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Toxicity of Zinc, Cadmium and Copper to the Shrimp *Callianassa australiensis*. I. Effects of Individual Metals*

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Abstract

The acute toxicity of zinc, cadmium and copper to *Callianassa australiensis* (Dana) was evaluated in static tests. Each test lasted up to 14 d and LC_{50} values were calculated for 4, 7, 10 and 14 d intervals. The toxicity of each metal increased with exposure time; thus the 4 d LC_{50} values of 10.20, 6.33 and 1.03 mg l^{-1} were considerably higher than the 14 d LC_{50} values of 1.15, 0.49 and 0.19 mg l^{-1} for zinc, cadmium and copper respectively. Toxicity curves reveal that none of the values were asymptotic, indicating that median lethal threshold concentrations were not reached for any of the metals. This suggests that 14 d is an insufficient time in which to complete meaningful, acute lethality tests for marine shrimps. Longer tests are necessary if lethal threshold concentrations are to be used with application factors to derive "safe" concentrations for the protection of *C. australiensis*.

Introduction

Many acute lethality studies have been conducted on the effects of metals on marine and freshwater invertebrates and fishes (Sprague, 1969). Such information is generally used in two ways: firstly, to compare the sensitivities of different species and potencies of chemicals using LC_{50} values, and secondly to derive "safe" environmental concentrations using LC_{50} values and application factors in the absence of chronic toxicity information on the tested species. Most acute studies have been conducted for 96 h as recommended by APHA (1971), Portmann (1972), and NAS/NAE (1973). However, the lethal threshold concentration of a toxicant may be a more appropriate quantity to characterize toxicity, since it represents a common point of lethal physiological response (Wuhrmann, 1952; Sprague, 1969; Brown, 1973). The lethal threshold concentration, therefore, serves as a better concentration from which relative sensitivities of different species to a toxicant can be determined than LC_{50} values based on an arbitrary time (Sprague, 1969). Some investigators (Eisler, 1971; Thorp and Lake, 1974) have also suggested that for certain toxicants, 96 h tests are too short to determine acutely toxic effects adequately.

The objectives of the present investigation were to determine the acute toxicity of zinc, cadmium and copper to the marine shrimp *Callianassa australiensis* in tests lasting up to 14 d; to compare the 4, 7, 10 and 14 d LC_{50} values for each metal; and to derive concentrations of zinc, cadmium and copper to be used in future laboratory studies on the rates of partitioning and accumulation of sublethal concentrations of metals in water, sediments and *C. australiensis* body tissues.

Callianassa australiensis is a sediment-dwelling thalassinidean shrimp which is widely distributed along the east and south coasts of Australia (Hailstone and Stephenson, 1961). Furthermore, it is a major prey organism of a commercially and recreationally important fish, the King George whiting *Sillaginodes punctatus* (Robertson, 1977). Zinc, cadmium and copper were studied because of their toxicity and common occurrence in effluent and riverine inputs into Australian coastal marine waters (Phillips, 1976a,b).

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Materials and Methods

Collection and Acclimation of *Callianassa australiensis* (Dana)

Shrimp were collected by the "Kiwi" method (Torres *et al.*, 1977) from the intertidal flats in the North Arm of Western Port, Victoria, Australia. This method involves jumping up and down in the mud until it liquefies and causes shrimp to move up to the surface. The shrimp were transported to the laboratory in aerated tanks containing 8 cm of sediment and 30 l of sea water. At the time of collection of shrimp during May and October 1976, the average water temperatures and salinities ranged between 12° to 16°C and 32 to 35‰, respectively. In the laboratory the shrimp were kept at 2 C° above the water temperature on the day of collection for one day. After that the temperature was raised to 19°C; the test temperature. Salinities during the experiments were kept similar to those at the time of collection. Acclimation to test conditions was restricted to 3 to 4 d to minimise loss of condition due to starvation. Preliminary investigations indicated that the shrimp could be maintained without food for up to 28 d; however, the maximum period of starvation in an experiment was 19 d. Only active adult males weighing between 1 and 1.50 g were used.

Toxicants

Stock solutions of 10 mg Zn l^{-1}, 50 mg Cd l^{-1} and 5 mg Cu l^{-1} were prepared daily using sea water and AR-grade $ZnCl_2$, $CdCl_2 . 2\frac{1}{2} H_2O$ and $CuSO_4 . 5H_2O$. Zinc and copper stock solutions were acidified slightly (pH $\cong$ 7) to prevent precipitation of the metals. Water from each experimental tank was taken immediately before redosing and chemically analysed daily. All data analyses were based on means of these measured total dissolved metal concentrations, from which was calculated the LC_{50} value.

Experimental Procedures

All tests were static and were conducted in accordance with the recommendations of Sprague (1973). The experimental apparatus was similar to that described by Ahsanullah (1976). In each experiment, 1 control and 5 different concentrations were used; in each concentration, 10 shrimp were exposed in a tank containing 10 litres of test solution. To avoid disturbing the shrimp, up to 9 litres of solutions were renewed daily by slowly siphoning water out of and into each experimental tank. Water quality in each tank was monitored daily for changes in pH, dissolved oxygen, temperature and salinity, which ranged from 7.9 to 8.2, 90 to 100% saturation, 18° to 20°C and 34.1 to 37.5‰, respectively.

Observations for mortality were made four times daily at regular intervals. The criterion for death was failure to respond to mechanical stimulation. Dead shrimp were removed at each observation.

The experiments were carried out between May and October 1976. Only zinc was tested both at the beginning and at the end of this experimental period to determine whether there was any change in the sensitivity of *Callianassa australiensis* collected during different seasons.

Data Analysis

The percentage mortalities in each concentration were corrected for control mortality using Abbott's formula (Finney, 1971); however, mortalities in controls were never greater than 10%.

Computation of 4, 7, 10 and 14 d LC_{50} values and fiducial limits, tests for parallelism of probit lines, and evaluation of potency ratios, where appropriate, were conducted as outlined in Finney (1971). χ^2 values were computed to test whether the probit lines were adequate representations of the data. To test the hypothesis that the LC_{50} values computed from two or more time intervals are identical, the assumption that the experiments are independent must be satisfied. Therefore only subjective comparisons of the 4, 7, 10 and 14 d LC_{50} values within metals have been made in the present study, as four independent and parallel experiments were not conducted, i.e., the 7 d LC_{50} values were dependent on the 4 d values, the 10 d values were dependent on the 4 d and 7 d values, and so on.

Results

Callianassa australiensis weakened progressively with time before death; generally, the pattern of mortality was similar for various concentrations of the three metals. LC_{50} values, slopes and relative potencies, together with 95% fiducial or confidence limits, are given in Tables 1 and 2.

Zinc

The toxicity of zinc increased between 4 and 14 d as LC_{50} values decreased from 10.20 to 1.15 mg l^{-1} (Table 1).

Substantial quantities of mucus were secreted by the shrimp when exposed to more than 10 mg l^{-1} of zinc. The shrimp usually died within 72 h after becoming weak in concentrations greater than 1.80 mg Zn l^{-1}, but in concentrations between 0.88 and 1.80 mg Zn l^{-1} mortality was further delayed. Mortalities of 10% or less were observed after 14 d exposure in concentrations of less than 0.88 mg Zn l^{-1}; however, many of the survivors were weak.

Table 1. *Callianassa australiensis.* LC_{50} values (mg l^{-1}) and slopes (b), with 95% fiducial (FL) and confidence (CL) limits, intercepts (a), and x^2 values of 4, 7, 10 and 14 d probit lines for shrimp exposed to zinc, cadmium and copper. LC 100% M: lowest concentration at which 100% mortality occurred after 14 d; HC 100% S: highest concentration in which all shrimp survived after 14 d

	Zinc	Cadmium	Copper
4 d LC_{50} (95% FL)	10.20(8.93–11.66)	6.33(5.25–7.61)	1.03(0.69–1.54)
b (95% CL)	6.74(4.20–9.27)	3.94(2.22–5.66)	1.95(1.10–2.80)
a	−1.79	1.84	4.97
χ^2	7.77 (NS)	17.86($P < 0.005$)	9.63 (NS)
7 d LC_{50} (95% FL)	1.98(1.61–2.45)	0.97(0.79–1.19)	0.34(0.25–0.46)
b (95% CL)	6.54(2.32–10.77)	2.94(2.07–3.81)	2.08(1.33–2.83)
a	3.05	5.04	5.97
χ^2	1.17 (NS)	27.96($P < 0.001$)	16.13 (NS)
10 d LC_{50} (95% FL)	1.54(1.38–1.71)	0.61(0.54–0.68)	0.22(0.17–0.30)
b (95% CL)	11.13(6.11–16.15)	6.90(4.45–9.35)	2.18(1.54–2.81)
a	2.92	6.50	6.41
χ^2	2.74 (NS)	15.00($P < 0.01$)	20.23($P < 0.025$)
14 d LC_{50} (95% FL)	1.15(1.08–1.22)	0.49(0.44–0.54)	0.19(0.14–0.26)
b (95% CL)	14.08(7.59–20.58)	9.67(5.35–13.99)	1.92(1.29–2.55)
a	4.15	8.04	6.39
χ^2	0.83 (NS)	6.76 (NS)	19.63 (NS)
LC 100% M	1.40	0.56	1.03
HC 100% S	0.44	0.32	0.06
Zinc experiment only (early spring)			
14 d LC_{50} (95% FL)	1.06(0.97–1.15)		
b (95% CL)	10.66(5.21–16.11)		
a	4.75		
χ^2	6.35 (NS)		

Table 2. *Callianassa australiensis.* Combined slopes (b) with 95% confidence limits, and potency ratios (PR) and 95% fiducial limits, for Cd/Zn and Cu/Cd 4, 7, 10 and 14 d dose-mortality curves. NP: does-mortality curves not parallel

	Cd:Zn	Cu:Cd
4 d		
b	5.03(4.31–5.75)	2.41(2.00–2.82)
PR	1.60(1.26–2.03)	7.06(4.61–10.81)
7 d	NP	
b		2.49(2.20–2.78)
PR		2.79(1.96–3.95)
10 d		NP
b	7.93(6.72–9.14)	
PR	2.52(2.08–3.05)	
14 d		NP
b	11.24(8.76–13.72)	
PR	2.33(2.08–2.61)	

The sensitivity of the shrimp tested in late autumn and early spring (Table 1) was very similar, with no significant difference occurring between LC_{50} values.

Cadmium

A 13-fold increase in the toxicity of cadmium was observed between 4 and 14 d, LC_{50} values decreasing from 6.33 to 0.49 mg Cd l^{-1} (Table 1). Some differences were also found between the 4, 7, 10 and 14 d slope values from the dose-mortality curves (Table 1). Mortalities, especially after 4 and 7 d, were particularly variable in cadmium experiments, as shown by large χ^2 values. However, the dose-mortality curves gave no indication of a non-linear fit.

Copper

LC_{50} values for copper varied from 1.03 mg l^{-1} at 4 d down to 0.19 mg l^{-1} after 14 d exposure (Table 1). The slopes of the dose-mortality curves for all time intervals were similar, which suggests that the toxic action of copper did not change with time or concentration.

At concentrations less than 0.56 mg l^{-1}, mortalities, after initial deaths, were delayed. At 0.52 mg Cu l^{-1},

Table 3. Lethal concentrations of zinc, cadmium and copper for some marine Crustacea. nd: no data

Species	Time (h)	Experimental T (°C)	Salinity (°/₀₀)	LC_{50} (mg l^{-1})	Source
Zinc					
Allorchestes compressa	96	20.5	34.5	0.6	Ahsanullah (1976)
Palaemon sp.	96	19.5	35.5	11.3	
Paragrapsus quadridentatus	96	19.6	34.2	11.0	
Pandalus montagui	48	15.0	sea water	~10.0	Portmann (1968)
Carcinus maenas	48	15.0	sea water	~20.0	
Crangon crangon	48	15.0	sea water	~100.0	
Pagurus longicarpus	96	20	20	0.4	Eisler and Hennekey (1977)
	168	20	20	0.2	
Callianassa australiensis	96	~19.0	~35.0	10.2	Present study
	336	~19.0	~35.0	1.2	
Cadmium					
Mysidopsis bahia	96	25–28	15–25	0.016	Nimmo *et al.* (1978)
Allorchestes compressa	96	16.8	34.5	0.2–0.4	Ahsanullah (1976)
Palaemon sp.	96	17.3	nd	6.6	
	168	18.7	32.1	1.9	
Paragrapsus quadridentatus	168	17.8	32.6	14.0	Ahsanullah (1976)
Crangon septemspinosa	96	20.0	20.0	0.3	Eisler (1971)
Palaemonetes vulgaris	96	20.0	20.0	0.4	
Pagurus longicarpus	96	20.0	20.0	0.3	
Pagurus longicarpus	96	20.0	20.0	1.3	Eisler and Hennekey (1977)
	168	20.0	20.0	0.7	
Carcinus maenas	96	20.0	20.0	4.1	Eisler (1971)
Eurypanopeus depressus	72	21.0 ± 2	25.0	4.9	Collier *et al.* (1973)
Callinectes sapidus	96	20–22	35	11.6	Frank and Robertson (1979)
Uca pugilator	96	20.0	30.0	37.0	O'Hara (1973)
	240	20.0	30.0	17.9	
	240	10.0	30.0	47.0	
Homarus americanus (larval Stage I)	96	20 ± 1	30.5 ± 1	0.078	Johnson and Gentile (1979)
Penaeus duorarum	96	25 ± 2	20 ± 2	4.6	Bahner and Nimmo (1975)
	720	25 ± 2	20 ± 2	0.72	Nimmo and Bahner (1976)
Palaemonetes vulgaris	96	25 ± 2	20 ± 2	0.76	Nimmo *et al.* (1977)
	696	25 ± 2	20 ± 2	0.12	
Callianassa australiensis	96	~19.0	~35.0	6.3	Present study
	336	~19.0	~35.0	0.5	
Copper					
Balanus balanoides	6		hypertonic sea water	0.23	Pyefinch and Mott (1948)
Balanus crenatus	6		hypertonic sea water	0.5–1.6	
Carcinus maenas	264–288		nd	1.2	Raymont and Shields (1964)
	48	15	sea water	100	Portmann (1968)
Pandalus montagui	48	15	sea water	0.2	
Homarus americanus	22–33	13	30	1.0	McLeese (1974)
	105	13	30	0.08	
	70	5	30	0.560	
Homarus americanus (larval Stage 1)	96	20 ± 1	30.5 ± 1	0.048	Johnson and Gentile (1979)
Crangon crangon	48	15	sea water	30.0	Portmann (1968)
Callianassa australiensis	96	~19	~35	1.0	Present study
	336	~19	~35	0.2	

for example, 50% mortality occurred within 5 d, but 40% of the shrimp survived for 14 d. At even lower concentrations, e.g. 0.06 mg l^{-1}, 100% of the test population survived for 14 d, although 50% of these were weak in their swimming activities at the end of the experiment.

Comparison of Individual Metals

Copper was the most toxic metal, followed by cadmium and then zinc. This order of toxicity did not change throughout the 14 d period of testing (Table 1).

Potency ratios for cadmium and zinc increased with time between 4 and 10 d and then decreased slightly between 10 and 14 d (Table 2). Those for copper and cadmium, on the other hand, decreased substantially between 4 and 7 d; after that, the dose-mortality curves diverged from parallelism (Table 2). Zinc and copper could not be compared because of significant differences in their slopes.

Discussion

Results indicated that copper was more toxic than either zinc or cadmium to *Callianassa australiensis* (Table 1), a finding which agrees with toxicity information reported for other crustaceans by Bryan (1971), Ahsanullah and Arnott (1978), and Arnott and Ahsanullah (1979). Comparisons of the slopes of dose-mortality curves showed some significant differences between metals (Table 2) which suggests a different toxic action for each of the three metals.

Callianassa australiensis appeared more resistant than either *Palaemonetes* or *Penaeus* species to cadmium, intermediate in tolerance to copper, and about as tolerant as most other species to zinc, based on available acute toxicity information for some marine Crustacea (Table 3).

The marked changes in LC_{50} values with time observed for all of the metals support the conclusion that 4 d is too short a time for the occurrence of median lethal threshold concentrations for some metals (e.g. Sprague, 1969; Thorp and Lake, 1974). In addition, the results suggest that even 14 d is inadequate to reveal such levels for *Callianassa australiensis*. The results of Bahner and Nimmo (1975) and Nimmo and Bahner (1976), on the effects of cadmium on the shrimp *Penaeus duorarum* showed that, between 4 and 30 d, there is a significant decrease in LC_{50} values from 4.6 to 0.72 mg Cd l^{-1}. Nimmo *et al.* (1977) also reported a similar result for cadmium on *Palaemonetes vulgaris* (Table 3).

Given that it is not possible to extrapolate exactly from the laboratory to the environment, the experimental conditions do not replicate those of the natural habitat of *Callianassa australiensis*. Nevertheless, the present results have important practical implications. If "safe" environmental concentrations for *C. australiensis* are derived using acute lethality data and application factors (EPA, 1979), concentrations from 4 d tests would be approximately 10 times higher than those concentrations derived from 14 d LC_{50} values. Application of the 0.01 factor to 4 d LC_{50} values for *C. australiensis* gives concentrations of 102 $\mu g\ l^{-1}$ for zinc, 63 $\mu g\ l^{-1}$ for cadmium and 10.3 $\mu g\ l^{-1}$ for copper. The minimal risk concentrations recommended by EPA (1979) for zinc, cadmium and copper are 20, 0.2 and 10 $\mu g\ l^{-1}$, respectively. It is obvious that only in the case of copper are derived and recommended minimal risk concentrations close to each other; for zinc and cadmium, the derived concentrations are higher. By using the application factor and 14 d LC_{50} values, safe concentrations of 11.5 $\mu g\ l^{-1}$ for zinc, 4.9 $\mu g\ l^{-1}$ for cadmium and 1.9 $\mu g\ l^{-1}$ for copper were derived. Cadmium values were still higher than the recommended minimal risk concentrations. This suggests that acute lethality tests for the comparison of sensitivities of different species and toxicants, and for the derivation of "safe" concentrations, especially for cadmium, should be continued until such time as median lethal thresholds are obtained.

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