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Accumulation features of trace elements in mass-stranded harbor seals (*Phoca vitulina*) in the North Sea coast in 2002: The body distribution and association with growth and nutrition status

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ABSTRACT

Body distribution and growth- and nutritional status-dependent accumulation of 21 trace elements were investigated in harbor seals (*Phoca vitulina*) stranded in the North Sea coast in 2002. Higher concentrations and burdens of Mn, Se, Mo, Ag, Sn, Hg, and Bi in the liver, Cd in the kidney, As in the blubber, and Co, Sr, and Ba in the bone were observed. Significant positive correlations of hepatic Se, Mo, Ag, Cd, Sn, Hg, Tl, and Bi with standard body length were found, while significant negative relationships were detected for Mn, As, Rb, Sr, and Sb in the liver. Concentrations of Co, Se, Sr, Sn, Hg, and Bi in the liver, V, Sr, Ag, Sn, and Hg in the kidney, V, Mn, Co, Rb, Sr, Sn, Ba, and Pb in the blubber increased with decreasing blubber thickness of harbor seals, indicating enrichment of these elements in the target tissue by emaciation.

1. Introduction

Increase in various diseases, mass mortalities, and abnormal stranding of aquatic mammals have been reported in various locations of the globe (Simmonds and Mayer, 1997; Harvell et al., 1999; Gulland and Hall, 2007). In 1988, about 18,000 harbor seals (*Phoca vitulina*) died in the North Sea (Dietz et al., 1989). Fourteen years later, in 2002, the mass mortality of about 22000 harbor seals occurred again (Rijks et al., 2005, 2008). Some investigations have provided rigid evidence that these events were directly caused by infection with phocine distemper virus (PDV) (Dietz et al., 1989; Mahy et al., 1988; Osterhaus et al., 1988; Jensen et al., 2002; Rijks et al., 2005, 2008). On the other hand, immunodeficiency by chemical pollutants in the seals has also been of great concern as an indirect factor of the outbreak.

It has been recognized that the North Sea is a metal-contaminated area due to historical mining and increased urban and industrial activities in the coastal regions, and waste disposal at the offshore (Ducrotoy et al., 2000). Anthropogenic releases of trace elements through river, atmosphere, and direct discharges have been under control in the past several decades, leading to a significant reduction of contamination in the North Sea environment (Scholten et al., 1998; OSPAR, 2000). However, higher concentrations over the reference criteria for trace elements have been observed in several environmental compartments (OSPAR, 2000).

Aquatic mammals that have a long life-span and occupy a higher trophic position in the aquatic ecosystem accumulate toxic contaminants including some trace elements. Previous studies have suggested that mass mortality and stranding of aquatic mammals are related to the accumulation of trace elements such as Hg and Zn in their tissues (Siebert et al., 1999; Bennett et al., 2001; Anan et al., 2002). Kakuschke et al. (2005) and Das et al. (2008) have reported immunological effects of trace elements in the blood of harbor seals from the North Sea. For harbor seals that were infected with PDV in 1988, Frank et al. (1992) found higher accumulations of Cr in the liver and Cu in the kidney and lower concentrations of Cu and Pb in the liver and Zn in the kidney, compared with non-infected specimens. However, no data is available on trace elements in PDV-infected harbor seals stranded along the coastal area of the North Sea in 2002.

Furthermore, it is predicted that rare trace elements such as Sb, Tl, and Bi are released into the environment due to their recently increased production, usage, and disposal. This may cause accumulation of these elements in aquatic mammals through the food

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web. However, only a few studies on accumulation status of trace elements in harbor seals from the North Sea and its around waters have been reported and also only selected elements such as Cd and Hg have been so far analyzed (Drescher et al., 1977; Reijnders, 1980; Frank et al., 1992; Skaare et al., 1994). Hence, contamination status and behavior of rare trace elements is not clear in the aquatic ecosystem.

The present study measured 21 trace elements (V, Cr, Mn, Co, Cu, Zn, Se, As, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Hg, Tl, Pb, and Bi) in harbor seals stranded in the North Sea coast in 2002. Here, we clarify the body distribution of trace elements and discuss the association with growth and nutritional status of the stranded seals.

2. Materials and methods

2.1. Samples

Fifty-eight dead harbor seals including juveniles ($n = 20$), subadults ($n = 18$), and adults ($n = 20$) were collected in the northwestern coastal zone in the Netherlands, the North Sea during May, 2002–February, 2003, and the liver ($n = 58$), kidney ($n = 20$), blubber ($n = 20$), and hair ($n = 20$) were sampled. The liver, stomach, heart, large and small intestines, lung, spleen, pancreas, kidney, urinary bladder, gonad (testis or ovary), brain, muscle, adrenal gland, thyroid gland, blubber, bone, and hair were sampled from two specimens of harbor seals (standard body length (SBL) = 139 cm and body weight (BW) = 43.2 kg for male; SBL = 128 cm and BW = 42.9 kg for female) to investigate the body distribution of trace elements. Growth stage (juvenile, subadult, and adult) was determined by measuring the SBL and weight of testis in male and size of uterine horn in female. By applying a polymerase chain reaction (PCR) method, it was confirmed that all 58 specimens employed in this study were infected by PDV. Information on SBL and blubber thickness is shown in Table S1 in the Supplementary material. All samples were kept at −25 °C in the Environmental Specimen Bank (*es*-BANK) at the Center for Marine Environmental Studies (CMES), Ehime University, Japan (Tanabe, 2006) until chemical analyses.

2.2. Chemical analyses

Trace element analyses were carried out following our previous methods (Asante et al., 2007; Ikemoto et al., 2004; Kubota et al., 2001). In brief, tissue and organ samples except hair were dried at 80 °C for 12 h and homogenized. Hair was ultrasonically cleaned with 3% polyoxyethylene lauryl ether for 40 min, washed with acetone and Milli-Q water to remove exogenous contaminants, and then dried at 80 °C for 12 h. For analysis of As, samples excluding blubber were digested with acid mixture (HNO_3, $HClO_4$, and H_2SO_4) and the concentration was measured by a hydride generation (HG) – atomic absorption spectrometry (AAS) with a Shimadzu HVG-1 hydride generation system coupled with a Shimadzu AA680 AAS (Shimadzu, Kyoto, Japan). For analyses of other trace elements, about 0.2 g of sample was digested in a microwave system (Ethos D, Milestone S.r.l., Sorisole, BG, Italy) with HNO_3 in Teflon vial. Digestion program was set as 2 min at 250 W, 3 min at 0 W, 5 min at 250 W, 5 min at 400 W, 5 min at 500 W, 10 min at 400 W, and 5 min for ventilation. An aliquot of the solution of blubber digested by microwave was treated with the acid mixture and its As level was quantified by HG-AAS. Concentrations of 18 trace elements (V, Cr, Mn, Co, Cu, Zn, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Tl, Pb, and Bi) were measured by an inductively coupled plasma-mass spectrometer (ICP-MS; Hewlett–Packard, HP-4500, Avondale, PA, USA). Matrix effects and instrumental drift for the ICP-MS analysis were corrected by the internal standard method using Yttrium. Mercury and Se were quantified by a cold vapor-AAS (CV-AAS; Model HG-3000, Sanso, Tsukuba, Japan) and HG-AAS (Model HFS-3, Hitachi, Tokyo, Japan), respectively.

Quality assurance of these analyses was done using a standard reference material, DORM2 (National Research Council, Canada). The recoveries of all the elements were in the range of 77–136% ($n = 3$). The analytical precision for the materials ($n = 3$) were within 10%. In the present study, trace element concentrations in tissues are expressed on a dry weight (DW) basis, if not specified.

2.3. Statistical analyses

Statistical analyses were executed by SPSS (version 12.0, SPSS Inc., Chicago, IL, USA), StatView (version 5.0, SAS® Institute, Cary, NC, USA), and EXCEL Toukei (Version 6.05, Esumi Co., Ltd., Tokyo, Japan). For trace elements whose concentrations were below the limit of determination, one-half of the value was substituted for further statistical analysis. Since almost all trace element concentrations showed non-normal distribution, the concentrations were logarithmically transformed for parametric analysis. Outliers (Cr (4.3 μg/g), Sr (1.13 μg/g), Ag (6.8 and 4.9 μg/g), Cs (0.01 μg/g) low Pb (0.866 μg/g) in liver, V (0.33 μg/g), Cu (29.6 μg/g), Se (17 μg/g), and Sr (1.29 μg/g) in kidney, Cu (0.728 μg/g), Zn (8.41 μg/g), As (0.67, 0.40, and 0.28 μg/g), and Cs (0.02 and 0.01 μg/g) in blubber, and Cd (0.008 μg/g) in hair) were removed by the Smirnov–Grubbs test. By using the Student's *t*-test and ANCOVA with SBL and blubber thickness as covariates, sex differences were assessed. The growth stage- (juvenile, subadult, and adult) and tissue- (liver, kidney, blubber, and hair) specific differences in trace element concentrations were tested by the Tukey–Kramer method, along with one-factor ANOVA. Pearson's correlation coefficient and linear, quadratic, and stepwise multiple regression analyses were used to evaluate relationships between trace element concentrations of tissues and SBL or blubber thickness. A p value of less than 0.05 is considered as statistically significant.

3. Results and discussion

3.1. Body distribution of trace elements

Concentrations of trace elements were measured in 18 tissues and organs of two harbor seals (Table 1). Manganese, Cu, Se, Mo, Ag, Sn, and Hg were highly accumulated in the liver. The present results agreed with previous studies on body distribution of trace elements in other seals (Watanabe et al., 1996; Watanabe, 1998).

Bismuth level was also the highest in the liver, followed by brain (Table 1). Only very few data are available on the body distribution of Bi in animals. Agusa et al. (2005a, 2007) reported that Bi level was less than detection limit in most of the muscle and liver in the marine fish from southeastern Asia. In BALBc/a female mice orally treated with Bi compounds, high accumulation of Bi was observed in the stomach, duodenum, ileum, and kidney during less than 10 h after exposure, and in the lymph nodes, liver, spleen, kidney, and thymus after 1 and 9 weeks following the exposure (Larsen et al., 2003). To our knowledge, this is the first report indicating high accumulation of Bi in the liver and brain of aquatic mammals.

Although information on body distribution of Ag is limited in aquatic mammals, the highest concentration was observed in the liver of northern fur seals in the northwestern Pacific (Saeki et al., 2001). Interestingly, the study on the northern fur seals (Saeki et al., 2001) also found that Ag concentrated in the cerebrum (arithmetic mean (AM), 0.073 μg/g) following the liver (AM, 0.44 μg/g). Oceanic sea birds exhibited higher accumulation of Ag in the fat (AM, 0.112 μg/g), liver (AM, 0.102 μg/g), and brain (AM,