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Acute Toxicity of Cadmium, Copper, Zinc, Ammonia, 3,3′-Dichlorobenzidine, 2,6-Dichloro-4-nitroaniline, Methylene Chloride, and 2,4,6-Trichlorophenol to Juvenile Grass Shrimp and Killifish

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The acute toxicity of several compounds was investigated while performing a toxicity evaluation of a complex chemical effluent. The tests were conducted for one or more of the following reasons: 1) data were not available for the chemical; 2) data were not available for the species; or 3) data were not available for the juvenile life stage of the species. Forty-eight hour acute toxicity tests were run on juvenile grass shrimp (Palaemonetes pugio) and juvenile killifish (Fundulus heteroclitus) exposed to the following compounds: cadmium, copper, zinc, ammonia, 3,3'-dichlorobenzidine, 2,6-dichloro-4-nitroaniline, methylene chloride (dichloromethane) and 2,4,6-trichlorophenol.

MATERIALS AND METHODS

Juvenile grass shrimp, which were <20 mm in length when tested, were taken from Parrish Creek at the mouth of the West River, Maryland. They were held in the laboratory under static renewal conditions at a density of <50/L and fed flake fish food daily. Juvenile mummichogs were reared from eggs obtained from adults also taken from Parrish Creek. The juveniles, which were <23 days old when tested, were held under static renewal conditions at a density of <25/L and fed daily with Artemia nauplii and dry trout food. Both grass shrimp and mummichogs were acclimated for a minimum of two weeks and tested under the following conditions: 20 (±2)°C; 10 (±1)^{o}/oo; dissolved oxygen >40% saturation; and 16h light: 8h dark photoperiod. Filtered Chesapeake Bay water (8-12 ^{o}/oo), adjusted as necessary with sea salts or de-ionized water, was used as rearing and diluent test water.

Both species were tested by definitive static acute toxicity procedures described in ASTM (1980). A minimum of five test concentrations was run for all compounds with the exception of 3,3'-dichlorobenzidine and 2,6-

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dichloro-4-nitroaniline which had fewer concentrations because the maximum concentration tested was at the solubility of the compound in the diluent water. Two replicates of 10 organisms each were run at each test concentration. No test solutions were aerated during the 48-h tests, with the exception of 2,4,6-trichlorophenol where aeration was initiated at the beginning of the 2nd 24-h period to avoid the possibility of the dissolved oxygen dropping below 40% saturation. Dissolved oxygen, pH, and temperature were measured daily during each test. Salinity was measured at the beginning and end of each test. The animals were not fed during testing.

Anhydrous cadmium chloride, hydrated copper sulfate ($CuSO_4 \cdot 5H_2O$) and zinc chloride were used as sources for the heavy metals. For each metal studied, water samples were taken from all test aquaria at the initiation of each bioassay and immediately preserved with concentrated nitric acid (100 uL/50 mL). The metals were later analyzed by direct aspiration atomic absorption spectrophotometry. The metals were measured and reported as total metal. All heavy metals were ACS reagent grade.

Ammonium chloride (anhydrous) was used as the ammonia source. Ammonia samples were quantified immediately after the samples were taken from each test aquarium at the beginning of each test by the ammonia-selective electrode method. The method of Emerson et al. (1975), which corrects for pH and temperature, was used to calculate the concentration of dissolved un-ionized NH_3. ACS reagent grade chemicals were used for ammonium chloride and all other organics tested in the study.

A solution of 8 mg/L of 3,3'-dichlorobenzidine (DCB) in 35% sulfuric acid was used as the stock solution. The highest concentration of DCB tested was at the solubility of the compound in the diluent water (no dispersing agent was used). Water samples were taken from each test concentration at the beginning and end of the 48-h test for chemical analysis. The samples were placed in 125 mL glass bottles with negligible head space, sealed with teflon lined screw caps and stored at 4°C until analysis. The gas chromatographic (GC) method for acid base neutral extractibles in water was used to quantify DCB.

A solution of 31.9 mg/L 2,6-dichloro-4-nitroaniline (DCNA) dissolved in acetone was used as the stock solution. The highest concentration of DCNA tested was at the solubility of the compound in the diluent water. An acetone control (0.0005 mL/L) was used in addition to the normal control. Water samples were stored and analyzed as described above for DCB. The DCNA was re-crystallized before being used. Stock solutions of methylene chloride and 2,4,6-trichlorophenol (TCP) were used. Water samples for chemical analysis were taken and stored as described above. Methylene chloride was analyzed by the purge and trap/GC method. The GC method for acid base neutral extractibles in aqueous solution was used to quantify TCP.

The measured concentrations of all compounds were used in the calculation of the LC50s. The mean concentration of the replicates at each dilution at the beginning of the tests was used for the heavy metals. The average concentrations of the replicates at each dilution at the beginning and end of the tests were used for the organic compounds since the concentrations of some of the test materials decreased substantially (in particular, 3,3'-dichlorobenzidine and methylene chloride) over the course of the 48-h studies. 3,3'-Dichlorobenzidine decreased approximately 50% in both the grass shrimp and mummichog tests; methylene chloride decreased an average of 67 and 54%, respectively, for the grass shrimp and mummichog tests over the 48-h test period.

LC50s were determined in most cases using the probit method. When the probit method could not be used because the goodness of fit probability was <0.95, the moving average angle method was used. Wherever the moving average angle method was used, the LC50 is labeled in Table 1.

RESULTS AND DISCUSSION

Dissolved oxygen, temperature and salinity fell within the limits given above. The mean (±S.D.) pH for the diluent water was 7.8 (± 0.35) (n=28); the range was 7.2 - 8.4. The mean pH of all sample dilutions was 7.5 (±0.39) (n=98); the range was 6.1 - 8.0. The 48-h LC50s and 95% confidence limits of juvenile grass shrimp and mummichogs exposed to all test compounds are given in Table 1. The 48-h LC50 values in Table 1 for cadmium are the means (± S.D.) of several reference toxicity tests run on each species.

Cadmium appears to be slightly more toxic to juvenile grass shrimp than adults. The 48-h LC50 for juvenile grass shrimp obtained in the present study was 1.3 mg/L. Eisler (1971) obtained a 48-h LC50 of 5.8 mg/L for adult P. vulgaris, a grass shrimp in the same genus. He obtained a 96-h LC50 for adults of 0.42 mg/L while Nimmo et al. (1977) obtained a similar 96-h LC50 of 0.76 mg/L for the same species. Ahsanullah (1976) obtained 96-h LC50s for adult Palaemon sp., an organism in the same family as grass shrimp, of 6.4 - 6.8 mg/L.

Cadmium, as well as copper and zinc, is more toxic to grass shrimp than mummichogs. No difference appears to

Table 1. Summary of the 48-h LC50 values and associated 95% confidence limits of juvenile *Palaemontes pugio* and *Fundulus heteroclitus*.

Compound (mg/L)	*Palaemontes pugio* 48-h LC50 (95% Confidence Limits)	*Fundulus heteroclitus* 48-h LC50 (95% Confidence Limits)
Cadmium (mg/L as Cd)	1.3 (±0.51)[a]	44.4 (±12.22)[b]
Copper (mg/L as Cu)	2.1 (1.52 - 3.03)	19.0 (15.94 - 22.55)
Zinc (mg/L as Zn)	11.3 (9.14 - 13.99)	96.5 (70.55 - 152.89)
Ammonia (un-ionized)	1.2 (0.96 - 1.35)	1.6 (1.35 - 1.99)[c]
3,3'-Dichlorobenzidine	10% mortality at 0.73[d]	50% mortality at 0.73[d]
2,6-Dichloro-4-nitro-aniline	1.9 (1.06 - 2.30)[c]	20% mortality at 2.7[d]
Methylene chloride	108.5 (92.37 - 130.90)	97.0 (89.44 - 105.14)[c]
2,4,6-Trichlorophenol	5.6 (4.30 - 6.84)	2.3 (1.99 - 2.62)[c]

[a] Grass shrimp 48-LC50 is the mean (± S.D.) of 23 tests.
[b] Mummichog 48-LC50 is the mean (± S.D.) of 10 tests.
[c] LC50 determined by the moving average angle method.
[d] Highest concentration tested was at the solubility of the compound in the diluent water (no dispersing agent was used).

exist between the sensitivity of juvenile and adult mummchogs exposed to cadmium. The 48-h LC50 for juvenile mummichogs exposed to cadmium in this study was 44.4 mg/L. No other data exist for juveniles. The 48-h LC50s of adult F. heteroclitus, F. majalis, and the closely related sheepshead minnow, Cyprinodon variegatus, range from 23 ->200 mg/L (Eisler, 1971; Voyer, 1975; Dorfman, 1977). Other studies conducted for 96 h support this conclusion (Eisler and Hennekey, 1977; Jackim et al., 1970). Eisler (1985) concluded in his synoptic review of cadmium hazards to fish and invertebrates that decapod crustaceans were the most sensitive marine group in short-term tests.

Copper was found to be more toxic to juvenile grass shrimp than juvenile mummichogs in this study. No data exist for the same life stages in the literature. Curtis et al. (1979) reported a 96-h LC50 of 35.9 mg/L for adult P. pugio exposed to cupric acetate, which suggests that adults may be substantially more resistant to copper than juveniles. The authors noted that at their test pH of 8.4 a great deal of cupric oxide precipitated in the test containers. Thus, the actual LC50 would be much lower than the value reported. Dorfman (1977) reported 48-h LC50s of 0.5 - 3.1 mg/L for adult F. heteroclitus, which are much lower than the value found for juveniles in this study. Jackim et al. (1970) reported an approximate 96-h LC50 for adult mummichogs of 3.2 mg/L mg/L; Hansen (1983) obtained a 96-h LC50 of 0.28 for adult sheepshead minnow. These values are similar to Dorfman's (1977) 96-h LC50s of 0.4 - 2.0 mg/L for adult mummichogs . This suggests that Dorfman's 48-h LC50 for copper may be appropriate. The acute toxicity value for mummichogs obtained in this study appears to be high; however, it is less than one order of magnitude higher than the values reported in the literature for similar tests.

The 48-h LC50 of zinc to juvenile grass shrimp was 11.3 mg/L. Ahsanullah (1976) obtained 96-h LC50s for adult Palaemon sp. of 9.5 - 13.1 mg/L. As is the case for cadmium, zinc is more toxic to juvenile grass shrimp than to juvenile or adult mummichog. Cardin (1985) reported a 96-h LC50 for larval F. heteroclitus of 83.0 mg/L. A 48-h LC50 of 96.5 mg/L was found for juveniles in this study. The LC50s for adult mummichogs exposed to zinc ranged from a high of 125 mg/L at 24 h to a low of 27.5 mg/L at 96 h (Dorfman, 1977; Eisler and Hennekey, 1977). Thus, no difference in sensitivity appears to exists between juvenile and adult mummichogs exposed to zinc.

The order of toxicity of the three metals to juvenile grass shrimp in this study was generally: Cd > Cu > Zn; the order for juvenile mummichogs was Cu > Cd > Zn. In

the toxicity review of the effects of temperature and salinity on heavy metals to marine and estuarine invertebrates, McLusky et al. (1986) reported that the order of toxicity of metals was generally: Hg > Cd > Cu > Cr > Zn > Ni > Pb > As. Eisler and Hennekey (1977) studied the acute toxicity of several heavy metals to estuarine macrofauna (including F. heteroclitus) and found the rank order of toxicity to be: Hg > Cd > Zn > Cr > Ni (copper was not studied). The order of toxicity in this study is the same as that reported by others.
The toxicity of un-ionized ammonia to grass shrimp and mummichogs was approximately the same (Table 1). The 48-h LC50 for grass shrimp was 1.2 mg/L, while the value for the mummichog was 1.6 mg/L. Un-ionized ammonia LC50 values have been reported for larval, juvenile, and adult grass shrimp (P. pugio). EA Engineering (1985) obtained a 96-h LC50 of 0.82 mg/L for larva. LC50s for juvenile grass shrimp range from the 48-h value of 1.2 mg/L obtained in this study to a 96-h LC50 of 2.57 mg/L (Fava et al., 1985). Hall et al. (1978) obtained 24-, 48-, and 96-h LC50s of 12.9, 9.7, and <0.97 mg/L for adults. No data exist in the literature for the toxicity of un-ionized ammonia to mummichogs. Poucher (1986) reported three 96-h LC50s for juvenile sheepshead minnows which ranged from 2.1 - 3.5 mg/L. EA Engineering (1985) reported four 96-h LC50s for adult sheepshead minnows which varied from 1.73 - 2.46 mg/L. Little difference appears to exist in the acute toxicity of un-ionized ammonia to juvenile and adult killifish.

3,3'-Dichlorobenzidine was not acutely toxic to juvenile mummichogs or grass shrimp at the solubility limit (0.73 mg/L) of the compound in the diluent water. Although no LC50s could be obtained for DCB, the data indicate that DCB may be slightly more toxic to mummichogs. For example, 50% of the mummichogs died at 0.73 mg/L in contrast to only 10% for grass shrimp at 0.73 mg/L. No other toxicity data exist for saltwater organisms.

2,6-Dichloro-4-nitroaniline was more toxic to grass shrimp than mummichogs. The 48-h LC50 for grass shrimp exposed to DCNA was 1.9 mg/L. Only 20% of the mummichogs tested at the solubility limit of DCNA in the diluent water (2.7 mg/L) died in 48 h. No other data exist for saltwater organisms exposed to DCNA.

The 48-h LC50 for grass shrimp exposed to methylene chloride was 108.5 mg/L. The only other acute data available for saltwater invertebrates are for the mysid shrimp, Mysidopsis bahia. A 96-h LC50 of 256 mg/L has been reported by LeBlanc (1984) for M. bahia. The 48-h LC50 for juvenile mummichogs exposed to methylene chloride was 97.0 mg/L. In contrast to grass shrimp which died throughout the 48-h period, all mummichogs died within one hour of the start of the test. Based on the measured concentration at the beginning of the test, the 1-h LC50 was 134.6 mg/L. Heitmuller et al. (1981) obtained 24-, 48-, and 96-h LC50s of 370, 360, and 330 mg/L, respectively, for the sheepshead minnow. The LC50s obtained by Heitmuller et al. (1981) were nominal methylene chloride concentrations. We found that the average measured methylene chloride values were 54-67% less than the nominal concentrations. Thus, if one assumes that similar decreases in concentration occurred over the 48-h test period in the Heitmuller et al. study, their 48-h LC50 would range from 119 to 166 mg/L, which is similar to the value obtained in this study for mummichogs.

The 48-h LC50 for juvenile grass shrimp exposed to 2,4,6-trichlorophenol was 5.6 mg/L. Mayer (1986) reported a 96-h LC50 of 4.0 mg/L for adult P. pugio exposed to TCP. A slightly lower 96-h LC50 (1.1 mg/L) was found by Mayer (1986) for molting adult grass shrimp. The 48-h LC50 for mummichogs was 2.3 mg/L. No other data exist for saltwater organisms exposed to TCP.

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