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Effects of waterborne uranium on survival, growth, reproduction and physiological processes of the freshwater cladoceran *Daphnia magna*

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Abstract

Acute uranium toxicity (48 h immobilisation test) for *Daphnia magna* was determined in two different exposure media, differing in pH and alkalinity. LC_{50} varied strongly between media, from $390 \pm 40\ \mu g\ L^{-1}$ U at pH 7 to $7.8 \pm 3.2\ mg\ L^{-1}$ U at pH 8. According to the free ion activity model uranium toxicity varies as a function of free uranyl concentration. This assumption was examined by calculating uranium speciation in our water conditions and in those reported in the literature. Predicted changes in free uranyl concentration could not solely explain observed differences in toxicity, which might be due to a competition or a non-competitive inhibition of H^+ for uranium transport and/or the involvement of other bioavailable chemical species of uranium.

Chronic effects of uranium at pH 7 on mortality, ingestion and respiration, fecundity and dry mass of females, eggs and neonates were investigated during 21-day exposure experiments. A mortality of 10% was observed at $100\ \mu g\ L^{-1}$ U and EC_{10} for reproduction was $14 \pm 7\ \mu g\ L^{-1}$ U. Scope for growth was affected through a reduction in feeding activity and an increase in oxygen consumption at $25\ \mu g\ L^{-1}$ U after 7 days of exposure. This had strong consequences for somatic growth and reproduction, which decreased, respectively, by 50% and 65% at $50\ \mu g\ L^{-1}$ U after 7 days and at $25\ \mu g\ L^{-1}$ U after 21 days. Uranium bioaccumulation was quantified and associated internal alpha dose rates from 2.1 to $13\ \mu Gy\ h^{-1}$ were estimated. Compared to the toxicity of other alpha-emitting radionuclides and stable trace metals, our results confirmed the general assumption that uranium chemical toxicity predominates over its radiotoxicity.

Keywords: *Daphnia magna*; Uranium; Chemical speciation; Scope for growth; Energy allocation

1. Introduction

Uranium is a naturally occurring metal from the actinide series and is composed of three alpha-emitting radioactive isotopes, ^{238}U, ^{235}U and ^{234}U, respectively, contributing 99.27%, 0.72% and 0.0055% of mass (Colle et al., 2001). Its behaviour in natural ecosystems has been extensively studied and described in several reviews (Colle et al., 2001; Ragnarsdottir and Charlet, 2000). Uranium is ubiquitous in natural waters at trace concentrations, ranging from 0.02 to $6\ \mu g\ L^{-1}$ U (Bonin and Blanc, 2001). Locally, higher concentrations may reach $2\ mg\ L^{-1}$ U, reflecting mainly the composition of surrounding rocks (Bonin and Blanc, 2001; WHO, 2001). Uranium concentration may also increase in some ecosystems due to anthropogenic activities such as mining, extraction and processing of uranium for nuclear fuel and weapons, as well as spent fuel reprocessing. For two decades, the chemical toxicity of uranium, as a heavy metal, has become of increasing concern (Environment-Canada and Health-Canada, 2000; Sheppard et al., 2005).

In freshwater organisms, ecotoxicological data for acute and chronic exposure concern a wide range of endpoints and show great variability, notably due to differences in the chemical composition of the exposure medium. In a recent review, Sheppard et al. (2005) showed that the sensitivity of organisms to uranium is dependent on several environmental parameters such as alkalinity (due to complexation of uranyl ion UO_2^{2+} with soluble

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carbonates), and hardness (due to its competition with calcium and magnesium). For example, in *Daphnia magna* water hardness and alkalinity reduce the acute toxicity of uranium, with 48 h LC_{50} increasing from 6.4 to 51.9 mg L^{-1} U (Poston et al., 1984). Barata et al. (1998) reported a similar value of acute LC_{50}, ranging from 8.3 to 22.4 mg L^{-1} U dependent on daphnid clone and water hardness. However, as pH, hardness and alkalinity varied concomitantly, the effects of complexation and/or competition on uranium toxicity are difficult to differentiate.

Discrepancies between ecotoxicity data on uranium originate also from the diversity in tested endpoints and time of exposure. In *D. magna*, standard ecotoxicological tests for the identification of chronic effects are based on the outcome of 21-day reproduction. As previously studied by Poston et al. (1984), reproduction inhibition was shown at uranium concentrations from 0.5 to 3.5 mg L^{-1} U. Reproduction was shown to be more sensitive for another freshwater cladoceran, *Moinodaphnia macleayi* with a 6-day LOEC of 20–49 μg L^{-1} U (Semaan et al., 2001). In *Ceriodaphnia dubia*, reported effects on reproduction strongly differed between studies, with a 7-day LOEC of 3.91 mg L^{-1} U (Kuhne et al., 2002) and a chronic (7-day) EC_{25} of 3 μg L^{-1} U (Pickett et al., 1993).

In the literature, effects of uranium are commonly reported to concentrations, as it is done for trace metals, in accordance with the assumption that chemotoxicity of uranium predominates over its radiotoxicity. A small part of toxicity of uranium, as a radioelement, might be imputable to the radiological exposure. Sublethal effects of chronic alpha and gamma irradiation were recently reported in *D. magna* (Alonzo et al., 2006; Gilbin et al., in press). A comparison with these works requires that dose rates are quantified for uranium, based on accumulated concentration in tissues. At dose rates ≥0.9 mGy h^{-1}, alpha irradiation induced a reduction in somatic growth with potential strong consequences for energy allocation in organisms. Such studies of individual energy budgets have increasingly been used over the last decade to link effects of pollutants on physiological processes to growth and reproduction (Calow and Sibly, 1990; Calow, 1991; Kooijman, 2000; Knops et al., 2001; Baillieul et al., 2005).

The objectives of this work are: (1) to modulate pH and alkalinity of exposure medium within the tolerance range of daphnid physiology, in order to increase bioavailability and acute toxicity of uranium to *D. magna*; (2) to determine chronic effects of uranium on *D. magna* survival, reproduction, somatic growth and individual endpoints governing energy budget (ingestion, respiration); (3) to quantify uranium uptake and associated dose rate in daphnids and compare toxicity with those of stable trace metals and radiological stressors.

2. Materials and methods

2.1. D. magna culture

D. magna cultures (clone obtained from INERIS Verneuil en Halatte, France) were maintained in continuous parthenogenic reproduction in artificial freshwater at pH 8 (Elendt, 1990; M4 medium, hereafter 'M4-pH8') and pH 7 (modified M4 medium, hereafter 'M4-pH7') renewed twice a week. Composition of M4-pH8 was: 2 mM Ca, 0.5 mM Mg, 0.87 mM Na, 0.081 mM K, 0.51 mM SO_4, 3.2 μM NO_3, 2.1 μM PO_4, 4.9 nM NH_4, 35 μM SiO_3, 46 μM B, 1.8 μM Mn, 7.2 μM Li, 0.59 μM Rb, 0.57 μM Sr, 0.16 μM Br, 0.31 μM Mo, 98 nM Cu, 50 nM Zn, 43 nM Co, 19 nM I, 11 nM Se, 4.9 nM V, 7.2 μM Fe, 13.4 μM EDTA and 4.1 mM Cl. Composition of M4-pH7 differed only in a Cl concentration of 4.8 mM. Media were at equilibrium with air ($pCO_2 = 3.16 \times 10^{-4}$ atm).

Daphnids were fed daily with green algae *Chlamydomonas reinhardtii* (Dangeard, strain 11/32B from CCAP, United Kingdom). Algae under exponential growth phase were centrifuged (15 min, 1000 × *g*) and resuspended in M4 to achieve a daily ration of 100 μg carbon per daphnid. Neonates were removed and the medium was renewed twice a week. Cultures were maintained at 20 °C (±1 °C), a photoperiod of 16 h-light:8 h-dark and a light intensity of 30 μE m^{-2} s^{-1}. Reproductive rates >60 neonates per adult over 21 days were confirmed in the two media during 1 year, as a criterion for population health. All experiments were started with juveniles (<24-h old) from the fourth brood.

2.2. Exposure conditions

Uranium was obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France) as uranyl nitrate hexahydrate in 0.2% nitric acid solution (1 g L^{-1} U). Uranium acute toxicity was studied at concentrations ranging from 100 μg L^{-1} to 100 mg L^{-1} in M4-pH8 and M4-pH7. Chronic toxicity was examined from 10 to 100 μg L^{-1} U in M4-pH7. For all test conditions including controls, nitrate concentration was adjusted to 18 μM in M4-pH7 or 1 mM in M4-pH8. This was done to eliminate differences in NO_3^- concentration associated with uranium spikes (added as uranyl nitrate hexahydrate). This addition of nitrate did not affect survival of daphnids in the control. Uranium and major ionic concentrations were quantified prior and after 48 h exposure in acute condition or daily in freshly renewed medium and twice weekly after 24 h exposure in chronic conditions (filtration 2 μm), by ICP-AES (Optima 4300DV, PerkinElmer—detection limit = 10 μg L^{-1} and 0.5 mg L^{-1}, for U and major cations, respectively) and ionic chromatography (Dionex DX-120, Sunyvale, CA, USA—quantification limit = 100 μg L^{-1} for major anions). All water samples were stored at 4 °C in darkness before analysis. All concentrations remained within 10% of nominal concentrations. pH was similarly monitored and remained within 0.1 unit of nominal pH.

2.3. Modeling of aqueous speciation

Because of a lack of practicable techniques to directly measure individual uranium chemical species in solution, uranium speciation in water conditions of the exposure medium was predicted using the geochemical speciation code J-Chess (Java Chemical Speciation Equilibrium Speciation with Surfaces, Van der Lee, 1998). A consistent thermodynamic database was compiled of the OECD-NEA (Organization for Economic Cooperation and Development-Nuclear Energy Agency, 1996)