



Effects of water hardness and alkalinity on the toxicity of uranium to a tropical freshwater hydra (*Hydra viridissima*)

N. RIETHMULLER^{1,2}, S.J. MARKICH^{3*}, R.A. VAN DAM¹
and D. PARRY²

¹ Wetland Risk Assessment, Environmental Research Institute of the Supervising Scientist (*eriss*), Locked Bag 2, Jabiru, NT, 0886, Australia

² School of Mathematics and Physical Sciences, Northern Territory University, Darwin, NT, 0909, Australia

³ Environment Division, Australian Nuclear Science and Technology Organization, Private Mail Bag 1, Menai, NSW, 2234, Australia.

In tropical Australian freshwaters, uranium (U) is of potential ecotoxicological concern, largely as a consequence of mining activities. Although the toxicity of uranium to Australian freshwater biota is comprehensive, by world standards, few data are available on the effects of physicochemical variables, such as hardness, alkalinity, pH and organic matter, on uranium speciation and bioavailability. This study determined the individual effects of water hardness (6.6, 165 and 330 mg l⁻¹ as CaCO₃) and alkalinity (4.0 and 102 mg l⁻¹ as CaCO₃), at a constant pH (6.0), on the toxicity (96 h population growth) of uranium to *Hydra viridissima* (green hydra). A 50-fold increase in hardness (Ca and Mg concentration) resulted in a 92% (two-fold) decrease in the toxicity of uranium to *H. viridissima* [i.e. an increase in the EC₅₀ value and 95% confidence interval from 114 (107–121) to 219 (192–246) µg l⁻¹]. Conversely, at a constant hardness (165 mg l⁻¹ as CaCO₃), the toxicity of uranium to *H. viridissima* was not significantly ($P > 0.05$) affected by a 25-fold increase in alkalinity (carbonate concentration) [i.e. EC₅₀ values of 177 (166–188) and 171 (150–192) µg l⁻¹ at 4.0 and 102 mg l⁻¹ as CaCO₃, respectively]. A knowledge of the relationship between water chemistry variables, including hardness and alkalinity, and uranium toxicity is useful for predicting the potential ecological detriment in aquatic systems, and can be used to relax national water quality guidelines on a site-specific basis.

Keywords: hardness, alkalinity, toxicity, uranium, freshwater, hydra.

Introduction

Metal speciation and bioavailability in fresh surface waters may be influenced by a variety of physicochemical 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 water hardness. These algorithms have been incorporated into the water quality guidelines of several countries for the protection of aquatic ecosystems (CCREM 1991, US EPA 1995, ANZECC and ARMCANZ 1999). Although several studies (Tarzwell and Henderson 1960, Parkhurst *et al.* 1984, Poston *et al.* 1984, Barata *et al.* 1998) have found that water hardness typically reduces the toxicity of uranium to freshwater biota (all temperate northern hemisphere species), insufficient and/or inconsistent data have precluded an algorithm being established.

* Corresponding author: Scott Markich, Environment Division, Australian Nuclear Science and Technology Organization, Private Mail Bag 1, Menai, NSW, 2234, Australia. e-mail: sjm@ansto.gov.au

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 (Ca and/or Mg concentration) with alkalinity (carbonate concentration) and pH (proton concentration), since an increase in Ca and/or Mg concentration is frequently associated with an increase in alkalinity (as Ca and/or Mg carbonate), and hence pH. It is important to separate the effects of hardness and alkalinity, since each variable has a different mechanism of toxicity. Calcium and/or Mg competitively inhibit the uptake, and hence, toxicity of trace metals at the cell membrane surface (Markich and Jeffree 1994), whereas complexation of trace metals with carbonate in the aquatic medium reduces the concentration(s) of toxic metal species (i.e. a change in metal speciation) (Hunt 1987).

No data are available on the effects of true water hardness (Ca and/or Mg concentration) on the toxicity of uranium to freshwater biota. In the only study to determine the effects of alkalinity on the toxicity of uranium to freshwater biota, at a constant hardness (4.0 mg l⁻¹ as CaCO₃) and pH (5.0), Markich *et al.* (1996) found that a five-fold increase in carbonate concentration (from 2.5 to 12.5 mg l⁻¹) decreased the duration of valve opening in the tropical freshwater bivalve, *Velesunio angasi*, by 20%.

The aim of this study was to separate the effects of true water hardness (6.6, 165 and 330 mg l⁻¹ as CaCO₃) and alkalinity (4.0 and 102 mg l⁻¹ as CaCO₃), at a constant pH, on the toxicity (96 h population growth) of uranium to *H. viridissima* (green hydra), which is both ubiquitous and abundant in the fresh surface waters of tropical Australia. Uranium is of potential ecotoxicological concern in the freshwaters of tropical northern Australia due to uranium mining. Based on a review of the toxicity of metals to freshwater biota in tropical Australia, Markich and Camilleri (1997) concluded that *H. viridissima* was one of the more sensitive test species.

Materials and methods

Toxicity tests used a 'synthetic' water (Markich and Camilleri 1997) that simulates the inorganic composition of a tropical Australian sandy-braided stream during the wet season. This water is very soft (2–4 mg l⁻¹ as CaCO₃), slightly acidic (mean, pH 6.0) and has a low buffering and complexation capacity. Synthetic water was prepared by adding analytical grade reagents to deionized water (< 1 µS cm⁻¹) in acid-washed polyethylene containers. The pH of the water was adjusted to pH 6.0 ± 0.2 with dilute acid (H₂SO₄) and/or base (NaOH). Uranium test solutions were freshly prepared for each run by serially diluting a 400 mg l⁻¹ uranium stock solution with pH-adjusted synthetic water. Initial range-finding uranium concentrations were determined from the results of Markich and Camilleri (1997).

Based on regional water quality data, three hardness levels (6.6, 165 and 330 mg l⁻¹ as CaCO₃, corresponding to 1:1, 25:25 and 50:50 Ca:Mg in mg l⁻¹, respectively) and two alkalinity levels (4.0 and 102 mg l⁻¹ as CaCO₃) were selected. Hardness was increased by adding Ca and Mg as nitrate, whereas alkalinity was increased by adding NaHCO₃. The pH of the test waters with elevated alkalinity (102 mg l⁻¹ as CaCO₃) was buffered with 0.25 M 2-morpholinoethanesulphonic acid (MES) (pK_a = 6.1). Preliminary experiments showed that the increased conductivity (due to the addition of NaHCO₃) and the addition of the MES buffer did not significantly (*P* > 0.05) affect population growth in the controls or alter uranium toxicity to hydra.

The toxicity testing protocols used for *H. viridissima* are described in detail by Hyne *et al.* (1996) and Markich and Camilleri (1997). In summary, the tests exposed asexually reproducing (budding) hydra to 14 uranium concentrations, ranging from 0.1 to 400 µg l⁻¹, for 96 h. To commence a test, each uranium concentration was aliquoted into a separate Petri dish, and ten hydra were randomly placed in each dish. Three replicates were used for each uranium concentration. The test dishes were kept in a constant temperature incubator at 27 ± 1°C with a 12:12 light:dark photoperiod. The number of intact hydroids was recorded daily, where one hydroid equalled a single animal plus any attached buds. Each hydroid was individually fed live brine shrimp nauplii (*Artemia franciscana*) for approximately 2 h before the test waters were renewed every 24 h. The pH, conductivity and dissolved oxygen in the

test waters were measured at the commencement and conclusion of each renewal. Mean measured values of these physicochemical variables did not significantly ($P > 0.05$) differ between days or experiments. After 96 h the test was terminated and the quantitative population growth response was evaluated.

The minimum detectable effect concentration (MDEC), an alternative measure to the lowest-observed effect concentration (LOEC), was estimated using the approach described by Ahsanullah and Williams (1991). The MDEC was calculated from a regression model and is defined as the metal concentration at which the response became significantly ($P = 0.05$) lower than in the 'controls'. The advantages of using a regression-based model to estimate low toxic effects (e.g. MDEC), rather than a hypothesis-testing approach (e.g. LOEC) are described elsewhere (e.g. Bruce and Versteeg 1992, Moore and Caux 1997). Sigmoidal concentration–response curves were fitted using a logistic regression model (Seefeldt *et al.* 1995). Using the model, the EC_{50} and its 95% confidence intervals were calculated for each experiment. Measured, not nominal, uranium concentrations were used for all experiments.

Physicochemical analyses of the test waters, including quality assurance, are described by Markich and Camilleri (1997). Uranium concentrations were measured using inductively coupled plasma mass spectrometry (Hewlett Packard 4500). Concentrations of Ca and Mg (and thus calculated hardness; APHA *et al.* 1998) were measured using inductively coupled plasma atomic emission spectrometry (Varian Vista). Alkalinity (bicarbonate) was measured using potentiometric titration (Metrohm 682 Titroprocessor). Uranium speciation in the test waters was calculated using the geochemical speciation code HARPHRQ (Brown *et al.* 1991), as described by Markich and Camilleri (1997). Differences in predicted uranium speciation between varying hardness and alkalinity levels were tested using the generalized *F* test (Ratkowsky 1990).

Results and discussion

Influence of hardness and alkalinity on the toxicity of uranium to H. viridissima

This study was designed to separate the effects of true hardness (Ca and Mg) and alkalinity (carbonate) on the toxicity of uranium to *H. viridissima*. The calculated MDEC and EC_{50} values for *H. viridissima* exposed to uranium at three hardness levels (6.6, 165 and 330 mg l⁻¹ as CaCO₃ at a constant alkalinity) and two alkalinity levels (4.0 and 102 mg l⁻¹ as CaCO₃ at a constant hardness) at pH 6.0 ± 0.2, are given in table 1.

Based on the EC_{50} values, a 25-fold increase in water hardness (from 6.6 to 165 mg l⁻¹ as CaCO₃) significantly ($P \leq 0.05$) reduced the toxicity of uranium to *H. viridissima* by 55% (an increase in the EC_{50} value from 114 to 177 µg l⁻¹; table 1). A 50-fold increase in water hardness (from 6.6 to 330 mg l⁻¹ as CaCO₃) significantly ($P \leq 0.05$) reduced the toxicity of uranium to *H. viridissima* by 92% (an increase in the EC_{50} value from 114 to 219 µg l⁻¹; table 1). A two-fold increase in water hardness (from 165 to 330 mg l⁻¹ as CaCO₃) significantly ($P \leq 0.05$) reduced the toxicity of uranium to *H. viridissima* by 24% (an increase in the EC_{50} value from 177 to 219 µg l⁻¹; table 1). The EC_{50} value of 114 ± 7 µg l⁻¹ at 6.6 mg l⁻¹ as CaCO₃ is consistent with that reported by Markich and Camilleri (1997) under identical experimental conditions (108 ± 6 µg l⁻¹).

The trends observed for the EC_{50} values are not consistent with the MDEC values given in table 1, due to differences in the slopes of the concentration–response curves. For example, the MDEC at a hardness of 165 mg l⁻¹ as CaCO₃ (90 µg l⁻¹), is higher than at 330 mg l⁻¹ as CaCO₃ (62 µg l⁻¹), due to the concentration–response curve of the former having a steeper slope. Nevertheless, the uranium toxicity between 6.6 and 330 mg l⁻¹ as CaCO₃ is reduced by a similar margin as the EC_{50} (table 1).

In contrast to the results for hardness, a 25-fold increase in alkalinity (from 4.0 and 102 mg l⁻¹ as CaCO₃), at a hardness of 165 mg l⁻¹ as CaCO₃, did not significantly ($P > 0.05$) affect the toxicity of uranium to *H. viridissima* (i.e. overlapping 95% confidence intervals of the EC_{50} values; table 1). This result suggests that water hardness (Ca and Mg concentration) is more important than alkalinity (bicarbonate concentration) in

Table 1. Toxicity endpoints (MDEC and EC₅₀ values) calculated for *H. viridissima* exposed to uranium (µg l⁻¹) at three hardness and two alkalinity levels at pH 6.0 ± 0.2 for 96 h.

Hardness (mg CaCO ₃ l ⁻¹)	Alkalinity (mg CaCO ₃ l ⁻¹)	MDEC	EC ₅₀ (95% CI)
6.6	4.0	32	114 (107–121)
165	4.0	90	177 (166–188)
165	102	42	171 (150–192)
330	4.0	62	219 (192–246)

reducing uranium toxicity to *H. viridissima*, at least under the experimental conditions described. Differences in the slopes of the concentration–response curves of the two alkalinity treatments precluded a reasonable comparison of the MDEC. That is, the alkalinity treatment at 4.0 mg l⁻¹ as CaCO₃ has a much steeper slope and a higher MDEC value (table 1) than the 102 mg l⁻¹ as CaCO₃ treatment, with the former having a larger range of response over a smaller concentration range than the latter.

Relationship between uranium toxicity and uranium speciation

Water quality guidelines to protect aquatic biota are typically reported as a total metal concentration. However, it is well established that the bioavailability of metals to aquatic organisms is critically dependent on the physico-chemical form(s) or speciation of these metals (Hamelink *et al.* 1994). Metals may occur as a variety of physico-chemical forms in aquatic ecosystems; the free metal ion (Mⁿ⁺) and metals complexed with a range of naturally occurring organic and inorganic compounds in soluble, colloidal or particulate forms (Pickering 1995). It is generally considered that metal toxicity is governed by the activity of the free hydrated metal ion (i.e. the free-ion activity model, FIAM) (Campbell 1995). Metals in strong complexes or adsorbed to colloidal or particulate matter are usually considered less toxic (Hunt 1987).

The predicted speciation (% distribution) of uranium in the test waters at pH 6.0 at the three hardness levels (6.6, 165 and 330 mg l⁻¹ as CaCO₃) is given in figure 1(a). No significant (*P* > 0.05) differences were found in the speciation of uranium between the three hardness levels. For example, the calculated activity of the free uranyl ion (UO₂²⁺) for the EC₅₀ value at each hardness was constant (6.6–6.8%, table 1). This result provides evidence to support the working hypothesis that uranium toxicity is inversely related to the concentrations of Ca and Mg, where Ca²⁺ and Mg²⁺ may competitively inhibit the uptake of UO₂²⁺ at the cell membrane surface. This is the subject of further work. Previous studies (Markich and Jeffree 1994, Issa *et al.* 1995, Erickson *et al.* 1996) have confirmed this hypothesis for other species of freshwater organisms (bivalves, fish and crustaceans) with trace metals (Cd, Cu, Mn, Pb and Zn).

The predicted speciation (% distribution) of uranium in the test waters at pH 6.0 at two alkalinity levels (4.0 and 102 mg l⁻¹ as CaCO₃) is given in figure 1(b). In contrast to the effects of increased Ca and Mg concentration, the increased alkalinity (bicarbonate concentration) altered the calculated uranium speciation through inorganic complexation. Based on the EC₅₀ value at each alkalinity (table 1), the calculated percentage of UO₂CO₃ increased by a factor of about four at

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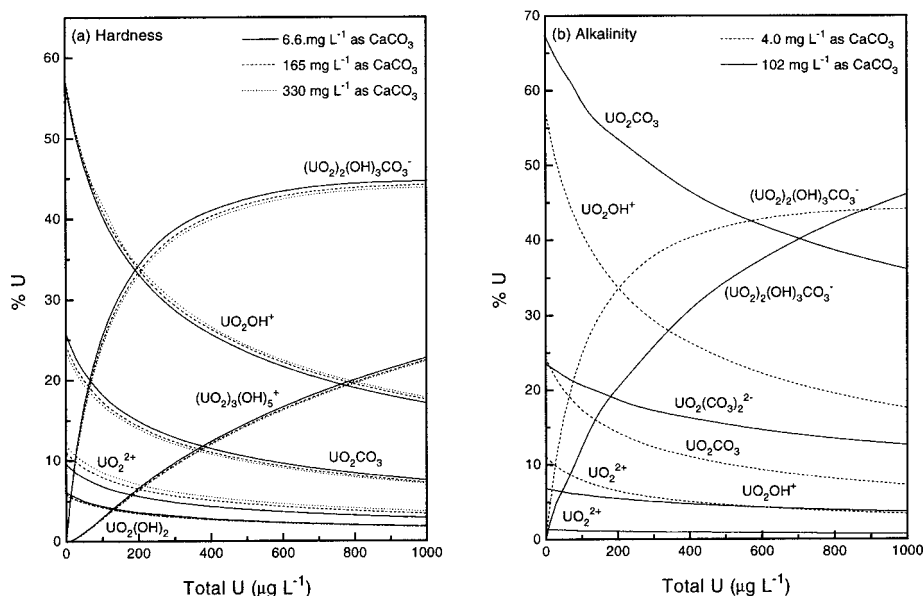


Figure 1. Predicted speciation (% distribution) of uranium in the tests water at pH 6.0 at (a) three hardness levels (6.6, 165 and 330 mg L⁻¹ as CaCO₃) and (b) two alkalinity levels (4.0 and 102 mg L⁻¹ as CaCO₃). Uranyl species comprising < 2% total uranium are excluded for clarity.

102 mg L⁻¹ as CaCO₃ (compared with the baseline alkalinity of 4 mg L⁻¹ as CaCO₃), whilst the percentages of UO₂²⁺ and UO₂OH⁺ decreased by about a factor of six. The polymeric uranium species, (UO₂)₂(OH)₃CO₃⁻, was also calculated to decrease by a factor of two.

The increased alkalinity also substantially increased the percentage of UO₂(CO₃)₂²⁻ from < 1 to 20%. Despite the changes in the calculated uranium speciation, there was no significant ($P > 0.05$) change in uranium toxicity (i.e. overlapping 95% confidence intervals, table 1). The absolute percentage change in UO₂²⁺ 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 uranium to *H. viridissima*, is governed by UO₂²⁺, then a minimal change in uranium toxicity would be expected. The FIAM could be further tested by creating a larger absolute percentage difference between the calculated activity of UO₂²⁺, by slightly reducing the pH of the test waters.

Implications for water quality guidelines

The draft Australian guideline for uranium for the protection of freshwater ecosystems is 3.5 μg L⁻¹ (ANZECC and ARMCANZ 1999). 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 relax the uranium guideline value to account for increased water hardness. This study provides evidence that the toxicity of uranium to *H. viridissima* is reduced with increasing hardness. However, further work is needed to determine the effects of uranium on other freshwater organisms

at varying hardness levels, to determine if such a generic relationship exists before an algorithm can be established that relaxes 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 and Krist 1992).

An increase in water hardness is frequently associated with an increase in alkalinity (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 mg l⁻¹ as CaCO₃) at a fixed water hardness (165 mg l⁻¹ as CaCO₃) and pH (6.0) did not significantly ($P > 0.05$) affect the toxicity of uranium to *H. viridissima*. In contrast, Markich *et al.* (1996) found that a five-fold increase in alkalinity, at a fixed hardness (3.5 mg l⁻¹ as CaCO₃) and pH (5.0), decreased uranium toxicity 20%. Further work, however, is needed to determine the effects of uranium on other freshwater organisms at varying alkalinity levels across a range of pH.

Overall, the effects of alkalinity on uranium toxicity to freshwater biota are directly governed by changes in uranium speciation (i.e. usually an increase in non-toxic uranyl carbonate species and a concomitant decrease in UO₂²⁺). In contrast, it is generally considered that an increase in hardness (i.e. Ca²⁺ and Mg²⁺) reduces uranium toxicity by reducing the uptake of UO₂²⁺ at the cell membrane surface. A mechanistic knowledge of metal toxicity is important for improving quality guidelines for the protection of freshwater biota on a site specific basis.

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