

The percent distribution of Cu^{2+} in the test waters differed by 5% between 4.0 mg L⁻¹ and 102 mg L⁻¹ alkalinity, supporting the fact that alkalinity did not affect Cu toxicity to *H. viridissima* at the pH and increased alkalinity used in this experiment. More specifically, the addition of carbonate ions did not alter the proportional relationship between Cu species, therefore not influencing the toxicity of Cu.

4.4.2 Influence of alkalinity on U toxicity to *H. viridissima*

The present study found that a 25-fold increase in alkalinity did not affect the toxicity of U to *H. viridissima*, at pH 6.0. This contrasts with the results of Markich et al (1996) who found a 5-fold increase in alkalinity reduced U toxicity by 20% in the freshwater bivalve, *Velesunio angasi*, at pH 5. The different observations made in these studies may be attributed to the use of different pH levels and/or use of different test organisms. Evidence of such reasoning is provided by Markich et al (1996), who found U toxicity decreased with increasing pH from 5.0 to 6.0, while the relative proportions of UO_2^{2+} and UO_2OH^+ declined, and several uranyl carbonates and hydroxides increased.

In contrast to the effects of increased Ca and Mg concentration, the increased alkalinity (ie bicarbonate concentration) altered the calculated U speciation through inorganic complexation. Despite the changes in the calculated U speciation, there was no change in U toxicity. The absolute percent change in UO_2^{2+} from 6 to 1% is minimal, given the errors associated with the selected stability constants used in the calculations. Therefore, according to the FIAM, which interprets that the toxic effect of U to *H. viridissima* is governed by UO_2^{2+} , then a minimal change in U toxicity would be expected. The FIAM could be further tested by creating a larger absolute percentage difference between the calculated activity of UO_2^{2+} , by slightly reducing the pH of the test waters.

4.5 Conclusions

MES biological buffer (4 mM at pH 6.0; alkalinity 4 and 102 mg CaCO_3 L⁻¹) was found to be a suitable and practical option of controlling the pH in the bioassay protocols used in this study. Although the buffer enhanced *M. mogurnda* survival in elevated levels of Cu, the buffer did not affect *H. viridissima* population growth or toxicity of Cu and U. A 25-fold increase in alkalinity at constant hardness and pH, was found to have no effect on the toxicity of either Cu or U to *H. viridissima*. The toxicity effects of Cu occurred without any change in speciation. In contrast, U speciation was altered with increasing alkalinity through inorganic complexation, but with no change in U toxicity. These results indicate that carbonate alkalinity does not affect Cu and U toxicity under the experimental conditions of this study.

5 General discussion

Metal speciation and bioavailability in fresh surface waters may be influenced by a variety of physico-chemical variables, particularly water hardness, alkalinity, pH, natural organic matter and redox potential (Hamelink et al 1994, Markich et al 2000). Quantitative relationships (algorithms) have only been established to describe the reduction in the bioavailability of Cd, Cr(III), Cu, Ni, Pb and Zn as a function of increasing hardness. Such algorithms have been incorporated into the water quality guidelines of several countries for the protection of aquatic organisms (CCREM 1991, US EPA 1995, ANZECC & ARMCANZ 2001).

Although several studies have found water hardness to reduce Cu (Gauss et al 1985, Belanger et al 1989) and U (Parkhurst et al 1984, Barata et al 1998) toxicity to freshwater biota, insufficient and/or inconsistent data have precluded an algorithm being established. These and

other studies that have investigated the effects of water hardness on the toxicity of metals to freshwater biota have confounded the effects of true water hardness (ie Ca and/or Mg concentration) with alkalinity (ie carbonate concentration) and pH (ie proton concentration), since water hardness is often positively correlated with alkalinity in natural waters (Stumm & Morgan 1981). The relative contribution of hardness and alkalinity in reducing metal toxicity is of importance, as each variable affects toxicity differently. Hardness (ie Ca and/or Mg) competitively inhibits the uptake and hence toxicity of trace metals at the cell membrane surface (Markich & Jeffree 1994), while alkalinity (ie carbonate) complexes with trace metals, reducing the concentration(s) of toxic metal species (ie a change in metal speciation) (Hunt 1987).

Several studies have reported that the toxicity and bioavailability of Cu is reduced with increasing water hardness (Miller & Mackay 1980, Mierle 1981, Horne & Dunson 1995 and Erickson et al 1996) and alkalinity (Andrew et al 1977, Miller & Mackay 1980, Laurén & McDonald 1986, Daly et al 1990a), without confounding parameters. The present study provides the first data concerning the effects of true water hardness on U toxicity to freshwater biota, while only one other study (Markich et al 1996) has described the effects of alkalinity on the toxicity of U to a tropical freshwater organism.

In the light of this information, the present study determined the individual effects of true water hardness and alkalinity on the 96 h toxicity of Cu and U to *H. viridissima* and *M. mogurnda*, at constant pH. Such data has provided a greater understanding of the relationship between water hardness and alkalinity, and hence provided a greater predictive ability of metal toxicity and bioavailability in tropical Australian freshwater systems.

5.1 Comparative sensitivity of test organisms to Cu and U toxicity

At baseline hardness (ie 6.6 mg CaCO₃ L⁻¹) and alkalinity (ie 4.0 mg CaCO₃ L⁻¹), *H. viridissima* was found to be more sensitive to both Cu and U compared with *M. mogurnda* (tables 11 & 12). However, the difference in organism sensitivity is not proportional for both metals. *H. viridissima* is approximately three-fold more sensitive to Cu than *M. mogurnda*, and about 16-fold more sensitive to U.

The relative sensitivity of *H. viridissima* and *M. mogurnda* to Cu and U can be compared with other tropical freshwater species. For comparative purposes, only toxicity data derived under similar experimental conditions (ie softwater, slightly acidic, low alkalinity and conductivity) to this study are reviewed (tables 11 and 12). For a tropical freshwater alga (*Chlorella* sp), Franklin et al (1998) reported a 72 h EC₅₀ value for growth inhibition of 35 µg L⁻¹ Cu at pH 5.7, and 1.5 µg L⁻¹ Cu at pH 6.5. Investigations using the valve movement of a freshwater bivalve (*Vesunio angasi*) found a 48 h EC₅₀ of 10 µg L⁻¹ Cu (pH 6.0) (Markich 1998). The present study found a 96 h EC₅₀ value of 4.6 µg L⁻¹ Cu for *H. viridissima* and a 96 h LC₅₀ value of 13 µg L⁻¹ Cu for *M. mogurnda* (pH 6.0). Although these studies were conducted at different pH levels, the sensitivity of these species can still be compared, as Cu speciation is similar between pH 5.7 and 6.0 (Franklin et al 1998). Based on EC₅₀ values, *H. viridissima* appears to be more sensitive to Cu than the bivalve and alga. Unfortunately, the 'sublethal' endpoint measuring the response of hydra, algae and bivalve to Cu is not directly comparable with the less sensitive 'lethal' endpoint measuring *M. mogurnda* survival (Hendriks 1995).

Table 11 Comparative toxicity of Cu to Australian tropical freshwater biota

Species	Endpoint	Cu toxicity ($\mu\text{g L}^{-1}$)	Reference
Cnidaria (<i>H. viridissima</i>)	96 h EC ₅₀	4.0 (pH 6.0)	Markich & Camilleri (1997)
		4.6 (pH 6.0)	This study
Mollusca (<i>V. angasi</i>)	48 h EC ₅₀	10 (pH 6.0)	Markich (1998)
Alga (<i>Chlorella</i> sp)	72 h EC ₅₀	35 (pH 5.7)	Franklin et al (1998)
		1.5 (pH 6.5)	
Chordata (<i>M. mogurnda</i>)	96 h LC ₅₀	22 ^a (pH 6.0)	Markich & Camilleri (1997)
		13 ^a (pH 6.0)	This study

a LC₅₀ values cannot be directly compared with the EC₅₀ values in this table

Table 12 Comparative toxicity of U to Australian tropical freshwater biota

Species	Endpoint	U toxicity ($\mu\text{g L}^{-1}$)	Reference
Alga (<i>Chlorella</i> sp)	72 h EC ₅₀	78 (pH 5.7)	Franklin et al (1998)
		44 (pH 6.5)	
Cnidaria (<i>H. viridissima</i>)	96 h EC ₅₀	95 (pH 6.0)	Markich & Camilleri (1997)
		114 (pH 6.0)	This study
Mollusca (<i>V. angasi</i>)	48 h EC ₅₀	210 (pH 6.0)	Markich et al (1996)
Chordata (<i>M. mogurnda</i>)	96 h LC ₅₀	1550 ^c (pH 6.0)	Markich & Camilleri (1997)
		1730 ^{a,c} and 1965 ^{b,c} (pH 6.0)	This study

^a LC₅₀ from first investigation

^b LC₅₀ from second investigation

^c LC₅₀ values cannot be directly compared to the EC₅₀ values in this table

The sensitivity of these organisms to U revealed a similar trend (table 12). Franklin et al (1998) reported a 72 h EC₅₀ value of 78 $\mu\text{g L}^{-1}$ U at pH 5.7, and 44 $\mu\text{g L}^{-1}$ U at pH 6.5 for the toxicity of U to the alga, *Chlorella* sp. Assessment of the toxicity of U to the freshwater bivalve found a 48 h EC₅₀ value of 210 $\mu\text{g L}^{-1}$ U at pH 5.8 (Markich et al 2000). In the present study, a 96 h EC₅₀ value of 114 $\mu\text{g L}^{-1}$ U was reported for *H. viridissima*, while a 96 h EC₅₀ value of 1730 and 1955 $\mu\text{g L}^{-1}$ U was reported for *M. mogurnda*, in the first and second investigation respectively (pH 6.0). Despite different test durations used, comparison of the EC₅₀ values suggest *H. viridissima* is less sensitive compared with the alga, but more than the bivalve. Although the acute response of *M. mogurnda* in this study cannot be directly compared with the chronic response of the other species listed in table 11, the 7 d chronic response of *M. mogurnda* to U investigated by Holdway (1992) may provide a comparison. Holdway (1992) reported a NOEC and LOEC of 920 and 1780 $\mu\text{g L}^{-1}$ U, respectively. These values are greater than the EC₅₀ values for alga, hydra and bivalve, suggesting *M. mogurnda* could potentially be the least sensitive species in the suite of organisms listed in table 12.

It should be noted that the trends described above are based on a single species from each phylum. Consequently, the comparisons made may not be a true indication of the relative sensitivities when multiple species are compared. However, it does suggest *H. viridissima* and *M. mogurnda* represent a range of metal sensitivities among tropical freshwater biota, and are therefore good indicators of metal contamination in tropical Australian freshwaters.

5.2 Effect of hardness on Cu and U toxicity

Increasing the true hardness of the test water (ie Ca and Mg concentration) had a variable effect, depending on the metal and test organism investigated. A 50-fold increase in hardness resulted in a 2-fold decrease in the toxicity of Cu to *M. mogurnda*, while it had no effect (ie in the second investigation) on U toxicity. The opposite was observed for *H. viridissima*, where increased hardness had no effect on Cu toxicity, but decreased U toxicity by approximately 2-fold. The observed effects of hardness on toxicity occurred without any change in the speciation of Cu or U. Such evidence supports the hypothesis that the protective effect of increased Ca^{2+} and/or Mg^{2+} involves a biological mechanism (Bradley & Sprague 1985, Part et al 1985, Markich & Jeffree 1994). Markich and Jeffree (1994) found that some metals (ie Pb, Cd, Mn and Co) are adsorbed as analogues of Ca from the aquatic medium, suggesting that Ca ions compete with the free ionic species for binding sites at the membrane surface. This biological mechanism may explain the effect hardness had on reducing Cu toxicity to *M. mogurnda* and U toxicity to *H. viridissima*. However, the competition mechanism is not amenable where Cu toxicity to *H. viridissima* or U toxicity to *M. mogurnda* was not reduced, suggesting there is another mechanism in place. Perhaps further research could provide evidence which would distinguish between physiological and toxicological effects. This could be achieved using metal tracers to compare the internal uptake and distribution of the metals by the organism with the external metal concentration and bioavailability.

Comparing the individual protective effects of Ca and Mg could further define the effect of total hardness on metal toxicity. Although the present study did not investigate this subject, those studies that have (Carroll 1979, Part et al 1985, Jeffree & Simpson 1986, Jackson et al 2000) reported Ca to be more effective than Mg in reducing the uptake and toxicity of trace metals to aquatic organisms. For example, Carroll et al (1979) reported 7-fold more Mg was needed to ameliorate the toxicity of Cd to brook trout, *Salvelinus fontinalis*, to the same extent as Ca. Likewise, Jackson et al (2000) reported increased Ca (ie from 2.0 to 150 mg L^{-1}) decreased Cd toxicity to *Hyaella azteca* 14-fold (ie LC_{50} increased from 3.8 to 55 $\mu\text{g L}^{-1}$), while increased Mg (ie from 1.2 to 83 mg L^{-1}) reduced Cd toxicity 3-fold (ie LC_{50} increased from 3.8 to 12 $\mu\text{g L}^{-1}$). The individual effects of Ca and Mg were not investigated in this study. Further experimental work is required to define the individual protective effects of Ca and Mg to tropical Australian freshwater biota.

5.3 Effect of alkalinity on Cu and U toxicity

An increase in water hardness is frequently associated with an increase in alkalinity (ie as Ca and/or Mg carbonate). For acidic waters (pH <6), hardness and alkalinity are typically uncoupled, whereas in neutral and alkaline waters (pH 6–9) both parameters may be closely coupled. In this study, a 25-fold increase in alkalinity (from 4.0 to 102 $\text{mg CaCO}_3 \text{ L}^{-1}$) at a fixed water hardness (165 $\text{mg CaCO}_3 \text{ L}^{-1}$) and pH (6.0) did not significantly ($P > 0.05$) affect the toxicity of Cu or U to *H. viridissima*. In contrast, Markich et al (1996) found that a 5-fold increase in alkalinity, at a fixed hardness (3.5 $\text{mg CaCO}_3 \text{ L}^{-1}$) and pH (5.0), decreased U toxicity by 20%. The difference between studies may be attributed to either/or the use of different test organisms and diluent physico-chemical constituents. The effect of alkalinity on Cu and U toxicity to *M. mogurnda* was not assessed as the biological buffer (ie MES), introduced to stabilise pH, enhanced sac-fry survival at elevated levels of Cu. Further work is needed to determine the effects of Cu and U toxicity on other freshwater organisms at varying alkalinity levels across a range of pH.

A 25-fold increase in alkalinity at constant hardness and pH, did not alter the toxicity of Cu or U to *H. viridissima*, implicating hardness (ie Ca and Mg concentration) as the influential

factor on Cu and U toxicity under the experimental conditions described. The toxicity effect of Cu occurred without any change in speciation. However, increasing alkalinity altered U speciation through inorganic complexation, despite no change in U toxicity. These results are in agreement with those reported by Playle et al (1992) who investigated Cu accumulation by fathead minnow (*Pimephales promelas*) gills. These authors found increasing Ca^{2+} reduced gill Cu accumulation, while increased carbonate did not. Similarly, Bradley and Sprague (1985) reported a 12-fold increase in hardness to reduce Zn toxicity to rainbow trout (*Salmo gairdneri*, renamed *Oncorhynchus mykiss*) by more than one order of magnitude, while a two-fold increase in alkalinity had no effect. On the contrary, Daly et al (1990b) found increasing alkalinity reduced Cu toxicity to an Australian freshwater shrimp, as did Markich et al (1996) for U toxicity to a freshwater bivalve. These authors attributed the reduction of the free cupric ion activity to the formation of metal-carbonate complexes, which subsequently decreases the uptake and toxicity of the metal.

5.4 Derivation of water quality guidelines

Currently, there is debate as to which statistical endpoints should be used to derive water quality guidelines and assess environmental risk (Hoekstra & van Ewijk 1993a,b, Denton & Norberg-King 1996, Dhaliwal et al 1997, Moore & Caux 1997). Much of this has been discussed by Markich and Camilleri (1997) and Camilleri et al (1998) with respect to tropical ecotoxicology and is summarised below. The concentration of a toxicant, which has no adverse biological effect, has traditionally been reported as a NOEC value. This endpoint has come under criticism because it is restricted to one of the test concentrations, suggesting it does not necessarily represent the actual toxicant concentration that causes no adverse biological effect (Hoekstra & van Ewijk 1993a,b, Chapman et al 1996, Moore & Caux 1997). The determination of the NOEC is also reliant on the statistical power of the test, that is, the probability (P) to correctly conclude that the control is significantly different from the treatment concentration. Ecotoxicological tests often possess low power (ie sometimes <30%), thus it is difficult to accept the NOEC as a true measure of no biological effect (Hoekstra & van Ewijk 1993a,b, Chapman et al 1996). For these reasons, Hoekstra and van Ewijk (1993a,b) have proposed the use of BEC_{10} as an alternative statistical measure to NOEC, where BEC_{10} is the highest concentration that can be claimed with 95% confidence that its biological effect does not exceed 10% of the observed effect (Hoekstra & van Ewijk 1993a). The process of deriving the BEC_{10} described by Hoekstra and van Ewijk (1993a) is summarised in section 2.5.

In this study, most estimates of the BEC_{10} were lower than the corresponding NOEC values, with the exception of Cu toxicity values for *H. viridissima* at 165 mg $\text{CaCO}_3 \text{ L}^{-1}$ hardness (table 12). However, the difference between BEC_{10} and NOEC values does not seem dependent on metal, test organism, hardness or alkalinity. For example, the BEC_{10} value for the effect of 102 mg $\text{CaCO}_3 \text{ L}^{-1}$ alkalinity on U toxicity to *H. viridissima* is 5-fold less than the NOEC, while the BEC_{10} for the effect of both 6.6 and 165 mg $\text{CaCO}_3 \text{ L}^{-1}$ hardness is 2-fold less than the NOEC (table 12). In contrast, Camilleri et al (1998) reported the difference between BEC_{10} and NOEC values to be a product of inadequate test power, reflected by low test replication, low number of treatments and large variability in organism response to the herbicide, Tebuthiuron. This trend enabled the same authors to compare the predictive ability of BEC_{10} and NOEC values and conclude that the BEC_{10} should be considered an appropriate statistical endpoint to evaluate a no adverse biological concentration. However, Camilleri et al (1998) warned that care should be taken that the BEC_{10} value does not result in an overly conservative estimate of the no adverse biological effect concentration.

The use of the LOEC as a measure of the lowest adverse biological effect concentration of a toxicant has come under the same criticism as the NOEC (Chapman et al 1996, Moore & Caux 1997). Ahsanullah and Williams (1991) proposed the minimum detectable effect concentration (MDEC) as an alternative statistical endpoint to the LOEC. The MDEC is calculated from a regression model and is defined as the metal concentration at which the response becomes significantly lower than in the 'control' treatment. The present study reported all estimates of the MDEC to be lower than the corresponding LOEC and EC₅₀/LC₅₀ (table 12). However, the LOEC estimates were sometimes close to or greater than the corresponding EC₅₀/LC₅₀ values. The difference between LOEC and EC₅₀/LC₅₀ values were generally less for *M. mogurnda* than those for *H. viridissima*. The small difference between the LOEC and LC₅₀ values for *M. mogurnda* is most likely due to the large inherent variability of response (ie survival) at each test concentration. However, this explanation cannot be applied where the LOEC equalled the EC₅₀ calculated for U toxicity to *H. viridissima* at 102 mg L⁻¹ alkalinity (table 12). It is difficult to confidently use such LOEC values as an estimate of the true lowest adverse biological effect concentration.

The Australian water quality guideline for Cu to protect moderately disturbed freshwater ecosystems is 1.4 µg L⁻¹ at a water hardness of 30 mg CaCO₃ L⁻¹ (ANZECC & ARMCANZ 2001). The Cu guideline value increases as the hardness of the water increases, according to the algorithm given in Section 1.2.1. The hardness modified Cu guideline values at 6.6 and 330 mg CaCO₃ L⁻¹ are 0.4 and 10.7 µg L⁻¹, respectively. In this study, *H. viridissima* detected Cu at 0.8 µg L⁻¹ and had an EC₅₀ of 4.6 µg L⁻¹, at 6.6 mg L⁻¹ hardness (table 13). These values were not affected by increasing hardness and exceed the guideline value (table 13). *Mogurnda mogurnda* detected Cu at 6.4 and 5.7 µg L⁻¹, at 6.6 and 330 mg CaCO₃ L⁻¹ hardness, respectively (table 13). The BEC₁₀ value at 6.6 mg CaCO₃ L⁻¹ exceeds the Cu guideline values, but at 330 mg CaCO₃ L⁻¹ is below the guideline value (table 13). At 165 mg CaCO₃ L⁻¹ hardness, *M. mogurnda* detected Cu at 9.3 µg L⁻¹, six times greater than the hardness modified Cu guideline (ie 6.0 µg L⁻¹). Considering these values were derived in a synthetic water, which lacks any organic chelating agents (ie DOC) and represents a high risk scenario, *H. viridissima* and *M. mogurnda* showed no adverse response to Cu at concentrations outside those listed in the 2000 guidelines.

An interim U guideline for the protection of Australian freshwater ecosystems is 0.5 µg L⁻¹ (ANZECC & ARMCANZ 2001). Unlike several other metals (Cd, Cr(III), Cu, Ni, Pb and Zn), there is currently no provision in the guidelines to use an algorithm to modify the U guideline value to account for increased water hardness. The present study found *H. viridissima* detected U at 14 µg L⁻¹ in water with a hardness of 6.6 mg CaCO₃ L⁻¹ (table 13), which is well above the interim guideline. However, *H. viridissima* detected U at 81 and 47 µg L⁻¹ in water with a hardness of 165 and 330 mg CaCO₃ L⁻¹ respectively (table 13). These BEC₁₀ values are approximately 160 times greater than the interim U guideline. *Mogurnda mogurnda* detected U at levels about 2000 times the interim U guideline (ie second investigation, table 13). Unlike the sensitivity of *H. viridissima* to U, increasing hardness did not affect the sensitivity of *M. mogurnda* (table 13). Although the interim U guideline appears conservative with respect to *M. mogurnda* toxicity data, the fact that *M. mogurnda* is eight-fold less sensitive to U than *H. viridissima* must be considered. Recently, a preliminary site-specific trigger value of 4.9 µg L⁻¹ was derived for Magela Creek, based on chronic toxicity data from five local species (R van Dam pers comm). The value, being that predicted to protect 99% of the species (see ANZECC & ARMCANZ 2001), appears more appropriate than the interim trigger value given the historical toxicity data.

Table 13 Toxicity endpoints (BEC₁₀, MDEC, NOEC, LOEC, EC₅₀, LC₅₀) calculated for *H. viridissima* and *M. mogurnda* exposed to Cu and U ($\mu\text{g L}^{-1}$) at three hardness levels and two alkalinity levels (pH 6.0 \pm 0.3) for 96 h

Species	Metal	Hardness (mg CaCO ₃ L ⁻¹)	Alkalinity (mg CaCO ₃ L ⁻¹)	BEC ₁₀	MDEC	NOEC	LOEC	Effect concentration (95% CI)	
Green hydra (<i>H. viridissima</i>)	Cu	6.6	4	0.8	1	0.9	1.9	4.6 ^a (4.1–5.1)	
		165	4	1.1	1.4	0.9	1.7	5.0 ^a (4.5–5.5)	
		165	102	1.2	1.4	0.7	1.8	6.0 ^a (5.5–6.5)	
		330	4	0.8	0.9	0.9	1.8	5.5 ^a (5.0–6.0)	
Purple-spotted gudgeon (<i>M. mogurnda</i>)	U	6.6	4	14	32	32	62	114 ^a (107–121)	
		165	4	81	90	150	162	177 ^a (166–188)	
		165	102	25	42	130	171	171 ^a (150–192)	
		330	4	47	62	62	87	219 ^a (192–246)	
		6.6	4	6.4	8.8	11.4	11.8	13.0 ^b (10.8–15.2)	
	First investigation	Cu	165	4	9.3	10.6	20	23.1	26.4 ^b (23.1–29.7)
			330	4	5.7	6.9	19.7	24.4	23.4 ^b (16.1–30.7)
			6.6	4	1410	1460	1450	1530	1730 ^b (1600–1860)
			165	4	570	860	1100	1310	1335 ^b (1165–1500)
			330	4	725	915	1050	1280	1270 ^b (1140–1400)
Second investigation	U	6.6	4	900	1220	1835	1950	1965 ^b (1600–2325)	
		165	4	1110	1240	1510	1770	1710 ^b (1400–2000)	
		330	4	860	1040	1530	1990	1770 ^b (1570–1970)	

^a 50% Effect concentration (EC₅₀)

^b 50% Lethal concentration (LC₅₀)

5.5 Conclusions

The influence of true water hardness and alkalinity on Cu and U toxicity to tropical Australian freshwater species was investigated, to help modify national water quality guidelines, and because such data are limited and/or ambiguous for tropical ecosystems.

The present study provides evidence that the toxicity of U to *H. viridissima* is reduced with increasing hardness, while U toxicity to *M. mogurnda* is not affected. In contrast, increasing hardness reduced the toxicity of Cu to *M. mogurnda*, but had no effect on the toxicity of Cu to *H. viridissima*. Further work is needed to determine the effects of Cu and U on other freshwater organisms at varying hardness levels, to determine if a generic relationship exists which will allow an algorithm to be established that can be used to modify the national guideline on a site-specific basis. Markich and Jeffree (1994) proposed that Ca concentration is a better choice than total hardness (Ca + Mg) for the protection of freshwater biota because Ca is far more effective at ameliorating metal toxicity at the cell membrane surface than Mg. They suggest that only in surface waters where the concentration of Mg considerably exceeds that of Ca will the joint hardness (Ca + Mg) be more useful. The German water quality guidelines actually use Ca concentration instead of total hardness with Cu, Zn and Cd for the protection of freshwater fisheries (Rump & Krist 1992). This study also found alkalinity had no effect on Cu and U toxicity to *H. viridissima*, suggesting that true water hardness is more important than alkalinity in reducing metal toxicity. Copper speciation did not significantly differ with increasing hardness or alkalinity, eliminating it as a confounding factor. In contrast, U speciation was altered through inorganic complexation. It is speculated that hardness (ie Ca²⁺ and Mg²⁺) reduced Cu and U toxicity by reducing the uptake of the free metal ion at the cell membrane surface. A mechanistic knowledge of metal toxicity is important for improving water quality guidelines for the protection of freshwater biota on a site-specific basis.

In summary, the information reported in this study will assist the development of water quality guidelines to protect tropical Australian freshwater systems. The separation of hardness and alkalinity effects provided a mechanistic knowledge of the influence these parameters have on Cu and U toxicity in tropical freshwaters. Such information is important for improving national water quality guidelines and for the protection of freshwater biota on a site-specific basis.