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Concentrations of Selected Essential and Non-essential Elements in Blood of Harbor Seal (*Phoca vitulina*) Pups of the German North Sea

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Abstract This study on harbor seal (*Phoca vitulina*) pups of the North Sea evaluated concentrations of 14 essential and non-essential elements (Al, As, Be, Ca, Cd, Cr, Fe, Mn, Mo, Ni, Pb, Se, Sn, and Zn) in whole blood samples. The essential elements are analyzed to give references for health status determinations of pups. The measurement of classic toxic metals, like Pb or Cd, and other elements that may be in toxic concentrations in blood, is important due to their influence on health, particularly on the immune system. Blood samples of six seal pups found on the German Wadden Sea coast of Schleswig-Holstein in 2004 and transported to the Seal Centre Friedrichskoog, Germany were collected. The blood sampling was performed three times, immediately after collection of the newborns, after 1.5 months, and after 2 months before their release back into the wild. Inductively coupled plasma mass spectrometry and total reflection X-ray fluorescence spectrometry were used to determine the element concentrations. We found higher concentrations of Al, As, Fe, Mo, and Zn in blood samples of newborns compared to samples collected later, probably due to transplacental and lactational transfer from mother to fetus. Furthermore, there is a high need for, in particular, Fe and Zn in the developing organism, which may cause reduced values after some month. In contrast, the concentrations of Be, Cd, Ca, Cr, Pb, Mn, Ni, Se, and Sn, which were low in newborns and increased during the study, may be due to the fish fed to infant pups. Compared to free-ranging adults, in pups, the concentrations of Al, Ni, and Pb were higher in contrast to lower concentrations of As, Mn, and Mo. This case study is the first report on element levels in harbor seal pups of the North Sea.

Keywords Harbor seal · Pups · Elements · Metals · Blood · Pollution · North Sea

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Introduction

The harbor seal, *Phoca vitulina*, is one important domestic marine mammal in the Wadden Sea and is considered as an indicator for changes in the ecosystem. Therefore, the "Seal Agreement" has been adopted, which include the monitoring of pollution and investigations on the effects of pollutants, as well as the assessment of the health status. Nevertheless, there is a lack of studies on seal pups. A useful way is the investigation of the newborn and infant pups living in the Seal Centre Friedrichskoog, Germany to get baseline information to health parameters of pups [1, 2].

As a monitoring tool, the multi-element measurement of elements in blood of animals provides both information to essential elements and to the levels of toxic elements. However, only few studies have performed a multi-element analysis in blood of marine mammals [3–6], in particular of the pinnipeds of the North Sea [7–10].

Since elements like Fe, Zn, or Ca have important functions in the body, i.e., Fe as component of cytochromes and oxygen-binding molecules, Zn as components of more than 70 metalloenzymes, or Ca as important element for building of bones and teeth, for blood clotting, muscle contraction, or maintenance of cell membranes, these essential elements were determined in blood serum during medical investigations, such as on marine mammals [11–17]. Some studies focused on Se due to the strong relationship to mercury and the importance of the element in the detoxification process. Most of them measured Se in tissues such as liver or kidneys [18, 19] but only few in blood [20]. Toxic elements like Cd were investigated in blood in several studies on whales and pinnipeds [20–24]. Nevertheless, all metals are toxic if they are present in living organisms in sufficiently high quantity, and there is a lack of multi-element investigations of blood in marine mammals.

Metal exposure for seal pups may result from the transplacental and lactational transfer from mother to fetus and later through contaminated prey [25–27]. Metal contamination is believed to adversely affect marine mammal health [28–30]. A relationship between stillbirths and nickel concentration in the hair of ringed seal (*Pus hispida saimensis*) pups from Finland was found [31]. Some marine mammal studies surmise that greater metal concentrations result in lesser resistance to diseases [32–34]. These relationships are substantiated by metal contamination affecting the immunocompetence and disrupt the immune homeostasis. Immunomodulation by metals in marine mammals are reported in several studies [9, 10, 35–39]. An *in vitro* study on lymphocytes of seal pups suggests that there immune cells are particularly susceptible to the toxic effects of metals during the newborn period and that this susceptibility decreases as the animals age [1]. Metals found in blood of the seal pups may have a potential influence on the immune status of the animals.

This pilot study is the first investigation that measured element levels in harbor seal pups from the German North Sea coast.

Materials and Methods

Animals

We analyzed blood samples of six harbor seal pups found on the German Wadden Sea coast of Schleswig-Holstein in 2004 (Table 1). The pups were transported to the Seal Centre Friedrichskoog, Germany and were examined by a veterinarian. This investigation included

Table 1 Harbour Seal (*Phoca vitulina*) Pups Analyzed in this Study

Seal	Arrival in the Seal Centre	Weight (kg)	Length (cm)	Sex	WBC ($*10^3$ mm^{-3})	Blood sampling	Age (days)
01	20.06.04	8.9	58	f	10	21.06.04	8
						03.08.04	51
						26.08.04	74
02	20.06.04	8.6	57	f	9	21.06.04	8
						03.08.04	51
						26.08.04	74
03	20.06.04	10.5	59	f	9	21.06.04	8
						03.08.04	51
						26.08.04	74
04	21.06.04	8.9	57	f	7	21.06.04	7
						03.08.04	50
						26.08.04	73
05	21.06.04	8.7	54	m	10	21.06.04	7
						03.08.04	50
						26.08.04	73
06	21.06.04	10.9	65	m	8	21.06.04	10
						03.08.04	53
						26.08.04	76

f Female, *m* male, *WBC* white blood cells

a full external examination, auscultation of heart and lung, and measurements of body temperature, mass, and length. Information on the intake of breast milk was not available. Blood samples were obtained from the epidural vertebral vein. The numbers of white blood cells were investigated (Dr. Driver, Kleintierpraxis, Reinsbuettel, Germany) and were in the normal range for harbor seals with 6–12 × 10^3 mm^{-3} [40]. The first blood sampling was performed in June immediately after their arrival at the Seal Centre (age of pups, 7–10 days). The second sampling took place in the beginning of August when these animals were between 50 and 53 days old. The seals were sampled a third time just before their release back into the sea after gaining a weight of 25 kg (age of pups, 73–76 days).

Multi-element Analysis

Concentrations of 14 elements (Al, As, Be, Ca, Cd, Cr, Fe, Mn, Mo, Ni, Pb, Se, Sn, and Zn) were determined in whole blood samples. Whole blood samples were collected in special lithium heparin monovettes for metal analysis (Sarstedt, Nümbrecht, Germany) and stored at −20 °C. For multi-element determination, a microwave digestion system (MarsXpress, CEM GmbH, Kamp-Lintfort, Germany) was used. Five hundred microliters of whole blood was pipetted into perfluoralkoxy vessels. Two milliliters sub-boiled nitric acid, 1 ml of hydrogen peroxide, and 50 µl internal standard (1 mg l^{-1} of yttrium, Merck, Darmstadt, Germany) were added and the vessels heated in a three-step program up to 180 °C.

Concentrations of 14 trace elements were determined with two different methods. Al, Be, Cd, Cr, Mn, Mo, Ni, Pb, and Sn were analyzed using an inductively coupled plasma–mass spectrometer (ICP-MS) with a collision cell (Agilent 7500c ICP-MS, Agilent Technologies, Tokyo, Japan). Matrix effects and instrumental drift of the ICP-MS were corrected by using yttrium as an internal standard. For calculation, external calibration was carried out with diluted standard solutions (Merck). The standard mode without collision gas was used for Al, Be, Pb, and Sn. For the other elements, better results were obtained with helium as the collision gas (flow rate, 3.0 ml min^{-1}).

Measurements of As, Ca, Fe, Se, and Zn were performed by total X-ray fluorescence spectrometry (TXRF; Atomika TXRF 8030 C, FEI Company, Oberschleissheim, Germany). Digested samples (20 µl) were pipetted onto the sample carrier and evaporated to dryness. The Mo Kα excitation was selected for the detection of the elements. Yttrium as internal standard was used to calculate the concentrations.

For internal quality control, the reliability of the analytical procedures was checked with the human reference material Clin Check® Whole Blood Control Level II lot no. 932 (Recipe, Chemicals + Instruments, Munich, Germany) and animal reference material IAEA-A-13 (International Atomic Energy Agency, Vienna, Austria; Table 2).

Statistical Analysis

For the results of the six pups investigated three times, we used the Friedman tests in comparing the element concentrations in the whole blood (Table 3). All indicated *P* values are two-tailed. Statistical significance was designated as $P \leq 0.05$.

Results

Two trends were found for elements in whole blood of harbor seal pups during the time of investigation. Lower concentrations of Be, Ca, Cd, Cr, Mn, Ni, Se, and Sn were measured directly after collection of the newborns compared to measurements in the following time when the animals were older (Fig. 1). All results are significant (Table 3). Although the results for Pb were not significant, for four of six pups, similar trends were found, and Pb therefore was included in Fig. 1.

Table 2 Concentrations of Elements (Mean Value, n=10) of the Reference Materials Clin Check (Human Whole Blood, Level II) Measured with ICP-MS and IAEA-A-13 (Animal Whole Blood) Measured with TXRF

Element	Certified mean value	Control range	Measured mean value (n=10)	±SD
ICP-MS measurement: ClinCheck, human whole blood, Level II, lyophilized (µg l^{-1})				
Cr	7.1	6.0–8.2	7.26	0.872
Mn	27	22–32	31.3	1.29
Co	4.7	3.7–5.7	5.06	0.251
Ni	8.3	6.4–10.2	8.24	1.06
Cd	4.3	3.5–5.1	3.42	0.210
Pb	304	258–350	272	11.3
TXRF measurement: IAEA-A-13, animal whole blood, freeze dried (mg kg^{-1})				
K	2,500	2,100–2,700	2,615	60
Ca	286	226–332	268	42
Fe	2,400	2,200–2,500	2,363	115
Cu	4.3	3.7–4.8	4.25	0.51
Zn	13	12–14	12.6	0.52
Se	0.24	0.15–0.31	0.25	0.02

Table 3 Statistical Analysis of Time-Dependent Differences Between Element Concentrations (N=14) in Whole Blood Samples Collected Three Times During 2 Months (Friedman Test, k=3, n=6)

Metal	F_r	P Value
Al	9.33	0.006*
As	6.33	0.052
Be	11.47	0.004*
Ca	9.33	0.006*
Cd	7.14	0.020*
Cr	12.00	<0.001*
Fe	8.33	0.012*
Mn	8.33	0.012*
Mo	2.33	0.430
Ni	9.33	0.006*
Pb	1.33	0.570
Se	9.33	0.006*
Sn	9.65	0.003*
Zn	8.33	0.012

*$P \leq 0.05$, significant differences

In contrast, higher concentrations of Al, Fe, and Zn were measured in the samples of the newborns compared to the samples collected when these animals were older (Fig. 2). All results are significant (Table 3). The results for As and Mo were not significant; nevertheless, similar decreasing trends were found, and the results of both elements are inserted in Fig. 2.

Compared to free-ranging adults of the North Sea, higher concentrations of Al, Ni, and Pb and lower concentrations of As, Mn, and Mo were found in the blood of the pups (Figs. 1 and 2).

Discussion

Blood is the medium for the transport of elements, including essential elements such as Ca, Fe, Mn, Se, or Zn, as well as several important contaminants such as As, Be, Cd, or Pb. In this study, the concentrations of 14 elements in blood of harbor seal pups were investigated during their first 2 months of life. Metals could be grouped into two classes: increasing and decreasing concentrations with age during juvenile stages.

The concentrations of the major element Ca and of the trace elements Be, Cd, Cr, Mn, Ni, Pb, Se, and Sn increased during the time of investigation. Young animals have an elevated Ca metabolism due to bone development and alteration processes, which cause elevated levels in blood. Similarly, increases of Ca concentrations in blood of harbor and gray seal pups during the first weeks of life were measured [13, 41]. Greater concentrations of Ca concentrations in serum were determined in harp seal pups compared to adult harp seals [42]. Furthermore, increasing concentrations of elements, e.g., of toxic metals like Be or Cd during the time of investigation, are probably caused by contaminated prey. In general, for marine mammals, the main intake of pollutants is due to contaminated prey [43]. The species fed to the harbor seal pups at the Seal Centre was herring (*Clupea harengus*) from the Baltic Sea (personal communication: Seal Centre Friedichskoog), which was probably the source of the metals [44–46].

Higher concentrations of Al, As, Fe, Mo, and Zn were determined in the blood samples of the newborns compared to samples taken 2 months later. Such elevated concentrations of elements in blood of newborns were probably caused by transplacental and/or lactational

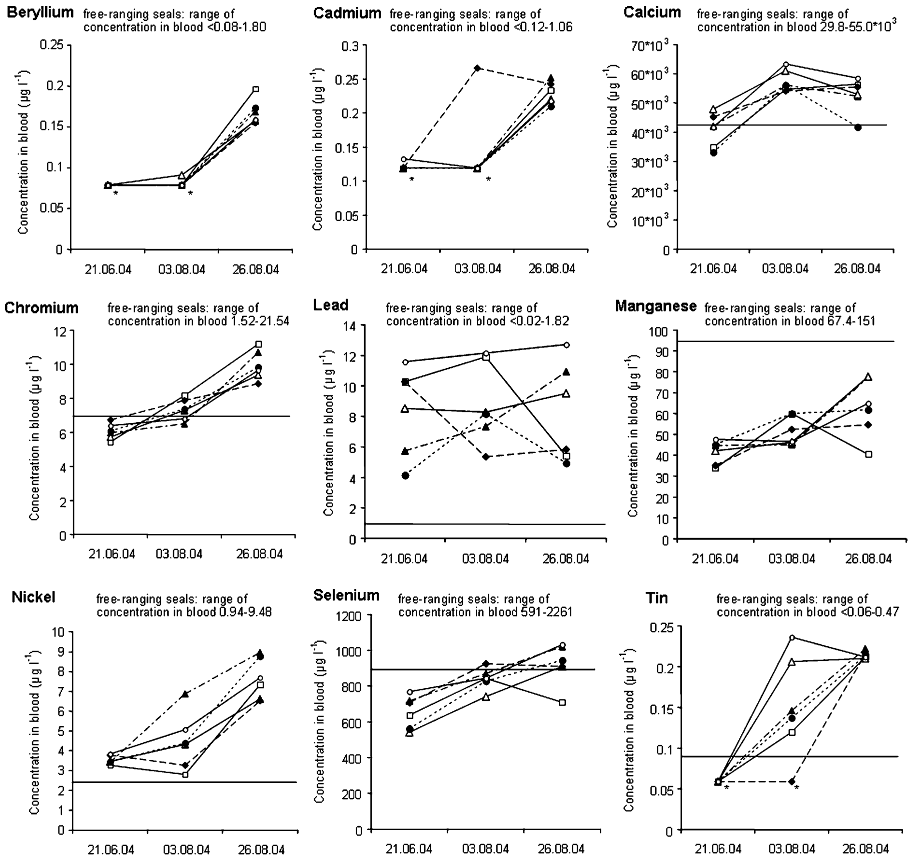

Fig. 1 Concentrations (µg l^{-1}) of Be, Cd, Ca, Cr, Pb, Mn, Ni, Se, and Sn in whole blood samples of six harbor seal pups (* values<LOD) measured three times. Mean values (*lines*; Be and Cd, no mean values published) and ranges of concentrations in blood of free-ranging seals of the North Sea, Lorenzenplate [8]

transfers from mother to pup. A study on a nursing *Tursiops truncatus* female revealed that metal pollutants pass from the tissue of the female into the milk [47]. A transplacental transfer from mother to pup was described for different marine mammals and several pollutants including metals [25–27, 31]. Furthermore, a decrease can be caused by a high consumption of several elements in the first time of life. Concentrations of Fe and Zn in newborn pups were comparable to free-ranging, older seals and decreased in the following 2 months, which may be caused by the utilization of these elements through the high metabolism and blood formation of the newborn organism. Additionally, higher Fe concentrations in newborns may have been caused by elevated hemoglobin concentrations during this period [41]. Human studies showed higher concentrations of Fe in newborns compared to nursing infants and children [48].

The concentrations of the majority of trace elements in blood of harbor seal pups are comparable to the values of free-ranging animals of the North Sea [8]. Nevertheless, metal-specific differences were found, which can be caused by ingestion of different metal-

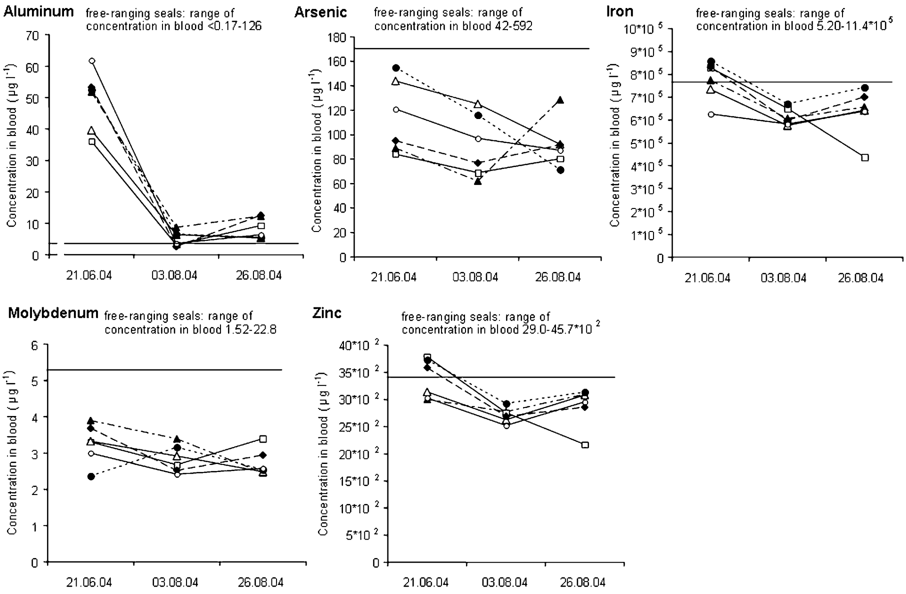

Fig. 2 Concentrations (µg l^{-1}) of Al, As, Fe, Mo, and Zn in whole blood samples of six harbor seal pups (* values<LOD) measured three times. Mean values (*lines*) and ranges of concentrations in blood of free-ranging seals of the North Sea, Lorenzenplate [8]

contaminated prey. While seals from the North Sea are mostly benthic feeders and the fish species vary with offer and feeding location [49, 50], the species fed to the seals in the Seal Centre was only one, the pelagic fish species, herring *C. harengus*.

Our results show that newborn seals in the North Sea have high concentrations in blood of selected metals, which may have a potential immunological impact on the pups. The immunosuppressive influence of metals on parameters of cellular immunity was investigated for several marine mammal species [35–39]. A study on harbor seal pups, in particular, suggests a higher susceptibility to toxic effects of metals on precursor stages of immune cells isolated from blood of newborns and a decreased susceptibility when the pups were older [1]. Metal pollutants may also induce an activation (e.g., inflammatory process) or dysregulations, e.g., hypersensitivity. Metals found in blood of the pups, e.g., Pb or Ni are known sensitizers for harbor seals [9]. Although a direct relationship to metal body burden cannot be shown, investigations of cytokines of harbor seal pups from the German North Sea coast suggest an activated immune system of the newborns directly after collection of the newborns [1, 2].

Summarizing this pilot study indicates that the multi-element measurement is a highly effective method to get baseline information to essential elements and to the levels of toxic elements in the blood of animals investigated and is the first study on harbor seal pups of the German Wadden Sea coast.

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