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Global assessment of cadmium concentrations in the skin of free-ranging sperm whales (*Physeter macrocephalus*)☆

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ABSTRACT

Cadmium is a non-essential, toxic metal found accumulated in the organs of stranded cetaceans. Currently, there is no baseline cadmium concentration reported in a free-ranging, pelagic cetacean. The aim was to determine cadmium concentrations in the skin of free-ranging sperm whales ($n = 340$) collected from 16 regions around the world during the voyage of the *Odyssey* (2000–2005) considering region, gender, and age in males. Cadmium was detected in 81% of skin biopsies with a mean of 0.3 ± 0.04 μg/g ww (0.02 to 12.4 μg/g ww). These concentrations were higher than reported in literature in toothed whale skin (0.002–0.1 μg/g ww). Concentrations by region were significantly different ($p < 0.0001$) with the highest mean in Maldives and the Sea of Cortez (0.8 and 0.6 μg/g ww, respectively). There was no significant difference in cadmium concentration by gender ($p = 0.42$). Cadmium is known to have a long biological half-life, and cadmium concentrations in males were significantly higher in adults with a mean of 0.3 μg/g ww compared to subadults with 0.2 μg/g ww ($p = 0.03$). Selenium, an element that binds to cadmium inhibiting its toxicity, had a moderately positive correlation with cadmium ($r = 0.41$). Mercury, a toxic metal that positively correlates with cadmium in cetacean tissue, had a weakly positive relationship ($r = 0.20$). The regional baselines reported in this study may be used to develop residue criteria for prediction of toxicological risk in sperm whale skin. Additionally, this study shows the extent of cadmium exposure in a pelagic cetacean that has global distribution.

1. Introduction

Increasing anthropogenic activities have released metals into the marine environment with increases both regionally and globally (Riget and Dietz, 2000; Riget et al., 2004; Braune et al., 2005; Braune, 2007). Many of these metals have toxic properties. The toxicological significance of metals found in cetacean tissues is largely unknown due to the difficult nature of investigating these species in the marine habitat, especially in pelagic species, and the inability of laboratory testing due to their protected status. Limited data are available from cell culture studies of cetaceans due to a lack of immortalized and primary cell lines (Wise et al., 2011b, 2012; Li Chen et al., 2012; Pabuwal et al., 2013). Tissue residue criteria for prediction of toxicological risk for metals in cetaceans are highly needed with established baseline concentrations of metals in cetacean tissue required. In response to these concerns, numerous studies have been conducted over the last several decades on trace metals in marine mammals (Krishna et al., 2003); however, none other than the authors of this dataset have investigated metal concentrations in a pelagic species that is widely distributed enabling regional comparisons in the species across the globe (Wise et al., 2009, 2011a,b; Savery et al., 2013a,b, 2014a,b).

Cadmium (Cd) is a recognized environmental pollutant and a human health hazard (Jarup and Akesson, 2009; Nordberg, 2009). Exposure to cadmium is primarily associated with kidney damage and bone effects (Jarup, 2003; Godt et al., 2006). Atmospheric cadmium is

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mainly present in the form of aerosols or small particulates (~1 μm diameter) that can transport very long distances (~1300 km within 3 days) (WHO, 2007). These particles later fall on oceans by dry or wet deposition (Davidson, 1980; Nriagu, 1980) accounting for 80–90% of oceanic cadmium input (Cullen and Maldonado, 2013), which is estimated to be up to 4000 t/year (Duce et al., 1991). Natural sources of cadmium emissions can be from volcanic eruptions, rock weathering, and production of marine biogenic aerosols (Nriagu, 1990; Pan et al., 2010). Anthropogenic sources of cadmium in the atmosphere can be from metal production, the manufacture of rechargeable batteries, production of pigments and dyes, and stabilization of plastics (Jarup, 2003; Cullen and Maldonado, 2013). Marine sediments are the ultimate sink for metal pollutants (Millward and Turner, 2001) and can become enriched with cadmium due to sinking and decomposition of biological material (Boyle et al., 1976). Cadmium is known to bioaccumulate in organisms particularly in squid, a main prey source for many cetaceans with relatively high cadmium concentrations in the digestive gland (Honda and Tatsukawa, 1983; Dorneles et al., 2006).

Marine mammals occupy a high trophic level in the marine food chain, have relatively long life spans and have been suggested to be a sentinel for monitoring spatial and temporal trends of contaminants and environmental health (Bossart, 2006; Wells et al., 2004; Das et al., 2003; Fair and Becker, 2000). Recently, free-ranging sperm whales have been used as an indicator species for worldwide ocean pollution of different metal contaminants (Wise et al., 2009; Savery et al., 2013a,b, 2014a,b). The sperm whale breaths air and feeds at the top of the food chain (Whitehead, 2003); thus, it can be exposed to contaminants in the air and water column. In addition, this species has a wide geographical range (Rice, 1989). All these characteristics make the sperm whale an excellent species to indicate overall environment health.

Accumulation of cadmium has been reported primarily in liver, kidney, and muscle tissue from stranded dolphins considering age, sex, tissue type, and region (Stavros et al., 2011). It is important to assess cadmium and other trace elements in free-ranging animals to develop baselines and subsequently criteria for prediction of toxicological risk. Studies have established that skin can be used as a non-invasive sampling method to evaluate trace element levels in cetaceans (Bryan et al., 2007; Stavros et al., 2007, 2011).

Selenium, an essential element, is known to bind to cadmium (Zwolak and Zaporowska, 2012) and has a protective effect against cadmium toxicity in mammals (Nishiyama et al., 1987; Czauderna and Rochalsa, 1989; Yin et al., 1991). A positive correlation between cadmium and selenium has been reported in striped dolphin liver and muscle (Monaci et al., 1998) and in the liver of belugas, narwhals, and pilot whales (Hansen et al., 1990; Caurant et al., 1994). It is unknown if selenium is positively correlated to cadmium in sperm whale skin, possibly preventing cadmium toxicity. Conversely, mercury has also been reported to be positively correlated with cadmium in all tissues of striped dolphins (Monaci et al., 1998). Mercury is of particular toxicological concern as it bioaccumulates and biomagnifies in the marine ecosystem and at a greater degree upon its conversion to methylmercury by microorganisms in the environment. Our recent study reports that mercury is positively correlated to selenium in sperm whale skin (Savery et al., 2013a), and research reports that selenium binds strongly to mercury into an inert end product (Venugopal and Luckey, 1978; Yang et al., 2008; Khan and Wang, 2009; Arai et al., 2014). It is unknown if selenium or mercury are positively correlated with cadmium in sperm whale skin.

Our previous studies investigated the concentrations of other trace elements, arsenic, barium, chromium, gold, lead, mercury, selenium, silver, strontium, and titanium, in the skin of free-ranging sperm whales during 2000–2005 from around the globe during the voyage of the *Odyssey* (Wise et al., 2009, 2011a,b; Savery et al., 2013a,b, 2014a,b). The objectives of this study were to: (1) examine the concentrations of cadmium in sperm whale skin by region; (2) assess sex and age related effects; and (3) determine if correlations exist between cadmium and selenium and cadmium and mercury. The established baseline in this study can be used to develop residue criteria for prediction of toxicological risk in skin and determine the extent of cadmium exposure in a pelagic cetacean with global distribution.

2. Materials and methods

We measured total cadmium concentrations in 340 free-ranging sperm whale skin samples collected from 16 regions around the globe (Fig. 1) with 117 samples in the Pacific Ocean, 160 in the Indian Ocean, 30 in the Mediterranean Sea and 33 in the Atlantic Ocean. We considered 227 female and 113 male sperm whales (52 adult and 61 subadult males).

2.1. Biopsies

Skin biopsies were collected from free-ranging sperm whales during the voyage of the research vessel *Odyssey*, between 2000 and 2005, as described previously (Wise et al., 2009). Sampling was carried out simultaneously with photo-identification of individual whales to minimize duplication. The behavior of all whales sampled appeared to be healthy. Samples were taken from the whale's flank, a location that has been shown to elicit the fewest reactions (Whitehead, 2003). We used a 50 mm stainless steel cylindrical biopsy dart. Samples were removed from the biopsy dart and divided into two pieces at the interface between the skin and blubber. These two pieces were stored separately for later genetic and metal analysis. All tissue samples were frozen at −20 °C within a few minutes of collection in a 20 ml amber glass vial. The samples were shipped frozen to the Wise Laboratory. Age classification of males as subadult or adult was estimated by the length of the whale; however, classification of females could not be reliably determined because of their overall smaller size compared to males. Additionally, sloughed skin samples from two sperm whales were collected with a net and genotyped and analyzed for cadmium concentrations.

2.2. Genotyping

Gender was determined by genotyping, as published in our methods in Wise et al. (2009). DNA was extracted from a piece of whale skin, and gender was determined by PCR amplification reactions in which the SRY (male determining factor) gene was amplified. The keratin gene was used as an amplification control for all samples. Male samples showed both the keratin and SRY (male) bands, at ~311 bp and ~152 bp, respectively. Female samples showed only the keratin band at ~311 bp. Primer sequences were the following:

SryPMF: 5′CATTGTGTGTGGTCTCGTGATC
SryPMR: 5′AGTCTCTGTGCCTCCTCGAA
KF: 5′AGATCAGGGGTTCATGTTTCTTTGC
KR: 5′TTTACAGAGGTACCCAAGCCTAAG.

2.3. Inductively coupled plasma mass spectroscopy

Whale skin samples were analyzed for total cadmium using a Perkin-Elmer/Sciex ELAN inductively coupled plasma-mass spectrometer (ICP-MS) according to our published methods (Wise et al., 2009; Savery et al., 2013a,b). Samples were rinsed with deionized water and allowed to air dry in a laminar flow hood to minimize contamination. Approximately 0.1 g of tissue was placed in a digestion vessel, 2 ml of Optima grade nitric acid (Fisher Scientific, Pittsburg, PA) was added, the vessel placed in a hot block, and refluxed at 95 °C for 4 h. The sample was cooled, 2 ml Optima grade hydrogen peroxide (Fisher Scientific, Pittsburg, PA) and deionized water (3:2 v/v) was added, heated until the effervescence subsided, cooled, and brought up to a final volume of 20 ml. Standard quality assurance procedures were employed (Table 1) and include the analysis of standard reference materials, a