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Retention and distribution of methylmercury administered in the food in marine invertebrates: Effect of dietary selenium

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ABSTRACT

Methylmercury is transported along aquatic food chains from the lower trophic levels and selenium modulates the biokinetics of mercury in organisms in complex ways. We investigated the retention of orally administered methylmercury in various marine invertebrates and the effect of selenium hereon. Shrimps (*Palaemon adpersus* and *P. elegans*), blue mussels (*Mytilus edulis*), shore crabs (*Carcinus maenas*) and sea stars (*Asterias rubens*) eliminated methylmercury slowly ($t_{½}$ = ½ to > 1 year) and the copepod (*Acartia tonsa*) faster ($t_{½}$ ~ 12–24 h). Orally administered selenite augmented elimination of methylmercury in the copepod (in one of two experiments) and blue mussels, but not in shrimps, crabs and sea stars. Selenium generally alters the distribution of the body burden of mercury, leaving more mercury in muscle and less mercury in digestive glands or rest of the body – also in the species where total body retention is not affected.

1. Introduction

Methylmercury is efficiently taken up and assimilated in most organisms and once assimilated, methylmercury is retained very efficiently in aquatic organisms, with biological half lives in various species typically ranging from days in copepods (Lee and Fisher, 2017) over one (Tsui and Wang, 2004a; b) to three (Karimi et al., 2007) weeks in daphnids and weeks to years in some fish (e.g. Amlund et al., 2007; Bjerregaard et al., 2011; Pickhardt et al., 2006; Ruohtula and Miettinen, 1975; Tillander et al., 1969; Van Walleghem et al., 2013; Van Walleghem et al., 2007) and decapod crustaceans (Bjerregaard and Christensen, 2012; Evans et al., 2000; Fowler et al., 1978; Headon et al., 1996; Larsen and Bjerregaard, 1995; Rouleau et al., 1999; Tillander et al., 1969).

The major amount of methylmercury enters the aquatic food chains at the lower trophic levels and the level of methylmercury attained by predators at the top of aquatic food chains is generally determined by biomagnification processes up through the food chain (Lavoie et al., 2013; Riget et al., 2007). Hence, the potential to eliminate methylmercury in the organisms along the food chain determines the levels of methylmercury attained at the upper end of the food chain where methylmercury may cause toxic effects in top predators among wildlife (Dietz et al., 2013; Scheuhammer et al., 2008, 2015, 2016) and neurological symptoms in children of women with a high fraction of aquatic organisms in their diet (Debes et al., 2016; Grandjean et al., 1997).

Selenium may interact with mercury in aquatic organisms in ways that are not fully understood. Treatment of experimental lakes with selenite resulted in decreased levels of mercury in fish (Paulsson and Lundbergh, 1989, 1991; Turner and Rudd, 1983) and crayfish (Turner and Rudd, 1983), and fish in areas with elevated levels of selenium contain reduced amounts of mercury (Belzile et al., 2006; Peterson et al., 2009; Southworth et al., 1994, 2000; Yang et al., 2010). Investigations in the laboratory on selenium-mercury interactions in fish and aquatic invertebrates have shown highly variable results (reviewed by Cuvinaralar and Furness, 1991; Pelletier, 1986); different exposure routes, chemical forms of the elements, timing of the exposure and concentration or dose may lead to different results and different organisms respond differently.

Selenium administered in the food has been shown to reduce the concentrations of methylmercury in liver, kidney and muscle of rainbow trout *Oncorhynchus mykiss* (Bjerregaard et al., 1999) and reduce whole body retention of radiolabelled methylmercury in zebrafish *Danio rerio* and goldfish *Carassius auratus* (Bjerregaard et al., 2011) and the brown shrimp *Crangon crangon* (Bjerregaard and Christensen, 2012).

The purpose of the present investigation was to further elucidate half-lives for mercury and the effect of selenium on methylmercury

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retention and distribution in various marine invertebrates - the copepod *Acartia tonsa*, the blue mussel *Mytilus edulis*, the sea star *Asterias rubens*, the shore crab *Carcinus maenas* and the shrimps *Paleamon elegans* and *P. adspersus*.

2. Materials and methods

2.1. Experimental principle

The animals were initially exposed to food labelled with $^{203}HgCH_3^+$ and subsequently they were exposed to the same type of food, half of them with selenite amended food and the other half without added selenite. The exposure concentrations for selenium were selected with respect to the possibility of identifying effects rather than environmental relevance. The use of the gamma emitter ^{203}Hg allows repeated counting of the individual animals and the radioactivity of the animals was determined for days or weeks to follow the retention of the mercury. At the termination of the experiments, the distribution of mercury among the organs was determined for the larger organisms.

2.2. Preparation of food

2.2.1. Labelling of the algae with $^{203}HgCH_3^+$

The algae were labelled by adding $^{203}HgCH_3^+$ to algal cultures with 500000 cells mL^{-1} *Rhodomonas salina* and 100,000 cells mL^{-1} *Thalassiosira weissflogii* and leaving them for 24 h. The cell cultures were centrifuged at 4500 rpm for 4 min to concentrate the algae and remove them from the $^{203}HgCH_3^+$ in the water phase. The radioactivity of the algae was determined and they were re-suspended in medium before they were presented to the mussels and copepods. Assuming a single cell dry weight of 92 pg (Brown et al., 1998), the *R. salina* had an activity of approximately 42 * 10^6 and 2.8 * 10^6 dpm g^{-1} when presented to the copepods and blue mussels, respectively.

2.2.2. Algal exposure to selenium

Cultures of *R. salina* (500000 cells mL^{-1}) were exposed to selenium as selenite for 24 h at concentrations of 500 µg Se-SeO_3^{2-} L^{-1} when fed to the copepods and 1000 µg Se-SeO_3^{2-} L^{-1} when fed to the blue mussels. Light intensity was at 60–90 µE with light intervals of 12 h light and 12 h dark; temperature was 15 °C. Non-exposed algae were kept under the same conditions as the exposed ones.

Algal cultures were filtered through a glass fiber filter, GC-50 for determination of their selenium content. The control algae contained approximately 1.84 µg Se g^{-1} wet weight (two determinations with 3 replicates each: 1.87 ± 0.23 and 1.81 ± 0.15). The algae exposed to 500 µg Se-SeO_3^{2-} L^{-1} for the copepods and 1000 µg Se-SeO_3^{2-} L^{-1} for the mussels contained 35 ± 7 and 103 ± 9 µg Se g^{-1} wet weight, respectively (3 replicates each).

2.2.3. Food for sea stars, crabs and shrimps

The food for the shrimps, sea stars and shore crabs was cubes of homogenized and solidified blue mussel soft parts with a weight of 6–12 mg wet weight for shrimps and sea stars and 1.6 g wet weight for the crabs. For the sea star and shore crab experiment, the blue mussels (*M. edulis*) were collected in Great Belt, Denmark and homogenate and solid cubes with added $^{203}HgCH_3^+$ or selenite were prepared as described by Bjerregaard and Christensen (2012). For the *Paleamon sp.* experiments, the food was made from commercially obtained, boiled soft parts of *M. chilensis*.

2.3. Experimental organisms

2.3.1. Rhodomonas salina culture

The food source for the copepods and blue mussels was the algae *R. salina*. Cultures were obtained from DTU Aqua, Chartottenlund, Denmark and the Marine Biological Research Centre, Kerteminde, Denmark. Semi-continuous cultures of these algae were maintained at 20 °C under a 12 h light (60–90 µE) and 12 h dark. Water for the experiment was obtained from Great Belt outside Kerteminde, Denmark, and the water was filtered through a glass fiber filter, GC-50. Nutrients added to the algae were based on Walne's medium for algae cultures (Walne, 1970). The cultures were not grown sterilely and between one and two weeks into the experiment with the blue mussels, the culture was contaminated by an unidentified microorganism and a new culture was initiated. One experiment was carried out with the diatom *Thalassiosira weissflogii* (supplied by DTU Aqua and grown in a similar way).

2.3.2. Acartia tonsa

The copepod was obtained as eggs from a semi-continuous culture of the copepod obtained from DTU Aqua, Charlottenlund, Denmark. Before starting the experiment, the eggs were stored at 4 °C. For a start-up culture eggs were placed in a 10 L container. After hatching, the copepods were fed *ad libitum* with the algae *R. salina* at a cell density of 50000 cells mL^{-1} (Kiorboe et al., 1985; Stottrup and Jensen, 1990).

2.3.3. Field collected animals

Blue mussels *M. edulis*, sea stars *A. rubens*, shore crabs *C. maenas* and shrimps *P. elegans, P. adspersus* were collected from the Kerteminde area, Great Belt, Denmark. The salinity in this area typically varies between 15 and 25‰.

2.4. Experiments

2.4.1. Acartia tonsa

Three weeks old, adult females were used in this experiment with 10 specimens placed in each 200 mL glass beaker at 20 °C. To be able to move the adults to a new solution, the adults were placed inside mesh-bottomed sieves (8.5 cm height, 4.5 cm diameter, 100 µm mesh size bottom panel). During the whole experiment, the copepods were fed with algae *R. salina* (50000 cells mL^{-1}). At the beginning of the experiment, the copepods were exposed to $^{203}HgCH_3^+$-labelled algae for 5 h. Thereafter, the copepods were moved to clean beakers where half of the beakers had selenium exposed *R. salina* added and the other half non-exposed algae. The following days the copepods were moved to new beakers with clean water and algae were added once a day. Copepods were taken out for determination of radioactivity at 0, 4, 7, 19, 30, 47, 101, 125 and 149 and 0, 4.5, 8, 21, 49, 72, 102 and 120 h, respectively, after the onset of feeding with selenium amended/control algae in the two experiments carried out. For each treatment and time, three replicate beakers were sampled. We succeeded in retrieving an average of 7.8 ± 0.13 (n = 183) out of the 10 added copepods from the beakers.

The influence of temperature on the retention of mercury was investigated in a separate experiment. $^{203}HgCH_3^+$ was presented to the copepods on *R. salina* at 15, 20 and 25 °C and the retention of mercury was monitored at the same temperatures by sampling copepods at 0, 21, 44, 74 and 91 h.

R. salina and *T. weissflogii* as the food source for the copepods were compared, also in a separate experiment. $^{203}HgCH_3^+$ was presented to the copepods on *R. salina* and *T. weissflogii* and the retention of mercury was monitored with the same species as food sources by sampling copepods at 0, 19, 43, 65 and 90 h.

2.4.2. Mytilus edulis

At the beginning of the experiment, 20 blue mussels (shell length 17–21 mm; kept individually in 200 mL glass beakers at 17‰ and 10 °C) were fed with $^{203}HgCH_3^+$ exposed *R. salina* at 1000 cells mL^{-1} five times at 2 h intervals. Thereafter, 10 of the mussels were fed control algae and the other 10 fed selenium exposed algae one to five times a day. Each day the mussels were moved to a new beaker with clean water. For the next 41 days, the radioactivity of the mussels was determined at 1–10 day intervals. Approximately 1–2 weeks into the