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Correlation and toxicological inference of trace elements in tissues from stranded and free-ranging bottlenose dolphins (*Tursiops truncatus*)

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ABSTRACT

The significance of metal concentrations in marine mammals is not well understood and relating concentrations between stranded and free-ranging populations has been difficult. In order to predict liver concentrations in free-ranging dolphins, we examined concentrations of trace elements (Al, As, Ba, Be, Cd, Co, Cu, Fe, Li, Mn, Ni, Pb, Sb, Se, Sn, total Hg (THg), V, Zn) in skin and liver of stranded bottlenose dolphins (*Tursiops truncatus*) from the South Carolina (SC) coast and the Indian River Lagoon, Florida (FL) during 2000–2008. Significantly higher concentrations of Zn, Fe, Se, Al, Cu and THg were found in skin while liver exhibited significantly higher Cu, Fe, Mn and THg concentrations for both study sites. Mean skin concentrations of Cu and Mn were significantly higher in SC dolphins while higher concentrations of THg and V were found in FL dolphins. In addition, liver tissues in SC dolphins exhibited significantly higher As concentrations while higher Fe, Pb, Se, THg, and V levels were found in FL dolphins. Two elements (Cu and THg) showed significant age-related correlations with skin concentration while five elements (Cu, Se, THg, Zn and V) showed age-related correlations with liver concentrations. Geographic location influenced age-related accumulation of several trace elements and age-related accumulation of THg in hepatic tissue was observed for both sites to have the highest correlations ($r^2 = 0.90$SC; $r^2 = 0.69$FL). Mean THg concentration in liver was about 10 times higher in FL dolphins (330 μg g^{-1} dw) than those samples from SC dolphins (34.3 μg g^{-1} dw). The mean molar ratio of Hg to Se was 0.93 ± 0.32 and 1.08 ± 0.38 for SC and FL dolphins, respectively. However, the Hg:Se ratio varied with age as much lower ratios (0.2–0.4) were found in younger animals. Of the 18 measured elements, only THg was significantly correlated in skin and liver of stranded dolphins and skin of free-ranging dolphins from both sites suggesting that skin may be useful in predicting Hg concentrations in liver tissue of free-ranging dolphins. Results indicate that 33% of the stranded and 15% of the free-ranging dolphins from FL exceed the minimum 100 μg g^{-1} wet weight (ww) (~400 dw) Hg threshold for hepatic damage while none from SC reached this level. Hepatic concentrations of As in SC dolphins and V in FL dolphins were also highly correlated with skin concentrations which may have some regional specificity predictive value. The present study provides the first application of trace element concentrations derived from stranded bottlenose dolphins to predict liver concentrations in free-ranging populations.

1. Introduction

Increasing human populations and related anthropogenic activities have resulted in the release of trace elements into the environment with temporal trends indicating increases in certain elements 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 and the toxicological significance of metals found in tissues for marine mammals is more problematic than other species due to the limited available information (Law, 1996). Metals may adversely affect mammalian health by modulation of immune homeostasis and metal-induced alterations of immune function have been demonstrated in captive and wild harbor seals (*Phoca vitulina*) providing insight into mechanisms of these toxicants (Kakuschke et al., 2005, 2008, 2009). In response to these concerns, numerous studies have been conducted over the last several decades on trace metals in marine mammals. Due to its

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persistence and high mobility in the marine ecosystem, mercury is of particular concern as it biomagnifies in the upper levels of the food web (Leonzio et al., 1992b; Cardellicchio et al., 2002; Braune et al., 2005). Ecosystem-scale simulations suggest that anthropogenic mercury pollution is a global problem with increased disposal and release of mercury, primarily from the US and China that will continue to affect future generations in all regions of the world (Booth and Zeller, 2005).

Elevated mercury levels have been observed in several mammalian species including river otters (*Lontra canadensis*) (Basu et al., 2005), mink (*Mustela vison*) (Wobeser and Swift, 1976), Florida panthers (Facemire et al., 1995b) and polar bears (*Urus maritimus*) (Dietz et al., 2006). The high concentrations of some non-essential metals, such as mercury, in wildlife species have raised concerns regarding their toxicity. Mercury is a potent developmental and neural toxin that can readily cross placental and brain barriers and has been shown to affect the brain, nervous system, kidneys and developing fetuses (ASTDR, 1999). In mammals, mercury has been documented to cause adverse physiological effects, endocrine (thyroid) function, reproductive problems and death (Facemire et al., 1995b; Basu et al., 2005).

Marine mammals occupy a high trophic level in the marine food chain, have relatively long life spans and have been suggested to be a representative sentinel for monitoring spatial and temporal trends of contaminants and environmental health (Fair and Becker, 2000; Das et al., 2003; Wells et al., 2004; Bossart, 2006). As a widely distributed cetacean that exhibits site fidelity to near coastal environments, along with a well-characterized life history and a capacity for bioaccumulating metals, bottlenose dolphins may serve as a bell weather species indicating changes in the environment. Accumulations of trace elements, such as cadmium and mercury, have been reported primarily in liver, kidney and muscle tissue from stranded dolphins (Leonzio et al., 1992a; Kemper et al., 1994b; Kuehl and Haebler, 1995; Beck et al., 1997; Meador et al., 1999; Durden et al., 2007). Recent studies evaluating trace metals in free-ranging dolphins along the US coasts have established that skin can be used as a non-invasive sampling method to evaluate trace element levels in these populations (Bryan et al., 2007; Stavros et al., 2007).

Exposure assessment in dolphins may indicate geographic areas where dolphins may face the greatest risk from metal toxicity and is an important aspect of the management and conservation of cetacean wildlife species. Most studies report metal concentrations in tissues of marine mammals as it relates to age, sex, tissue type, and geographic area, which can vary greatly among marine mammals (Wagemann and Muir, 1984; Kemper et al., 1994a; Law, 1996; Das et al., 2003). However, studies investigating tissue concentrations of elements as measures of health outcome are needed to develop tissue residue criteria for prediction of toxicological risk in these species. The majority of studies on levels of trace metals in marine mammals are based on samples obtained from stranded animals and liver tissue is one of the most frequently reported. Generally, the liver has the highest concentrations of mercury and other metals and it has been used as the indicator organ for metals (Lockhart et al., 2005). It is important to assess trace elements in free-ranging dolphins that have the potential to cause toxicity. However, this is not possible using skin biopsy samples from free-ranging dolphins as there are no toxicological indicators available to assess metal concentrations in skin. Liver tissue accumulates the highest levels of some elements such as mercury which causes liver damage at high concentrations (ASTDR, 1999). Further, liver abnormalities have been reported to be associated with chronic mercury accumulation in Atlantic bottlenose dolphins (Rawson et al., 1993) and there is an estimated mercury limit threshold for hepatic damage in liver tissue of marine mammals (Wagemann and Muir, 1984).

Our previous studies investigated the concentrations of trace metals in skin and blood from free-ranging dolphins during 2003–2005 from South Carolina (SC) and the Indian River Lagoon, Florida (FL) (Stavros et al., 2007, 2008). In order to compare liver concentrations in dolphins from these same areas, we measured trace elements in liver and skin samples from stranded dolphins inhabiting similar locations during 2000–2008. The objectives of this study were to: (1) examine the relationship of trace element concentrations between skin and liver in stranded bottlenose dolphins and compare to levels in skin of free-ranging dolphins with the aim of predicting liver concentrations; (2) assess age-related associations of trace elements in skin and liver tissues from stranded dolphins; and (3) compare mercury levels in dolphins with mercury toxicological reference levels.

2. Materials and methods

2.1. Sample collection

Samples of liver and skin tissues were collected from stranded bottlenose dolphins between 2000 and 2008 from two study sites, South Carolina coastal area ($n = 12$) and the Indian River Lagoon, FL ($n = 15$) by the South Carolina Marine Mammal Stranding Network and Hubbs-Sea World Research Institute, FL, respectively (Table 1). Tissue samples were collected during necropsies from fresh dead (code 2) or early moderate decomposition (code 3) carcasses. Approximately 250 g of liver was collected from the mid-lateral section of one lobe and ~3 g of skin tissue was collected for trace metal analysis from the mid-lateral side in the lumbar region. Samples were stored at −80 °C until further analysis. Total length was measured as straight-line length from the tip of the upper jaw to the fluke notch. Sex was determined by external and internal examination. Teeth were decalcified, sectioned using a freezing microtome, and stained with hematoxylin using standard methods (Myrick et al., 1983). Age was determined by counting post-natal dentine layers in an extracted tooth (Hohn et al., 1989).

2.2. Sample preparation

Liver and skin tissues were cleaned with deionized water (18 MΩ Milli-Pore Billerica, MA) before being processed as detailed in Stavros et al. (2007). Samples of skin and liver tissue (approximately 1–2 g) were dried for 12 h at 80 °C. Sub-samples of ca 0.1–0.05 g of both tissues were prepared for total mercury (THg) analysis. Dry tissue samples were digested in a Teflon digestive vessel with ultrapure nitric acid (J.T. Baker, Philipsburg, NJ) in a microwave (CEM MDS-2100, Matthews, NC). After the first digestion, ultrapure 30% hydrogen peroxide (J.T. Baker) was added for an additional 10 min microwave digestion to complete oxidation of organic matter. The final solution was diluted with deionized water for trace element analyses.

2.3. Trace element determination

Concentrations of 17 trace elements (Al, As, Ba, Be, Cd, Co, Cu, Fe, Li, Mn, Ni, Pb, Sb, Se, Sn, V, Zn) were measured as described in Stavros et al. (2007) using Perkin Elmer ICP-MS 6100 (Wellesley, MA). Internal standards (45Sc, 72Ge, 103Rh, 175Lu) were added to each sample and calibration standard solutions. Total mercury (THg) concentration was determined by Milestone direct mercury analyzer (DMA-80, Shelton, CT). Methods were previously described for trace metal analysis in skin and blood (Stavros et al., 2007, 2008). Several reference materials including 1566b (oyster tissue, NIST, USA), TORT-2 (lobster hepatopancreas), DOLT-3 (dogfish liver) and DORM2 (dogfish muscle, NRC Canada) were used for