Acute Toxicities of Selected Heavy Metals to the Softshell Clam, *Mya arenaria*

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Introduction

Increasing concern regarding the use of the oceans and coastal zone as repositories for anthropogenic wastes has prompted our laboratory to initiate a continuing series of investigations on toxicological hazards of selected waste components to representative species of marine biota. This account reports on results of static acute toxicity tests using selected heavy metals and adults of softshell clam, Mya arenaria, a locally abundant and economically important species. Specifically, bioassays were conducted with salts of copper, cadmium, zinc, nickel, manganese and lead in raw seawater under ambient summer conditions (30 $^{o}/oo$-22^{o}C). Additional tests were conducted with Cu^{2+} and Zn^{2+} in raw seawater (30 $^{o}/oo$) during autumn (17^{o}C) and winter (4^{o}C) in order to ascertain seasonal effects on survival.

Methods

All experiments were conducted during 1975 aboard the U.S. EPA research barge located in Jerusalem, R.I. Softshell clams were collected from Narragansett Bay, R.I. and maintained in flowing raw seawater at 30±1 $^{o}/oo$ and ambient water temperatures for 20-25 days before use. For all collections the average height and wet weight of soft parts was 45 mm (range 38-49 mm) and 5.4 g (range 3.6 to 6.0 g), respectively. No food was offered at any time although some was available in the flow-through holding system. Deaths during the first 48 hours of holding was less than 3%; afterwards it was less than 1%.

All static bioassays described herein were conducted in unfiltered seawater of 30±1 $^{o}/oo$ salinity, pH 7.95± 0.1 unit, and dissolved oxygen always >4.0 mg/ℓ. Bioassay results from studies conducted in raw seawater were not markedly different from those in a synthetic seawater medium of identical pH, D.O, salinity and temperature. Toxicity studies with Mya arenaria and

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several species of marine fishes and invertebrates were conducted in the two media using a variety of toxicants; survival curves after 168 hours were essentially the same for both media and all species (EISLER et al, 1972).

Test aquaria were widemouth glass jars of 4 liter capacity containing 2 liters of raw seawater and floated in a water bath of flowing seawater at ambient temperatures. During the summer studies this was 22.0±5°C; for fall and winter studies it was 17.5±0.8°C, and 4.0±2.0°C, respectively. A single Mya was added to each jar about 60 minutes before the appropriate toxicant. A minimum of 6 concentrations, five to ten replicates per concentration, plus 10 controls was used in each assay (minimum of 40 clams per assay); no animal was used more than once. Compounds tested were analytical grade reagents of $CdCl_2 \cdot 2\frac{1}{2} H_2O$, $NiCl_2 \cdot 6H_2O$, $ZnCl_2$, $Pb(NO_3)_2$, $CuCl_2 \cdot 2H_2O$, and $MnCl_2 \cdot 4H_2O$. A number of stock solutions were prepared in distilled water for each metal so that total volume added to each jar was between 10 and 20 mℓ. Concentrations of individual stock solutions were confirmed analytically by atomic absorption; all were within 2% of expected values.

Dead clams were removed and recorded every 24 hours. Concentrations of each metal lethal to 50% (LC-50) of the test population was determined by graphical interpolation at different exposure periods using the plot: log concentration vs. percent dead (AMERICAN PUBLIC HEALTH ASSOC., 1971). The highest concentrations tested producing no deaths (LC-0), and lowest concentrations tested fatal to all (LC-100) were also recorded daily. Assays were terminated at 168 hours for summer studies or when control mortality exceeded 10% in fall and winter experiments.

It is emphasized that all LC values computed were based on initial concentrations of metals added to the assay medium, and as such were conservative. They do not reflect known loss rates of individual metals from seawater with increasing time through physical, chemical, and biological processes that include precipitation, adsorption, and physiological detoxification.

Results

LC-50 (168 hr) values, in mg/ℓ, at 22°C were 0.035 for Cu, 0.150 for Cd, 1.55 for Zn, 8.80 for Pb, 300.0 for Mn, and >50.0 for Ni. LC-0 and LC-100 values tended to confirm this rank order in sensitivity (TABLE 1). In every case there were deaths recorded between 96 and 168 hours, as evidenced by a decline in

TABLE 1

Concentrations of Cu, Cd, Zn, Pb, Mn, and Ni salts fatal to 0, 50, and 100% of *Mya arenaria* at 30 o/oo salinity and 22.0 ± 0.5° at three time intervals. Values are in mg/l (ppm) metal added to medium at start.

Metal	48 hours			96 hours			168 hours		
	LC-0	LC-50	LC-100	LC-0	LC-50	LC-100	LC-0	LC-50	LC-100
Copper	0.150	5.000	15.000	0.025	0.039	0.100	0.025	0.035	0.050
Cadmium	1.500	3.400	15.000	0.500	0.850	1.500	0.050	0.150	1.500
Zinc	30.000	52.000	90.000	3.000	5.200	9.000	0.900	1.550	3.000
Lead	50.000	>50.000	>50.000	15.000	27.000	50.000	5.000	8.800	15.000
Manganese	300.000	>300.000	>300.000	300.000	>300.000	>300.000	30.000	300.000	>300.000
Nickel	50.000	>50.000	>50.000	50.000	>50.000	>50.000	50.000	>50.000	>50.000

LC-50 values during this interval, suggesting that 168 hours may not be sufficient to adequately evaluate metals toxicity to *Mya*.

Mya subgroups exhibited increasing survival to given concentrations of zinc and copper with decreasing temperatures. For example, the LC-50 (168 hr) value for Zn at 22°C was 1.55 mg/ℓ but at 17°C and 4°C LC-50 (336 hr) values were 2.65 and >25.00 mg/ℓ respectively. A similar pattern was observed for Cu (TABLE 2).

Discussion

The present study demonstrated that Cu and Cd were the most toxic metals tested to softshell clam; similar results were reported by SHUSTER and PRINGLE (1968) in 20-week flowing water studies with oysters and quahaug clams. Copper was especially toxic to softshell clam; concentrations greater than 0.020 mg/ℓ were fatal over a continuous exposure of several weeks (PRINGLE et al, 1968). Copper was also the most toxic metal tested to bivalve embryos in 48 hours but cadmium was less toxic than Zn, Ni, and Pb, in that order (CALABRESE et al, 1973; CALABRESE and NELSON, 1974). Of the six metals assayed, Mn and Pb were among the least toxic to softshell clam, and this is in good agreement with comparative studies of others. For example, with embryos of quahaug clam and oyster the rank order in toxicity was Cu-Zn-Ni-Pb-Cd-Mn (CALABRESE et al, 1973; CALABRESE and NELSON, 1974); for adults of quahaug clam it was Cu-Cd-Zn-Pb, and for oysters Cd-Cu-Zn-Pb (SHUSTER and PRINGLE, 1968).

Assays with Cu and Zn at different temperatures (seasons) demonstrated that softshell clams are considerably more resistant at lower temperatures. Increasing resistance to Cu and Zn with decreasing temperature is well documented for many species of fishes and invertebrates, but data for marine molluscs are lacking (EISLER, 1973; EISLER and WAPNER, 1975). The physiological mechanisms to account for this phenomenon in bivalves appears to warrant additional research effort.

For comparison purposes, acute toxicity data for salts of Cu, Cd, Mn, Ni, Pb and Zn to various species and life stages of marine bivalve molluscs are summarized in TABLE 3. It is underscored that these studies were conducted under a variety of physiochemical conditions and for different test intervals. Accordingly, the wide range in LC values shown was expected. Part of this variability was attributed to inherent

TABLE 2

Concentrations of $ZnCl_2$ and $CuCl_2 \cdot 2H_2O$ fatal to 0, 50, and 100% of *Mya arenaria* at 30 o/oo salinity and seasonal water temperatures. Values are in mg/l (ppm) metal added to medium at start.

Metal (season)	Mean water temp. °C	168 hours			336 hours			504 hours		
		LC-0	LC-50	LC-100	LC-0	LC-50	LC-100	LC-0	LC-50	LC-100
Zinc										
(summer)	22.0	0.900	1.550	3.000	--	--	--	--	--	--
(fall)	17.5	10.000	>10.000	>10.000	1.750	2.650	3.000	1.500	2.000	2.500
(winter)	4.0	25.000	>25.000	>25.000	25.000	>25.000	>25.000	--	--	--
Copper										
(summer)	22.0	0.025	0.035	0.050	--	--	--	--	--	--
(fall)	17.5	0.075	0.086	0.100	0.075	0.086	0.100	0.075	0.086	0.100
(winter)	4.0	3.000	>3.000	>3.000	3.000	>3.000	>3.000	--	--	--

TABLE 3

Summary of published data on acute toxicities of salts of copper, cadmium, nickel, lead, zinc and manganese to marine bivalve molluscs. All values are in mg metal/l (ppm) medium lethal to indicated percentages at designated test interval.

	Copper			Reference
Mytilus edulis	LC-0	(30 days)	0.012 mg/l	(1)
Mercenaria mercenaria	LC-91	(20 weeks)	0.025 mg/l	(15)
Mya arenaria	LC-100	(several weeks)	>0.02 mg/l	(12)
Mytilus edulis	LC-50	(19 days)	0.025 mg/l	(1)
Mya arenaria	LC-50	(168 hours)	0.035 to >3.000 mg/l	This study
Mytilus edulis	LC-100	(10 days)	0.045 mg/l	(1)
Crassostrea virginica	LC-6	(20 weeks)	0.05 mg/l	(15)
Venerupis decussata	LC-100	(50 days)	0.1 mg/l	(16)
Crassostrea virginica embryos	LC-50	(48 hours)	0.103 mg/l	(3)
Mytilus edulis	LC-55	(7 days)	0.2 mg/l	(14)
Cardium edule	LC-50	(48 hours)	~1.0 mg/l	(11)
Japanese oysters	LC-50	(96 hours)	1.9 mg/l	(9)
Crassostrea virginica larvae	LC-100		>1.25 mg/l	(13)
Rangia cuneata	LC-50	(48 hours)	14.7 mg/l	(10)
Mytilus edulis planulatus larvae	LC-50	(2 hours)	22.5 mg/l	(17)
	Manganese			
Crassostrea virginica embroys	LC-50	(48 hours)	16.0 mg/l	(3)
Mya arenaria	LC-50	(168 hours)	300.0 mg/l	This study
	Nickel			
Mercenaria mercenaria embryos	LC-50	(48 hours)	0.31 mg/l	(4)
Crassostrea virginica embryos	LC-50	(48 hours)	1.18 mg/l	(3)
Mya arenaria	LC-50	(168 hours)	>50.00 mg/l	This study
Cardium edule	LC-50	(48 hours)	500.0 mg/l	(11)

TABLE 3 (CONTINUED)

				Reference
	Cadmium			
Mercenaria mercenaria	LC-3	(20 weeks)	0.1 mg/l	(15)
Crassostrea virginica	LC-32	(20 weeks)	0.1 mg/l	(15)
Mya arenaria	LC-50	(168 hours)	0.150 mg/l	This study
Mercenaria mercenaria	LC-38	(20 weeks)	0.2 mg/l	(15)
Crassostrea virginica	LC-45	(20 weeks)	0.2 mg/l	(15)
Mya arenaria	LC-50	(96 hours)	2.2 mg/l	(5)
Crassostrea virginica embryos	LC-50	(48 hours)	3.8 mg/l	(3)
Mytilus edulis	LC-50	(96 hours)	25.0 mg/l	(5)
	Lead			
Mercenaria mercenaria	LC-0	(20 weeks)	0.2 mg/l	(15)
Crassostrea virginica	LC-1	(20 weeks)	0.2 mg/l	(15)
Mercenaria mercenaria embryos	LC-50	(48 hours)	0.78 mg/l	(4)
Crassostrea virginica embryos	LC-50	(48 hours)	2.45 mg/l	(3)
Mya arenaria	LC-50	(168 hours)	8.80 mg/l	This study
	Zinc			Reference
Mercenaria mercenaria embryos	LC-50	(48 hours)	0.166 mg/l	(4)
Crassostrea virginica	LC-1	(20 weeks)	0.2 mg/l	(15)
Mercenaria mercenaria	LC-0	(20 weeks)	0.2 mg/l	(15)
Crassostrea virginica embryos	LC-50	(48 hours)	0.31 mg/l	(3)
Mya arenaria	LC-50	(168 hours)	1.55 to >25.00 mg/l	This study
Cardium edule	LC-50	(48 hours)	200.0 mg/l	(11)

differences in resistance among species; for example, oysters were consistently more resistant to copper than clams. Dissimilarities in thermosaline test regimes probably accounted for much of the variability in LC values; other factors contributed the remainder.

Despite the economic importance of many species of bivalve molluscs, there is a paucity of data on acute and comparative toxicities of many common metals to this group. Also, additional research effort appears warranted on effects of complex metals wastes upon shellfish survival and metabolism under different environmental conditions. Studies in both problem areas are now in progress at this laboratory.

Summary

Static acute toxicity bioassays with adult softshell clams and salts of copper, cadmium, zinc, lead, manganese, and nickel were conducted at 30 ^{o}/oo salinity and 22^{o}C. Concentrations fatal to 50% in 168 hours, in mg/ℓ (ppm) metal added at start, were 0.035 for Cu, 0.150 for Cd, 1.55 for Zn, 8.80 for Pb, 300.0 for Mn, and >50.0 for Ni. Additional tests were conducted with Zn^{2+} and Cu^{2+} at 30 ^{o}/oo during fall (17.5^{o}C) and winter (4^{o}C); clams displayed increasing survival with decreasing temperature. For Cu, LC-50 (336 hr) values at 17^{o}C and 4^{o}C were 0.086 and >3.00 mg/ℓ, respectively; for Zn these were 2.65 and >25.0, respectively.

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