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Assessment of mercury and selenium tissular concentrations and total mercury body burden in 6 Steller sea lion pups from the Aleutian Islands

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ABSTRACT

Concentrations of total mercury ([THg]) and selenium ([TSe]) were measured in several tissue compartments in Steller sea lion (*Eumetopias jubatus*) pups; in addition we determined specific compartment and body burdens of THg. Compartmental and body burdens were calculated by multiplying specific compartment fresh weight by the [THg] (summing compartment burdens equals body burden). In all 6 pup tissue sets (1) highest [THg] was in hair, (2) lowest [THg] was in bone, and (3) pelt, muscle and liver burdens contributed the top three highest percentages of THg body burden. In 5 of 6 pups the Se:Hg molar ratios among compartments ranged from 0.9 to 43.0. The pup with the highest hair [THg] had Se:Hg molar ratios in 9 of 14 compartments that were $\leqslant$0.7 potentially indicating an inadequate [TSe] relative to [THg].

1. Introduction

Mercury (Hg) is a naturally occurring element with known toxic effects in humans and terrestrial mammals. Recently, there has been increasing concern over total Hg concentrations ([THg]) found in some Steller sea lions (*Eumetopias jubatus*) in the western Aleutian Islands (Fig. 1) (Rea et al., 2013). Steller sea lions are piscivorous marine mammals that biomagnify relatively high Hg concentrations through the diet and easily transfer Hg through the placenta to the developing fetus (Castellini et al., 2012) similar to other piscivorous mammals (Basu and Head, 2010; Basu, 2012). Steller sea lions from the Aleutian Islands are part of the western distinct population segment (DPS) which are genetically different from the Steller sea lions found in Southeast Alaska. The Steller sea lion populations in the western DPS, particularly those in the western Aleutian Islands, have been slow to recover from dramatic population declines that occurred during the 1970s and 1980s (Atkinson et al., 2008; DeMaster, 2011; Merrick et al., 1995; Lander et al., 2012). Several causes have been hypothesized, such as nutritional stress, fisheries competition, and chemical pollution among others, but no studies have produced conclusive evidence (Atkinson et al., 2008; Holmes et al., 2008; Loughlin and York, 2000; Trites and Donnelly, 2003). Chemical pollution, including exposure to contaminants such as polychlorinated biphenyls (PCB) and Hg, has been under study for several years as a potential cause for the lack of recovery of Steller sea lions in the western DPS (Rea et al., 2013; Castellini et al., 2012; Holmes et al., 2008; Beckmen et al., 2002). Determining Hg distribution in Steller sea lion tissues (and in piscivorous marine mammal tissues in general) is an important step in understanding Hg toxicity in this species, especially in the fetus and neonate as the main cohorts of concern (e.g., exposure of the dam during gestation).

Toxicity of Hg is dependent on the bioavailability and chemical form of Hg which dictates distribution among tissues. Monomethyl mercury ($MeHg^+$) has been known to have adverse effects on reproductive, immunological, and neurological functions in humans and rats (Eto, 2000; Bennett et al., 2001; Clarkson and Magos, 2006; Zalups, 2011). $MeHg^+$ can cross the blood–brain barrier as well as other organs such as the placenta and gastrointestinal tract, and >90% of ingested $MeHg^+$ can be absorbed into blood (Basu and Head, 2010; Basu, 2012; Scheuhammer et al., 2007). Thus, $MeHg^+$ distribution is systemic, reaching all vital organs including the brain, and accumulates in several tissues including erythrocytes, muscle, and hair (Zalups, 2011). On the other hand, inorganic mercury (Hg^{2+}) is generally found in greater concentrations in two target organs, the liver and kidney. Several studies have found that both liver and kidney have demethylating mechanisms that convert $MeHg^+$ to Hg^{2+} (Zalups, 2011; Wintle et al., 2011; Dietz

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et al., 2000). Demethylating mechanisms develop with increasing age (Wintle et al., 2011; Dehn et al., 2005) leaving fetal and very young mammals more vulnerable to $MeHg^+$ toxic effects when compared to adult mammals.

Hair has been commonly used as an indicator tissue for $MeHg^+$ exposure for piscivores. Hair total Hg concentration ([THg]) is highly correlated with [THg] in blood and is thought to be a good indicator of [THg] in circulation (Rea et al., 2013; Castellini et al., 2012; Lieske et al., 2011). Keratin is the main structural protein found in hair, nails and epidermis and contains multiple disulfide cross linkages as well as cysteine residues. Cysteine is a sulfur-containing amino acid and is likely the binding site for $MeHg^+$ in hair. It is estimated that approximately 80% of total mercury (THg) in hair is in the form of $MeHg^+$ (Cernichiari et al., 1995). In recent studies [THg] in hair of Steller sea lion pups have been found to be higher in the Aleutian Islands when compared to Southeast Alaska (Rea et al., 2013; Castellini et al., 2012) and in some cases have exceeded numerous human and wildlife thresholds for Hg adverse effects (Dietz et al., 2013; Poulin and Gibb, 2008). Steller sea lion pups are born with a natal pelage (lanugo) that is molted when they are 4–6 months old. Therefore, Hg in the hair of pups under 4 months of age represents Hg exposure via placental transfer from the mother during gestation. Since it is more difficult to sample tissues such as muscle, liver, kidney and brain in live pups, this study will determine how representative hair [THg] is of other tissue compartments from pups found dead on rookeries.

Some investigators have hypothesized that Hg toxicosis in humans and marine mammals can be diminished through the association of the essential trace element selenium (Se) and Hg particularly when the Se:Hg molar ratio is greater than 1 in the kidney, liver and possibly other tissues (Koeman et al., 1973, 1975; Wang et al., 2001; Khan and Wang, 2009). The antioxidant role of Se in the mammalian diet is under homeostatic control (Battin and Brumaghim, 2009; Papp et al., 2007) but we also likely need to consider direct interactions with toxic elements such as Hg (covalent linkages, Hg–Se). Selenium can increase Hg half-life in the blood and liver, make it less reactive, and have a significant effect in organ distribution and excretion of Hg (Khan and Wang, 2009; Gailer, 2007). Selenium tends to be higher in marine mammals when compared to terrestrial mammals likely due to a greater intake of Se in the marine fish diet. High Se intake is also an advantage for marine mammals in that it can play a physiological role as an antioxidant for diving mammals (Zenteno-Savín et al., 2002, 2012) as well as a role in ameliorating adverse effects of Hg (Khan and Wang, 2009).

Evaluating THg body burden along with individual tissue compartment burdens and concentrations in pinnipeds such as Steller sea lion pups will provide understanding about Hg distribution and storage in various biological tissues. In particular, this will put [THg] measured in traditionally sampled tissues (e.g., hair, liver and skeletal muscle) into better perspective (e.g., % of total body burden). We compare [THg] from all tissues collected from pup carcasses, taking into account mass of tissue, to determine rank order of tissues from the highest to lowest [THg] and THg burden. We evaluate the [TSe] in order to determine Se:Hg molar ratio among tissues of the body to provide insight on the possible protective role of Se within the whole body as well as specific tissue compartments.

2. Materials and methods

2.1. Sample collection

Steller sea lion pups ($n = 6$) found dead on their natal rookeries in the Aleutian Islands, Alaska (Fig. 1) in 2011, 2012 and 2013 were collected by the Alaska Department of Fish and Game (ADF&G) (MMPA/ESA Permit No. 14325). Pup age was estimated by using collection dates (June23–July2) and assumed birthing dates (May15–July15) as determined by Pitcher et al. (2001). Necropsies of thawed animals were performed under an educational outreach setting with university student volunteers assisting in total body measurements (weight, length, girth, etc.), external examination, sex determination, tissue collection, and dissection under professional guidance and instruction. All tissues were processed and analyzed whole except pelt, muscle and bone. All subsamples were collected using stainless steel disposable scalpels and cutting knives. A subsample of hair was removed from the pelt using battery operated grooming clippers (Wahl® Super Pocket Pro® Clippers). Hair samples were washed in 1% Triton X-100 following a previously published hair washing protocol for metals analysis (Rea et al., 2013; Castellini et al., 2012; Lieske et al., 2011). A 5 g subsample of the pelt (hair, epidermis, and dermis) was collected to represent whole pelt [THg]. Muscle subsamples for THg analysis were collected in equal proportion by mass from the scapular region and the pelvic region of each pup. All remaining skeletal muscle was removed from bone and weighed. Total skeletal muscle mass was a combination of the scapular region subsample, pelvic region subsample, and skeletal muscle removed from bones.

One femur and a cartilaginous rib (4th rib) were used to represent bone (Brookens et al., 2008). Cartilage was separated from bone and its weight was added to the final bone mass. Bone was placed in distilled water for 3 weeks to soften the remaining fascial tissue for removal. Bone was then air dried under a fume hood and dry weight was recorded. Freeze drying of samples was performed using a Freezone 4.5 Freeze Dry System (Labconco, Kansas City, MO). Percent moisture of most tissues was calculated: [(wet weight − dry weight)/wet weight] × 100. Homogenization of complete tissue compartments and subsampled tissues was performed using a Retsch Cryomill (Retsch Inc, Newton, PA). All samples were stored in polyethylene Whirlpaks® and 1 gallon Ziploc® bags at −20°F and −80°F prior to freeze drying. Dry homogenized samples were stored short-term at room temperature and returned to ADF&G for archiving after chemical analysis.

2.2. Mercury analysis

Approximately 0.010–0.020 g of homogenized powdered tissues were analyzed for [THg] using the Direct Mercury Analyzer (Milestone, Inc, Shelton, CT; EPA Method 7473) (Rea et al., 2013; Castellini et al., 2012; Lieske et al., 2011; Knott et al., 2011). Approximately 6.0 mg of hair was analyzed separately from the total pelt. All samples were analyzed in triplicate and were considered acceptable with a 15% error from the mean. Each run included one blank, a liquid standard (0.001 μg/g $HgCl_2$ or 1 μg/g $HgCl_2$ standard; Perkin Elmer, Waltham, Massachusetts) and two certified reference materials (DORM 3 = 0.382 μg/g and DOLT 4 = 2.58 μg/g; National Research Council Canada, Institute for National Measurement Standards, Ottawa, Canada). The detection limits were 0.075 μg/g (0.374 μM) for 0.010 g of tissue and 0.038 μg/g (0.189 μM), for 0.020 g of tissue. Recovery range of standard and certified reference materials were 87–101% (0.010 μg/g $HgCl_2$ standard), 94–104% (1 μg/g $HgCl_2$ standard), 91–118% (DORM 3) and 102–114% (DOLT 4).

2.3. Selenium analysis

Approximately 0.030–0.050 g of each homogenized powdered tissue was digested by microwave using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) and analyzed for total selenium concentration ([TSe]) following previous methods (Moses et al., 2009; Knott et al., 2011; Correa et al., 2013). For each set of digestions,