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Tolerance of Five West Coast Marine Toxicity Test Organisms to Ammonia

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Toxicity tests are widely used to estimate the biological impacts of pollutants found in waste effluents and ambient waters. Most effluents and environmental samples contain complex mixtures of numerous anthropogenic and naturally derived chemicals. Toxicity test organisms exposed to such samples may respond to individual contaminants or to the combined dosage of multiple compounds. It is often necessary to determine the chemical cause of toxicity through toxicity identification evaluations (TIEs), or through chemical analysis and reference to previously derived toxicity thresholds.

Ammonia frequently occurs in municipal effluents and sediment interstitial waters, and is a commonly characterized toxicant in marine TIEs (Burgess 2000). Total ammonia is made up of toxic unionized ammonia (NH_3) and the relatively non-toxic ammonium ion (NH_4)(US EPA 1989). The percentage of total ammonia that is in the unionized form is dependent upon pH, temperature, and salinity (Whitfield 1974; US EPA 1989). The marine TIE process characterizes ammonia toxicity through graduated pH treatments and addition of the marine alga, *Ulva lactuca* (US EPA 1996), but the weight of evidence used to identify ammonia as the cause of toxicity also considers the threshold response values of the organisms being tested.

Previous studies have determined the toxicity of ammonia to a variety of test species, but few data were available for some commonly used west-coast marine test organisms: *Atherinops affinis* (topsmelt) larvae, *Holmesimysis costata* (mysid) juveniles, *Haliotis rufescens* (red abalone) embryos, *Macrocystis pyrifera* (giant kelp) spores, and *Mytilus galloprovincialis* (bay mussel) embryos. These comprise the most commonly used test species for effluent and ambient toxicity monitoring in California, Oregon, and Washington. We determined no observed effect concentrations (NOEC), lowest observed effect concentrations (LOEC), and median effect concentrations (EC50) of unionized ammonia for these species.

MATERIALS AND METHODS

All tests were performed at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL), Carmel, California. Toxicity tests were conducted as

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summarized in US EPA (1995), with the following exceptions. Short-term chronic tests were performed for *H. rufescens* (48-h embryo development), *M. pyrifera* (48-h embryo gametophyte germination and growth), and *M. galloprovincialis* (48-h embryo development), but 96-hour acute (mortality) tests were performed for *H. costata* and *A. affinis*. Adult *H. rufescens* brood stock was maintained at MPSL throughout the year. Carlsbad Aquafarms (Carlsbad, CA) supplied *M. galloprovincialis* brood stock, and Aquatic Biosystems (Fort Collins, CO) supplied larval *A. affinis*. MPSL staff collected *H. costata* broodstock and *M. pyrifera* sporophylls from local kelp forests in Monterey, CA. All organisms were maintained in aquaria supplied with continuously flowing and aerated seawater at ambient sea surface temperature and salinity.

Ammonia test solutions were diluted using 1-µm filtered natural seawater and reagent stock (0.1 M ammonium chloride, Orion Research Inc., Beverly, MA). Total ammonia concentrations were determined using either an ion-specific ammonia electrode or the spectrophotometric salicylate method (Hach Company, Loveland, CO). Total ammonia detection limits were 0.1 mg/L for the electrode and 0.01 mg/L for the spectrophotometric method. Ammonia concentrations were measured at test initiation and termination, and before and after test solution renewal for *A. affinis* and *H. costata*. Unionized ammonia concentrations (mg/L NH_3) were calculated from total ammonia concentrations (Whitfield 1974), and averaged for statistical calculations.

Three definitive short-term toxicity tests were performed for each species, at 5 concentrations plus seawater controls, with 3 to 5 lab replicates per treatment. NOECs, LOECs, and appropriate median effect concentrations were calculated from mean measured unionized ammonia concentrations and organism response (ToxCalc™ Statistical Software, Tidepool Software, McKinleyville, CA). Statistical procedures followed US EPA (1995), and included the trimmed Spearman-Karber method (Hamilton et al. 1977) or linear interpolation method (*M. pyrifera* growth) for calculating median effect statistics.

RESULTS AND DISCUSSION

Control responses in all experiments met test acceptability criteria (US EPA 1995), and dissolved oxygen, pH, and salinity were all within tolerance limits for the organisms tested. The accuracy of the ammonia spikes varied considerably with mean percent recovery ranging from 61% to 113%. There was a slight trend toward ammonia loss during the exposures (data not shown).

Test species exhibited a range of sensitivity to unionized ammonia (Table 1), and were comparable to the sensitivities of other organisms (Table 2). *H. rufescens* and *M. galloprovincialis* were the most sensitive to unionized ammonia, and their results were similar to previous studies with larval mussels and purple urchins (Table 2). *A. affinis* and *H. costata* were more sensitive to unionized ammonia than their east coast counterparts *Menidia beryllina* and *Americamysis bahia*. The

Table 1. Unionized ammonia NOEC, LOEC and EC50 values for the five species tested. Values represent the mean of three tests.

			Mean Unionized Ammonia (mg/L) (SE)		
Test Species	Test Duration	Test Endpoint	NOEC	LOEC	EC50
A. affinis	96 hour Acute	Mortality	<0.424	0.587 (0.105)	0.560 (0.018)
H. rufescens	48 hours Chronic	Larval Development	0.043 (0.001)	0.102 (0.018)	0.082 (0.004)
H. costata	96 hours Acute	Mortality	0.757 (0.119)	1.179 (0.165)	0.839 (0.113)
M. pyrifera	48 hours Chronic	Germination	0.614 (0.041)	1.013 (0.125)	1.248 (0.086)
		Germ Tube Elongation	<0.545	0.848 (0.267)	1.405 (0.174)
M. galloprovincialis	48 hours Chronic	Larval Development	0.090 (0.003)	0.152 (0.005)	0.120 (0.003)

Table 2. Unionized ammonia NOEC, LOEC and EC50 values for other marine and estuarine species.

	Unionized ammonia (mg/L NH_3)		
Species	NOEC	LOEC	EC50
Amphipod Survival *Ampelisca abdita*	0.4 [a]		0.830 [b]
Amphipod Survival *Eohaustorius estuarius*	0.8 [a]		2.490 [b]
Amphipod Survival *Rhepoxynius abronius*	0.4 [a]		1.590 [b]
Mussel Development *Mytilus galloprovincialis*	0.097 [c]	0.182 [c]	0.231 [c]
Polychaete Survival *Neanthes arenaceodentata*	0.680 [d]	1.250 [d]	
Purple Urchin Development *Strongylocentrotus purpuratus*	0.050 [e] 0.012 [c]	0.010 [e]	0.070 [e] 0.036 [c]
Purple Urchin Fertilization *Strongylocentrotus purpuratus*	> 1.4 [e]		> 1.4 [e]

[a] US EPA 1994; [b] Kohn et al. 1994; [c] Tang et al. 1997; [d] Dillon et al. 1993; [e] Bay et al. 1993

A. affinis LC50 was 0.560 mg/L compared to the *M. beryllina* acute value of 1.77 mg/L at 30‰, and the *H. costata* LC50 was 0.839 mg/L compared to the *A. bahia* acute range of 1.47 – 3.41 mg/L at 30‰ (US EPA 1989). The giant kelp *M. pyrifera* was less sensitive than the algal species reported in US EPA (1989). The germination and growth EC50s for *M. pyrifera* were comparable at 1.248 and 1.405 mg/L, respectively, whereas the growth of seven diatom species was retarded at 0.247 mg/L (Admiraal 1977).

Unionized ammonia concentrations above the thresholds derived in this study occur in wastewater effluents (SCCWRP 2003), and ambient water and sediment samples (Hunt et al. 2001). The presence of unionized ammonia at or near the threshold concentrations might exacerbate toxicity caused by anthropogenic contaminants. The data presented here can be used along with TIE treatments in a weight of evidence approach to identify whether unionized ammonia is solely or partially responsible for toxicity. These data may also be useful in the selection of appropriate test species for testing samples of known or suspected composition.

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