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Selenium accumulation and the effects on the liver of topmouth gudgeon *Pseudorasbora parva* exposed to dissolved inorganic selenium

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ABSTRACT

Selenite(IV) and selenate(VI) are the major forms of Se in aquatic ecosystem. In this study, *Pseudorasbora parva* were exposed to 10, 200 and 1000 μg L^{-1} selenite and selenate for 28 days. Selenium accumulation, antioxidant enzyme levels, glutathione concentrations, lipid peroxidation and histology were evaluated in livers following exposure. Our results showed that Se(IV) and Se(VI) caused different accumulation patterns in the liver, with a more rapid accumulation of Se with Se(IV) treatment. Both Se species increased hepatic lipid peroxidation after 14 and 28 d (~ 30%). Among the antioxidants examined, the activity of SOD (except day 28) and the cellular levels of GSH were induced by 72–137% at lower concentrations, while the activity of GST was at least 24% lower than that of the control at 200 and 1000 μg L^{-1} for both Se species at all sampling points. Both forms of Se reduced the hepatosomatic index at 1000 μg L^{-1} after 28 d. In addition, marked histopathological alterations (10–31%) were observed in the liver of *P. parva* after exposure to both Se species, with higher frequency in the Se (IV) exposed fish. Liver local necrosis was observed only in the liver of fish exposed to 1000 μg L^{-1} of Se(IV) (~ 20%). Our results suggest that the ecological impacts of dissolved Se in this freshwater species may also contribute to overall toxicity.

1. Introduction

Selenium (Se) is an essential micronutrient to many organisms including fish , but can be toxic at levels slightly above the nutritional requirement (Lavado et al., 2012). Weathering of rocks, combustion of fossil fuels and agricultural drainage from irrigation of Se rich soils contribute the most to Se in aquatic ecosystems (Chapman et al., 2010a). Se originating from natural and anthropogenic sources enters the aquatic ecosystems mainly in the form of oxidized inorganic anions (i.e., Se(VI) and Se(IV)). In natural waters, the Se concentration is generally in the range of 0.1–10 μg L^{-1} (Sohrin and Bruland, 2011). However, much higher Se concentrations have been found in Se contaminated aquatic ecosystems with over 100 μg L^{-1} of Se observed in the drainage water flowing into the San Luis Drain and the wetlands at Kesterson in California's San Joaquin Valley (Ohlendorf et al., 1986). Even higher concentrations of Se (up to 1000 μg L^{-1} or higher) have been reported in waters receiving effluents from agricultural irrigation or coal mining (Pond et al., 2008).

Aquatic organisms can absorb Se from both aqueous and dietary exposures. Se in water and sediments can bioaccumulate in lower trophic level species (such as algae and benthic organisms) within aquatic food chains (Schlenk et al., 2003; Xie et al., 2016b) with subsequent transfer to higher trophic level species, resulting in toxic concentrations of Se in apex predators such as predatory fish and birds (Hamilton, 2004; Kupsco and Schlenk, 2016a; Lemly, 2004). Trophic transfer in aquatic food chains is complicated due to Se speciation, biotransformation and bioavailability differences within animal species as well as environmental impacts that alter Se fate (Chapman et al., 2010a; Hamilton, 2004). Following uptake by organisms, oxidized Se undergoes a series of transformations eventually resulting with

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incorporation into selenoproteins (including many important Se-containing enzymes) which are essential for normal physiology (Fairweather-Tait et al., 2010). Numerous studies have demonstrated that dietary exposure is the predominant route for Se accumulation in upper trophic level species such as fish and birds (Chapman et al., 2010a; Kupsco and Schlenk, 2014, 2016b; Lemly, 2004; Ohlendorf et al., 1986; Vidal et al., 2005). In the Belews Lake episode, 19 of the 20 species in Belews Lake were eliminated due to the teratogenic and reproductive toxicity of dietary Se. Meanwhile, dietary Se caused death and deformity of embryos of several waterfowl nesting at the wetland habitat of the Kesterson Reservoir (Lemly, 2002a). In addition, both field and laboratory studies have demonstrated that deleterious effects such as alterations of the antioxidants, mortality, impaired reproduction, teratogenesis, reduced growth and histopathological lesions can occur in fish and birds consuming Se contaminated prey (Chapman et al., 2010b; Lemly, 2002b). Therefore, more attention has been paid to the study of the effects of dietary Se in aquatic organisms (Schlenk et al., 2003; Xie et al., 2016b). However, given that selenite and selenate are the major forms of Se originating from natural and anthropogenic sources and probably the dominant Se species encountered in aqueous exposures by aquatic organisms, the accumulation of inorganic Se from water in fish is relatively less studied.

In this study, the bioaccumulation and hepatocellular effects of Se (IV) and Se(VI) from dissolved exposure were examined in topmouth gudgeon *Pseudorasbora parva*. *P. parva* is a small freshwater cyprinid found in Northeastern China surface waters within Heilong Jiang catchments as well as neighboring countries. Gudgeon have invaded waterways of many countries throughout the world (Gozlan et al., 2010). Its biological characteristics, including small body size (~ 3–8 cm at sexual maturity), relatively long life span of approximately 3–4 years, multiple spawning behavior and robust fecundity, make it an ideal fish species for ecotoxicological study (Gozlan et al., 2010). To the best of our knowledge, the bioaccumulation and effects of Se have been never studied in this species. The aims of this study were to determine the accumulation of Se, to evaluate the effects of Se on the antioxidant physiology, and to examine the histopathological alterations in the livers of *P. parva* exposed to dissolved inorganic selenium.

2. Material and methods

2.1. Chemicals and reagents

All glassware and plastic containers were acid washed in 10% HNO_3, thoroughly rinsed with deionized water, dried in an oven at 40 °C, and wrapped in aluminum foil until use. Selenite (in the form of Na_2SeO_3) and selenate (in the form of Na_2SeO_4) were purchased from Sigma-Aldrich. All other chemicals used in this study were of analytical grade or better. All solutions were freshly prepared with double deionized water (Milli-Q, Millipore; 18.2 MΩ cm^{-1} resistivity).

2.2. Test animals

P. parva with similar size (4.7 ± 0.6 cm, 735.4 ± 32.5 mg, measured in 30 individuals) were purchased from a local market, which provided fish originally collected from the Dahuofang Reservoir, a pristine reservoir used as a drinking water source for Shenyang city (a city with over 6 million residents (Wu et al., 2017)). Species was identified by Dr. Chenghui Wang from Shanghai Ocean University. The fish were acclimated to experimental conditions (see below) for 15 d in the laboratory. The fish were fed freshly hatched brine shrimp (*Artemia franciscana*) nauplii at a daily ration of 5% (on a wet weight basis) during the acclimation.

2.3. Determination of LC50-96h

An acute toxicity test was conducted to determine the LC50-96h of Se(IV) and Se(VI) in *P. parva*. The fish were exposed to a negative control without added Se, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 mg L^{-1} of Se(IV) or, 10.0, 20.0, 40.0, 80.0, 160.0, 320.0 mg L^{-1} of Se(VI), in plastic aquaria containing 10 L of moderately hard reconstituted water (MHR) (96 mg L^{-1} of $NaHCO_3$, 47.5 mg L^{-1} of $CaSO_4$, 123.0 mg L^{-1} of $MgSO_4$ $7H_2O$, and 4.0 mg L^{-1} of KCl). Each concentration had 3 replicates each with 10 fish. The exposure media were renewed totally every other day. Food was withheld during the acute exposure. The light period was 16: 8-h light: dark, and the temperature was maintained at 25 ± 1 °C. The mortality was recorded every 12 h and dead fish were removed from the exposure aquaria. The exposure lasted for 96 h.

2.4. Determination of Se accumulation and effects

Fish were divided randomly into seven groups (i.e., control, three Se (IV) groups and three Se(VI) groups), each with 120 fish. The fish were exposed to 10 (an environmentally relevant concentration), 200 and 1000 μg L^{-1} of Se(IV) or Se(VI). These concentrations of Se are representative of the upper limit of the Se concentrations in the waters of Se-contaminated environments (Chapman et al., 2010a). Each treatment had 3 replicates, each containing 40 fish in a plastic aquarium with 40 L of MHR water. A negative control consisted of 3 replicates with 40 fish each but without added Se. The exposure media were renewed totally every 2 d. The exposure conditions were maintained as follows: temperature: 25 ± 1 °C; light: dark: 16 h: 8 h; pH: 8.02 ± 0.16; dissolved organic carbon: 2.1 ± 0.3 mg L^{-1}; conductivity: 271 ± 15.6 μS cm^{-1}; dissolved oxygen: 8.98 ± 0.12 mg L^{-1} (mean ± S.E., n = 3). The fish were fed freshly hatched nauplii during the exposure. The exposure lasted for 28 d. The exposure duration we chose was based on the exposure duration of other fish species exposed to Se (Kim and Kang, 2014). The Se concentrations in the exposure media were checked prior to and after the water renewal by Atomic Fluorescence Spectrometer (AFS) (see below). The measured concentrations of Se for the negative control, 10, 200 and 1000 μg L^{-1} were 1.2 ± 0.03 (control), 9.3 ± 0.5, 242.9 ± 20.3, 1076.3 ± 10.1 μg L^{-1} for Se (IV) and 9.3 ± 0.4, 184.9 ± 2.5, 1011.7 ± 35.2 μg L^{-1} for Se (VI), respectively (n = 3). The average Se concentration in the brine shrimp was determined to be 0.78 ± 0.15 μg g^{-1} (wet wt, n = 3).

During the exposure, 12 fish were removed and sacrificed on day 4, 14 and 28. Livers were dissected out on ice and weighed. Livers of 6 fish were combined as one composite sample for total Se accumulation due to limited accumulation of Se at the lowest exposure concentration (i.e., 10 μg L^{-1}). The livers of the remaining fish were stored at − 20 °C for subsequent biochemical analysis. Histopathological analysis of the livers was carried out on day 28 only (see below).

2.5. Biochemical analysis

The activities of antioxidants (superoxide dismutase (SOD), glutathione *S*-transferase (GST)), levels of reduced glutathione (GSH) and lipid peroxidation (using thiobarbituric acid reactive substances (TBARS) as a surrogate biomarker) in *P. parva* were assayed using methods in previous studies (Chen et al., 2016b; Habig et al., 1974; Hansen et al., 2006; Li et al., 2016; Sun et al., 1988; Xie and Buchwalter, 2011). Enzyme activity was normalized to total protein concentration, which was evaluated by the Bradford method (Bradford, 1976). The activity of each enzyme was determined as mU mg $protein^{-1}$ (Xie and Buchwalter, 2011). Details for these assays can be found in the supplementary file.

2.6. Histopathological analysis

Histopathological analysis of the liver was conducted using procedures described previously (Cao et al., 2013; Chen et al., 2016a, 2016b). Briefly, liver was fixed in Bouin's solution right after dissection.