

Short Communication

COPPER TOXICITY TO *PARATYA AUSTRALIENSIS*: II. INFLUENCE OF BICARBONATE AND IONIC STRENGTH

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(Received 29 June 1989; Accepted 24 October 1989)

Abstract—The acute toxicity of copper to the Australian freshwater shrimp, *Paratya australiensis*, was shown to decrease in solutions of increasing alkalinity brought about by the addition of NaHCO_3 . This effect could not be explained merely in terms of changes in copper speciation in the solutions. Estimates of the free copper(II) ion concentration of the LC50 values were made using both copper ion selective electrodes measurements and chemical equilibrium computer calculations. Whereas there were discrepancies between the estimates obtained using these two methods, both indicated an apparent increase in tolerance of *P. australiensis* to the free copper(II) ion in more alkaline waters. An additional set of experiments, in which NaCl was added to test waters in place of NaHCO_3 , demonstrated that the increased tolerance of *P. australiensis* to copper in higher alkalinity waters was caused by a combination of physiological effects associated with increased ionic strength of the test waters and, to a lesser extent, changes in metal speciation in test waters. Further study of the reason for the discrepancy between estimates of copper(II) concentrations derived from ion selective electrode measurements and computer calculations is required.

Keywords—Copper toxicity Speciation Ionic strength Bicarbonate
Paratya australiensis

INTRODUCTION

The experiments reported in this paper were conducted as part of a study on the relationship between the physico-chemical forms of copper and its toxicity to the Australian freshwater shrimp, *Paratya australiensis*. The experimental program was designed to test the hypothesis that the free copper(II) ion (Cu^{2+}) was the major copper species causing acute toxicity to *P. australiensis*. Acute toxicity tests were conducted using copper in Melbourne tap water (no added complexing ligands), and in the presence of two well characterized organic complexing ligands (nitrilotriacetic acid (NTA) and glycine) [1], a representative inorganic ligand (bicarbonate) and natural dissolved organic matter collected from three aquatic sites in Victoria, Australia [2]. The results of the bicarbonate experiments are reported here.

In the copper-NTA and copper-glycine experiments described by Daly et al. [1], the concentrations of Cu^{2+} , and CuOH^+ and CuCO_3 were highly correlated and could not be separated to in-

dicade which was/were the toxic species. For this reason, it was decided to conduct a pair of experiments with copper and NaHCO_3 to determine whether or not CuCO_3 contributes to the toxicity of copper to *P. australiensis*. Unfortunately, a similar group of experiments with the hydroxide ion could not be run without changing the pH of the test waters, making it difficult to distinguish between physiological and metal speciation effects.

Most studies on acute copper toxicity have indicated that CuCO_3 is nontoxic to aquatic organisms (e.g., [3-5]). On the other hand, Shaw and Brown [6] argued that copper carbonate and the free copper(II) ion were equally toxic to rainbow trout. However, it is difficult to reconcile this interpretation with the results of their experiments, which demonstrate that copper toxicity was significantly reduced in high alkalinity test waters (in which there would have been considerable complexation of copper by the carbonate ion).

In the present study, *P. australiensis* was exposed to copper in the presence of two concentrations of NaHCO_3 . Preliminary experiments indicated that the change in ionic strength of the test waters caused by the addition of NaHCO_3 had a physiological effect on test organisms, which altered their sensitivity to copper. It was therefore necessary to

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run a parallel copper plus NaCl experiment for each copper plus NaHCO₃ experiment conducted.

MATERIALS AND METHODS

General procedures

The test procedure has been described by Daly et al. [1]. Briefly, each experiment was conducted as a 6-d continuous-flow test, in which test organisms were exposed to a range of copper concentrations at 15°C in continuously aerated solutions. Test concentrations were run in duplicate. If mortalities in the control exceeded 10%, the test was rejected. At the end of each experiment, LC50 values were calculated using a computer program for probit analysis by the maximum likelihood iterative method [7]. Results were expressed in terms of total copper concentrations and (where possible) both measured and calculated copper(II) ion concentrations.

For the bicarbonate experiments, sufficient NaHCO₃ (Univar) was added to stock solutions to obtain test waters with alkalinities of approximately 50 and 100 mg/L as CaCO₃. The quantity of NaHCO₃ required was calculated using a method for total carbonate and pH control described by Roberts and Allen [8]. Initially some difficulties were encountered with the continuous-flow bicarbonate experiments because of precipitation of malachite (Cu₂(OH)₂CO₃) in the stock solutions, which caused a reduction in the concentration of copper being delivered to the test tanks. The problem was solved by bubbling CO₂ through the stock solutions to lower the pH and thereby stop precipitation. This did not have any significant effect on the final pH of test solutions because of the small toxicant volume to diluent volume ratio.

A pair of copper plus NaCl experiments was run subsequently, in which sufficient NaCl was added

to stock solutions to obtain test waters with conductivities corresponding to those in the two alkalinity experiments. Mean values for physicochemical characteristics of all test waters are presented in Table 1. Corresponding values for Melbourne tap water [1] are included for comparison.

An Orion copper selective electrode (ISE; Model No. 94-29A) was used to measure copper(II) ion activities in solutions containing a range of total copper concentrations at each of the two bicarbonate and chloride concentrations used in the tests. From these results, the free copper(II) ion concentrations that would have been present in the test waters of each experiment were estimated. In addition, copper(II) ion concentrations were calculated from measured total copper concentrations using the MINEQL chemical equilibrium computer program devised by Westall et al. [9].

RESULTS

The 48-, 96- and 144-h LC50 values for *P. australiensis* with copper in two NaHCO₃ concentrations and two NaCl concentrations are presented in Table 2. Results are expressed in terms of total copper concentrations and of calculated (MINEQL) and measured (ISE) copper(II) ion concentrations.

In Figure 1 the 96-h LC50 values for *P. australiensis* with copper in Melbourne tap water, two NaHCO₃ concentrations and two NaCl concentrations are shown in terms of total copper and calculated copper(II) ion concentrations. The 95% fiducial limits for each LC50 value are indicated by diagonal lines; the horizontal component of these lines represents the fiducial limits for the LC50 values on a total copper basis, whereas the vertical component represents the fiducial limits on a copper(II) ion basis. It is apparent from Figure 1 that the presence of NaHCO₃ and NaCl in test so-

Table 1. Mean values for physicochemical characteristics of test waters in bicarbonate/chloride experiments

Indicator	Units	NaHCO ₃ ^a	NaCl ^b	NaHCO ₃ ^c	NaCl ^d	Melbourne tap water
Conductivity	µS/cm	109.5 (3.9)	121.4 (5.2)	167.5 (7.7)	172.4 (0.1)	59.7
Hardness	mg/L as CaCO ₃	11.9 (0.2)	13.5 (0.3)	12.7 (0.2)	13.2 (0.2)	17.0
pH		6.9 (0.2)	6.7 (0.4)	6.9 (0.2)	6.5 (0.1)	7.0
Alkalinity	mg/L as CaCO ₃	46.5 (2.9)	3.8 (0.2)	95.4 (3.0)	6.5 (0.2)	9.1
DOC	mg C/L	3.4 (0.3)	3.1 (0.2)	3.1 (0.2)	2.8 (0.2)	2.4

DOC, dissolved organic carbon concentration; values in parentheses are standard deviations ($n = 14$). Mean values for Melbourne tap water are included for comparison [1].

^aNominal alkalinity of 50 mg/L as CaCO₃.

^bNaCl solution with equivalent conductivity to ^a.

^cNominal alkalinity of 100 mg/L as CaCO₃.

^dNaCl solution with equivalent conductivity to ^c.

Table 2. 96-h LC50 values for *P. australiensis* exposed to copper in the presence of NaHCO₃ or NaCl

Test	Time (h)	Total Cu LC50 (µg Cu/L)		Cu(II) ion LC50 (µg Cu/L)			
		LC50	(95% FL)	Calculated		Measured	
				MINEQL	(95% FL)	ISE	(n, sd)
1	48	248	(147, 2,340)	99	(58, 931)	128	(5, 13)
2		161	(120, 566)	93	(69, 325)	135	(3, 10)
3		294	(197, 1,190)	81	(54, 328)	154	(5, 19)
4		183	(140, 477)	101	(80, 274)	169	(3, 12)
1	96	120	(99, 173)	48	(39, 69)	52	(3, 3)
2		99	(85, 125)	57	(49, 72)	73	(3, 3)
3		178	(137, 288)	49	(38, 79)	80	(5, 12)
4		112	(98, 138)	61	(55, 79)	89	(3, 10)
1	144	106	(90, 137)	42	(35, 55)	44	(5, 7)
2		85	(72, 102)	49	(41, 59)	59	(3, 4)
3		140	(113, 182)	40	(31, 52)	56	(3, 9)
4		103	(90, 122)	57	(51, 70)	79	(3, 9)

FL, fiducial limits; n, number of ISE measurements; sd, standard deviation of ISE measurements.

Test 1: NaHCO₃ (alk. = 50 mg/L as CaCO₃);

Test 3: NaHCO₃ (alk. = 100 mg/L as CaCO₃);

Test 2: NaCl solution, conductivity same as 1;

Test 4: NaCl solution, conductivity same as 3.

(See Table 1 for other chemical characteristics of test waters.)

lutions substantially reduced copper toxicity to *P. australiensis*.

However, this effect cannot be explained merely in terms of changes in copper speciation. For example, in Melbourne tap water, the 96-h LC50 value in terms of free copper(II) ion is approximately 20 µg/L, whereas in the presence of around 10⁻³ M NaHCO₃ (alkalinity = 100 mg/L) or NaCl it is more than 45 µg/L. This suggests that the free copper(II) ion is actually less toxic to *P. australiensis* in the presence of added sodium salts.

DISCUSSION

With the possible exception of results from the 50 mg/L alkalinity test waters, estimates of copper(II) ion concentrations obtained using the MINEQL chemical equilibrium program were substantially lower than their corresponding ISE-measured values (Table 2). One possible explanation for this result is that the copper ISE responds to species other than just the free copper(II) ion. In a study of copper speciation in certain Canadian

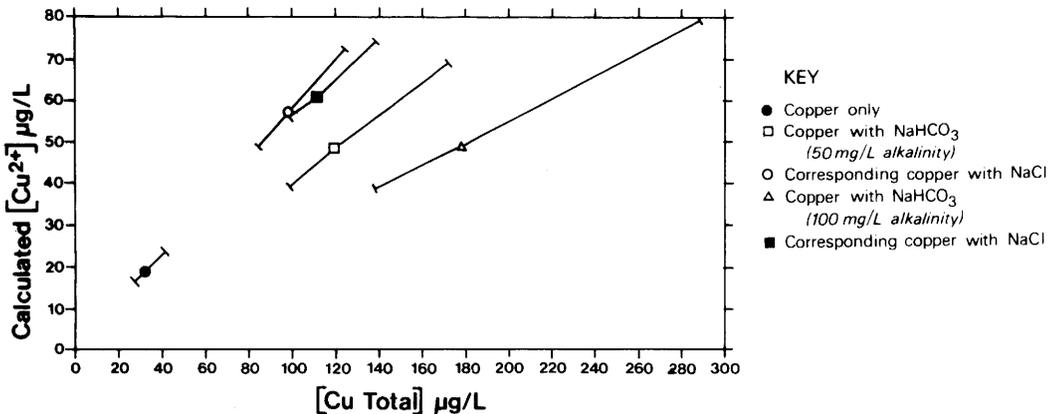


Fig. 1. LC50 values for *P. australiensis* exposed to copper in the presence of NaHCO₃ and NaCl, in terms of total copper concentration and calculated copper(II) ion concentration: 96-h test.

natural waters, Barica [10] found that the response of a copper ISE deviated significantly from the theoretical (Nernstian) response. He suggested that the electrode responded to singly charged copper complexes, such as CuOH^+ or CuHCO_3^+ , in addition to the free copper(II) ion. Similarly, Wagemann [11] argued that under certain conditions copper ISEs respond to positively charged inorganic complexes of copper, and that this can lead to significant deviations from expected values.

A basic assumption made in speciation measurements is that all of the species in the system are at thermodynamic equilibrium. Results of a recent study by Gulens et al. [12] suggest that this is not always so. They found that the speciation of micromolar solutions of copper was dominated by the formation of a copper-hydroxy colloid, which only slowly converted to thermodynamically more stable species. The presence of this solid phase controlled the concentration of free copper(II) ions in solution. This could obviously lead to an error in interpreting the results of ISE measurements where it is assumed that equilibrium has been attained.

When copper ISEs are used in the presence of high chloride ion activities, changes in the response slope and apparent potential of the electrode, slow response and sensitivity to stirring have been observed [9,13,14]. However, it seems unlikely that the chloride ion concentrations in the present set of experiments would be sufficient to cause such interference.

A comparison of the percentage of copper present as copper(II) ions in Melbourne tap water (determined by ISE) with that in tap water containing NaHCO_3 or NaCl supports the hypothesis that the ISE is responding to some factor other than just the activity of the free copper(II) ion. In Melbourne tap water, the free copper(II) was shown to constitute 50 to 60% of the total copper in the water [1]. As the alkalinity of the test water is increased, one would expect that more copper would be complexed as carbonate species, resulting in a significant reduction of the percentage of copper present as free copper(II). This is indicated for the computer calculated values (Table 2). In the lower alkalinity test waters there is reasonable agreement between the calculated and ISE-measured free copper(II) concentrations at the 96-h and 144-h LC50 values. However, there is little reduction in the ISE-measured percentage of free copper(II) when the alkalinity is increased to 100 mg/L as CaCO_3 . In the presence of NaCl there is, according to the ISE measurements, an increase in the percentage of copper present as copper(II) ions compared with

that present in tap water, whereas one would expect a slight reduction. The ISE measurements are contrary to what would logically be expected from a basic understanding of the aqueous system. Because of this, calculated rather than measured copper(II) concentrations were used to interpret the toxicity test results.

The data in Figure 1 demonstrate a substantial reduction in copper toxicity to *P. australiensis* when either NaCl or NaHCO_3 is added to the test water. Two likely reasons for this result are: (a) that changes in metal speciation of the test waters have altered the concentration of the toxic form(s) of copper; and (b) that the sensitivity of *P. australiensis* to copper is reduced in waters of high ionic strength (at least up to conductivities of 170 $\mu\text{S}/\text{cm}$).

At the concentrations of NaCl used in these experiments, complexation of copper by chloride ions was negligible, resulting in virtually no difference in metal speciation between the Melbourne tap water and NaCl test waters. Therefore, speciation effects cannot be used to explain the differences in copper toxicity observed between the NaCl and tap water experiments.

A number of workers have observed a decrease in copper toxicity with increasing alkalinity, and attributed this to the formation of nontoxic copper complexes, such as CuCO_3 and CuOH^+ , with a corresponding reduction in the concentration of the toxic free copper(II) ion [3-5, 15]. If CuCO_3 were making a substantial contribution to copper toxicity in the present set of experiments, one would expect that the LC50 in terms of the free copper(II) ion would be significantly lower in the bicarbonate experiments than in the NaCl experiments. Whereas the copper(II) LC50's from the bicarbonate experiments appear to be slightly lower (Table 2, Fig. 1), there is considerable overlap of the 95% fiducial limits, and the differences could not be considered significant. Therefore, it appears that CuCO_3 does not contribute substantially to the acute toxicity of copper to *P. australiensis*.

When the results of toxicity tests in waters containing added bicarbonate or chloride salts are compared with those in Melbourne tap water alone (Fig. 1), it is apparent that the copper(II) ion is significantly more toxic in the lower ionic strength tap water. This increased toxicity is evident as a lower LC50 value (in terms of both total copper and free copper(II) ion concentrations) in tap water compared with $\text{NaHCO}_3/\text{NaCl}$ test waters. Possible reasons for the heightened sensitivity of *P. australiensis* to copper(II) ions in waters of low ionic

strength include: (a) changes in the rate of active uptake of ions associated with the low ionic concentration of the water, which would increase the potential for movement of copper across the gill surface; (b) the effects of copper toxicity and ionic stress acting synergistically to decrease the shrimp's tolerance to heavy metal exposure. Lake et al. [16] found that cadmium was significantly less toxic to the freshwater amphipod *Austrochiltonia australis* in waters with high total ion concentrations. They attributed this result to a reduction in uptake of cadmium at higher conductivities, largely due to the presence of increased calcium and magnesium ion concentrations. However, the changes in toxicity observed in the present study were associated with increased sodium, chloride and bicarbonate concentrations rather than calcium and magnesium.

The reduction in an animal's tolerance to one environmental factor due to another has been reported frequently in the literature [e.g., 16–20]. Walker [17] showed that the tolerance of *P. australiensis* to extremes of temperature was reduced significantly in waters of low ionic strength. Studies on various Crustacea have demonstrated increased toxicity of metals in "ionically stressful" environments [20–22]. Whereas it is not suggested that the ionic strength of Melbourne tap water is sufficiently low to affect the survival of *P. australiensis* on its own, it does seem likely that the sensitivity of the shrimp to copper exposure could have been heightened under the conditions of the tap water experiments.

Acknowledgement—This study was funded by the Australian Water Resources Council.

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