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Exposure-dose-response of *Tellina deltoidalis* to contaminated estuarine sediments 3. Selenium spiked sediments

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ABSTRACT

The metalloid selenium is an essential element which at slightly elevated concentrations is toxic and mutagenic. In Australia the burning of coal for power generation releases selenium into estuarine environments where it accumulates in sediments. The relationship between selenium exposure, dose and response was investigated in the deposit feeding, benthic, marine bivalve *Tellina deltoidalis*. Bivalves were exposed in microcosms for 28 days to individual selenium spiked sediments, 0, 5 and 20 μg/g dry mass. *T. deltoidalis* accumulated selenium from spiked sediment but not in proportion to the sediment selenium concentrations. The majority of recovered subcellular selenium was associated with the nuclei and cellular debris fraction, probably as protein bound selenium associated with plasma and selenium bound directly to cell walls. Selenium exposed organisms had increased biologically detoxified selenium burdens which were associated with both granule and metallothionein like protein fractions, indicating selenium detoxification. Half of the biologically active selenium was associated with the mitochondrial fraction with up to 4 fold increases in selenium in exposed organisms. Selenium exposed *T. deltoidalis* had significantly reduced GSH:GSSG ratios indicating a build-up of oxidised glutathione. Total antioxidant capacity of selenium exposed *T. deltoidalis* was significantly reduced which corresponded with increased lipid peroxidation, lysosomal destabilisation and micronuclei frequency. Clear exposure–dose–response relationships have been demonstrated for *T. deltoidalis* exposed to selenium spiked sediments, supporting its suitability for use in selenium toxicity tests using sub-lethal endpoints.

1. Introduction

Selenium is an essential element within a fairly narrow concentration range, above which it is both mutagenic and toxic and below which selenium deficiency occurs (Hodson, 1988; Hoffman, 2002). Selenium studies which examined selenium dietary requirements, in the trout *Salmo gairdneri* showed that plasma glutathione peroxidase homeostasis was maintained at intakes of up to 1.25 μg/g dry food and toxicity occurred at 13 μg/g dry food. The authors speculated that dietary concentrations in excess of 3 μg/g in dry food over long periods might be toxic (Hilton et al., 1980; Hodson et al., 1980; Hodson and Hilton, 1983; Hicks et al., 1984). Eisler (2000) and Puls (1994) have reported similar responses in other fish species, birds and mammals in relation to selenium dietary requirements, deficiency, and toxicity. Selenium is released into aquatic environments through industrial activity such as metal smelting, overflow and leaching from ash dams and stack emissions associated with coal fired power stations and through sewage effluent (Davies and Linkson, 1991; Peters et al., 1999a). Sediments contain most of the total estuarine selenium inventory because of sorption and/or precipitation mechanisms (Peters et al., 1999a). Selenium biotransformation, bioaccumulation, and transfer through both sediment and water column foodwebs constitute major biogeochemical pathways in aquatic ecosystems (Lemly, 1999; Fan et al., 2002; Hamilton, 2004; Luoma and Rainbow, 2008; Maher et al., 2010). To acquire sufficient essential elements from environments with low ambient concentrations, aquatic organisms have evolved highly efficient uptake mechanisms, coupled with detoxification storage and excretion strategies (Phillips and Rainbow, 1989). Selenium accumulation by sediment dwelling deposit feeding bivalves may be from the interstitial water, sediment ingestion or from food (Luoma and Rainbow, 2005). The route of uptake may influence the organism's selenium handling and therefore its toxicity (Rainbow, 2007). Physiological effects and toxicity of metals and metalloids strongly depend on their intracellular localisation and binding to organelles and ligands (Sokolova et al., 2005) and selenium appears to be bound and incorporated differently according to the selenium species (Ewan, 1989; Burk, 1991; Hortensia et al., 2006).

To understand the fate and effects of such toxicants in aquatic environments the causal relationships between contaminant exposure, internal dose and associated biological effects need to be established (Widdows and Donkin, 1992; Adams et al., 2011). The evaluation of contaminant exposure, uptake and ecotoxicological effects is now an essential component of sediment quality assessment in Australia

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(Simpson et al., 2005) and toxicity data for local species along with suitable routine test protocols is necessary to develop relevant local exposure dose response toxicity guidelines. The current developments in ecotoxicological assessment are moving to the evaluation of sublethal endpoints for determining toxicant guideline exposure concentrations. To this end the development of biomarkers of exposure and effect for application in environmental assessment have been progressively developed and refined for a range of toxicants and aquatic species (Cajaraville et al., 2000; Adams, 2001; van der Oost et al., 2003; Galloway et al., 2004; Moore et al., 2004; Farmer, 2006; Batley et al., 2007; Damiens et al., 2007; Hagger et al., 2009; Taylor and Maher, 2010). Biomarker measurements can provide evidence that organisms have been exposed to contaminants at levels that exceed their detoxification and repair capacity establishing links between toxicant exposure and ecologically relevant effects (Koeman et al., 1993). Proteins contain the majority of selenium in organisms and of the known selenoproteins, cellular and plasma glutathione peroxidase, which is involved in redox metabolism, has the highest selenium content (Burk, 1991; Fan et al., 2002). The oxidative system has been shown to be sensitive to selenium through perturbations in the glutathione cycle (Hoffman, 2002). Lysosomes are involved in contaminant sequestration and are also susceptible to oxidative damage (Viarengo, 1989; Winston et al., 1996; Ringwood et al., 2002), while the frequency of micronuclei occurrence is an effective measure of DNA damage (Burgeot et al., 1996; Bolognesi et al., 2004).

Tellina deltoidalis is a sediment dwelling bivalve which is widely distributed in coastal estuaries around Australia where it lives buried in the sediments at a depth several times its shell length, of between 15–25 mm, and extends its siphons to the sediment surface to feed (Beesley et al., 1998). It satisfies most of the basic requirements to be an effective biomonitor being hardy, representative of the area of interest and an accumulator of bioavailable metals (Phillips, 1990; Phillips and Rainbow, 1994). The suitability of *T. deltoidalis* for use in whole sediment toxicity tests has been investigated by King et al. (2004, 2005, 2010) who found they were tolerant of a wide range of sediment types and salinities and easy to handle in a laboratory setting, while being sensitive to metal contamination. A protocol for the use of *T. deltoidalis* in whole-sediment acute toxicity tests has been included in the Australian Handbook for Sediment Quality Assessment (Simpson et al., 2005).

The purpose of this study was to examine the exposure–dose–response relationship to selenium in *T. deltoidalis* using 28 day sediment bioaccumulation tests (USEPA, 2000; ASTM-E1688–10, 2010) to develop useful biomarkers of effect, and further evaluate their potential for sediment toxicity testing in Australia using sublethal endpoints. There are no Australian sediment quality guideline concentrations for selenium so the exposure concentrations, 5 and 20 μg/g, chosen where based on those previously measured in contaminated Australian estuarine sediments (Peters et al., 1999a; Roach, 2005). Internal selenium exposure was measured in whole tissues, and subcellular tissue fractionation used to determine the active and detoxified selenium. Biomarker measurements of oxidative stress included total antioxidant scavenging capacity of cells, total glutathione concentrations, the ratio of reduced to oxidised glutathione, glutathione peroxidise and the extent of lipid peroxidation. Cellular damage was assessed using a lysosomal destabilisation assay and DNA damage through the presence of micronuclei. Measurement of enzymatic biomarkers in the glutathione cycle along with the cellular and genotoxic biomarkers of lysosomal membrane integrity and micronuclei occurrence provides a weight of evidence approach for selenium toxicity at the individual organism level which may indicate the potential for population level effects.

2. Materials and methods

2.1. Organism and sediment collection

Sediments were collected from a NSW Office of Environment and Heritage reference site in Durras Lake NSW, and stored at 4 °C until use. *T. deltoidalis* of 15–20 mm in size were collected from Durras Lake and Lake Tabourie, NSW in July 2005 and January 2006 and placed in coolers with sediment and water from the collection sites for transportation. Organisms were maintained for a maximum of two weeks at 22 °C in uncontaminated sediments, depth 15 cm, in glass aquaria with filtration and aeration to allow acclimation before experimentation. Overlying water used in aquaria was collected from coastal waters near Murramurrang National Park, NSW and adjusted from 35‰ to 28‰ with deionised water to match the salinity of the estuarine water from which organisms were collected.

2.2. Sediment selenium spiking

Sediments were sieved through a 2 mm stainless steel sieve, to remove large pieces of organic matter and organisms, prior to the addition of selenium. Sub samples of the collected sediments were measured for moisture content and grain size. To ensure the sediment matrix was suitable for organism burrowing and feeding, sediment was mixed with clean beach sand so that the 63 μm fraction was not greater than 20% mass/mass. To ensure added selenium was rapidly adsorbed and strongly bound to the sediment particles a method developed by Simpson et al. (2004) for producing metal spiked marine sediments, was followed. Wet sediment was added to mixing containers. Na_2SeO_3, (AR grade Sigma-Aldrich) was added to concentrations of 5 and 20 mg/kg dry mass of sediment. All containers were topped up with clean deoxygenated sea water and the final mixture was completely deoxygenated by bubbling with nitrogen for 2 h. Head spaces of containers were filled with nitrogen prior to sealing. Any pH adjustments were made immediately after the addition of the selenium using 1 M NaOH, (AR grade BDH), prepared in seawater, checked weekly and maintained at 7–8.2. Sediments were mixed on a Cell-production Roller Apparatus (Belco, USA) for several hours each day. Sediments were maintained at room temperature 22–25 °C. The time required for equilibration of added metals will be affected by the sediment properties, equilibration pH and the concentration and properties of the metal (Simpson et al., 2004). To determine when the added selenium was completely bound to sediment particles, pore waters were collected and acidified to 1% v/v with nitric acid (AristaR, BDH, Australia) and selenium was measured using an ELAN® 6000 ICP-MS (PerkinElmer SCIEX, USA). Once pore water selenium concentrations had fallen below instrument detection limits 0.001 μg/L the sediment was ready for use. Time to full absorption was 4 to 6 weeks. Unspiked sediments were treated in the same way and used for control treatments. Sediment selenium concentrations were measured by ICP-MS after digestion of 0.2 g of lyophilised sediment in 3 mL of nitric acid (AristaR, BDH, Australia) in polyethylene 50 mL centrifuge tubes for 60 min at 115 °C (Maher et al., 2003). Selenium in NRCC Certified Reference Materials, BCSS-1 marine sediment measured along with samples was 0.41 ± 0.10 μg/g ($n = 10$) and in agreement with certified values 0.43 ± 0.06 μg/g. Sediment selenium concentrations were measured prior to and at the end of the 28 day exposure period. Preexposure concentrations were <0.001, 5.00 ± 0.05 and 20 ± 1 μg/g and post exposure were <0.001, 5.00 ± 0.15 and 19 ± 2 μg/g.

2.3. Microcosm experiment design

Procedures for conducting the exposures were adapted from the test method for conducting 28 day sediment bioaccumulation tests (USEPA, 2000). Spiked and control sediments (500 g wet mass) were placed in each of three replicate 770 mL polypropylene containers (Chanrol # 01C30, Australia) per treatment. The containers were filled with fresh seawater adjusted to a salinity of 28‰. Containers were placed in random order on a tray in an incubator set at 22 °C with a day/night light cycle of 14/10 h to reflect spring/summer conditions. Aeration was introduced and the treatments were left for 24 h to allow them settle and the temperature to equilibrate. Fifteen *T. deltoidalis* were then introduced into each treatment container. Organisms were not