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Effects of depleted uranium on the reproductive success and F1 generation survival of zebrafish (*Danio rerio*)

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ABSTRACT

Despite the well-characterized occurrence of uranium (U) in the aquatic environment, very little is known about the chronic exposure of fish to low levels of U and its potential effect on reproduction. Therefore, this study was undertaken to investigate the effects of environmental concentrations of depleted U on the reproductive output of zebrafish (*Danio rerio*) and on survival and development of the F1 embryo-larvae following parental exposure to U. For that purpose, sexually mature male and female zebrafish were exposed to 20 and 250 μg/L of U for 14 days and allowed to reproduce in clean water during a further 14-day period. At all sampling times, whole-body vitellogenin concentrations and gonad histology were analyzed to investigate the effects of U exposure on these reproductive endpoints. In addition, accumulation of U in the gonads and its genotoxic effect on male and female gonad cells were quantified. The results showed that U strongly affected the capability of fish to reproduce and to generate viable individuals as evidenced by the inhibition of egg production and the increased rate of mortality of the F1 embryos. Interestingly, U exposure resulted in decreased circulating concentrations of vitellogenin in females. Increased concentrations of U were observed in gonads and eggs, which were most likely responsible for the genotoxic effects seen in fish gonads and in embryos exposed maternally to U. Altogether, these findings highlight the negative effect of environmentally relevant concentrations of U which alter the reproductive capability of fish and impair the genetic integrity of F1 embryos raising further concern regarding its effect at the population level.

1. Introduction

Uranium (U) is naturally found as a mixture of three isotopes: ^{234}U, ^{235}U, and ^{238}U. These isotopes are all alpha emitters and contribute to 0.005%, 0.720% and 99.274% of natural uranium (NU) mass composition, respectively (Madic and Genet, 2001). U is naturally present in the earth's crust at concentrations of 2–4 g t^{-1}, and is dispersed throughout the biosphere in soil, water, air, plants, and animals through natural biogeochemical processes (Ribera et al., 1996; Bleise et al., 2003). Natural concentrations of U in water vary from a few ng L^{-1} to more than 12 mg L^{-1} (World Health Organization, 2001; Salonen, 1994). However, U concentrations can increase due to various anthropogenic contributions, such as industrial activities linked to nuclear fuel production, numerous military applications (Miller and McClain, 2007), or accidental discharge (Gagnaire et al., 2011). For example, high concentrations of U from 10 to 20 mg L^{-1} have been measured in water close to mining sites in the United States (Ragnarsdottir and Charlet, 2000).

Depleted uranium (DU) is a byproduct of NU enrichment. Its widespread use in armor-penetrating weapons has raised environmental and human health concerns (World Health Organization, 2001). The (eco)toxicological risks associated with U potentially originate from both its chemical and radiological properties, depending on the specific activity of the different isotopes. However, for DU and NU, the risks are greater due to chemical rather than radiological toxicity (Mathews et al., 2009).

The ecotoxicological effects of U are not fully known. Studies describing the effects of U in fish have primarily focused on bioaccumulation and acute toxicity induced by waterborne exposure to U. For instance, lethal concentrations at 50% (LC_{50s}) (96 h) ranging from 0.7 to 135 mg L^{-1} have been reported depending on biotic (species, life stage) and abiotic factors (temperature, water hardness, pH) (Poston, 1982; Bywater et al., 1991; Labrot et al., 1999; Roex et al., 2002). More recently, it was shown that exposure of

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adult fish to low concentrations of U led to a broad range of biological responses in various target tissues including induction of oxidative stress (Labrot et al., 1999; Cooley et al., 2000; Barillet et al., 2007), genotoxicity (Barillet et al., 2005) and neurotoxicity (Lerebours et al., 2010). These studies are relevant with regard to the mechanisms of U toxicity at molecular and cellular levels, but they are not informative in terms of the impact on individuals or populations. Conversely, a study which would explore key developmental processes and critical steps in fish life cycle could provide such information. However, this type of information is limited in the case of U. We recently showed that low concentrations of U affected the embryo-larval development of fish by negatively affecting their growth and survival (Bourrachot et al., 2008). To further understand the effect of U on fish life cycle, we studied the toxicity of low concentrations of U on fish reproduction, using the zebrafish as a model organism. This species is commonly used as a biological model in ecotoxicology (Nagel, 2002; Hill et al., 2005; McAleer et al., 2005), particularly in studies evaluating the reproductive effects of endocrine disrupting chemicals (Fenske et al., 2005). Endocrine effects can be assessed by monitoring vitellogenin (Vtg), a large serum phospholipoglycoprotein normally produced in the liver of female zebrafish in response to circulating endogenous estrogen (Han et al., 2011). It is a precursor of egg yolk proteins, and once produced in the liver, travels in the bloodstream to the ovary, where it is taken up and modified by developing eggs (Heppell et al., 1995). Little is known about the impact of U on reproduction and particularly whether it can affect Vtg production and gonad cells (structure and DNA integrity) in genitors. In addition, the mechanism by which U may decrease fertility and impact embryo survival has never been studied.

To study the effects of U on reproduction, male and female zebrafish were exposed over 20 days to two concentrations of U, respectively: 20 $\mu g\,L^{-1}$, a low concentration close to the threshold concentration recommended by World Health Organization (15 $\mu g\,L^{-1}$) for drinking water and 250 $\mu g\,L^{-1}$, a concentration that is often measured close to U mining areas (Antunes et al., 2007). After exposure to DU, ecologically relevant parameters (laying, egg fertilization and survival) were recorded during a 14-day period in clean water. Furthermore, bioaccumulation of U was measured in the gonads of the genitors as well as in their progeny (at embryo and larval stages) and the F1 development was studied to assess whether maternal transfer of U can affect the F1 development.

2. Materials and methods

2.1. Fish maintenance

The zebrafish (*Danio rerio*) was used as a test organism in this partial life cycle study. Adult mature fish (4 months-old) were obtained from Aquasylva, Pertuis, France and were maintained in aerated tap water at a mean density of 4 fish per liter. Water was manually renewed by changing 50% of the total volume each week. The tank was kept in a room with a 12-h light:12-h dark photoperiod and a temperature of 25 ± 1 °C. Fish were fed with dry flake food (Tetramin®, Germany), twice a day supplemented with live neonates of *Daphnia magna* twice a week. Fish were gradually acclimatized to the artificial water used in the experiments (composition in $mg\,L^{-1}$: K^+ = 5.9; Na^+ = 7.5; Mg^{2+} = 4.7; Ca^{2+} = 11.6; Cl^- = 32.6; NO_3^- = 19.5; SO_4^{2-} = 9.6; pH 6.5 ± 0.2) for at least 3 weeks prior to the exposure phase. Water composition was a compromise between the conditions necessary for healthy fish physiology and optimal U bioavailability (Denison, 2004).

2.2. Exposure conditions to depleted uranium

Female and male zebrafish were exposed to 0, 20 and 250 $\mu g\,L^{-1}$ of waterborne depleted uranium ($UO_2(NO_3)_2{\cdot}6H_2O$, Sigma Aldrich, France) for 20 days under the same experimental conditions as those used during the acclimation phase. Contaminated water was continuously renewed by means of a flow-through water system (Supporting information 1), ensuring a daily renewal of half the volume in each tank. During the experiment, temperature and pH were monitored once a day. pH was maintained at 6.5 by the addition of HNO_3 (10^{-3} M) via peristaltic pumps linked to pH stats (Consort R301, IllKirch, Belgium). Fish were fed twice a day with dry flake food (Tetramin®, Germany). To avoid the build-up of excessive bacterial colonies, residual organic matter (food, feces) was removed daily by aspiration. Following 20 days exposure to U, fish were transferred into small spawning aquaria containing clean water for 15 days to study their reproductive output (see Section 2.4.1).

2.3. Uranium quantification

2.3.1. Contaminated water

Raw water samples were collected twice a day. Uranium and major cation concentrations were measured after acidification (2% v/v, HNO_3 15.3 M) by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Optima 4300 DV, Perkin Elmer, Wellesley, MA, USA; detection limits: U, 10 $\mu g\,L^{-1}$; Mg^{2+}, 1 $\mu g\,L^{-1}$; Na^+ and Ca^{2+}, 5 $\mu g\,L^{-1}$; K^+, 10 $\mu g\,L^{-1}$). Major anion concentrations were also analyzed by ionic chromatography (Dionex DX-120, Sunnyvale, CA, USA; detection limit = 100 $\mu g\,L^{-1}$ for major anions) to monitor water quality.

2.3.2. Uranium quantification in organisms

Measurement of U in the whole fish and in gonads was carried out after 20 days of exposure (n = 3 for whole body and n = 9 for gonads) and after 15 days of reproduction in clean water (n = 3 for whole body and 10 for gonads).

Just after dissection, tissue samples were dried until constant mass in a dry off oven (at least 48 h at 55 °C) and weighed using a microbalance (ultra-microbalance, Sartorius, Göttingen, Germany, precision 0.1 μg).

Tissues were then digested in 3 mL of HNO_3 (15.3 M) over heating at 90 °C (180 min) on a sand bath. After complete digestion, samples were then evaporated to incipient dryness (100 °C). The digestion process was completed by the addition of 2 mL of H_2O_2 (1 M) and evaporation to incipient dryness (60 min, 100 °C). Before measurement by inductively coupled plasma-mass spectrometry (ICP-MS; Varian 810-MS, detection limit: 10 $ng\,L^{-1}$), acidified ultrapure water (2%, v/v, HNO_3, 15.3 M) was added: 5 mL for whole-body samples and 10 mL for tissue samples.

Uranium accumulation was also measured in eggs of the first laying in each condition and in eggs of the last laying. Samples were digested in 1 mL of HNO_3 (15.3 M) and 1 mL of H_2O_2 (1 M). They were then dried on a sand bath (180 min, 90 °C) and dissolved in 10 mL of acidified ultrapure water (2%, v/v, HNO_3) before analysis by ICP-MS. In addition, U accumulation was measured 48 h post fertilization (hpf) at the chorion and embryo levels after manual dissection using microscopic needles.

Before digestion and U measurements, all the samples were rinsed in 3 successive baths containing artificial clean water, placed in an aluminum pan and dried for 48 h at 55 °C. After cooling, they were weighed using an ultra-microbalance (SE2 ultra-microbalance, Sartorius, Goettingen, Germany, precision of 0.1 μg).

2.4. Biological analysis

2.4.1. Reproductive output

2.4.1.1. Biometry. At the end of the exposure and reproduction periods, the length and weight of the fish were measured. The