COPPER TOXICITY TO *PARATYA AUSTRALIENSIS*: III. INFLUENCE OF DISSOLVED ORGANIC MATTER

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Abstract — The influence of dissolved organic matter on the toxicity of copper to the Australian freshwater shrimp, *Paratya australiensis*, was measured using water collected from three sites in Victoria, Australia. A two-ligand model was used to describe copper complexation in these test waters. The binding characteristics of the ligands (i.e., total ligand concentrations and conditional stability constants) were determined using a method combining ion selective electrode and anodic stripping voltammetry.

Ion selective electrode measurements and the two-ligand model were each used to estimate the concentration of copper(II) (the major toxic species) at the LC50 values. Both methods overestimated by a factor of approximately 1.5 to 3 the expected copper(II) concentration at the LC50 values, based on earlier experiments in Melbourne tap water. The agreement seems remarkably good given the many assumptions used in making the comparison and the difficulties encountered with the use of ion selective electrodes in natural waters.

Keywords — Copper toxicity   Speciation   Complexing capacity   Dissolved organic matter
Paratya australiensis

INTRODUCTION

The experiments reported in this paper were conducted as part of a study on the relationship between the physicochemical 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 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) [2] and natural dissolved organic matter. The results of the experiments using natural waters are reported here.

Data from the tests conducted in well-characterized waters (Melbourne tap water alone, or tap water with added NTA, glycine (Gly) or bicarbonate)

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indicate that, of the copper species tested, the free copper(II) ion was most acutely toxic to *Paratya australiensis* [1,2]. However, at least one other species ($CuGly^+$) appeared to be mildly toxic also [1]. Experiments in the presence of bicarbonate demonstrated that the ionic strength of the test waters influenced copper toxicity to *P. australiensis*, with toxicity decreasing as ionic strength was increased [2].

In metal speciation studies there are obvious advantages in using a well-defined medium containing ligands that form known complexes with the metal. However, eventually it is necessary that the toxicity of metals in natural waters be studied, since these are the systems that we intend to protect.

Most natural waters have an ability to complex metals (known as the complexing capacity), which, in many fresh waters, appears to be associated mainly with the presence of organic matter [3–7]. A number of workers have shown that metal toxicity to organisms in natural waters is reduced by the complexing capacity of the waters [3,8–14].

In the present study, three pairs of experiments were conducted in which *P. australiensis* was exposed to copper in the presence of natural dissolved organic matter collected from three sites in Victoria, Australia. The results of the experiments

were interpreted in the light of complexing capacity measurements made on each of the test waters.

MATERIALS AND METHODS

General procedures

The test procedure used has been described by Daly et al. [1]. Because of the difficulty of transporting large quantities of the natural waters from the field to the laboratory, flow-through experiments were not undertaken and instead each experiment was run as a 6-d semi-static test with half of the test volume changed daily. Solutions were continuously aerated to ensure mixing and to maintain saturated dissolved oxygen condition. The natural waters were filtered through a Whatman GF/C glass fiber filter (approx. 1 μm particle size retention) to remove any coarse particulate matter prior to the experiments.

The natural waters were collected from three sites in Victoria, Australia:

1. the Inkpot in Western Victoria;
2. a Forests Commission fire dam on the Errinundra Plateau in East Gippsland;
3. Redwater Creek in the Otway Ranges (Western Victoria).

Further details of the sites are given by Daly [15].

In each experiment, a tap water control, a natural water control and seven (Inkpot experiments) or eight (Errinundra and Redwater Creek experiments) copper concentrations were run in duplicate at 15°C. Laboratory tap water and distilled water were mixed with each natural water in appropriate quantities to result in test waters with dissolved or-

ganic carbon concentrations of approximately 7 to 8 mg/L and 12 to 13 mg/L, and similar pH, hardness, conductivity and alkalinity to the laboratory tap water used in other tests [1]. Dissolved organic carbon measurements were made on a Sybron Barnstead Photochem organic carbon analyzer. Standard methods [16] were used for determining the alkalinity, hardness, pH and conductivity of test waters. The chemical characteristics of the test waters for each experiment are shown in Table 1. Mean values for Melbourne tap water [1] are included for comparison. During the experiments with Inkpot and Redwater Creek waters, the pH of test waters was adjusted to around 7 with 0.1 M NaOH.

An Orion copper ion selective electrode (ISE) (Model 94–29A) was used to measure copper(II) ion activities in solutions containing a range of total copper concentrations at each of the "natural water" concentrations used in the toxicity experiments.

Complexometric method

The complexing capacity of each of the test waters was determined by complexometric titration using the anodic stripping voltammetry (ASV) and ion selective electrode (ISE) methods described by Hart and Jones [17] and Jones [18], except that the pH of the samples was maintained at 7.0 rather than 6.0. The essence of the method is that a sample is titrated with ionic copper and, at certain intervals, the uncomplexed copper is measured using either ASV or ISE. In the early stages of the titration there is very little free copper(II) determined because most of the added metal is complexed. However, once the complexing capacity is exceeded, the added metal remains uncomplexed and

Table 1. Mean values for physicochemical characteristics of test waters in natural water experiments

Indicator	Units	Redwater 1:7[a]	Redwater 1:3	Errinundra 1:3	Errinundra 1:1	Inkpot 1:9	Inkpot 1:4	Melbourne tap water
Conductivity	μS/cm	64.0 (0.4)	70.4 (0.8)	51.6 (0.4)	49.2 (0.8)	64.3 (0.7)	60.7 (0.5)	59.7
Hardness	mg/L as $CaCO_3$	16.0 (0.4)	16.4 (0.6)	17.5 (0.5)	15.6 (0.3)	14.7 (0.4)	14.1 (0.3)	17.0
pH		6.79 (0.04)	6.70 (0.02)	7.00 (0.04)	7.04 (0.06)	6.91 (0.09)	6.73 (0.04)	6.96
Alkalinity	mg/L as $CaCO_3$	6.0 (0.3)	4.9 (1.0)	9.4 (0.7)	7.5 (0.3)	5.5 (0.2)	4.4 (0.4)	9.1
DOC	mg C/L	8.3 (0.6)	12.6 (1.0)	7.4 (0.3)	13.8 (0.5)	8.0 (0.4)	13.0 (1.0)	2.4

DOC, dissolved organic carbon concentration; values in parentheses are standard deviations ($n = 18$ for Redwater Creek and Errinundra; $n = 16$ for Inkpot). Mean values for Melbourne tap water are included for comparison [1].
[a]1:7 = 1 part natural water to 7 parts diluent.

the concentration of the free copper(II) rises sharply.

If a two-ligand system is assumed, the ISE can be seen to give a measure of the complexing capacity of both ligands, because it responds only to the free copper(II) ion (Fig. 1). The ASV determines labile copper, which includes free copper(II) ions, most inorganic complexes and weaker organic complexes. The ASV complexing capacity therefore consists only of the stronger binding ligand, L_1. By using the results of the ASV and ISE titrations in the MINEQL chemical equilibrium computer program [19], modified for a two-ligand system, it is possible to estimate the free copper(II) concentration present in each of the natural waters at the LC50 value.

RESULTS

Results of complexing capacity measurements made on each of the test waters are given in Table 2. These data were used, in conjunction with a modified version of the MINEQL program [19], to estimate the copper(II) concentrations present in test waters at the LC50 values.

The 96-h LC50 values for *P. australiensis* exposed to copper in the six natural water experiments are presented in Table 3. Results are expressed in terms of total copper concentration and the corresponding copper(II) ion concentration, based on both MINEQL calculations and direct ISE measurements. LC50 data from experiments conducted in Melbourne tap water [1] are included for comparison.

The data in Table 3 indicate that the presence of natural organic matter significantly reduces the toxicity of copper to *P. australiensis*. For example, the 96-h LC50 values for *P. australiensis* exposed to copper in Melbourne tap water is 34 μg Cu/L, compared with values of 141 μg Cu/L in 1:7 Redwater Creek water (1 part natural water to 7 parts diluent) and 317 μg Cu/L in 1:4 Inkpot water.

Table 2. Complexing capacity of natural waters used in toxicity experiments assuming a two ligand system

Test water	$[L_1]$ (μM)	log $*K_1$	$[L_2]$ (μM)	log $*K_2$
Redwater Creek				
1:7[a]	0.27	8.0	6.0	5.8
1:3	0.54	8.0	12.1	5.7
Errinundra				
1:3	0.12	7.6	3.2	5.7
1:1	0.23	7.6	6.4	5.7
Inkpot				
1:9	0.37	7.6	7.9	5.4
1:4	0.74	7.6	15.8	5.4

$[L_n]$ = concentration of ligand n; $*K_n$ = conditional stability constant for the reaction $Cu + L_n = CuL_n$.
[a]1:7 = 1 part natural water to 7 parts diluent.

However, when LC50 values are expressed as copper(II) ion rather than total copper concentrations, the variability is substantially reduced.

DISCUSSION

In this series of experiments, the presence of natural dissolved organic matter in the test waters was shown to considerably reduce the toxicity of copper to *P. australiensis*, presumably through the formation of nontoxic copper organic complexes. Various other workers [3,8–14] have similarly demonstrated a reduction in metal toxicity to aquatic organisms in the presence of natural organic matter. This suggests that natural organic matter in aquatic systems may provide substantial protection to aquatic organisms from the potentially harmful effects of heavy metals.

On the basis of the hypothesis that the copper(II) ion is the (major) toxic form of copper, one would expect that the 96-h LC50 values in terms of the copper(II) ion in the natural water experiments would approximate that derived from the Melbourne tap water experiments (Table 3). There is
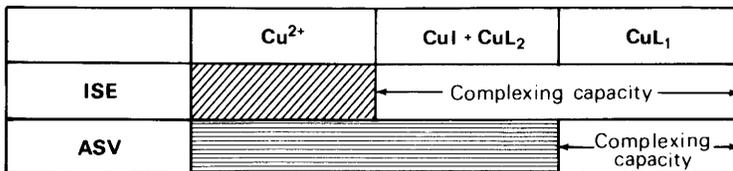

Fig. 1. Diagrammatic representation of the differences in copper complexing capacity measurements by anodic stripping voltammetry (ASV) and ion selective electrode (ISE). CuI, copper inorganic complexes; CuL_2, ASV–labile copper–organic complexes; CuL_1, copper–inorganic complexes not detected by either ISE or ASV.

Table 3. 96-h LC50 values for *P. australiensis* exposed to copper in the presence of natural organic matter

Condition	Total copper LC50 (μg Cu/L)		Cu(II) ion LC50 (μg Cu/L)			
			Calculated		Measured	
	LC50	(95% FL)	LC50	(95% FL)	ISE	(n, SD)
Redwater Creek						
1:7[a]	141	(127, 157)	34	(29, 40)	21	(3, 7)
1:3	238	(214, 264)	34	(31, 39)	28	(3, 6)
Errinundra						
1:3	108	(96, 121)	38	(33, 44)	33	(4, 8)
1:1	184	(172, 197)	46	(43, 49)	54	(4, 9)
Inkpot						
1:9	196	(174, 235)	56	(49, 68)	71	(3, 14)
1:4	317	(275, 377)	54	(46, 65)	74	(3, 9)
Melbourne tap water[b]	34	(28, 41)	20	(17, 24)[c]	16	(3, 2)

FL, fiducial limits; *n*, number of ISE measurements; SD, standard deviation of ISE measurements.
[a]1:7 = 1 part natural water to 7 parts diluent.
[b]Ref. [1].
[c]Calculated using MINEQL and log *K* values estimated from the literature.

good agreement between the mean of the ISE-determined copper(II) ion concentrations at the 96-h LC50 for the Redwater Creek experiments and the expected value of 16 to 20 μg Cu^{2+}/L (based on ISE-measured and computer-calculated estimates; Table 3). The ISE measurements for both the Errinundra and Inkpot waters give estimates that are higher than the expected values. Note that there are a number of problems associated with the use of ion selective electrodes in natural waters. Poor reproducibility, unstable potentials and relatively long response times have been reported for a copper(II) ion selective electrode in the presence of humic and fulvic acids [20]. We found there was substantial variability in the copper(II) ion activity measured by ISE for a given total copper concentration and water type. For example, the mean estimated 96-h LC50 in terms of the copper(II) ion for *P. australiensis* in 1:7 Redwater Creek water was 21 μg Cu^{2+}/L with a standard deviation of 7 μg/L on the basis of three measurements (Table 3). Electrode response was also slow in the natural waters used for toxicity experiments. In Errinundra water, in particular, the electrode potential took several hours to stabilize sufficiently to take a reading. The slow response of the electrode may be indicative of interference from some component of the natural water or of slow complexation reactions taking place.

In solutions free from interfering ions, the copper(II) ion selective electrode is generally considered to respond specifically to copper(II) ions. This has led to the widespread use of the ISE for measurement of copper(II) ion activities in a variety of aqueous systems. However, there are now some doubts about the specificity of the electrode response in natural waters [4,20,21]. For example, copper ion selective electrodes have been shown to respond to the presence of organic complexing agents [20,22]. In the series of experiments reported here, the ISE was calibrated in UV irradiated water, free from organic matter, before being used to measure copper(II) ion activities in the waters containing natural humic substances. This could have some effect on the accuracy of the ISE measurements if the electrode responded to organic material in the natural waters which was absent from the calibration water. An obvious focus for further studies would be the investigation of responses of ion selective electrodes in a variety of natural waters under a range of conditions. Until then, note that there are some uncertainties involved in the use of ion selective electrodes in natural waters.

In addition to directly measuring the copper(II) ion activities in the natural waters used for our toxicity experiments, we used a combination of the ASV- and ISE-measured complexing capacities to estimate the free copper(II) ion concentration present at the 96-h LC50. These complexing capacity determinations indicate the presence of (at least) two copper-binding ligands in each of the test waters, one (L_1) which is present at lower con-

centrations, but binds copper relatively strongly (log) $^*K = 7.6$–8.0), and the other (L_2), which is present at much higher concentrations, but forms weaker complexes with copper (log) $^*K = 5.4$–5.7; Table 2). The estimates of $[Cu^{2+}]$ at the LC50 value for the test water, obtained by using the complexing capacity data in MINEQL, are approximately 1.5 to 3 times greater than the "expected" values of 20 $\mu g/L$, based on results from the Melbourne tap water experiments (Table 3). Nevertheless, the agreement is remarkably good, given the assumptions made in making this comparison.

We have assumed that, with the exception of the presence of extra organic matter, the waters used in this group of experiments have the same physicochemical characteristics as Melbourne tap water. Although the test waters were made up to simulate Melbourne tap water in many respects (e.g., pH, conductivity and hardness; Table 1), there are undoubtedly some differences which could have affected the sensitivity of *P. australiensis* to the free metal. In addition, the time allowed for the ISE and ASV determinations of copper(II) ion activity was assumed to be sufficient for equilibrium to be reached. If this were not the case, the amount of copper complexation taking place may have been underestimated. To determine whether or not this was so, the ISE was placed in a sample of 1:3 strength Errinundra water containing 130 $\mu g/L$ added copper. The pH of the solution was adjusted to 7.0 (± 0.1) using a Radiometer pH-stat, and EMF readings were taken at regular intervals over an 8-h period. The results indicate that 60% of the copper was complexed rapidly (within 20 min), followed by a further 10% complexation over the next 7 to 8 h. The concentration of complexed copper was still increasing slowly when the experiment was terminated. Thus, it seems that the ISE and ASV methods used to determine free copper(II) ion activities in solution would underestimate the amount of complexation taking place. It is also possible that there were additional ligands present which were not detected by the techniques used, and this would again lead to overestimation of the amount of free copper present.

Preliminary calculations were run with MINEQL to see whether the inclusion of complexation of calcium and magnesium by the natural organic ligands would significantly alter calculated copper(II) ion concentrations. Stability constants of 10^5 and 10^3 for CaL_1 and CaL_2, respectively, and 10^5 and $10^{2.5}$ for MgL_1 and MgL_2, respectively, were used in the calculations. These values are

probably slightly higher than the "real" values, and, if anything, would overestimate the effect of calcium and magnesium complexation on copper(II) ion concentrations. Nevertheless, calculations for 1:7 Redwater Creek and 1:4 Errinundra waters showed that inclusion of complexation reactions between calcium and magnesium and the natural organic ligands increased the calculated copper(II) ion concentrations by only 5%. This difference·was considered insignificant, and subsequent calculations did not include these reactions.

Finally, in our expectation that LC50 in terms of the free copper(II) should be approximately 20 $\mu g/L$, we have assumed that the complexes CuL_1 and CuL_2 are nontoxic to *P. australiensis*. If either or both of these complexes were toxic, the LC50 expressed as copper(II) would decrease as the concentration of organic matter, and consequently of copper–organic complexes, was increased. However, the substantial protection from copper toxicity provided by the presence of the natural humic substances suggests that these copper–organic complexes have negligible toxicity to *P. australiensis*.

Although recognizing that there are limitations in the use of a simple two-ligand model to describe a largely uncharacterized natural system, we believe that there is sufficient agreement between the free copper concentrations predicted by the model and those predicted from the toxicity experiments to warrant further use of the model to estimate copper speciation in natural waters.

REFERENCES

1. **Daly, H.R., I.C. Campbell** and **B.T. Hart.** 1989. Copper toxicity in *Paratya australiensis* I. Influence of nitrilotriacetic acid and glycine. *Environ. Toxicol. Chem.* **9:** 997–1006.
2. **Daly, H.R., I.C. Campbell** and **B.T. Hart.** 1989. Copper toxicity to *Paratya australiensis* II. Influence of bicarbonate and ionic strength. *Environ. Toxicol. Chem.* **9:** 1007–1011.
3. **Chau, Y.K.** and **P.T.S. Wong.** 1976. Complexation of metals in natural waters. In R.W. Andrew, P.V. Hodson and D.E. Konasewich, eds., *Proceedings*, Workshop on the Toxicity to Biota of Metal Forms in Natural Waters. International Joint Commission, Windsor, Ontario, Canada, pp. 187–196.
4. **Florence, T.M.** 1982. The speciation of trace elements in waters. *Talanta* **29:** 345–364.
5. **Florence, T.M.** and **G.E. Batley.** 1980. Chemical speciation in natural waters. *CRC Crit. Rev. Anal. Chem.* **9:** 219–296.

6. **Hart, B.T.** 1981. Trace metal complexing capacity in natural waters – A review. *Environ. Technol. Lett.* **2**: 95–110.

7. **Mantoura, R.F.C., A. Dickson** and **J.P. Riley.** 1978. The complexation of metals with humic materials in natural waters. *Estuarine Coastal Mar. Sci.* **6**: 387–408.

8. **Barber, R.T.** and **J.H. Ryther.** 1969. Organic chelators: factors affecting primary production in the Cromwell current upwelling. *J. Exp. Mar. Biol. Ecol.* **3**: 191–199.

9. **Erickson, S.J.** 1972. Toxicity of copper to *Thalassiosira pseudonana* in unenriched inshore seawater. *J. Phycol.* **8**:318–323.

10. **Lewis, A.G., P.H. Whitfield** and **A. Ramnarine.** 1972. Some particulate and soluble agents affecting the relationship between metal toxicity and organism survival in the calanoid copepod *Euchaeta japonica.* *Mar. Biol.* **6**: 387–408.

11. **Sunda, W.G.** and **J.M. Lewis.** 1978. Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga *Monochrysis lutheri.* *Limnol. Oceanogr.* **23**: 870–876.

12. **Fisher, N.S.** and **D. Frood.** 1980. Heavy metal and marine diatoms: influence of dissolved organic compounds on toxicity and selection for metal tolerance among four species. *Mar. Biol.* **59**: 85–93.

13. **Srna, R.F., K.S. Garrett, S.M. Miller** and **A.B. Thum.** 1980. Copper complexation capacity of marine water samples from southern California. *Environ. Sci. Technol.* **14**: 1482–1486.

14. **Knezovich, J.P., F.L. Harrison** and **J.S. Tucker.** 1981. The influence of organic chelators on the toxicity of copper to embryos of the Pacific oyster *Crassostrea gigas.* *Arch. Environ. Contam. Toxicol.* **10**: 214–248.

15. **Daly, H.R.** 1985. An investigation of the relationship between copper speciation and toxicity to the freshwater shrimp *Paratya australiensis.* M.A.S. thesis. Chisholm Institute of Technology, Melbourne, Australia.

16. **American Public Health Association, American Water Works Association** and **Water Protection Control Federation.** 1975. *Standard Methods for the Examination of Water and Wastewater*, 14th ed. American Public Health Association, Washington, DC.

17. **Hart, B.T.** and **M.J. Jones.** 1984. Measurement of trace metal complexing capacity of Magela Creek waters. In C.J.M. Kramer and J.C. Duinker, eds., *Complexation of Trace Metals in Natural Waters.* Martinus Nijhoff/W. Junk Publishers, The Hague, The Netherlands, pp. 201–211.

18. **Jones, M.J.** 1987. Development of Methods to Measure the Complexing Capacity of Natural Waters and their Application to Magela Creek Waters. M. App. Sci. thesis, Chisholm Institute of Technology, Melbourne, Australia.

19. **Westall, J.C., J.L. Zachory** and **F.M. Morel.** 1976. MINEQL, a computer program for the calculation of chemical equilibrium composition of aqueous systems. Technical Note 18. Ralph M. Parsons Water Quality Lab, Massachusetts Institute of Technology, Cambridge, MA.

20. **Sekerka, I.** and **J.F. Lechner.** 1978. Response of copper(II) selective electrode to some complexing agents. *Anal. Letts.* **A11**: 415–427.

21. **Wagemann, R.** 1980. Cupric ion-selective electrode and inorganic cationic complexes of copper. *J. Phys. Chem.* **84**: 3433–3436.

22. **El-Taras, M.F.** and **E. Pungor.** 1976. The application of some organic complexing agents on the potential of copper(II) selective electrodes. Application of the silicone rubber-based electrode to the determination of citrate ion and 8-hydroxyquinoline. *Anal. Chim. Acta.* **82**: 285–292.