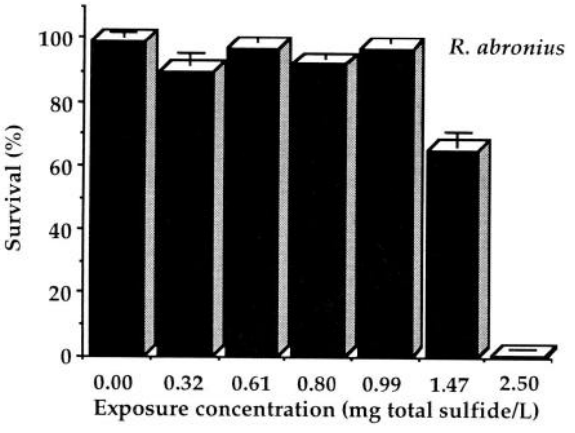

Figure 3. Results of the 48-hr flow-through experiments conducted with *R. abronius* (error bars represent standard errors). Exposure concentrations are means of measured values.

Results of the 48-hr flow-through exposures conducted with *E. estuarius* (Fig. 4) indicate that this amphipod was less sensitive to sulfide than *R. abronius.* Significant mortality was not observed until a concentration 1.92 mg total sulfide/L and complete mortality did not occur at the highest exposure concentration of 4.35 mg total sulfide/L.

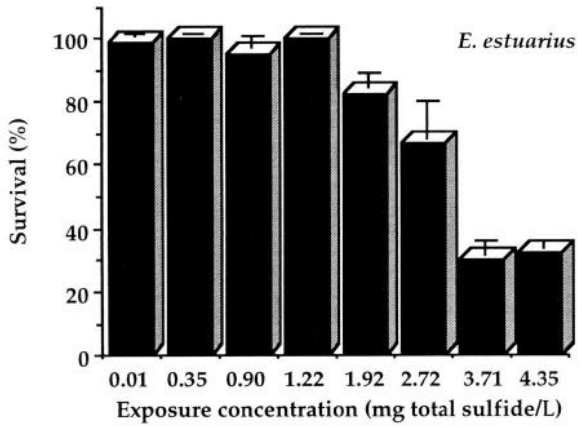

Figure 4. Results of the 48-hr flow-through experiments conducted with *E. estuarius* (error bars represent standard errors). Exposure concentrations are means of measured values.

Table 2. Summary of EC50, LC50, NOEC and LOEC values for flow-through experiments.[a]

	Total sulfides (mg/L)[b]			
	EC50	LC50	NOEC	LOEC
M. edulis	0.10		0.05	0.09
S. purpuratus	0.19		0.10	0.13
R. abronius		1.60		1.47
E. estuarius		3.32		1.92

[a] Results are based on measured values.

[b] The concentration of hydrogen sulfide is approximately 9-10% of the total sulfide concentration under the conditions of these tests (pH = 8.0 ± 0.1).

The presence of sulfides in natural sediments may produce confounding results in sediment and pore-water toxicity tests. The results of these studies indicate that total sulfide concentrations as low as 0.09 to 0.13 mg/L can be toxic, to embryos used in pore-water toxicity tests (Table 2). In addition, relatively short exposure periods at the initiation of experiments can contribute to toxicity observed at the end of the incubation period. For example, the influence of extended exposure time had little influence on the toxicity of sulfides to *M. edulis* embryos. This is evidenced by the fact that embryos exposed to

83 µg/L total sulfides for 48 hr (Fig. 2) did not exhibit an increase in abnormal development when compared to embryos exposed to a similar concentration (i.e., 96 µg/L) during the first 10 hr of development (Fig. 1). Caldwell (1975) reported that the development of *Crassostrea gigas* embryos was also significantly inhibited following 2-hr exposures to 290 µg total sulfide/L. Collectively, these results indicate that the initial period of exposure is critical in determining impacts on development that is assessed at 48 hr. These results also indicate that sulfide volatilization during the course of 48-hr exposures may not be sufficient to preclude sulfide toxicity to developing embryos.

Adult amphipods that are used to determine bulk-sediment toxicity were less sensitive to sulfides than the bivalve and echinoderm embryos and exhibited the onset of toxicity at values of 1.47- to 1.92-mg total sulfide/L. Because sulfide concentrations in bulk sediment and pore-water may exceed these values (Anderson et al. 1995; Bagarinao 1992), care must be taken to ensure that sulfides are not contributing to toxicity observed when using these test organisms. In addition, even lower sulfide concentrations may affect amphipods during the course of longer (i.e., 10 d) exposures that are typically conducted in the evaluation of bulk-sediment toxicity.

Although sulfides are volatile and will oxidize during the course of toxicity tests, it is apparent that sufficient sulfide may be present at the initiation of bulk sediment and pore-water toxicity tests to elicit toxic effects in test organisms. It is important, therefore, that routine analysis of total sulfides in sediment pore water be performed prior to the initiation and at the termination of toxicity tests.

In addition, the values reported here for total sulfides may not be conservative because sediments typically have a lower pH than overlying water, which favors the formation of the toxic hydrogen sulfide species. For example, the total-sulfide LOEC for *M. edulis* development (i.e., 90 µg/L) would result in a hydrogen-sulfide LOEC of approximately 9 µg/L at a pH of 8.0. In porewater at a pH of 7.5, however, a lower concentration of total-sulfide (i.e., 40 µg/L) would produce the hydrogen-sulfide LOEC. It is critical, therefore, that the pH of the pore water be measured and reported so that the concentration of hydrogen sulfide can be determined and test results can be accurately interpreted.

Acknowledgments. This work was supported by the U.S. Army Corps of Engineers under Contract MIPR E86 92 3026 and was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-Eng-48 and by the Lawrence Berkeley Laboratory under Contract DE-AC03-76SF00098.

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