Impact of Environmentally Based Chemical Hardness on Uranium Speciation and Toxicity in Six Aquatic Species

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(Submitted 2 May 2014; Returned for Revision 13 June 2014; Accepted 30 November 2014)

Abstract: Treated effluent discharge from uranium (U) mines and mills elevates the concentrations of U, calcium (Ca), magnesium (Mg), and sulfate (SO₄²⁻) above natural levels in receiving waters. Many investigations on the effect of hardness on U toxicity have been experiments on the combined effects of changes in hardness, pH, and alkalinity, which do not represent water chemistry downstream of U mines and mills. Therefore, more toxicity studies with water chemistry encountered downstream of U mines and mills are necessary to support predictive assessments of impacts of U discharge to the environment. Acute and chronic U toxicity laboratory bioassays were realized with 6 freshwater species in waters of low alkalinity, circumneutral pH, and a range of chemical hardness as found in field samples collected downstream of U mines and mills. In laboratory-tested waters, speciation calculations suggested that free uranyl ion concentrations remained constant despite increasing chemical hardness. When hardness increased while pH remained circumneutral and alkalinity low, U toxicity decreased only to Hyalella azteca and Pseudokirchneriella subcapitata. Also, Ca and Mg did not compete with U for the same uptake sites. The present study confirms that the majority of studies concluding that hardness affected U toxicity were in fact studies in which alkalinity and pH were the stronger influence. The results thus confirm that studies predicting impacts of U downstream of mines and mills should not consider chemical hardness.

Keywords: Uranium Acute toxicity Chronic toxicity Speciation Freshwater

Introduction

Uranium (U) is an element that occurs naturally in the environment. Once dissolved in natural surface water, the concentration of free (UO₂²⁺) and hydrated (UO₂OH⁻) uranyl ions depends on the water chemistry, including pH, dissolved organic carbon (DOC), hardness, and alkalinity [1]. First, the pH of the water determines the proportion of free and hydrated uranyl ions [2]. As pH ranges from 5.5 to 8.5 in natural surface waters, the proportion of free and hydrated uranyl ions decreases with an increase in pH. Second, depending on the pH, free uranyl ions can also form dissolved complexes with DOC [3]. Third, natural hardness, which is the content of Ca and Mg salts in equilibrium with bicarbonate and carbonate, also will affect the speciation of U. As the concentrations of Ca and Mg increase, their proportion bound with carbonate ions increases, which decreases the amount of U bound to carbonate species. Finally, alkalinity, which is the concentration of hydroxide, bicarbonate, and carbonate in surface waters, can also affect U speciation. As the alkalinity increases, more free uranyl ions can become hydrated and/or bind to carbonate and bicarbonate ions and eventually precipitate. The speciation of U in natural surface waters is therefore quite complex.

Treatment technology for U mining and milling effluents consists of a complex process whereby (CaO, CaOH), barium chloride, and sulfuric acid are added at different treatment steps [4]. The addition of Ca, Mg, and SO₄²⁻ during the chemical treatment process elevates these ions above natural levels in receiving water. The predominance of Ca and Mg in equilibrium with SO₄²⁻ downstream of U mines and mills is referred to as chemical hardness [5]. The wide range in chemical hardness (13–1550 mg CaCO₃ L⁻¹, Table 1), rather than natural hardness, could likely have the most influence on the speciation of U downstream of U mines and mills.

Because U has little affinity to SO₄²⁻ [6], SO₄²⁻ will have a limited effect on U speciation. Similarly, because effluent mainly contains Ca, Mg, and SO₄²⁻, pH downstream of U mines and mills will remain circumneutral (pH 6.2–7.8), similar to reference areas where pH ranges from 6.2 to 7.4 (Table 1). In addition, treated effluent slightly increases alkalinity downstream of U mines and mills by a factor of 2 (3–24 mg CaCO₃ L⁻¹) compared with reference areas, where alkalinity ranges from 2 mg CaCO₃ L⁻¹ to 11 mg CaCO₃ L⁻¹ (Table 1). Alkalinity could therefore affect U speciation downstream of U mines and mills; but because it is narrow in range in comparison with chemical hardness, alkalinity likely is less important in predicting U speciation.

The difference in U speciation in natural waters versus waters receiving treated effluent from U mines and mills is important in predicting U toxicity to aquatic organisms. If the free and hydrated uranyl ions are the species of ecotoxicological concern [1], aquatic organisms will take up U mainly depending on the levels of Ca and Mg. However, there is limited evidence on the importance of this mechanism for U uptake; only 1 study has demonstrated that algae take up less UO₂²⁺ because Ca and Mg compete for similar uptake sites [2].

In contrast, several studies have investigated the effect of hardness on U toxicity; unfortunately, different chemicals were used to set hardness at a desired level. As a result, most of the
available U toxicity studies are more suited to investigate the effect of natural hardness. Examples of such studies are cases in which hardness is set by either dilution of natural [7] or synthetic water [8,9] or addition of a combination of sodium carbonate, calcium and magnesium sulfate, and potassium chloride [10]. Few studies have been suited to investigate the effect of chemical hardness that would be encountered downstream of U mines and mills. Examples of such studies are when hardness was set by adding calcium and magnesium sulfate [11], calcium and magnesium chloride [12], or calcium and magnesium nitrate salts [2].

Experiments better suited to investigate the effect of natural hardness by diluting natural or synthetic water affected U toxicity to fish [7], to *Daphnia magna* clones [8], and to *Hyalella azteca* [9]. Experiments that added a mixture of carbonate, sulfate, and chloride salts also affected U toxicity to invertebrates [10]. In these experiments, an increase in hardness decreased U toxicity because the carbonate ions bound U, thereby limiting exposure of U to the tested organisms. It appears that changes in hardness in these experiments [7–10] are confounded by changes in alkalinity. Sheppard et al. [13] conducted a literature review of U toxicity to different fish species and indeed found a marked difference in U toxicity to fish between soft, low-alcaline water and harder, higher alkaline water, suggesting that it was the change in alkalinity that affected U toxicity to fish.

In contrast, experiments better suited to investigate the effect of chemical hardness had limited impacts on U speciation and toxicity. For instance, Charles et al. [11] found only a minor influence of CaSO4 and MgSO4 on U toxicity to the algae *Chlorella* sp., whereas the effect of hardness added as calcium nitrate was unclear on U toxicity to *Hydra viridisstima* [14].

Because the current published literature on experimental U toxicity data to pelagic organisms has been derived using several types of salts in different combinations to adjust hardness, there are few available U toxicity values from which impacts downstream of treated effluent discharge from U mines and mills can be predicted. Therefore, more studies investigating the effects of elevated Ca and Mg while keeping low alkalinity and circumneutral pH are needed to better predict U impacts on aquatic organisms downstream of U mines and mills. Hence, the objective of the present study was to investigate the toxicity of U to 6 freshwater species exposed to water-borne U at chemical hardness, pH, and alkalinity levels representative of freshwaters receiving treated U mine and mill effluent.

**METHODS**

**Solution preparation**

All glassware was soaked in an acid bath for at least 3 h and washed thoroughly with deionized water before use (>18 MΩ cm). Solutions for all toxicity tests were prepared with uranyl nitrate hexahydrate (UO2[NbO3]2 · 6H2O; 502.13 g mol⁻¹; CAS number 13520-83-7; ACS grade; Spectrum Products, lot# MJ0385). The test solutions were stored in 20-L carboys at room temperature during the test. The test solutions did not require pH adjustment prior to testing.

Carr and Neary [15] indicated that water hardness typically ranges from 17 mg L⁻¹ to 180 mg L⁻¹ as CaCO3. At some mine sites, chemical hardness averages around 240 mg L⁻¹ as CaCO3, but it can go up to 1500 mg L⁻¹ as CaCO3 depending on the distance from the point of effluent discharge (Table 1). Hence, we conducted standard U toxicity tests at various chemical hardness values within this natural and anthropogenic range (5–240 mg L⁻¹ as CaCO3) while maintaining constant pH (7.0 ± 0.5) and alkalinity (5 mg as CaCO3 L⁻¹ ± 20%). To achieve variability in chemical hardness in the tested synthetic solutions, we modified standard water recipes to achieve a specific hardness and alkalinity (Table 2). To achieve the target bicarbonate levels, the concentration of NaHCO₃ was reduced to 8.4 mg/L for all water recipes. To vary hardness without covarying bicarbonate levels, Ca and Mg sulfate were adjusted as described in Table 2. In addition, the ratio of Ca and Mg in the
water recipes was adjusted to achieve a constant molar ratio for all recipes. The same 1:1 Ca:Mg ratio (or 3:1 in the case of *Hyalella*) was maintained, because these ratios are currently used in the standard water recipes for the test species. Measured concentrations of the different elements of each synthetic solution are provided in Table 3.

### Toxicity testing

Six aquatic species were used to test the influence of chemical hardness using either static (*Lemna minor, Pseudokirchneriella subcapitata, Oncorhynchus mykiss, Pimephales promelas*) or static-renewal (*H. azteca, Ceriodaphnia dubia, O. mykiss embryos*) setups.

### Quality assurance and quality control

All experiments met the test validity criteria established in the test methods, unless otherwise stated. Reference toxicant tests were performed using sodium chloride for the fathead minnow and *C. dubia*, phenol for the rainbow trout, copper sulfate for *H. azteca*, and zinc sulfate for *P. subcapitata*, all in accordance with the Environment Canada test protocols. For all reference toxicant tests, the lethal concentration percentage (LCₚ) or inhibition concentration percentage (ICₚ), depending on the test, were within the acceptable range (±2 standard deviation [SD]) of previous tests conducted at the Vizon SciTec laboratory (Vancouver, BC, Canada).

### Statistical analyses

The LCₚ or ICₚ and their 95% confidence limits were calculated using the maximum likelihood probit or log-logit nonlinear interpolation method for survival data and the linear interpolation method for the growth data, with Toxcalc™ (Ver 5.0), an Excel-based software application (Tidepool Scientific Software 1994–1996).

### Table 2. Water recipes modification from original standard solution for each toxicity tests

| Chemical | Fathead minnow/rainbow trout | Hyalella azteca | Pseudokirchneriella subcapitata | Lemna minor | Ceriodaphnia dubia |
|----------|-------------------------------|----------------|---------------------------------|-------------|-------------------|
| NaHCO₃   | 156                           | 101            | 9.38                            | 10.4        | 0.0025            |
| CaSO₄    | 76.5                          | 69.7           | 2.76                            | 10.1        | 0.00025           |
| MgSO₄    | 97.5                          | 31.6           | 9.19                            | 4.149       | 0.000025          |
| KCl      | 6.5                           | 4.0            | 0.89                            | 0.0078      | 0.02           |
| NaBr     | 0.0                           | 4.0            | 0.0                             | 0.000009    | 0.02           |
| NaHCO₃   | 101                           | 69.7           | 2.76                            | 10.1        | 0.00025           |
| CaSO₄    | 69.7                          | 31.6           | 9.19                            | 4.149       | 0.000025          |
| MgSO₄    | 31.6                          | 4.0            | 0.89                            | 0.0078      | 0.02           |
| KCl      | 4.0                           | 4.0            | 0.0                             | 0.000009    | 0.02           |
| NaBr     | 0.0                           | 4.0            | 0.0                             | 0.000009    | 0.02           |
| NaHCO₃   | 9.38                          | 2.76           | 0.89                            | 0.0078      | 0.02           |
| CaCl₂•2H₂O | 62.5                       | 3.39           | 0.89                            | 0.0078      | 0.02           |
| MgSO₄•7H₂O | 9.19                        | 2.30           | 0.89                            | 0.0078      | 0.02           |
| CoCl₂•6H₂O | 0.08                            | 0.008          | 0.008                           | 0.000009    | 0.02           |
| CuCl₂•6H₂O | 0.08                           | 0.008          | 0.008                           | 0.000009    | 0.02           |
| H₂BO₃    | 0.11595                       | 0.11595        | 0.11595                         | 0.11595     | 0.11595           |
| K₂HPO₄   | 0.65                          | 0.65           | 0.65                            | 0.65        | 0.65              |
| MnCl₂•4H₂O | 0.1875                      | 0.1875         | 0.1875                          | 0.1875      | 0.1875            |
| Na₂EDTA•2H₂O | 0.00454                    | 0.00454        | 0.00454                         | 0.00454     | 0.00454           |
| Na₂MoO₄•2H₂O | 0.0726                    |                |                                | 0.0726      | 0.0726            |
| NaN₃     | 15.94                         | 15.94          | 15.94                           | 15.94       | 15.94            |
| ZnCl₂    | 0.0025                        | 0.00205        | 0.00205                         | 0.00205     | 0.00205           |
| NaHCO₃   | 10.4                          |                 |                                |             |                  |
| KCl      | 10.1                          |                 |                                |             |                  |
| NaHCO₃   | 150                           |                 |                                |             |                  |
| NaNO₃    | 255                           |                 |                                |             |                  |
| CaCl₂•2H₂O | 44.1                        |                 |                                |             |                  |
| MgCl₂•6H₂O | 121.7                       |                 |                                |             |                  |
| FeCl₃•6H₂O | 1.6                          |                 |                                |             |                  |
| MnCl₂•4H₂O | 4.149                       |                 |                                |             |                  |
| CuCl₂    | 0.0078                        |                 |                                |             |                  |
| CuCl₂    | 0.00009                       |                 |                                |             |                  |
| H₂BO₃    | 1.86                          |                 |                                |             |                  |
| MgSO₄•7H₂O | 147                         |                 |                                |             |                  |
| Na₂MoO₄•2H₂O | 0.0726                    |                 |                                |             |                  |
| NaN₃     | 15.94                         |                 |                                |             |                  |
| ZnCl₂    | 0.0327                        |                 |                                |             |                  |

*a*Values are reported in μg L⁻¹.

*b*Hardness set at 30 mg/L.

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### References

1. Goulet, R.R. et al. Environ Toxicol Chem 34, 2015.
The relationship between U toxicity endpoints and hardness was quantified with linear regression analysis using Sigma-Plot V.8.0 linear regression analysis.

The next sections provide descriptions of each of the toxicity experiments.

H. azteca survival and growth static renewal tests

Hyalella azteca were obtained from Aquatic BioSystems. The chronic toxicity of U to H. azteca was determined with the following modifications to the Environment Canada test method [16]. The 14-d test was conducted as a water-only experiment, with nylon mesh as the substrate instead of sediments, and the reconstituted water recipe (Table 2) was altered to achieve low alkalinity (~5 mg L\(^{-1}\)) and specific water hardness of 15 mg L\(^{-1}\), 60 mg L\(^{-1}\), 120 mg L\(^{-1}\), and 240 mg L\(^{-1}\) as CaCO\(_3\).

Organisms were acclimated for 6 d, during which approximately 25% of the water was replaced daily with hardness-adjusted control water. At 8 to 9 d old, 10 organisms were introduced in 250-mL glass beakers containing 200 mL of test solutions spiked at different U concentrations (0.005 mg L\(^{-1}\), 0.01 mg L\(^{-1}\), 0.03 mg L\(^{-1}\), 0.07 mg L\(^{-1}\), 0.2 mg L\(^{-1}\), and 0.5 mg L\(^{-1}\)). Also, the Ca:Mg ratio was set at 3:1. Bromide was also added to the H. azteca water recipe in a Ca:Br ratio of 15:1 to alleviate the potential toxicity of CaCl\(_2\) to H. azteca [17]. Bromide has low affinity with U [6]. Six replicates of each solution were tested. The test solutions were renewed 3 times weekly, and each individual was fed 0.5 mL of Yeast, Cereal Leaves, and Tetramin (YCT) after each renewal. Gentle aeration was provided to each test vessel using glass pipettes. Tests were conducted in an environmental chamber, which was maintained at 23 ± 1 °C and had full-spectrum lighting with a 16:8-h light:dark photoperiod.

P. subcapitata growth inhibition static tests

Pseudokirchneriella subcapitata, strain UTCC 37, was obtained from the University of Toronto Culture Collection (ON, Canada). Tests were conducted using a P. subcapitata culture maintained at Vizon SciTec. Microscopic examination of the culture was performed regularly to ensure that test organisms were free of contamination. The toxicity of U to P. subcapitata was determined with a modification to the Environment Canada test method [18]. The modification was made to achieve low alkalinity (~5 mg L\(^{-1}\)) and specific water hardness values of 5 mg L\(^{-1}\), 15 mg L\(^{-1}\), 60 mg L\(^{-1}\), 120 mg L\(^{-1}\), and 240 mg L\(^{-1}\) as CaCO\(_3\). Ethylenediamine tetraacetic acid (EDTA) was added at 8.06 × 10\(^{-5}\) M.

Algae were cultured under aseptic conditions in a filter-sterilized growth medium, and were not acclimated to the altered media prior to testing. Sterile 96-well round-bottom microplates were inoculated with 10 000 cells mL and exposed to a range of U concentrations (0.005 mg U L\(^{-1}\), 0.009 mg U L\(^{-1}\), 0.018 mg U L\(^{-1}\), 0.036 mg U L\(^{-1}\), 0.073 mg U L\(^{-1}\), 0.145 mg U L\(^{-1}\), 0.29 mg U L\(^{-1}\), 0.58 mg U L\(^{-1}\), and 1.16 mg U L\(^{-1}\)) for 72 h. Four replicates of each concentration were tested.

It was not possible to measure water quality or other parameters in the microplate wells; therefore, solutions were prepared that simulated the test well solutions. These simulated test solutions were prepared by adding 300 mL of test solution to 30 mL of a nutrient spike/reagent water mixture (50:50). The control and the 0.009 mg U L\(^{-1}\), 0.073 mg U L\(^{-1}\), and 1.164 mg U L\(^{-1}\) treatments were subsampled for U, alkalinity, and hardness. The pH, conductivity, temperature, and dissolved oxygen concentrations were also measured in these solutions, using the appropriate meters, as per the test method. Samples for redox potential, chloride/sulfate concentrations, and total metals were collected from the control and the 0.005 mg U L\(^{-1}\) and 1.164 mg U L\(^{-1}\) treatments.

The test plates were incubated at 24 ± 2 °C for 72 ± 1 h under cool white fluorescent light with a 24-h photoperiod. Algal cell numbers were counted using a microscope and a hemacytometer.

The tests met the validity criteria set out in the test protocol; however, the coefficient of variation in the standard control counts was slightly higher than 21% than the test validity criteria of 20% in 2 of the 5 tests. The 72-h reference toxicant test was conducted with reagent water and a nutrient spike that was prepared according to standard procedures (standard water).

L. minor static growth inhibition tests

The original L. minor Linnaeus culture was obtained from the University of Toronto Culture collection (UTCC #492, Landolt clone 7730) and has been maintained in axenic culture by weekly subculture in Hoagland’s E+ medium at Vizon SciTech since 1999. Toxicity of U to L. minor was determined with a modification to the Environment Canada test method [19], to achieve a constant low alkalinity (~5 mg L\(^{-1}\)) and specific water hardness values of 30 mg L\(^{-1}\) and 120 mg L\(^{-1}\) as CaCO\(_3\).

To start the experiment, 2 plants (6 fronds) were introduced into 270-mL transparent polystyrene disposable cups, with matching lids, containing 150 mL of the test solutions spiked at different U levels (10 mg U L\(^{-1}\), 16 mg U L\(^{-1}\), 25 mg U L\(^{-1}\), 40 mg U L\(^{-1}\), 63 mg U L\(^{-1}\), 100 mg U L\(^{-1}\), and 160 mg U L\(^{-1}\)) based on the results of 2 separate 7-d range-finding tests (1 test for each water hardness). There were 4 replicates per treatment. Tests were conducted without water renewal (static) and without aeration, under continuous full-spectrum lighting. Test temperature was maintained at 25 ± 2 °C.

At the end of the 7-d test, total frond numbers per cup were recorded, and the fronds were dried at 60 °C. Frond increase (fronds at 7 d minus initial fronds) and dry weight data for each replicate were used to estimate effective concentration, 25% and 50% (EC25 and EC50) values.

Rainbow trout early life stage static renewal tests

The gametes of rainbow trout (O. mykiss) were obtained from the Fraser Valley Trout Hatchery in Abbotsford, British Columbia, Canada. Toxicity of U to embryo/alevin stages of O. mykiss was determined with a modification to the Environment Canada protocol [20] to achieve a constant low alkalinity (~5 mg L\(^{-1}\)) and specific water hardness values of 5 mg L\(^{-1}\) and 60 mg L\(^{-1}\) as CaCO\(_3\).

Eggs from 5 females were dry-fertilized with sperm from 4 males and placed in the weigh boats containing test solutions on the same day the gametes were obtained. Within 30 min of fertilization, 30 embryos were transferred into 800-mL plastic beakers with slits placed in 4-L food grade polyethylene pails containing 2.5 L of stock solutions spiked at different U concentrations (0.31 mg U L\(^{-1}\), 0.63 mg U L\(^{-1}\), 1.3 mg U L\(^{-1}\), 2.5 mg U L\(^{-1}\), and 5.0 mg U L\(^{-1}\)). The embryo/alevin tests were conducted with 5 U concentrations and a control of dilution water, with 4 replicates per concentration. A laboratory control containing standard laboratory water only was also tested. No pH adjustment of the test solutions was necessary. The test solutions were partially (~80%) renewed 3 times weekly. Tests were conducted in the dark for the first week of testing and then in subdued lighting for the remainder of the test. Test temperature was maintained at 14 ± 1 °C. Gentle aeration was provided.
Table 3. Measured or estimated (only UO$_2^{2+}$ and UO$_2$OH$^+$) water chemistry parameters in all test solutions as hardness was increased using CaSO$_4$ and MgSO$_4$

| Parameters | Target hardness$^b$ | FHM/RBT | Ceriodaphnia dubia | Hyalella azteca | Lemna minor | Pseudokirchneriella subcapitata |
|------------|---------------------|----------|-------------------|----------------|-------------|-------------------------------|
| pH         |                     |          |                   |                |             |                               |
| 5          | 6.5–6.9             | 6.5–6.9  | —                 | —              | —           | 6.8–7.8                       |
| 15         | 6.3–7.0             | 6.6–7.0  | 6.4–7.0           | —              | 7.8–8.2     |
| 30         | 6.5–6.8             | —        | 6.4–6.9           | 6.8–7.4        | —           |                               |
| 60         | 6.4–6.8             | 6.6–7.3  | 6.5–7.1           | 7.2–7.3        | —           |                               |
| 120        | 6.4–6.8             | 6.7–7.1  | 6.5–7.0           | 7.2–7.4        | —           |                               |
| 240        |                     |          |                   |                |             |                               |
| Hardness   |                     |          |                   |                |             |                               |
| 5          | 5                   | 6.6–6.9  | —                 | —              | —           | 5                            |
| 15         | 23                  | 7.0–7.0  | 6.4–7.0           | 2.5            | —           |                               |
| 30         | —                   |          | 6.4–7.0           | 5.9            | —           |                               |
| 60         | 61                  | —        | 6.4–7.0           | 10.6           | —           |                               |
| 120        | 123                 | 6.4–7.0  | 28.8              | 24.9           | 20.9        |
| 240        | 238                 | —        | 58.9              | 41.9           | —           |                               |
| Calcium    |                     |          |                   |                |             |                               |
| 5          | 1.1                 | 2.8      | 1.4               | —              | 0.7         |
| 15         | 5.2                 | 1.9      | 1.4               | —              | 1.9         |
| 30         | —                   | —        | 1.4               | 4              | —           |
| 60         | 10.4                | —        | 5.6               | —              | 7.9         |
| 120        | 20.1                | 15.5     | 10.4              | 16.6           | 15.7        |
| 240        | 50.2                | 51.1     | 21.2              | 29.1           | —           |                               |
| Magnesium  |                     |          |                   |                |             |                               |
| 5          | 0.7                 | 2        | 1                 | —              | 0.7         |
| 15         | 2.4                 | 1.9      | 1.4               | —              | 1.9         |
| 30         | —                   | —        | 1.4               | 4              | —           |
| 60         | 10.4                | —        | 5.6               | —              | 7.9         |
| 120        | 20.1                | 15.5     | 10.4              | 16.6           | 15.7        |
| 240        | 50.2                | 51.1     | 21.2              | 29.1           | —           |                               |
| Sulfate    |                     |          |                   |                |             |                               |
| 5          | 2                   | 2        | —                 | —              | 1           |
| 15         | 15                  | 12       | 9                 | —              | 3           |
| 30         | —                   | —        | 8                 | —              | —           |
| 60         | 60                  | —        | 36                | —              | 11          |
| 120        | 118                 | 111      | 73                | 35             | 21          |
| 240        | 235                 | 234      | 150               | —              | 45          |                               |
| Phosphate  |                     |          |                   |                |             |                               |
| 5          | 0.09                | 0.09     | —                 | —              | 0.57        |
| 15         | 0.09                | 0.05     | 0.05              | —              | 0.41        |
| 30         | —                   | —        | 0.05              | —              | —           |
| 60         | 0.13                | —        | 0.06              | 5.93           | —           |
| 120        | 0.14                | 0.05     | 0.07              | 6.29           | 0.41        |
| 240        | 0.16                | 0.05     | 0.07              | —              | 0.41        |                               |
| Bicarbonate HCO$_3^-$ |     |          |                   |                |             |                               |
| 5          | 6.71                | 3.04     | —                 | —              | 4.27        |
| 15         | 6.71                | 2.43     | 4.27              | —              | 4.27        |
| 30         | —                   | —        | 4.27              | —              | 4.27        |
| 60         | 7.92                | —        | 4.27              | —              | 4.27        |
| 120        | 6.1                 | 4.27     | 5.49              | —              | 4.27        |
| 240        | 6.1                 | 4.27     | 5.49              | —              | 4.27        |                               |
| Uranium$^c$|                     |          |                   |                |             |                               |
| 5          | 2.42                | 0.489    | —                 | —              | 0.96        |
| 15         | 0.464               | 0.032    | 0.032             | —              | 0.965       |
| 30         | —                   | —        | 134               | —              | —           |
| 60         | 2.83                | —        | 0.187             | —              | 0.921       |
| 120        | 1.75                | 0.482    | 0.301             | 131            | 0.91        |
| 240        | 1.77                | 0.47     | 0.347             | —              | 0.892       |                               |
| UO$_2^{2+}$|                     |          |                   |                |             |                               |
| 5          | 1.29e-9             | 5.75e-10 | —                 | —              | 1.03e-11    |
| 15         | 4.47e-10            | 8.87e-11 | —                 | —              | 4.76e-12    |
| 30         | —                   | —        | 4.04e-9           | —              | —           |
| 60         | 1.46e-9             | —        | 5.45e-10          | —              | 1.70e-10    |
| 120        | 1.19e-9             | 5.36e-11 | 8.40e-10          | 1.36e-9        | 1.70e-10    |
| 240        | 2.06e-9             | 9.17e-11 | 2.63e-9           | —              | 1.23e-10    |                               |
| UO$_2$OH$^{+}$|                |          |                   |                |             |                               |
| 5          | 1.14e-7             | 1.00e-8  | —                 | —              | 8.42e-10    |
| 15         | 9.52e-9             | 5.65e-10 | —                 | —              | 6.04e-10    |
| 30         | —                   | —        | 1.96e-7           | —              | —           |
| 60         | 1.19e-7             | 3.25e-9  | 3.22e-9           | 3.14e-9        | 2.67e-9     |
| 120        | 9.30e-8             | 3.18e-9  | 4.75e-9           | 4.78e-8        | 3.14e-9     |
| 240        | 1.20e-7             | 4.03e-9  | 8.86e-9           | —              | 2.67e-9     |                               |

$^a$All parameters except for pH measured in mg L$^{-1}$.

$^b$Nominal hardness in mg CaCO$_3$L$^{-1}$.

$^c$Maximum exposure concentrations are shown; concentrations in the control treatment were below detection limit of 0.0001 mgL$^{-1}$.

$^d$Estimated concentrations obtained with the PHREEQC speciation code.

FHM/RBT = fathead minnow/rainbow trout; — = experiments were not conducted at that hardness level.
The experiments lasted for 31 d for the 5 mg L\(^{-1}\) as CaCO\(_3\) hardness test and 30 d for the 60 mg L\(^{-1}\) as CaCO\(_3\) hardness test. The difference in test duration was because of the test method requirement for terminating the test 7 d after 50% of the control organisms had hatched. Embryos were observed daily. Dead embryos were removed starting on day 21. When the embryos began hatching, embryo and alevin mortalities and alevin deformities were recorded.

Rainbow trout acute lethality static tests

Rainbow trout (O. mykiss) fry were obtained from the Sun Valley Trout Farm, Mission, British Columbia, Canada. Uranium toxicity was determined with a modification to the Environment Canada test method [21]. The protocol was modified to maintain a constant low alkalinity (~5 mg L\(^{-1}\)) and specific water hardness of 15 mg L\(^{-1}\), 60 mg L\(^{-1}\), 120 mg L\(^{-1}\), and 240 mg L\(^{-1}\) as CaCO\(_3\).

At the start of the experiment, 10 fry were introduced into glass aquaria with plastic liners containing 15 L of test solutions spiked at different U concentrations (1.0 mg L\(^{-1}\), 2.7 mg L\(^{-1}\), 6.7 mg L\(^{-1}\), 16.7 mg L\(^{-1}\), and 41.7 mg L\(^{-1}\)). Loading density was 0.39 g L\(^{-1}\)/test vessel. There was 1 replicate per test concentration, as per the test method. Tests were conducted without water renewal, with gentle aeration (provided by air stones), and under full-spectrum lighting with a 16:8-h light:dark photoperiod. Test temperature was maintained at 15 ± 1°C. Samples were taken at test initiation and termination (4 d later) to measure hardness, alkalinity, and U concentration. The larvae were not fed. The number of dead fish in each test chamber was recorded daily.

Fathead minnow static renewal 7-d survival and growth tests

Fathead minnow (P. promelas) embryos were obtained from Aquatic BioSystems. Toxicity tests were initiated with larvae that were less than 24 h old, according to the Environment Canada test method [22]. The test method was modified to acclimate fathead minnow embryos and hatched larvae to the dilution waters prior to testing. In addition, the water was renewed 4 times during the 7-d experiment. Tests were conducted without water renewal, with gentle aeration (provided by air stones), and under full-spectrum lighting with a 16:8-h light:dark photoperiod. Test temperature was maintained at 25 ± 1°C. Samples were taken at test initiation and termination (4 d later) to measure hardness, alkalinity, and U concentration. The larvae were not fed. The number of dead fish in each test chamber was recorded daily.

Chemical analyses

Samples were collected for analysis of total U, hardness, and alkalinity in the control and low, medium, and high concentrations; samples were collected before and after water renewal in the static renewal tests, and at the start and end of the static tests. In the static renewal tests, samples were not collected after every water renewal; they were collected at specific water renewals such that the new (initial) solution was sampled at the start of the water renewal, and the old (final) solution was sampled at the following water renewal.

Uranium concentrations in the test solutions were analyzed by inductively coupled plasma–mass spectroscopy (ICP/MS), with a detection limit of 0.0001 mg L\(^{-1}\) at the Saskatchewan Research Council accredited laboratory in Saskatoon, Saskatchewan, Canada. Total elements were measured using ICP/optical emission spectroscopy (OES) and ICP/MS [24], also at the Saskatchewan Research Council. The water samples were preserved with ultrapure nitric acid before analysis. At least 1 control, a standard, and 1 duplicate sample were analyzed with each batch of samples run through the ICP.

Hardness was measured in aqueous samples by EDTA titration according to method 2340 C (EDTA titrimetric method) in Standard Methods for Examination of Water and Wastewater [24]. Alkalinity was measured in aqueous samples by automated colorimetric analysis using the Cobas Fara (Roche Diagnostic Systems) Automated Analytical System according to the procedure developed by Fenwick Laboratories (Halifax, NS, Canada). Sulfate and chloride concentrations were measured by ion chromatography, according to method 4110-B (ion chromatography with chemical suppression of eluent conductivity) in Standard Methods for the Examination of Water and Wastewater [24]. Dissolved oxygen concentrations, temperature, and pH were measured in all the definitive test solutions—before and after each water renewal in static-renewal tests, and at the beginning and end of the test in static tests.
Effects of chemical hardness on U toxicity

Effect of chemical hardness on U toxicity

Quantifying cation competition with U for uptake sites

We quantified cation competition with U for similar uptake sites by calculating the ratio of the sum of free and hydrated U toxicity to the total U toxicity (Table 4). A constant ratio with increasing hardness would indicate that Ca and Mg were not competing with U for the same uptake sites. In contrast, a change in this ratio would indicate that Ca and Mg compete with the free and hydrated ions for the same uptake sites, which would reduce the toxicity of the uranyl ions.

RESULTS

Calculated U speciation in exposure media

Initially, we verified that the calculated levels of UO₂OH⁺ and UO₂²⁻, the assumed species of ecotoxicological concern [1, 26, 27], remained constant despite varying chemical hardness within the range encountered downstream of U mines and mills in the test waters. Overall, the speciation calculation indicated that UO₂(OH)₀⁻⁻⁻ likely dominated the U species, followed by UO₂PO₄⁺⁻⁻, (UO₂)₂(OH)₂CO₃⁻, UO₂HPO₄⁻⁻, UO₂CO₃⁻, UO₂OH⁺, and lastly UO₂²⁻⁻⁻ (Figure 1). The proportion of UO₂PO₄⁺⁻⁻ decreased by an order of magnitude from the low Ca and Mg exposure to the high exposure media, which slightly increased the proportion of other U species. The changes in the calculated proportion of UO₂(OH)₀⁻⁻⁻ and UO₂²⁻⁻⁻ in the laboratory test media were not significant. Therefore, calculated levels of UO₂OH⁺ and UO₂²⁻⁻⁻ remained the same regardless of Ca and Mg concentrations (Figure 1) in all tested waters except perhaps for the P. subcapitata test.

Figure 2 indicates that pH was controlled between pH 6.5 and 7.5 to limit changes in calculated concentrations of UO₂(OH)⁺ and UO₂²⁻⁻⁻ within most tests. In general, an increasing pH from pH 6.5 to 7.5 decreased the calculated concentrations of UO₂(OH)⁺ and UO₂²⁻⁻⁻ in all tests, typically by a factor of 10. However, pH may not have been adequately controlled in the P. subcapitata tests because the calculated ratio of UO₂²⁻⁻⁻ to total U decreased by at least 2 orders of magnitude as pH increased from 6.8 to 8.2. Similarly, the calculated ratio of UO₂(OH)⁺ to total U decreased by 1 order of magnitude as pH increased from 6.8 to 8.2. Hence, pH influenced the toxicity response curves obtained more for this algal species at different chemical hardness levels than for other species tested.

Effect of chemical hardness on U toxicity

Variations in Ca and Mg concentrations in the exposure media did not affect survival of fathead minnows over 4 d and 7 d, survival of rainbow trout fry over 4 d, early life stage development of rainbow trout over 30 d, survival, and reproduction of C. dubia over 7 d and frond number and dry weight of L. minor for 7 d (Figure 3). However, all response endpoints for H. azteca increased (i.e., showed a decrease in toxicity) as Ca and Mg increased (Figure 4). The changes in Ca and Mg concentrations explained 72% to 92% of the changes in U toxicity (Figure 4). Similarly, the no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), and inhibitory concentration, 25% (IC25) for P. subcapitata increased with an increase in Ca and Mg (Figure 5). The changes in Ca and Mg in the exposure media explained 85% to 93% of the changes in U toxicity to the algae.

Quantification of competitive U uptake with Ca and Mg

Absence of an effect of chemical hardness on U toxicity suggested that cations did not compete with U for similar uptake sites. For H. azteca, there was a distinct change in the ratio of free and hydrated U to total U above 123 mg L⁻¹ (Table 4). In contrast, the ratios of free and hydrated U toxicity to total U toxicity to P. subcapitata and L. minor did not change with increasing hardness (Table 4). For fish species, Table 4 also showed that the ratio of free and hydrated uranyl toxicity to total U toxicity remained unchanged with increasing hardness. Finally, the ratio of free and hydrated U toxicity to total U toxicity for C. dubia changed at 124 mg L⁻¹ and higher even though C. dubia survival and reproduction were not affected by hardness.

Sensitivity of aquatic species tested

Table 5 reports U toxicity values for the different endpoints of the 6 species tested.

Growth and frond number endpoints for L. minor ranged from 4.7 mg U L⁻¹ (IC25) to 16.4 mg U L⁻¹ (IC50) and from 6.4 mg U L⁻¹ (IC25) to 35.5 mg U L⁻¹ (IC50), respectively. Growth endpoints for P. subcapitata ranged from a NOEC of 0.01 mg U L⁻¹ to an IC50 of 0.2 mg U L⁻¹. Survival endpoints of C. dubia ranged from a NOEC of 0.06 mg U L⁻¹ to a LC50 of 0.16 mg U L⁻¹, whereas reproduction endpoints ranged from a NOEC of 0.02 mg U L⁻¹ to an IC50 of 0.11 mg U L⁻¹. Survival endpoints of H. azteca ranged from a NOEC of 0.006 mg U L⁻¹ to a LC50 of 0.34 mg U L⁻¹. Larval development endpoints of rainbow trout ranged from a NOEC of 0.28 mg U L⁻¹ to an EC50 of 0.64 mg U L⁻¹ while survival endpoints of rainbow trout fry ranged from a NOEC of 2.4 mg U L⁻¹ to a LC50 of 4.2 mg U L⁻¹. For fathead minnows, we conducted 4-d and 7-d survival tests. The survival endpoints ranged from a NOEC of 1.2 mg U L⁻¹ to a LC50 of 2.1 mg U L⁻¹. The 7-d tests yielded similar survival endpoints, ranging from a NOEC of 0.81 mg U L⁻¹ to a LC50 of 2 mg U L⁻¹.

In summary, our results indicated species-specific differences in sensitivity to U in the following order from most to least sensitive: H. azteca, C. dubia, P. subcapitata, O. mykiss alevis/egg stage, P. promelas, O. mykiss fry stage, and L. minor.

DISCUSSION

Review of interspecies variability in sensitivity to uranium

As can be seen in Table 5, our toxicity results for most species and endpoints ranged from 0.006 mg U L⁻¹ (NOEC, H. azteca) to 6.7 mg U L⁻¹ (LOEC, O. mykiss), which is within the range of toxicity values reported in the literature (0.008–8 mg U L⁻¹). However, the toxicity results for L. minor fall outside of this range. Growth and frond number endpoints for L. minor ranged from 4.7 mg U L⁻¹ (IC25) to 16.4 (IC50) mg U L⁻¹ and from 6.4 mg U L⁻¹ (IC25) to 35.5 mg U L⁻¹ (IC50), respectively. The addition of a considerable quantity of...
phosphorus (5.9–6.3 mg L$^{-1}$; Table 3) to support growth is likely the reason for this apparent tolerance. Phosphorus has a strong affinity with the free uranyl ion, which likely decreased its availability in the test media. Figure 6 supports this hypothesis, as it shows concentrations of UO$_2$OH$^+$ and UO$_2^{2+}$ decreasing 5 and 8 times, respectively, in the L. minor test water compared with levels in bioassays conducted with other species.

For the remaining species tested, differences in U toxicity values from values found in the literature are mostly because of differences in water quality parameters. In particular, at similar hardness levels, the pH and alkalinity appear to explain these differences, likely because the proportion of UO$_2^{2+}$ and UO$_2$OH$^+$ in the test media changed. For example, at similar hardness, growth of P. subcapitata was inhibited more in the present study (IC50 of 0.1 mg U L$^{-1}$) than in experiments done by Liber et al. [28] (IC50 of 0.5–2.2 mg U L$^{-1}$) with the same species. The higher tolerance of P. subcapitata to U in the study of Liber et al. [28] is likely because of the higher alkalinity.

The NOEC of 0.07 mg U L$^{-1}$ for C. dubia survival endpoint was higher than the NOEC of 0.008 mg U L$^{-1}$ reported by Pickett et al. [29]. These authors [29] measured lower pH than in the present study, which could explain their lower NOEC (Table 5). Reproductive endpoints yielded lower U toxicity values to C. dubia in the present study. The range of U concentrations (0.01–0.18 mg U L$^{-1}$) that affected reproduction in C. dubia in the present study was much lower than the concentrations (1.5–3.9 mg U L$^{-1}$) reported by Liber et al. [28]. The reproductive toxicity endpoints from Liber et al. [28] were higher because pH and alkalinity in their experiments were 10 times higher. In contrast, U reproductive endpoints reported by Pickett et al. [29] were lower than in the present study because they used more acidic water (Table 5).

For H. azteca, at similar hardness, Kuhne et al. [30] obtained a higher LC50 of 1.5 mg U L$^{-1}$ than in the present study (LC50 1.7 mg U L$^{-1}$). In contrast, mortality of H. azteca in the present study was much lower than the NOEC of 0.008 mg U L$^{-1}$ reported by Pickett et al. [29]. As for H. azteca, at similar hardness, growth of P. subcapitata was inhibited more in the present study (IC50 of 0.1 mg U L$^{-1}$) than in experiments done by Liber et al. [28] (IC50 of 0.5–2.2 mg U L$^{-1}$) with the same species. The higher tolerance of P. subcapitata to U in the study of Liber et al. [28] is likely because of the higher alkalinity.

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Effect of chemical hardness on U toxicity

The results of the present study indicated that when pH remained circumneutral and alkalinity low, as encountered downstream of U mines and mills (Table 1), chemical hardness did not have a strong and consistent effect on U toxicity to all aquatic organisms. Chemical hardness affected U toxicity only to the invertebrate *H. azteca* and the algae *P. subcapitata*. The positive effect of chemical hardness on *P. subcapitata* was consistent with the results of Fortin et al. [2], who also report that Ca and Mg, added as nitrate salts, inhibit uranyl uptake by the algae *Chlamydomonas reinhardtii*.

The lack of a general effect of chemical hardness on aquatic organisms (Figure 3) was likely because, as suggested by the ratio of U species to total uranium calculations, Ca and Mg did not have a strong and consistent effect on U toxicity to all aquatic organisms. Chemical hardness affected U toxicity only to the invertebrate *H. azteca* and the algae *P. subcapitata*. The positive effect of chemical hardness on *P. subcapitata* was consistent with the results of Fortin et al. [2], who also report that Ca and Mg, added as nitrate salts, inhibit uranyl uptake by the algae *Chlamydomonas reinhardtii*.

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not compete with U for the same uptake sites (e.g., Ca channels in the cell membranes). Instead, it appeared more plausible that the increase in Ca, Mg, and sulfate increased the tolerance of the species to U. For instance, an increase in sulfate could have provided enough sulfur supply to cells for the synthesis of phytochelatins and metallothioneins to bind an increase in U. For instance, an increase in sulfate could have provided enough sulfur supply to cells for the synthesis of phytochelatins and metallothioneins to bind an increase in U.

The present study further confirms what Sheppard et al. [13] reported, that all the previous studies indicating that hardness affected uranium toxicity were in fact studies indicating that alkalinity mostly affected U toxicity. This is particularly the case when different hardness levels are set by diluting synthetic water [8,9] and when a mixture of carbonate, sulfate, and chloride salts is added [10]. These experiments are less relevant to water downstream of U mines and mills because alkalinity in these downstream waters remains low and stable despite the release of treated effluent (Table 1). Therefore, U toxicity tests relevant to Canadian U mines and mills are experiments that ideally use Ca and Mg sulfate salts.

**Impacts downstream of Canadian operating mines and mills**

The Nuclear Safety and Control Act and its regulations require that effluents be treated to prevent or minimize impacts to the environment. Table 1 reports U concentrations with distance from the treated effluent discharge point for different operating mines and mills as well as legacy mine sites that operated prior to the enactment of the Nuclear Safety and Control Act. At reference locations, U was present in surface waters at a concentration of 0.0001 mg L\(^{-1}\) (Table 1). In contrast, concentrations of U at currently operating U mines and mills ranged from 0.0001 mg L\(^{-1}\) to 0.075 mg L\(^{-1}\) and generally decreased with distance from the point of effluent discharge. At decommissioned mine sites, U concentration remained elevated relative to reference locations, with concentrations varying between 0.038 mg L\(^{-1}\) and 0.171 mg L\(^{-1}\) (Table 1). The lower U concentrations at currently operating U mines and mills demonstrate that modern environmental regulations have improved the quality of water downstream of U mines and mills.

Based on the U exposure levels in locations impacted by U mines and mills (Table 1) and by considering the U toxicity results of the present study (Table 5), we concluded that levels of U in receiving waters were below concentrations that are toxic to fish. Toxic U effects to fish species only appear at 0.280 mg U L\(^{-1}\) in our toxicity tests and in other data [36], which is higher than the current U levels downstream of all treated effluent discharge points including at decommissioned mine sites.

Concentrations of U downstream of operating U mines and mills and at some decommissioned sites may represent a risk to invertebrate and algal species. For example, based on the toxicity endpoints derived in the present study for *H. azteca* and *C. dubia*, certain sites have measured U concentrations that have the potential to negatively impact survival or reproduction of invertebrate species. If such a risk exists in the natural environment, it is limited to localized areas close to the point of effluent discharge (Table 1). Any potential toxicity of U in the pelagic compartment may be mediated or reduced by the presence of DOC or elevated hardness, as shown in the present study for *H. azteca* and *P. subcapitata*. However, data from the present study indicated a variable effect of chemical hardness on U toxicity.

**Uncertainties and future research**

Our study did not consider the presence of DOC in natural surface waters. Several studies indicate that DOC alleviates U toxicity because U bound to DOC is less bioavailable to aquatic organisms. It is likely that DOC was present in our study because *H. azteca*, *P. promelas*, and rainbow trout larvae were fed during the experiments. It is uncertain, however, what the actual levels of DOC in these tests were. It is likely that if DOC...
| Organism | Test | Endpoint | Duration (d) | pH | HCO$_3^-$ (mg L$^{-1}$) | 5 mg L$^{-1}$ | 15 mg L$^{-1}$ | 120 mg L$^{-1}$ | 200 mg L$^{-1}$ | Ref. |
|----------|------|----------|-------------|----|--------------------------|-------------|-------------|----------------|-------------|------|
| L. minor | Fixed no. | IC$_{25}$ (95% CI) | 7 | 5.8–7.4 | 3.1–3.7 | — | — | 4.3 (3.6–5.5)$^b$ | — | — | Present study |
| L. minor | Dry wt | IC$_{25}$ (95% CI) | 7 | 5.8–7.4 | 3.1–3.7 | — | — | 6.4 (4.9–8.5)$^b$ | — | — | Present study |
| P. subcapitata | Growth | NOEC | 3 | 6.8–8.2 | 4.3 | 0.01 | 0.06 | 0.06 | — | — | Present study |
| P. subcapitata | Growth | LOEC | — | — | — | 0.03 | 0.11 | 0.11 | — | — | Present study |
| P. subcapitata | Growth | IC$_{25}$ (95% CI) | — | — | — | 0.09 (0.08–0.12) | 0.06 (0.08–0.08) | 0.10 (0.08–0.11) | 0.20 (0.11–0.30) | 0.20 (0.16–0.21) | Present study |
| C. Dubia | Survival | NOEC | 7:1 | 6.5–7.3 | 2.4–4.3 | 0.07 | 0.06 | — | — | — | Present study |
| C. Dubia | Survival | LOEC | — | — | — | 0.18 | — | 0.44 | — | — | Present study |
| C. dubia | Reproduction | NOEC | 7 | 5.9–6.2 | 2 | 0.03 | — | — | — | — | Present study |
| C. dubia | Reproduction | LOEC | — | — | — | 0.02–0.007 | — | — | — | — | Present study |
| C. Dubia | Reproduction | IC$_{25}$ | — | — | — | 0.05 (0.05–0.06) | 0.08 (0.07–0.08) | — | — | — | Present study |
| C. Dubia | Reproduction | IC$_{50}$ (95% CI) | — | — | — | 0.09 (0.08–0.10) | 0.11 (0.11–0.11) | — | — | — | Present study |
| H. azteca | Survival | NOEC | 14 | 6.4–7.1 | 4.3–5.5 | — | — | 0.06 | — | — | Present study |
| H. azteca | Survival | LOEC | — | — | — | 0.11 | — | 0.44 | — | — | Present study |
| H. azteca | Survival | LC$_{25}$ (95% CI) | — | — | — | 0.005 (0.001–0.01) | 0.10 (0.08–0.12) | 0.13 (0.09–0.16) | 0.34 (0.19–1.8) | — | Present study |
| H. azteca | Survival | LC$_{50}$ (95% CI) | — | — | — | 0.02 (0.009–0.04) | 0.14 (0.12–0.18) | 0.20 (0.17–0.24) | 0.34 (0.19–1.8) | — | Present study |
| H. azteca | Survival | LC$_{50}$ | 14 | 7.9 | 137 | — | — | — | — | — | Present study |
| O. mykiss | Early life stage | NOEC | 30–31 | 6.4–7.0 | 6.1–7.9 | <0.28 | — | 0.31 | — | — | Present study |
| O. mykiss | Early life stage | LOEC | — | — | — | 0.28 | — | 0.61 | — | — | — | — | Present study |
| O. mykiss | Early life stage | LC$_{25}$ (95% CI) | — | — | — | 0.35 (0.28–0.39) | 0.55 (0.49–0.59) | — | — | — | Present study |
| O. mykiss | Early life stage | LC$_{50}$ (95% CI) | — | — | — | 0.46 (0.40–0.51) | 0.64 (0.56–0.65) | — | — | — | — | — | Present study |
| O. mykiss | Survival | NOEC | 4 | 6.4–7.0 | 6.1–7.9 | — | — | 2.6 | — | — | Present study |
| O. mykiss | Survival | LOEC | — | — | — | 0.67 | — | 1.3 | — | — | Present study |
| P. promelas | Survival | NOEC | 4 | 6.4–7.0 | 6.1–7.9 | — | — | 1.3 | — | — | Present study |
| P. promelas | Survival | LOEC | — | — | — | 1.2 | — | 0.81 | 1.2 | — | Present study |
| P. promelas | Survival | LC$_{25}$ | — | — | — | 2.1 | 1.9 | — | — | — | — | — | Present study |
| P. promelas | Survival | LC$_{50}$ | — | — | — | 2.0 (1.8–2.2) | 2.0 (2.0–2.1) | 2.1 (2.0–2.3) | 1.8 (1.5–2.0) | — | Present study |
| P. promelas | Growth | NOEC | 7 | 6.4–7.0 | 6.1–7.9 | — | — | 0.84 | 1.9 | 20 | — | Present study |
| P. promelas | Growth | LOEC | — | — | — | 1.3 | 1.9 | — | — | — | — | — | Present study |
| P. promelas | Growth | IC$_{25}$ (95% CI) | — | — | — | 1.4 (1.1–1.5) | 1.6 (1.3–1.9) | 1.2 (1.0–1.6) | 1.2 (1.0–1.4) | — | — | Present study |
| P. promelas | Growth | IC$_{50}$ (95% CI) | — | — | — | 1.6 (1.5–1.8) | 2.1 (2.0–2.1) | 2.0 (2.0–2.1) | 1.5 (1.3–1.7) | — | — | Present study |

$^a$Organisms were *Lemna minor*, *Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia*, *Hyalella azteca Oenochelys mykiss*, and *Pimephales promelas*.

$^b$Lemna minor was tested at hardnesses of 30 mg L$^{-1}$ and 120 mg L$^{-1}$ as CaCO$_3$.

NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; LC$_{25}$ and LC$_{50}$ = lethal concentration at 25% and 50%; IC$_{25}$ and IC$_{50}$ = inhibitory concentration at 25% and 50%.
Chemical hardness, uranium speciation, and toxicity

would have been added to the test. U toxicity based on total U would have decreased, whereas U toxicity values based on the free and hydrated uranyl ions would have remained the same.

However, U toxicity values based on free and hydrated uranyl ions rely on the assumption that the ionic form is the most bioavailable and on the accuracy of the selected mean thermodynamic constant values of the U complexes [6]. In future studies, attempts to measure the free uranyl ion using the diffusive gradient in thin film [37] or by ion exchange techniques [38] would help validate these speciation calculations.

Our observations that Ca and Mg did not appear to compete with U for uptake sites was based on the ratio of U species to total ions. Hence, conducting competition studies, and manipulation of Ca and Mg uptake rates, keeping alkalinity and pH as constant as possible.

The results of the present study apply mainly to receiving waters with low alkalinity and circumneutral pH, as encountered downstream of U mines and mills in Canada. The use of these toxicity results to predict the impact of uranium should take into consideration site-specific water chemistry downstream of the proposed treated effluent discharge relative to the water chemistry being evaluated in the present study. As U speciation is especially sensitive to pH, alkalinity, and DOC, knowledge of how treated effluent will affect these parameters should be considered carefully.

Finally, our toxicity results focused on water-borne U exposure to pelagic organisms. An important proportion of the U released from U mines and mills will eventually partition to sediments. Several investigations have linked total concentration of U to effects thresholds in sediments near U mines and mills [39–41]. However, considerable variability remains among these effects thresholds. Thompson et al. [39] derived lowest-effect levels ranging from 32 µg U g⁻¹ to 104 µg U g⁻¹, while Burnett-Seidel and Liber [40,41] derived no-effects levels ranging from 839 µg U g⁻¹ to 2296 µg U g⁻¹. This variability can be explained partly by U partitioning in sediments and the relative proportion of overlying water versus sediment as a source of U to benthic organisms [42]. Some investigators have explored the use of critical body concentrations of metals as predictors of toxic effects [43,44]. The advantage of this approach is that metal accumulation is a true measure of exposure and is less dependent on water chemistry [45]. For instance, Alves et al. [46] indicated that U accumulation in H. azteca predicted toxicity with more accuracy than water or sediment concentrations. Therefore, more research is warranted to better predict the impacts of U and other metals on benthic organisms.

CONCLUSIONS

Overall, the present study confirmed that the majority of studies concluding that hardness affected uranium toxicity were in fact studies in which alkalinity and pH were the stronger influence. Indeed, the toxicity tests performed on 6 freshwater species in which the free and hydrated uranyl ions were estimated to be held constant with increasing chemical hardness in waters of circumneutral pH and low alkalinity did not identify a consistent effect of chemical hardness on U toxicity. Also, our data did not provide unequivocal evidence that U competes with Ca and Mg for the same uptake sites. This finding is somewhat surprising because the biotic ligand model suggests that cations compete with metals for the uptake sites. Hence, conducting risk assessments assuming that chemical hardness will alleviate U toxicity to aquatic organisms is not warranted at this time.

Acknowledgment—This article is dedicated to the memory of S. Munger, who co-managed the contract along with G. Bird. The Canadian Nuclear Safety Commission funded the present study through a contract (87055–020395) with Vizon SciTec.

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