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Concentrations of selected chemical elements in organs and tissues of polar bears (*Ursus maritimus*) from East Greenland

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Environmental Toxicology and Chemistry

Submission date: July 2013

Supervisor: Bjørn Munro Jenssen, IBI

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Acknowledgement

This Master's thesis was carried out at the Department of Biology, at the Norwegian University of Science and Technology (NTNU) as a final part of the degree in Master of Science in Environmental Toxicology and Chemistry, with specialization in Toxicology. This thesis was performed in cooperation with Syverin Lierhagen (Department of Chemistry, NTNU), Christian Sonne and Rune Dietz (both of the Department of Bioscience, Arctic Research Centre, Aarhus University, Denmark). My supervisors during this project were Professor Bjørn Munro Jenssen and Tomasz Maciej Ciesielski.

First, I would like to thank Tomasz M. Ciesielski for his help and guidance throughout the entire process of this study. The invaluable suggestions and contributions you made during our discussions and my laboratory work played a huge part in the successful completion of this study. I can never thank you enough. I also want to thank Prof. Bjørn M. Jenssen for his support and suggestions during my writing. Special thanks also go to Syverin Lierhagen for his contributions, suggestions and assistance during my lab work. I also want to thank everyone who at some point had to help in securing my samples, gave me impromptu lectures on statistics; without your efforts this project would not have seen daylight.

Finally, I'd like to say thank you to my family (far, far away) and friends here in Trondheim; these last two years have been a blessing, and I wouldn't trade it for anything in the world! Thank you all for the memories.

Trondheim, July 2013

Ikenna Uba

Abstract

Selenium (Se), cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn) and arsenic (As) concentrations were analyzed in tissues of polar bears (*Ursus maritimus*) caught in Scoresby Sound, East Greenland between 2011 and 2012. Higher levels of Se, Cd, Hg and Pb were present in kidney compared to liver, while Zn and As levels were higher in the liver. Mean Cd levels in muscle, liver and kidney were lower in polar bears compared to ringed seals (*Phoca hispida*) from the same area, possibly due to low Cd levels in seal blubber. Analysis of variance (ANOVA) revealed no significant difference in element concentrations in the neck, sternal and hind muscle regions of the polar bear. No correlation was found for all elements with the different groups, probably due to the small polar bear sample size in this study. Significant correlations between elements were observed for Se with Hg, Cd and Zn in the kidney, Se with Hg and Zn in liver, Zn with Hg and Pb in liver, and Zn with Cd in the muscle. No significant correlation was found for any element in plasma and hair with other tissues. This study found Se:Hg molar ratios slightly above unity in polar bear liver and kidney while Zn:Cd molar ratio was over 60:1 in liver and almost 3:1 in the kidney. The Se:Cd molar ratio was less than unity in the kidney.

Estimated polar bear exposures to Hg, Cd and Pb through seal blubber consumption were estimated, and were shown to be below the effect threshold level. The polar bear and seal tissues, especially meat and liver, form a main part of the native Inuit diet in Greenland. The estimated human intake levels of Se, Hg and Cd were high and exceeded the tolerable limits set by WHO/FAO. The main source of Hg and Cd in the Inuit is seal liver, while the whale skin is responsible for high levels of Se. Lead intake was low, and below the approved consumption limit in humans.

Abbreviations

d.w	Dry weight
EG	East Greenland
HR - ICP- MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
IDL	Instrumental detection limit
MDL	Method detection limit
MT	Metallothionein
NTNU	Norwegian University of Science and Technology
POPs	Persistent Organic Pollutants
SPSS	Statistical Package for the Social Sciences
w.w	Wet weight

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1. Introduction

The evidence that pollution is a problem in Arctic areas and species inhabiting the region is accumulating (AMAP, 2002). Many pollutants, including chemical elements and persistent organic pollutants (POPs), are taken up by organisms, either directly from their surroundings or via food, and they possess the tendency to bioaccumulate in tissues and organs of the animals, if not excreted. This has resulted in increased apprehension among the native settlers in these regions who depend, almost entirely, on arctic marine mammals for source of food (Wheatley and Paradis, 1995). For instance, polar bears were found to exhibit some of the highest concentrations of different pollutants among arctic (or even world-wide distributed) species, which was attributed to biomagnification processes (Braune et al., 2005). High exposure to contaminants can have adverse effects on wildlife, including effects on reproduction, development, and resistance to disease (Macdonald and Bowers, 1996).

Over the past decades, bioaccumulation of certain metals have been observed and recorded in the Arctic region (Pacyna and Winchester, 1990). Sources of these contaminants include natural (e.g. biogeochemical) and anthropogenic (e.g. mining) activities. Mercury (Hg) and cadmium (Cd) have been found to accumulate in the polar bears due to their high trophic levels in the ecosystem (Dietz et al., 1996). It has been reported that 94% of Hg in the East Greenland polar bears is of anthropogenic origin (Dietz et al., 1991). There are no indications that lead (Pb) increases in higher trophic levels as reported in Dietz et al, (1996). Copper (Cu) and zinc (Zn) are essential trace elements and their concentration in the marine mammal tissues is regulated homeostatically (Dietz et al., 1995), up to a certain extent. Increased exposure to some of these chemical elements is associated with petroleum exploration and industrial activities which are planned for the study area, thus increasing anthropogenic load of these elements. Vanadium (V) and nickel (Ni) are constituents of crude oil, and chromium

(Cr), copper (Cu), zinc (Zn) and nickel (Ni) are associated with drilling fluids from produced water.

Human exposure to contaminants is higher in Greenland than in Europe and North America (Johansen et al., 2004). This is largely due to the traditional marine food items, e.g. fish, seals, whales and seabirds, which are more important in Greenland, and because most of these food items contain high contaminant levels, like Hg and Cd (Johansen et al., 2004). Dietary habits also differ among regions in Greenland, with local food from hunting and fishing of marine mammals more prevalent in local settlements of the north and east, as compared to the towns in the south-west where the consumption of imported food is high (Johansen et al., 2000).

1.1 Eastern Greenland polar bears

Greenland, the biggest island in the world, stretches from Nunap Isua (Kap Farvel) in the south at 59°46' N to Odaap Qeqertaa (Odak Island) at 83°40' N (UNEP, 2004) (**Fig 1**). The ice-free parts alone have a topography dominated by alpine areas and cover an area of 2 175 600 km². Some of the world's largest fjord complexes are found in East Greenland, e.g. Kejser Franz Josephs Fjord and Scoresby Sund, leading out north of the Denmark Strait (UNEP, 2004).

Polar bears (*Ursus maritimus*) are of interest from the ecotoxicological point of view due to their position as the top trophic level carnivore in the Eastern Greenland environment. They feed mainly on seals, of which the ringed seal (*Phoca hispida*) is the most relevant (Stirling and Achibald, 1977; Smith, 1980). Other seal preys include, to a lesser extent, bearded (*Erignathus barbatus*), harp (*Phoca groenlandica*), hooded (*Cystophora cristata*) and harbor (*Phoca vitulina*) seals. They also feed on whale, walrus, and seal carcasses they find along the coast. (Derocher et al., 2002; Grahl-Nielsen et al., 2003; Thiemann et al., 2008). Due to their longevity, top trophic position and demand of large energy reserves as subcutaneous fat,

polar bears are consistently exposed to high levels of potentially toxic chemicals (Becker, 2000).

Tissue samples from polar bears have, overtime, been used as indicators of levels of exposure to chemical elements. However, certain organs and tissues tend to be selective for accumulation of metals, e.g. kidney for cadmium, liver for inorganic mercury, bone for lead, muscle for methyl-mercury, and bone and fat for vanadium (Becker, 2000). Polar bear hair has also served as indicator for mercury body burden (Renzoni and Norstrom, 1990; Born et al, 1991), while liver and kidney tissues were used to assess chemical elements present in polar bears found dead or hunted at Svalbard (Norheim et al, 1992).

1.2 Aim of study

In this study, I present the results of selected chemical element analyses in liver, kidney, muscles, hair, gonad and adipose (fat) tissues collected from polar bears from Scoresby Sound area (69°00' - 73°30'N, 20°30' - 25°00'W) in Eastern Greenland. Cadmium, mercury, lead and arsenic were selected because of their significance as contaminants in the environment, while selenium and zinc were chosen because of their relationship with mercury and cadmium respectively. Ringed seals (*Phoca hispida*) were sampled in February/March 2012 in Scoresby Sound, and were used in establishing the feeding relationship between polar bears and ringed seals through element analyses.

The objectives of this study were (1) to evaluate the organ/tissue specific distribution of selected elements in tissues of polar bears and ringed seals harvested by the Inuit hunters; (2) to investigate the concentration of these elements in the polar bear hair and blood tissues, and their correlation with other tissues and organs; (3) to estimate the daily exposure levels of East Greenland polar bears to these chemical elements (4) to estimate the human exposure to Se, Cd, Hg and Pb.



Fig.1. Location of sampling site in East Greenland (World factbook, USA, 2007)

2. Material and method

2.1 Samples

Ten polar bears (*Ursus maritimus*) were sampled for liver, kidney, muscles, adipose, hair, gonads and plasma. These tissues were collected from Inuit subsistence hunters in the Scoresby Sound area in Central East Greenland. Nine polar bears were sampled in February and March 2011, while one polar bear was sampled in early 2012. Analyzed samples included 3 adult males, 4 sub-adults (2 males, 2 females) and 3 female juveniles; see **Appendix II**. Collection of samples was carried out by a team of Inuit hunters and scientists. These samples were stored in pre-marked polyethylene plastic bags together with information on the body weight, body length, date, location, sex and estimation of the mammal's age.

Samples were kept in temperatures between -5°C and -30°C until they were stored in the freezer. The temperature was maintained at -20°C during sample shipment to Norway from Scoresby Sound via Copenhagen, Denmark.

Age estimation was performed according to Stirling et. al. (1977), where annual layering in the cementum of the first lower premolar tooth was counted. Due to hunting regulations, no cubs of the year or females with cubs less than 1 year were killed (Anonymous, 1988).

Muscles samples were collected from three different locations; the neck (*Musculus splenius capitis*), sternum (*Latissimus dorsi*) and hind (*Gluteus maximus*) muscle regions, in the polar bears. No particular preference was placed in the location of liver and kidney collected.

Ringed seals (*Phoca hispida*) samples collected in late February and early March 2012 in Scoresby Sound were used for trophic comparisons and exposure levels between chemical elements analyses of polar bears and ringed seals. Samples analyzed included 9 adults (5 males, 4 females), 3 juveniles (2 males, 1 female) and 4 pups (2 males, 2 females)

(**Appendix II**). The ringed seals tissues harvested include muscles, kidney, liver, blubber, hair and plasma.

2.2 Chemical element analysis

After removal from the freezer, samples were collected using titanium scalpels, vinyl gloves and a plastic cutting board. Approximately 1.4 – 1.6 g was weighed for muscle, gonad, kidney and liver samples; 0.5 - 0.7 g for adipose; 0.13 - 0.15 g for hair and 0.4 g for plasma samples were also weighed. The samples were transferred into 18 mL PTFE-Teflon vials. Subsequently, 50 % v/v concentrated nitric acid (Scan Pure, equal to pure grade, Chemscan, Elverum, Norway) was added to each vial as follows; 6 mL for adipose, muscle, gonad, kidney and liver samples, 4 mL for hair and 1 mL for plasma samples. Digestion of the samples was performed in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany), with steadily increased temperature to a maximum 240°C within one hour. After cooling, the samples were transferred to 15 mL screw-cap polyethylene vials, and adjusted to 61 g for blubber, muscle, gonad, kidney and liver; 46.4 g for hair and 14.64 g for plasma, using ultrapure water. All samples were diluted to a final acid concentration of 0.6 M and transferred to polypropylene vials (11 mL) for analyses.

High Resolution-Inductively Coupled-Mass Spectrometry (HR-ICP-MS) analysis was performed using a Thermo Finnigan model Element 2 instrument (Bremen, Germany). The radio frequency power was set to 1400 W. Samples were introduced using a SC-FAST flow injection analysis system (ESI, Elemental Scientific, Inc. Omaha, USA) with a peristaltic pump (1 mL/min). The instrument was equipped with a PFA-ST nebulizer, spray chamber (PFA Barrel 35 mm), demountable torch, quartz standard injector and Al sample skimmer and X skimmer cones. The nebulizer argon gas flow rate was adjusted to give a stable signal with maximum intensity for the nuclides ^7Li , ^{115}In and ^{238}U . Methane gas was used in the

analysis to minimize interference from carbon and to provide enhanced sensitivity, especially for Se and As. The instrument was calibrated using 0.6 HNO₃ solutions of matrix-matched multi-element standards. A calibration curve consisting of 5 different concentrations was made from these standards. To check for instrument drift, one of these multi-element standards were analyzed for every ten samples. The accuracy of the method was verified by analyzing the certified reference materials Bovine Liver NIST 1577b and Chicken GBW-10018 (National Institute of Standards and Technology, Gaithersburg, MD).

Method detection limits (MDL) (**Appendix III**) was determined by choosing the highest value after comparing the instrument detection limit (IDL) and 3 times the standard deviation of the blanks. Calculation of IDL was done by analysis of solutions for decreasing concentrations of each chemical element. Concentrations resulting in a relative standard deviation of approximately 25% (n = 3 scans) were used as IDL, applying baseline corrections for these values. Chemical analyses results that were below the limit of detection (LOD) were replaced with half the value of this detection limit for the measured element.

2.3 Statistics

A one way analysis of variance (ANOVA) was performed to determine the difference between the mean concentrations of chemical elements between age groups in polar bears. Where required, a Tukey honestly significant difference (HSD) test was also performed for significant differences between the means of the age groups. An ANOVA was also applied to determine the difference in chemical element concentration in the different muscle regions sampled in the polar bears. Pearson's correlation was applied to determine the relationship between chemical elements, Se:Hg molar ratio and biometric data (length, weight).

The ANOVA and Pearson's correlation were executed using SPSS (version 20 for Windows, SPSS Inc., Chicago, IL, USA). Significance level was set at $p < 0.05$ for all analyses. For comparison of chemical element concentration in other studies, wet weight (ww) was

converted to dry weight (dw) using a factor of 3.66 (muscle), 3.17 (liver) and 3.67 (kidney) as suggested by Dietz et al (1995) for polar bears, and 3.3 for cetaceans (Yang and Miyazaki, 2003).

3. Results

3.1 Chemical element concentrations

3.1.1 Polar bear

The mean concentrations of Se, Cd, Hg, Pb, Zn and As are presented in **Table 1**. Element concentration range and median levels in all analyzed samples for polar bears are presented in **Appendix IV**.

Selenium

The Se levels in the liver ranged from 2.34 $\mu\text{g/g ww}$ to 7.38 $\mu\text{g/g ww}$ (**Appendix IV**), with a mean concentration 5.50 $\mu\text{g/g ww}$. These were all above the detection limit (0.005 $\mu\text{g/g ww}$). In the kidney, concentrations ranged from 0.374 $\mu\text{g/g ww}$ to 30.4 $\mu\text{g/g ww}$, with mean element level 9.28 $\mu\text{g/g ww}$. Mean selenium levels were lowest in the adipose (0.0605 $\mu\text{g/g ww}$) and plasma (0.185 $\mu\text{g/g ww}$). The muscles contained mean concentrations 0.460 $\mu\text{g/g ww}$, 0.477 $\mu\text{g/g ww}$ and 0.488 $\mu\text{g/g ww}$ in the neck, sternal and hind regions, respectively (**Table 1**).

Cadmium

The mean Cd levels in all samples in the polar bear ranged from 0.0142 $\mu\text{g/g ww}$ in the neck muscle to 20.3 $\mu\text{g/g ww}$ in the kidney (**Table 1**). Mean renal concentrations ranged from 1.22 $\mu\text{g/g ww}$ to 41.2 $\mu\text{g/g ww}$ (**Appendix IV**). The lowest concentrations were present in the three sampled muscle regions, but all were above the detection limit (0.005 $\mu\text{g/g ww}$). Mean Cd levels in other samples were in the decreasing order; liver, hair, gonad, plasma and adipose (**Table 1**).

Mercury

Mean mercury levels are presented in **Table 1**. The highest mean concentration was present in the kidney (23.8 $\mu\text{g/g ww}$) and lowest in the adipose (0.0286 $\mu\text{g/g ww}$). Liver (11.5 $\mu\text{g/g ww}$) and hair (7.11 $\mu\text{g/g ww}$) contained higher Hg concentrations than plasma and adipose, while the muscle concentrations ranged from 0.057 $\mu\text{g/g ww}$ in the neck region to 0.191 $\mu\text{g/g ww}$ in the hind muscle region (**Appendix IV**).

Lead

The highest mean Pb level, 1.31 $\mu\text{g/g ww}$, occurred in the hind muscle region in the polar bear, with a range from 0.0031 $\mu\text{g/g ww}$ to 10.01 $\mu\text{g/g ww}$ (**Appendix IV**). Other high mean concentrations were found in the kidney (0.295 $\mu\text{g/g ww}$), liver (0.176 $\mu\text{g/g ww}$) and hair (0.163 $\mu\text{g/g ww}$) (**Table 1**). Adipose tissue contained the lowest mean Pb levels, 0.00405 $\mu\text{g/g ww}$, which was below the detection limit (0.02 $\mu\text{g/g ww}$). A mean concentration of 0.0985 $\mu\text{g/g ww}$ was present in the gonads (**Table 1**).

Zinc

Zinc was present in higher concentrations in all samples when compared to the other analyzed chemical elements in this study (**Table 1**). The highest mean level was present in hair samples (130 $\mu\text{g/g ww}$) with levels ranging from 104 $\mu\text{g/g ww}$ to 150 $\mu\text{g/g ww}$ (**Appendix IV**). Adipose tissues contained the lowest concentration (2.058 $\mu\text{g/g ww}$), then plasma (2.91 $\mu\text{g/g ww}$) and gonads (12.0 $\mu\text{g/g ww}$) (**Table 1**). The mean muscle concentrations were 46.9 $\mu\text{g/g ww}$, 44.7 $\mu\text{g/g ww}$ and 50.4 $\mu\text{g/g ww}$ in the neck, sternal and hind regions, respectively. The liver had a higher mean level than the kidney (**Table 1**).

Arsenic

The mean arsenic levels in the polar bears ranged from 0.035 µg/g ww (in hair) to 0.162 µg/g ww (in liver) (**Appendix IV**). Similar mean concentrations were present in the 3 sampled muscle regions: neck (0.784 µg/g ww), sternum (0.0654 µg/g ww) and hind (0.0731 µg/g ww) regions (**Table 1**). An increasing order in the mean As levels in the other samples was thus; plasma, gonad, adipose and kidney (**Table 1**).

3.1.2 Ringed seals

The data on mean concentration of selenium (Se), cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn) and arsenic (As) in samples are presented in **Table 2**. Element concentration range and median levels in all analyzed samples for ringed seals are presented in **Appendix V**.

Blubber

Zinc was present at highest mean concentration, 1.62 µg/g ww, in the blubber (**Table 2**). The Zn levels ranged from 0.829 µg/g ww to 3.23 µg/g ww (**Appendix V**). Lead had lowest mean level 0.00324 µg/g ww which was below the detection limit (0.02 µg/g ww). The mean element levels were in the order: Zn > As > Se > Cd > Hg > Pb (**Table 2**).

Muscles

The mean Zn concentration was highest in the ringed seal muscle (21.7 µg/g ww), with a range from 3.39 µg/g ww to 31.9 µg/g ww (**Appendix V**). The lowest mean element concentration was Pb, 0.00811 µg/g ww (**Table 2**). Cadmium and mercury had mean levels 0.0902 µg/g ww and 0.305 µg/g ww respectively. The mean element levels in the muscle decreased in the order Zn, As, Se, Hg, Cd and Pb (**Table 2**).

Liver

The element with the highest hepatic concentration was Zn, with mean level 57.6 $\mu\text{g/g ww}$ (**Table 2**) and a range of 33.2 $\mu\text{g/g ww}$ – 112 $\mu\text{g/g ww}$ (**Appendix V**). The order of the mean levels of other elements was Cd > Hg > Se > As > Pb. Lead occurred at lowest mean concentration 0.0125 $\mu\text{g/g ww}$ (**Table 2**).

Kidney

Zinc (38.2 $\mu\text{g/g ww}$) and lead (0.0108 $\mu\text{g/g ww}$) had the highest and lowest respective mean levels in the ringed seal kidney. The renal element mean levels were in the order Zn > Cd > Se > As > Hg > Pb (**Table 2**). Selenium had mean concentration 2.56 $\mu\text{g/g ww}$ while mercury was present at mean level 0.866 $\mu\text{g/g ww}$.

Plasma

The mean Zn concentration (3.033 $\mu\text{g/g ww}$) was highest in the plasma while Pb level was lowest (0.0027 $\mu\text{g/g ww}$). Cadmium and mercury also had low mean levels, 0.0168 $\mu\text{g/g ww}$ and 0.0217 $\mu\text{g/g ww}$, respectively (**Table 2**).

Hair

Arsenic had the lowest mean concentration in the hair (0.663 $\mu\text{g/g ww}$), while zinc was present at the highest level, 125 $\mu\text{g/g ww}$ (**Table 2**), with range from 82.9 $\mu\text{g/g ww}$ to 157 $\mu\text{g/g ww}$ (**Appendix V**). Other chemical elements were present thus; Hg > Cd > Se > Pb (**Table 2**).

Table 1: Element concentration ($\mu\text{g/g ww}$) found in East Greenland polar bears (*Ursus maritimus*), (N) number of samples

Tissue/metal statistics		Se	Cd	Hg	Pb	Zn	As
Neck muscle (<i>Musculus splenius capitis</i>)	Mean	0.460	0.0142	0.0962	0.00439	46.9	0.0784
	SD	0.198	0.00513	0.0328	0.00232	13.9	0.0347
	N	7	7	7	7	7	7
Sternal muscle (<i>Latissimus dorsi</i>)	Mean	0.477	0.0204	0.113	0.00538	44.7	0.0654
	SD	0.123	0.00536	0.0345	0.00623	14.6	0.0277
	N	8	8	8	8	8	8
Hind muscle (<i>Gluteus maximus</i>)	Mean	0.488	0.0199	0.120	1.31	50.4	0.0731
	SD	0.135	0.00813	0.0454	1.31	5.66	0.0235
	N	8	8	8	8	8	8
Kidney	Mean	9.28	20.3	23.8	0.295	33.4	0.154
	SD	8.42	10.8	23.7	0.297	15.08	0.0965
	N	10	10	10	10	10	10
Liver	Mean	5.50	1.77	11.5	0.176	64.7	0.162
	SD	1.72	0.920	3.92	0.220	12.8	0.107
	N	10	10	10	10	10	10
Adipose	Mean	0.0605	0.0159	0.0286	0.00405	2.058	0.0925
	SD	0.0323	0.0102	0.0186	0.00239	0.901	0.0447
	N	10	10	10	10	10	10
Gonad	Mean	0.268	0.104	0.141	0.00985	12.0	0.0439
	SD	0.0483	0.0512	0.0687	0.0127	5.66	0.0151
	N	9	9	9	9	9	9
Hair	Mean	0.598	2.23	7.11	0.163	130	0.0354
	SD	0.0763	0.0209	1.12	0.101	13.3	0.0285
	N	10	10	10	10	10	10
Plasma	Mean	0.185	0.0160	0.0561	0.0182	2.91	0.0363
	SD	0.0310	0.0891	0.0877	0.0341	3.50	0.0275
	N	10	10	10	10	10	10

Table 2: Element concentration ($\mu\text{g/g ww}$) found in East Greenland ringed seals (*Phoca hispida*). (N) number of samples

Tissue/Element statistics		Se	Cd	Hg	Pb	Zn	As
Muscle	Mean	0.390	0.0902	0.305	0.00811	21.7	0.537
	SD	0.105	0.142	0.131	0.0180	7.00	0.187
	N	16	16	16	16	16	16
Kidney	Mean	2.56	28.3	0.866	0.0108	38.2	1.10
	SD	0.803	28.02	0.595	0.0142	15.5	0.453
	N	16	16	16	16	16	16
Liver	Mean	2.79	11.7	3.78	0.0125	57.6	1.89
	SD	2.17	11.7	4.087	0.0107	23.3	0.849
	N	16	16	16	16	16	16
Blubber	Mean	0.180	0.0467	0.0169	0.00324	1.62	1.57
	SD	0.0754	0.0559	0.00907	0.00529	0.701	0.548
	N	11	11	11	11	11	11
Hair	Mean	1.49	1.33	3.45	0.776	125	0.663
	SD	0.326	2.0073	1.79	1.19	21.9	0.801
	N	11	11	11	11	11	11
Plasma	Mean	0.476	0.0168	0.0217	0.00297	3.033	0.567
	SD	0.240	0.0415	0.0298	0.00461	4.12	0.194
	N	16	16	16	16	16	16

3.1.3 Correlation of element concentration with biometric data and age group

An analysis of variance (ANOVA) revealed no significant difference for any chemical element between age group in polar bears (**Appendix VI**). However, several significant differences were found between age groups in ringed seal samples (**Table 3**). Applying the post hoc test for pairwise comparisons between age groups, significant difference in the kidney existed between pups and juveniles for Cd, Hg, Zn and As, all with $p < 0.05$, and in the liver between pups and juveniles for Se (Tukey HSD, $p < 0.05$) and between pups and adults for Cd (Tukey HSD, $p < 0.05$).

The correlation between biometric data and chemical element concentration was measured in the muscle, kidney, liver and blubber of ringed seals (**Table 4**). Blubber thickness correlated significantly with Se in the muscles ($p < 0.01$), while measured seal weight correlated with Pb in muscles ($p < 0.01$) and kidney ($p < 0.05$).

Table 3: Influence of age group on chemical element concentration in ringed seals from East Greenland (ANOVA). Only significant values displayed.

Tissue/Element	f-value	p-value
Kidney		
Cd	6.79	0.01
Hg	5.24	0.021
Zn	5.78	0.016
As	5.08	0.023
Liver		
Se	4.85	0.027
Cd	3.71	0.047

Table 4: Significant correlations between ringed seal biometric data and chemical elements in muscle, kidney and liver

Biometric data	Muscles	Kidney	Liver
Standard length (SL)	(+)Cd*, (-)Pb*, (+)As*	(-)Pb**	
Zoological length (ZL)	(-)Pb**	(-)Se**, (-)Hg**, (-)Pb*	
ZL/SL ratio	(-)Cd*, (+)As**	(-)Hg**, (-)Pb*, (+)As*	
Circumference	(+)Cd*	(-)Pb*	(+)Cd*
Measured/Calculated weight	(+)Pb*	(+)Se**, (+)Cd*, (+)Pb**, (+)Zn*	(+)Se*, (-)Zn*
Blubber thickness	(+)Se*		

*p < 0.01; **p < 0.05; (+) positive correlation; (-) negative correlation

Table 5: Pearson correlation coefficients of Se, Cd, Hg, Pb, Zn and As in muscle (M), kidney (K) and liver (L) in polar bears. Number of samples (N) = 10 (statistically significant values in bold).

	Se _M	Se _K	Se _L	Cd _M	Cd _K	Cd _L	Hg _K	Hg _L	Pb _M	Pb _K	Pb _L	Zn _M	Zn _K	Zn _L	As _M	As _K	As _L
Se _M	1	-0.188	0.0258	0.238	-0.129	0.258	-0.223	-0.021	-0.456	-0.51	-0.461	-0.102	-0.239	0.209	-0.343	-0.382	-0.252
Se _K	-0.188	1	0.127	0.499	0.735*	-0.506	0.998**	0.151	-0.232	0.262	0.094	-0.407	0.706*	0.247	0.522	0.123	0.268
Se _L	0.0258	0.127	1	0.11	-0.04	0.519	0.149	0.990**	-0.0504	-0.38	-0.519	-0.0237	0.0801	0.803**	-0.0363	0.233	0.438
Cd _M	0.238	0.499	0.11	1	0.491	0.273	0.505	0.149	-0.31	-0.124	-0.173	-0.797**	-0.107	0.322	-0.25	-0.514	-0.121
Cd _K	-0.129	0.735*	-0.0403	0.491	1	-0.437	0.723*	0.0447	-0.145	0.198	0.0528	-0.379	0.629	0.0604	0.0336	-0.25	0.146
Cd _L	0.258	-0.507	0.519	0.273	-0.437	1	-0.48	0.512	-0.136	-0.558	-0.471	-0.165	-0.724*	0.456	-0.556	-0.262	-0.0864
Hg _K	-0.223	0.998**	0.149	0.505	0.723*	-0.48	1	0.172	-0.214	0.277	0.115	-0.406	0.679*	0.249	0.521	0.119	0.262
Hg _L	-0.0208	0.151	0.990**	0.149	0.045	0.512	0.172	1	-0.062	-0.379	-0.528	-0.101	0.0961	0.766**	-0.0532	0.215	0.498
Pb _M	-0.456	-0.232	-0.0504	-0.31	-0.145	-0.136	-0.214	-0.062	1	0.518	0.436	0.328	-0.0681	-0.217	-0.312	-0.0558	-0.194
Pb _K	-0.509	0.262	-0.379	-0.124	0.198	-0.558	0.277	-0.379	0.518	1	0.954**	-0.0312	0.212	-0.56	0.302	0.209	0.067
Pb _L	-0.461	0.0941	-0.519	-0.173	0.053	-0.471	0.115	-0.528	0.436	0.954**	1	0.0405	0.01	-0.684*	0.26	0.112	-0.101
Zn _M	-0.103	-0.407	-0.0237	-0.797**	-0.379	-0.165	-0.406	-0.101	0.328	-0.0312	0.0405	1	0.121	0.008	-	0.128	-0.345
Zn _K	-0.239	0.706*	0.08	-0.107	0.629	-0.724*	0.679*	0.096	-0.0681	0.212	0.01	0.121	1	0.202	0.531	0.419	0.39
Zn _L	0.209	0.247	0.803**	0.322	0.06	0.456	0.249	0.766**	-0.217	-0.56	-0.684*	0.00775	0.202	1	-0.118	0.0338	0.0981
As _M	-0.343	0.522	-0.04	-0.25	0.034	-0.556	0.521	-0.053	-0.312	0.302	0.26	-0.009	0.531	-0.118	1	0.771**	0.521
As _K	-0.382	0.124	0.23	-0.514	-0.25	-0.262	0.119	0.215	-0.0558	0.209	0.112	0.128	0.419	0.0338	0.771**	1	0.753*
As _L	-0.252	0.268	0.44	-0.121	0.146	-0.086	0.262	0.498	-0.194	0.067	-0.101	-0.345	0.39	0.0981	0.521	0.753*	1

*p < 0.05; **p < 0.01; (-) negative correlation

Table 6: Significant correlations between chemical elements in blubber, muscle, kidney and liver of ringed seal

Elements	Tissue	Blubber	Muscles	Kidney	Liver
Selenium	Blubber	(+) Zn*		(+)Cd*	(+)Se**, (+)Cd*, (+) Hg*, (+)As**
	Muscle		(+)Hg**	(+)As*	(+)As**
	Kidney	(-)Cd*	(+)Pb*	(+)Cd**, (+)Hg*, (+)Zn*, (+)As**	
	Liver		(+)Se**, (+)Hg**	(+)Se*, (+)Cd*, (+)Hg**, (+)Zn*, (+)As*	(+)Hg**, (+)As*
Cadmium	Blubber			(-)Se*, (-)Zn*, (-)As*	(+)Cd**, (+)Zn**, (-)As*
	Muscle				(+)Cd**, (+)Pb*
	Kidney	(+)Se*	(+)Pb**	(+)Se**, (+)Zn**, (+)As**	(+)Se*, (+)As**
	Liver	(+)Cd**, (-)As**	(+)Se*, (+)Cd**, (+)Zn*		(+)Pb**, (+)As*
Mercury	Muscle		(+)Se**	(+)Hg**, (+)As*	(+)Se**, (+)Hg**
	Kidney		(+)Hg**	(+)Se*, (+)Zn*, (+)As**	(+)Se**, (+)Hg**
	Liver		(+)Se*, (+)Hg**	(+)Hg**	(+)Se**
Lead	Muscle			(+)Se*, (+)Cd**, (+)Pb**, (+)Zn*	(+)Pb*
	Kidney		(+)Pb**, (+)Zn*	(+)Zn*	
	Liver		(+)Cd*, (+)Pb*	(+)Pb*	(+)Cd**
Zinc	Blubber	(+)Se*			
	Muscle	(-)As*			(+)Cd*
	Kidney	(-)Cd*	(+)Pb*	(+)Se**, (+)Cd**, (+)Hg*, (+)Pb*, (+)As**	(+)Se*, (-)Zn*
	Liver	(+)Cd*		(+)Se*, (-)Zn*	
As	Blubber		(-)Zn*		(-)Cd*
	Kidney	(+)Se*, (-)Cd*	(+)Hg*	(+)Se**, (+)Cd**, (+)Hg**, (+)Zn**	(+)Se*, (+)As**
	Liver	(-)Cd*	(+)Se**	(+)Cd**, (+)As**	(+)Se*, (+)Cd*

*p < 0.05; **p < 0.01; (+) positive correlation; (-) negative correlation

3.1.4 Intra- tissue correlations in muscle

An analysis of variance (ANOVA) revealed no statistically significant difference in the concentrations of detected chemical elements in the neck, sternal and hind muscles in the sampled polar bears. Selenium, cadmium, zinc and arsenic had respective p values of 0.97, 0.87, 0.52 and 0.92 (**Appendix VII**). A mean chemical element level was thus used in all muscle samples calculations in this study.

3.1.5 Inter-tissue correlation

Pearson's correlation was performed on the muscle, kidney and liver of the polar bear samples. Statistically significant positive correlations were found between element concentration in liver and kidney samples (**Table 5**). For arsenic, significant positive correlations were found between concentrations in muscle and liver, and kidney and liver. Positive correlation also existed between kidney and liver for Pb levels (**Table 5**).

In the polar bear liver, selenium strongly correlated with mercury ($r = 0.990$) and zinc ($r = 0.803$). Significant correlations in the kidney include Se-Cd, Se-Hg and Se-Zn, while Hg also correlated with Cd ($r = 0.723$) and Zn ($r = 0.679$). Renal zinc concentrations negatively correlated with hepatic cadmium ($r = -0.724$) and lead correlated with zinc ($r = -0.684$) in the liver.

No significant relationship was found between element concentration in polar bear hair and any other tissue, except Zn in hair which was negatively correlated with Pb in kidney ($r = -0.695$, $p < 0.05$). In plasma, Se showed significant negative relationships with hepatic Se ($r = -0.700$, $p < 0.05$) and Hg ($r = -0.650$, $p < 0.05$) (**Appendix VIII**).

3.1.6 Molar ratios

The Se:Hg molar ratio in the polar bears ranged from 7.36 – 16.1 (mean 10.5) in muscle, 0.795 – 1.39 (mean 1.045) in kidney and 1.11 – 1.37 (mean 1.23) in the liver (**Table 7**). Selenium showed molar ratios close to unity with mercury in both the liver and kidney (**Fig 2**). The molar ratio of selenium and cadmium was also calculated. The muscle, kidney and liver samples had mean Se: Cd values 40.3, 0.639 and 4.98 respectively. **Figure 3** shows the molar ratio relationship between Se and Cd in muscle, kidney and liver of polar bears. Zinc and cadmium molar ratios were calculated for the muscle, kidney and liver of polar bears (**Fig 4**).

Table 7: Relative factors between molar concentration of Se, Cd, Hg and Zn in liver and kidney tissues (nmol/g, from data in **Table 1**)

Element	Se/Hg	Se/Cd	Zn/Cd
<i>Tissues</i>			
Liver	1.23	4.98	62.9
Kidney	1.045	0.639	2.84

3.2 Estimation of polar bear exposure levels

Results from calculations of the estimated mean daily element exposure of the sampled polar bears in East Greenland from consumption of ringed seal blubber are presented in **Table 8**. It is seen that the increasing order of polar bear daily exposure level, Zn > As > Se > Cd > Hg > Pb (**Table 8**), is similar to the order of all analyzed element concentration in the adipose tissue of the polar bears (**Table 1**) and blubber of the ringed seals (**Table 2**), except for cadmium and mercury,

Data on possible human intake of the chemical elements from both seals and bears is presented in **Table 10**. Here, the mean levels of Se, Cd, Hg and Pb in muscle (meat) and liver which constitute the main food sources in Greenlanders is compared to mean levels reported in other studies.

Table 8: Estimated daily element exposure of East Greenland polar bears to ringed seal blubber in relation to human Proposed Tolerable Weekly Intake (PTWI) levels. Daily average consumption of ringed seal blubber is 2740g^a

Elements	Element concentrations in ringed seal blubber (µg/g ww)	Daily element exposure (µg/kg bw)	PTWI ^b (µg/kg bw)	References
Se	0.18	2.47	400	Yang et al (1989)
Cd	0.0467	0.640	7	FAO/WHO (1989)
Hg	0.0169	0.232	5	FAO/WHO (1972)
Pb	0.00324	0.0444	25	FAO/WHO (1993)
Zn	1.62	22.2	300 - 1000	FAO/WHO (1982)
As	1.57	21.5	15 ^c	FAO/WHO (1988)

a = Stirling and McEwan (1975)

b = Provisional Tolerable Weekly Intake for humans (average 60kg)

c = The PTWI level of 15µg/kg has been withdrawn

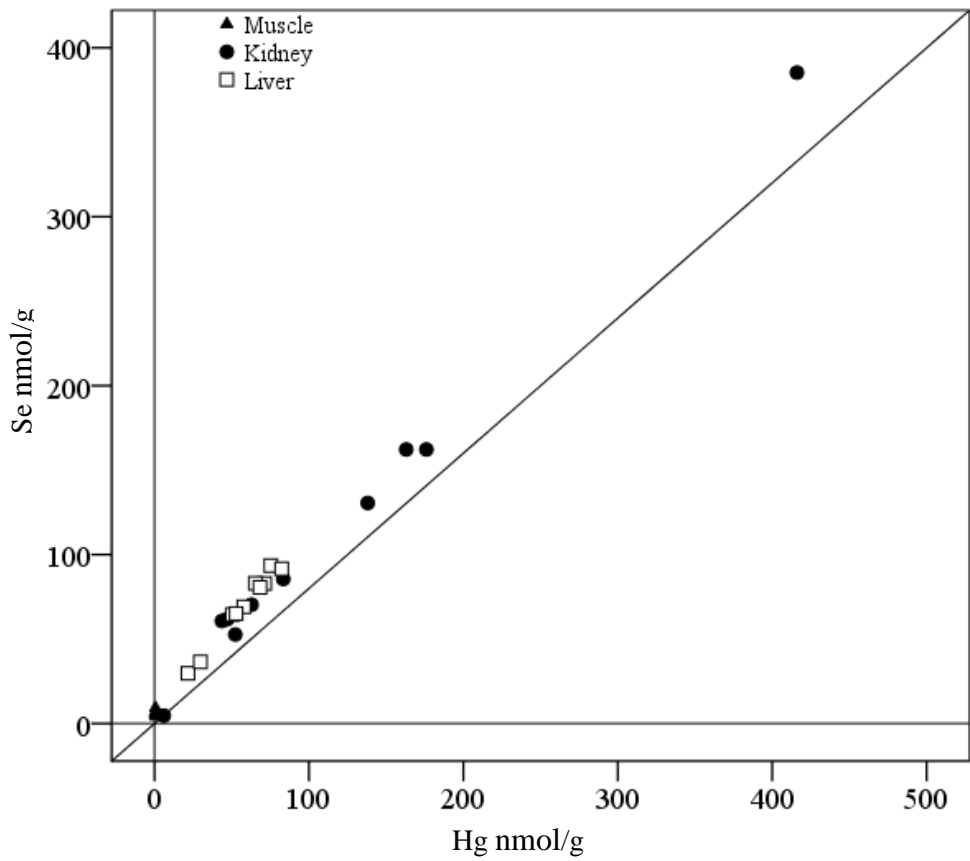


Fig 2 Molar selenium (Se) versus molar mercury (Hg) in muscle, kidney and liver of polar bears. Signatures presented in legend. The line represents a 1:1 ratio

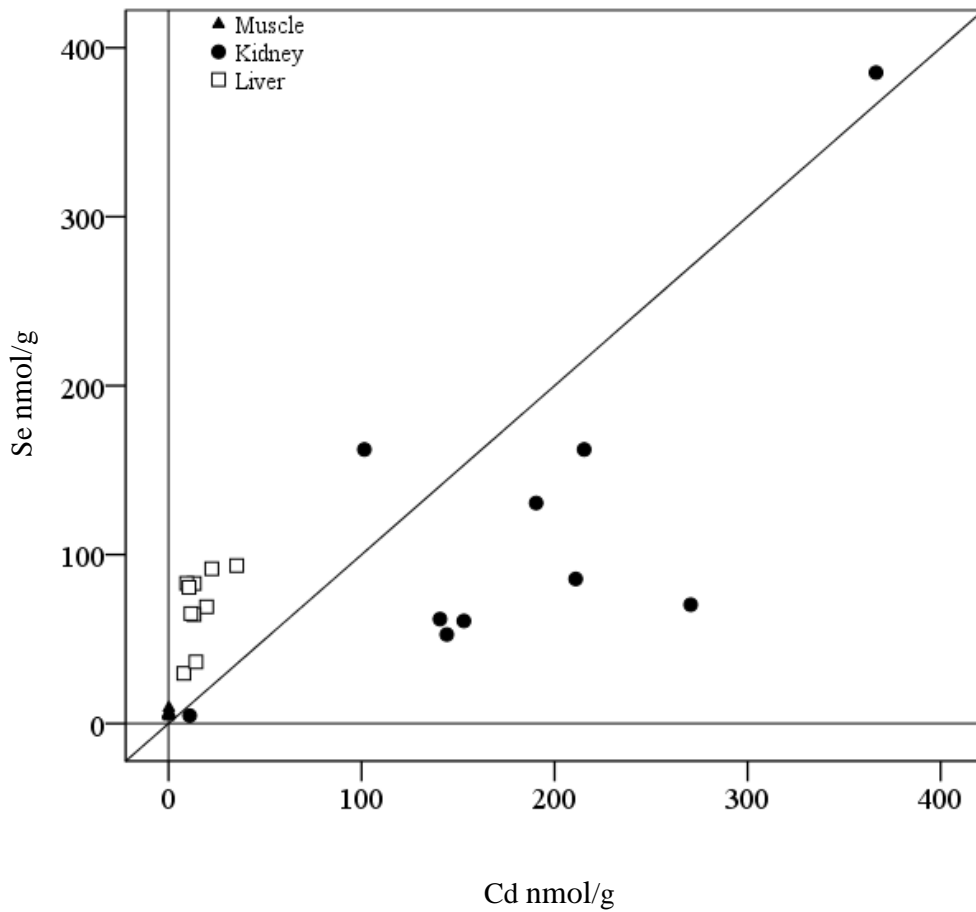


Fig 3 Molar selenium (Se) versus molar cadmium (Cd) in muscle, kidney and liver of polar bears. Signatures presented in legend. The line represents a 1:1 ratio

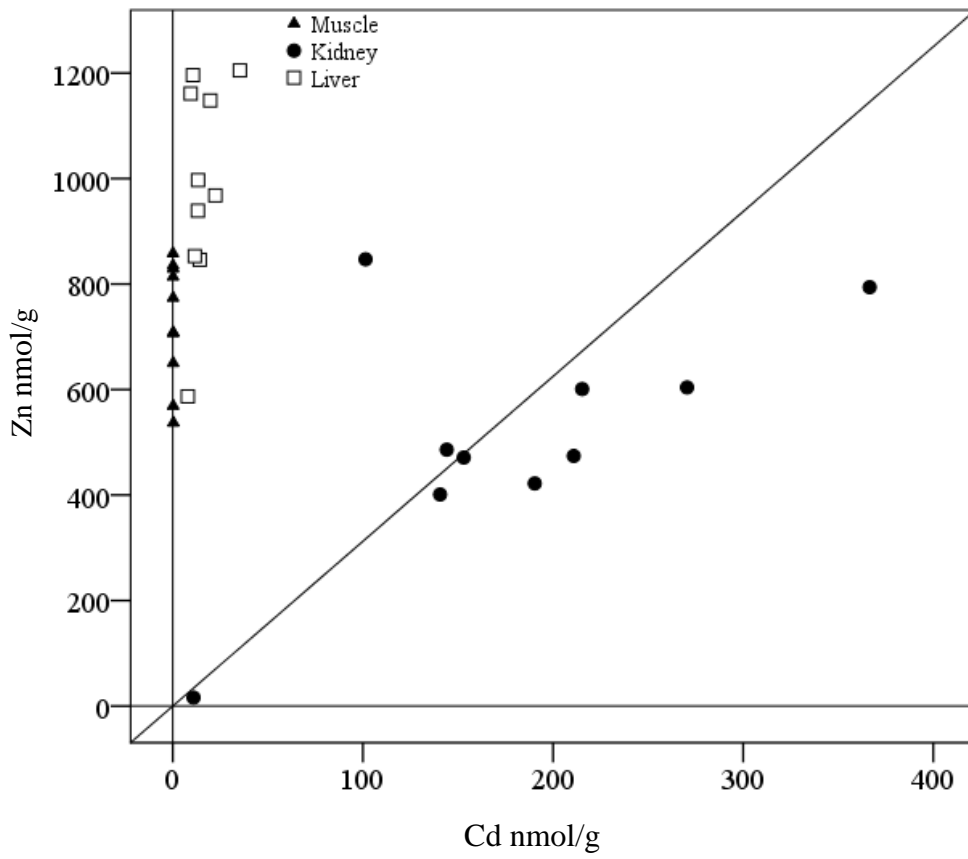


Fig 4 Molar zinc (Zn) versus molar cadmium (Cd) in muscle, kidney and liver of polar bears. Signatures presented in legend. The line represents a 1:1 ratio

Table 9: Comparison of the concentrations of cadmium, mercury and lead in liver and kidney from this study to the effect threshold levels reported in AMAP, (1998)

Tissue/Metal	Concentration ($\mu\text{g/g ww}$)	Effect threshold level (mg/g ww)	References
Liver			
Cd	1.77	20 - 200	Law, 1996
Hg	11.5	> 60	Law, 1996
Pb	0.176	> 30	Ma, 1996
Kidney			
Cd	20.3	50 - 400	Law, 1996
Hg	23.8	< 25	Law, 1996
Pb	0.295	> 90	Ma, 1996

Table 10: Comparison of calculated mean element level to estimated mean concentrations ($\mu\text{g/g ww}$) in marine mammals consumed by Inuit of Greenland, as reported in Johansen et al, (2000)

			Calculated mean ($\mu\text{g/g ww}$) from (Tables 1 and 2)	Estimated mean ($\mu\text{g/g ww}$) from Johansen et al., (2000)
Seal	Meat	Se	0.39	0.313
		Cd	0.0902	0.151
		Hg	0.305	0.355
		Pb	0.0081	< 0.03
	Liver	Se	2.79	3.36
		Cd	11.7	11
		Hg	3.78	8.04
		Pb	0.0125	0.032
Polar bear	Meat	Se	0.475	0.34
		Cd	0.018	0.017
		Hg	0.109	0.086
		Pb	0.439	-
	Liver	Se	5.5	4.4
		Cd	1.77	1.7
		Hg	11.5	10.3
		Pb	0.176	-

(-) No data reported

4. Discussion

4.1 Chemical element concentrations

4.1.1 Selenium

The mean Se concentrations in this study are within the ranges reported in marine mammals found in Canadian Arctic (Nostrom et al., 1986), Northwest Canada (Braune et al., 1991) and Central East Greenland (Dietz et al., 1995). This could be due to role of selenium as a major component of most enzymes, e.g. seleno-enzymes, in mammals (Yang et al., 1989). It has been proposed that selenium's ameliorative actions towards most metals, especially Hg and Cd, occur via an antioxidant mechanism (Rana and Verma, 1996). This assumption is due to the similar method of protection offered by other known antioxidants, e.g. vitamin E and glutathione (GSH) (Rana and Verma, 1996). In this study, the highest mean Se level in polar bears was found in the kidney, as is common in terrestrial mammals (Dietz et al, 1995), while in ringed seals, it was highest in the liver. Due to selenium's high affinity for certain metals, especially mercury, it shows co-accumulative characteristics with these metals in tissues like liver. Woshner et al, (2001a) reported a 3-fold higher concentration of Se in the liver over kidney samples in ringed seals of Arctic Alaska. A probable reason for this difference in Se speciation in tissues of both polar bears and ringed seals is due to the terrestrial ancestry of polar bears (Dietz et al, 1995) which could result in a different mechanism of the element toxicokinetics compared with other marine mammals. Another possible reason for this reported difference could be due to a difference in food source. Ringed seals feed on mostly fish, e.g. cod, which contain high level of selenium while the polar bear diet comprises mainly blubber of seals and other marine mammals (Dietz et al., 1995). The ringed seal blubber in this study contained mean Se concentration 0.18µg/g ww which is significantly

lower than levels reported in arctic cod (0.28 µg/g) and polar cod (0.90 µg/g) in Dietz et al, (2000).

No accumulation of selenium (or any other analyzed element) with age groups and sex in polar bears was found in this study. This is possibly due to the small sample size of only 10 polar bears used. Nielsen and Dietz, (1989) have shown that significance level of correlation between some elements concentrations in different tissues to be dependent upon the sample size. In ringed seals, however, Se accumulated with age in liver from pups to juveniles. This could be due to the increasing level of exposure and contamination to metals, e.g. Hg and Cd, in pups as they grow into juveniles. The absence of significant accumulation of elements, especially Hg, in the seal liver of this study could also be due to capacity of the ringed seals to reduce the levels of contamination, e.g. of Hg, due to the selenium activity (Skaare, 1994). An absence of significant difference between sexes in Se levels has been reported, and could be due to low levels of the element transferable across the placenta or excreted through milk in females (Wagemann et al., 1988).

4.1.2 Cadmium

The mean cadmium concentration in this study was almost 20 times higher in the kidney than the liver in polar bears, but was still lower than mean levels in ringed seal kidney. Similar mean level variations of this metal for both mammals were reported in Woshner et al (2001a). Ringed seal kidney samples, in this study, had significantly higher Cd level than the polar bear. Cadmium levels are generally higher in the marine environment and its mammals than in the terrestrial environment (Johansen et al., 2004). Thus, this difference could be due to the preference of the bears to eating mainly seal blubber and muscles (Stirling and Archibald, 1977), though Cd preferentially accumulates in kidney and liver of the seals (Johansen et al., 1980). The sampled seal blubber and muscles contained low Cd concentration, thus

eliminating the possibility of its biomagnification or presence to a toxicological risk level in polar bears (Woshner et al, 2001a).

Cadmium did not significantly accumulate with age in the polar bears in this study. In ringed seals, Cd accumulated with age in kidney and liver. Similar correlations have been reported in other marine mammals (Dietz et al., 1995; Braune et al., 1991). The lack of age correlation for this metal in ringed seal muscle tissue could be due to the very low levels present in the tissue.

4.1.3 Mercury

Mercury concentrations in the sampled polar bears were high in the kidney, liver and hair, at levels considered potentially toxic to domestic species (Puls, 1994). These levels were however consistent with reported mean levels for marine mammals (Braune et al., 1991; Dietz et al., 1996). The highest concentration was present in the kidney, as occurs in land mammals in general (WHO, 1976). The high Hg concentration in polar bear hair is consistent with reports in Renzoni and Nostrom (1990), as hair represents a good biomarker of Hg exposure since it accumulates methylmercury from the blood in mammals (Dietz et al., 2010). The ringed seal Hg levels in the liver and kidney were significantly lower than in the same organs in polar bears. This could possibly indicate a substantial exposure and intake of the metal in polar bears. Mercury levels in the muscle was however higher in the seals than in polar bears, as was also reported in Dietz et al., (2000). The reason for the low concentration in the bear muscles could show that an efficient catabolic function occurs in the polar bear liver (Dietz et al., 2000).

Mercury accumulated with age in the kidney of ringed seals, but not in any tissues in the polar bears. This non-accumulation in polar bears in relation to age is unusual, and could be as a result of the small sample size used in this study. Adult seals have been found to have

higher mercury levels, as this metal accumulates due to higher intake from the food and environment through the lifetime of the mammal (Noström, 1986).

4.1.4 Zinc

In this study, the mean zinc levels present in the polar bear liver and kidney were close to levels found in the ringed seal. Mean Zn levels are usually similar across muscle, liver and kidney within species, e.g. ringed seals (Wagemann et al., 1996). The reason for this consistent Zn level is probably due to the natural ability of mammals to regulate and maintain physiologically relevant levels of zinc, and other essential elements (Dietz et al., 1995) through homeostasis. The absorption and distribution of zinc to tissues in marine mammals is usually regulated relative to the general zinc concentration (Dietz et al., 2000) ingested in the food source. When intake of Zn is high, the low molecular weight proteins, metallothionein (MT), synthesis is induced which results in the retention of these zinc proteins in mucosa layers of the intestine (Elinder, 1986).

Zinc accumulation with age was significant in ringed seal kidney. Higher levels of this element in seal pups compared to other age groups could be an indication of a higher demand for Zn which is important in a variety of biochemical processes in the growing pups, or as a result of the reduced ability for pups to efficiently excrete Zn (Das et al., 2003).

4.1.5 Lead

Low Pb levels were present in all samples examined in both polar bears and ringed seals. Concentrations in the adipose, plasma and gonads of polar bears and in the blubber, plasma, muscles tissues in the ringed seals were below the detection limit (0.02 µg/g). The highest concentration in polar bear, 0.162 µg/g ww in the liver, was lower than concentrations associated with toxic effects of liver in animals (Puls, 1994). The kidney had similar levels of Pb to the liver, thus confirming the terrestrial ancestry of polar bears which favors higher concentration of Pb in the kidney (Woshner et al, 2001a) unlike in cetaceans (Woshner et al, 2001b). Mean concentrations of this metal were higher in polar bears than in ringed seals for all tissues. In the polar bear muscle, the sampled hind muscle region had a mean Pb concentration over 250 times greater than the neck and sternal muscle regions. This difference in mean level is due to an individual polar bear with hind muscle Pb concentration 10.013 µg/g ww. This is extremely high compared to all other sampled marine mammal, and is possibly due to the presence of lead bullet fragments from the hunters still present in the tissue at the time of sampling. When the mean Pb concentration in the sampled hind muscle tissues is calculated without this individual bear, the resulting concentration is clearly within similar range as present in both the neck and sternal muscle regions.

No lead accumulation with age groups was observed in both seal and bear samples. Norheim et al., (1992) reported no indication of age accumulation of lead in liver and kidney of polar bears from Svalbard, while Wagemann et al., (1983) found no correlation between lead levels and animal size in narwhals.

4.1.6 Arsenic

Arsenic levels were generally low in all sampled tissues. The highest concentration was found in the liver and blubber of polar bears and ringed seals respectively. The seal levels were similar to concentrations measured by Woshner et al., (2001b) in beluga whales but lower than levels reported in grey seals (Frank et al., 1992). Polar bear mean As concentration in the liver was more than 10 times less than levels in the ringed seals, and this is consistent with reports in Norstrom (1986). The blubber of ringed seals is the major food source for polar bears but it contained higher As levels than all other sampled tissues of the bear. This probably confirms the non-toxic, non-accumulative nature of this chemical element (Woshner et al, 2001a). Arsenic levels were correlated with age groups in ringed seals, and As is prevalent in some marine mammals (e.g. seals) as arsenobetaine (Fujihara et al., 2003) which has similar biosynthesis to choline (a neurotransmitter). It is also found in fish as arsenocholine, which like acetobataine, is non-toxic and usually excreted unchanged in urine (Donohue et al, 1999). Seals feed on both pelagic and benthic fishes, with the benthic fish possibly exposing them to potential As uptake due to As presence in marine sediments. These different forms of As have, however, not been investigated in polar bears (Routti et al, 2012). It has been reported that these present arsenic concentrations in marine mammals would probably represent normal physiological levels (Skaare et al., 1990).

4.2 Inter-organ correlations and molar ratios relationships

The correlations between chemical elements observed in different tissues in this study are consistent with other reports on marine mammals. Selenium was strongly correlated, positively, with mercury in the kidney and liver (**Table 5**). This is largely due to the characteristic relationship between both elements, usually resulting in the formation of a metal-selenide complex. The specific structure, function and distribution of this complex is still unclear (Pelletier, 1985; Nigro and Leonzio, 1996), and its occurrence in other tissues, aside the liver, has not fully been addressed (Woshner, 2001a). This Se-Hg complex has been shown to mitigate toxic effects of Hg in controlled experiments (Ridlington and Whanger, 1981; Cuvin-Aralar and Furness, 1991). Selenium and mercury showed a molar concentration ratio close to unity (1:1) in the liver and kidney tissues in polar bears (**Fig 2; Table 7**). Similar results have previously been reported in Hansen et al., (1990), Nielsen and Dietz (1990) and Dietz et al., (1995) in marine mammals from the Greenland and Canadian areas. Selenium was reported in Koeman et al., (1975) to be involved in a chemical mechanism able to detoxify methyl-mercury. This protective capacity of Se, however, occurs when the element concentration in relation to Hg is in excess in the mammals. In situations where the Hg level is higher, an inhibition of biologically important seleno-enzymes may occur due to the high affinity Hg has for Se (Hansen et al., 1990). The Se:Hg molar ratio in this study is slightly above unity, in favor of Se, thus eliminating the possibility of Hg accumulation at the measured concentration in the sampled marine mammals

Selenium in kidney correlated positively with cadmium and zinc, while in the liver Se correlated with Zn in the polar bears (**Table 5**). Selenium has been reported to form a mercury-like complex with cadmium, which has a role in detoxification of Cd in marine mammals (Ridlington and Whanger, 1981). Our results show a Se:Cd ratio in excess of Se in

the liver, and less than 1:1 in the kidney of the polar bears (**Fig 3; Table 7**). These ratios could potential limit the toxic effects of Cd in the liver due to presence of higher Se levels. However, the threat of toxic effects of Cd in the kidney could occur due to its presence in excess of Se, and its ability to antagonize and induce deficiency of essential elements, like selenium (Walker et al., 1996). The cadmium affinity to selenium, coupled with its accumulation with age in mammals, would likely explain the strong positive correlation between cadmium and mercury in the kidneys of East Greenland polar bears. The correlation between selenium and zinc could therefore be as a result of their mutual relationship with cadmium.

Zinc and cadmium were strongly correlated in muscle and liver (**Table 5**), and had molar ratios over unity in muscles, liver and kidney of the polar bears (**Fig 4; Table 7**). When comparing molar ratios, Zn levels exceeded Cd levels in liver (over 60 times), as was also reported in Paludan-Müller et al., (1993) and kidney (almost 3 times) in polar bears. A negative correlation was found between Cd in liver and Zn in kidney. This relationship could be due to the mutual binding to the inducible metallothionein (MT) protein in marine mammals. Cadmium binds to MT which in turn induces the synthesis of new MT (Wagemann and Holden, 1986; Tohyama et al., 1986; Bremner, 1987), which then binds available zinc.

Zinc also correlated positively with Hg in both liver and kidney (**Table 5**) in polar bears. This result is possibly due to the ability of Hg in polar bears, like in other terrestrial mammals, to induce MT which in turn accounts for the increased level of Zn in both liver and kidney (Woshner et al., 2001a). In the ringed seals, Zn correlated strongly with Cd, Se and As in the kidney, and with Se in the blubber (**Table 6**). Similar to relationships observed in the polar bear tissues, Zn interacts with Cd due to their mutual affinity for MT, which also results in its association with Se in seal tissues.

Arsenic concentration in the polar bear kidney correlated with As in the liver and muscles (**Table 5**). However, in ringed seals As correlated positively with Se, Cd, Hg and Zn in the kidney and Cd and Se in the liver (**Table 6**). These interactions could possibly be due to age accumulation of As with these elements in the seals.

Renal lead correlated strongly with Pb in the liver in polar bears (**Table 5**). Since most reports found no accumulation of Pb with age in marine mammals (Norheim et al., 1992; Wagemann et al., 1983), the absence of its correlation with other known age-accumulating metals, e.g. Hg and Cd, and their associating elements, e.g. Se and Zn, is understandable. In ringed seals, inter tissue correlation of Pb in muscle with renal Se, Cd, and Zn was observed (**Table 6**). This could possibly be due to the seals food source which includes cods and other benthic fauna like blue mussels. These organisms may contain elevated levels of certain elements, like Pb, when they are present in polluted environment conditions, e.g. in mining sites, located at distances of up to 30 km away (Asmund et al., 1991; Johansen et al., 1991).

4.3 Exposure estimates for polar bears

Utilizing the estimations of average seal blubber consumption by polar bears reported in Stirling and McEwan (1975), this study attempted to estimate the level of exposure of polar bears sampled from East Greenland. Based on the chemical element concentration in ringed seal blubber from East Greenland sampled in this study, the mean daily exposure of a polar bear of average body weight 397 kg was estimated (**Table 8**). Based on estimations in Stirling and McEwan (1975), a polar bear of average weight 200 kg would consume 1000 kg of seal blubber per year. The calculated average weight of polar bears from our study was 397 kg; hence the amount of estimated seal blubber consumed would probably be doubled. The total daily chemical element intake in polar bear is compared to the WHO reports on the proposed tolerable weekly intake (PTWI) in a human with average body weight of 60 kg. Proposed tolerable weekly intake (PTWI) is the acceptable level of chemical contaminant that can be ingested on a weekly basis without the risk of toxic effects, as determined by the World Health Organization and the Food and Agriculture Organization (WHO/FAO). The PTWI data is not available for polar bears, hence the comparison to human WHO values.

From the data on **Table 8**, it is evident that though significant levels of essential and non-essential chemical elements were present in the sampled polar bear tissues, these concentrations were clearly below the approved threshold intake levels in humans. It is also important to acknowledge the body weight discrepancy between humans and polar bears because the polar bear would experience a higher size dilution of the chemicals compared to the relatively smaller human, further justifying the presence of these chemical elements in possibly non – toxic levels in the bears. In a recent AMAP report (AMAP, 1998), the effect threshold for some non-essential elements in marine mammals were presented. In **Table 9**, the metal concentration observed in the East Greenland polar bears in this study are compared

to the designated effect threshold levels in liver and kidney of mammals. All concentrations measured in our study were lower than these reported effect levels. This also further asserts the relatively lower contamination level in East Greenland compared to results from studies on other Arctic regions, e.g. Northwest Greenland (Dietz et al., 2000) and Southwest Melville Island (Dietz et al., 1998).

4.4 Human exposure to elements in East Greenland

In estimating the human exposure and intake level of Se, Cd, Hg and Pb in Greenlanders from meat and liver of marine mammals, the mean concentrations of these elements were compared to estimated concentration levels reported in Johansen et al., (2000). In their study, they used a larger contaminant data base from Dietz et al., (1996) and Riget et al., (2000), and data from studies on dietary habits in the Disko Bay region by Pars (2000) to estimate Inuit Greenlanders exposure to chemical contaminants in food. Marine mammals they sampled, as common Inuit food sources include marine fish, seals, baleen whales, toothed whales, polar bears, sea birds, shrimps, Icelandic scallop and Blue mussels. The mean levels of Pb, Cd, Hg and Se in meat (i.e. muscles) and liver in some of these organisms were calculated and reported in Johansen et al., (2000).

In **Table 10**, the mean element values in meat and liver for seals and polar bears in Johansen et al., (2000) were compared to the mean values found in muscles (meat) and liver of the polar bears and ringed seals in our study. Due to the similarity of the mean element values from both studies, as seen in **Table 10**, and to avoid underestimating the contaminant intake levels in Greenlanders when estimating their exposure level in our study, mean values reported in Johansen et al., (2000) were substituted with mean values from our results for liver and muscle tissues. Hence, assuming the Greenlanders still consume a similar amount

and variety of marine organisms as food, as reported in Johansen et al., (2000), the intake levels of the chemical elements reported in our study should not be significantly different from levels in Johansen et al., (2000).

The Se intake from the local food is high in Greenland (Johansen et al., 2000). From **Table 10**, our calculated mean Se levels in the consumed tissues of both seals and polar bears are close to levels estimated in Johansen et al., (2000). The calculated mean Se intake, 4569 $\mu\text{g}/\text{week}/\text{person}$ reported in Johansen et al., (2000), is higher than the proposed maximum daily safe level of 2800 $\mu\text{g}/\text{week}$ (Yang et al., 1989). This Se intake level in the Inuit could however, be exaggerated because the calculations were based solely on the consumption of the harbor porpoise skin, which constitutes a high percentage of calculated Se intake in Greenland food (Johansen et al., 2000). It is however expected that where other marine organisms, e.g. seals, are available as food source, the amount of porpoise skin consumed by Greenlanders would be reduced. As such, the estimated level of Se intake would also decline due to the considerable lower levels of the element present in tissues of all other marine mammals.

The mean Hg intake level in the Greenlander is estimated at 846 $\mu\text{g}/\text{week}/\text{person}$, in Johansen et al., (2000). Our reported mean concentrations for Hg in ringed seal and polar bear tissues are similar to the values in Johansen et al., (2000) (**Table 10**). This Hg level clearly exceeds the PTWI of 300 $\mu\text{g}/\text{week}$ for a 60 kg person. The major source of Hg intake in the local Greenland diet is the seal liver and meat, and these are consumed in considerable large amounts (Pars, 2000). Almost 80 % of ingested Hg in Greenlanders diet is through consumption of seal liver and meat (Johansen et al., 2000). The presence of large populations of seals in the Greenland environment would suggest a higher number would possibly be hunted and eventually consumed by the Inuit, thus resulting in the high intake level of this metal.

The mean Cd intake level in the Inuit is estimated at 1004 $\mu\text{g}/\text{week}/\text{person}$ (Johansen et al., 2000). This exceeds the PTWI of 420 $\mu\text{g}/\text{week}$ for a 60 kg person, with the calculated Cd intake more than double the PTWI. Our calculated mean concentration for Cd is similar to the values reported in Johansen et al., (2000) (**Table 10**) for the bears and seals. As with Hg, the seal liver comprises the dominant source for Cd intake in the local diet in Greenland (Pars, 2000). Almost 70 % of ingested Cd from the local diet is from the seal liver (Johansen et al., 2000). More seal tissues are consumed than any other marine organism (Johansen et al., 2000), hence this would possibly explain the high Cd intake in Greenlanders.

The total Pb intake in the Greenlanders is 15 $\mu\text{g}/\text{week}/\text{person}$, according to reports in Johansen et al., (2000). However, considering that they did not include the Pb levels for polar bear muscle and liver, the addition of our mean values (**Table 10**) would likely increase the total Pb intake in Greenlanders. This increase, however, is not expected to exceed the Pb PTWI level of 25 $\mu\text{g}/\text{kg}$ body weight for humans. However, considering our sampled polar bear with high muscle Pb concentration 10 $\mu\text{g}/\text{g}$ ww is consumed along with the other known sources of Pb contamination in Greenlanders, then, the possibility of exceeding the PTWI for humans will be increased.

5. Conclusion

It is still unknown if the estimated body burdens of these elements pose any significant health risks to the polar bears. Most concentration levels obtained for essential elements depend mainly on the physiological and exposure levels of the individual marine mammal. Zinc and arsenic have been shown to be relevant in mammalian enzyme activity in marine species. The ability for non-essential elements to have potential toxic effects on the physiology of these marine mammals has not been fully understood. Renal and hepatic concentrations of mercury and cadmium is more prominent than in other tissues in mammals, and the kidney has repeatedly been identified as the primary target organ for potential metal toxicity. Our analyzed levels for cadmium, mercury and lead however, are lower than the effect threshold level in the liver and kidney of East Greenland polar bear. The molar concentration ratios for Se and Hg are slightly above 1:1 in liver and kidney of polar bear, thus, this interaction has been proposed to possibly reduce the potential toxicological effects of Hg. Lead and cadmium levels in kidney, liver and muscles of marine mammals in this study are also below the effect threshold levels associated with toxicological effects in marine mammals. The native Inuit feed on polar bear and ringed seal tissues, thus are possibly exposed to various chemical contaminants. The Greenlanders exposure to the chemical elements analyzed in this study are considered higher than the PTWI levels for cadmium and mercury, but below PTWI levels for selenium and lead. This high intake for Cd and Hg could potentially result in detrimental health effects in the individuals involved.

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Appendix

Appendix I - Accuracy of the method

Table A1: Measured concentration in reference material (Bovine Liver) with standard Deviation (SD), standard reference values (Bovine Liver 1577b) and recovery

Element	Mass number	Concentration ($\mu\text{g/g}$)	SD	Certified value ($\mu\text{g/g}$)	Estimated uncertainty	Recovery (%)
Arsenic	75	0.045	0.002	0.05	0.002	90
Cadmium	114	0.48	0.01	0.5	0.015	96
Lead	208	0.112	0.008	0.129	0.012	87
Mercury	202	0.0047	0.0002	0.003	0.000148	157
Selenium	78	0.739	0.043	0.73	0.06	101
Zinc	67	131	6	127	8.8	103

Table B1: Measured concentration in reference material (Chicken GBW) with standard Deviation (SD), standard reference values (Chicken GBW-10018) and recovery

Element	Mass number	Concentration ($\mu\text{g/g}$)	SD	Certified value ($\mu\text{g/g}$)	Estimated uncertainty	Recovery (%)
Arsenic	75	0.115	0.004	0.109	0.006	106
Cadmium	114	0.0063	0.0052	0.005	0.007	127
Lead	208	0.048	0.008	0.11	0.011	44
Mercury	202	0.0019	0.0004	0.0036	0.000441	54
Selenium	78	0.55	0.01	0.49	0.02	112
Zinc	67	25.4	0.2	26	0.4	98

Appendix II – Biometric data of polar bears and ringed seals from East Greenland

Table A2: Biometric data of polar bear and ringed seal samples collected in East Greenland

ID	Date of collection	Sex	Age group	Standard length (cm)	Zoological length (cm)	Ratio ZL/SL	Circumference (cm)	Blubber thickness (mm)
Polar bears								
43101	22-Feb-11	M	Adult	233	252	1.08	210	-
43102	25-Feb-11	M	Juvenile	212	233	1.1	185	-
43103	26-Feb-11	F	Pup	153	165	1.08	124	-
43104	27-Feb-11	M	Juvenile	205	221	1.08	140	-
43105	10-Mar-11	F	Juvenile	200	210	1.05	170	-
43106	11-Mar-11	F	Juvenile	180	195	1.08	138	-
43107	11-Mar-11	F	Pup	164	181	1.1	130	-
43108	12-Mar-11	F	Pup	185	199	1.08	125	-
43109	13-Mar-11	M	Adult	219	229	1.05	215	-
43171	-	M	Adult	-	-	-	-	-
Ringed seals								
43166	24-Feb-12	M	Juvenile	103.3	-	-	30	55
43167	24-Feb-12	M	Adult	107	130	1.11	107	-
43181	24-Feb-12	F	Adult	113	132	1.08	105	49
43182	24-Feb-12	M	Pup	52	122	1.14	98	65
43183	26-Feb-12	M	Adult	113	107	1.04	81	30
43184	27-Feb-12	F	Juvenile	93	122.5	1.08	99	72
43185	27-Feb-12	F	Adult	105	52.5	1.01	30.5	-
43186	27-Feb-12	M	Pup	43	121	1.07	89	60
43187	27-Feb-12	M	Adult	106.5	99	1.06	87	48
43188	27-Feb-12	M	Juvenile	100	117	1.11	96	55
43189	29-Feb-12	M	Adult	119	44	1.02	30	-
43190	29-Feb-12	M	Juvenile	102	110	1.03	96.5	40
43191	1-Mar-12	M	Adult	225	104	1.04	78	45
43192	1-Mar-12	F	Adult	109	122	1.03	79	60
43193	1-Mar-12	F	Pup	45	110	1.08	87	40
43194	5-Mar-12	F	Adult	116	242	1.08	172	72
43200	5-Mar-12	F	Pup	44	115	1.06	92	57

(-) Data not available

Appendix III – Method detection limit

Table A3: Method detection limit (MDL)*

Element	MDL1	MDL2	MDL3	MDL4	MDL5	MDL6	MDL7	MDL8	MDL9	MDL10
	hair (0.13g)	hair (0.14g)	hair (0.15g)	plasma (0.4g)	adipose/blubber (0.5g)	adipose/blubber (0.6g)	adipose/blubber (0.7g)	muscle, gonad, kidney, liver (1.4g)	muscle, gonad, kidney, liver (1.5g)	muscle, gonad, kidney, liver (1.6g)
As	0.002	0.043	0.04	0.042	0.001	0.017	0.017	0.021	0.023	0.019
Cd	0.00033	0.00033	0.00033	0.007	0.003	0.0081	0.008	0.0013	0.008	0.006
Pb	0.0008	0.0008	0.00045	0.0067	0.02	0.02	0.03	0.000447	0.03	0.024
Hg	0.228	0.228	0.228	0.228	0.228	0.228	0.228	0.228	0.228	0.228
Se	0.006	0.015	0.015	0.05	0.05	0.05	0.15	0.023	0.005	0.033
Zn	0.151	0.151	0.151	0.151	0.151	0.151	0.151	0.151	0.151	0.151

*Method Detection Limit (MDL) were derived from the calculated average weight of the group of tissues and organs.

Appendix IV – Concentration of elements in polar bears from East Greenland

Table A4: Element concentration ($\mu\text{g/g}$) found in East Greenland polar bears (*Ursus maritimus*). (N) number of samples

Tissue/metal statistics		Se	Cd	Hg	Pb	Zn	As
Neck muscle (<i>Musculus splenius capitis</i>)	Min	0.228	0.00927	0.057	0.00134	20.9	0.05
	Mean	0.46	0.0142	0.0962	0.00439	46.9	0.0784
	SD	0.198	0.00513	0.0328	0.00232	13.9	0.0347
	Median	0.412	0.0128	0.102	0.00419	46	0.0658
	Max	0.851	0.0244	0.135	0.00831	61.7	0.141
	N	7	7	7	7	7	7
Sternum muscle (<i>Latissimus dorsi</i>)	Min	0.328	0.0147	0.0765	0.00194	21.03	0.0273
	Mean	0.477	0.0204	0.113	0.00538	44.7	0.0654
	SD	0.123	0.00536	0.0345	0.00623	14.6	0.0277
	Median	0.473	0.0188	0.104	0.00302	43.7	0.0622
	Max	0.655	0.0312	0.169	0.0205	66.2	0.112
	N	8	8	8	8	8	8
Hind muscle (<i>Gluteus maximus</i>)	Min	0.288	0.0115	0.0691	0.0031	40.5	0.0479
	Mean	0.488	0.0199	0.12	1.31	50.4	0.0731
	SD	0.135	0.00813	0.0454	1.31	5.66	0.0235
	Median	0.493	0.0192	0.118	0.00755	50.3	0.0721
	Max	0.657	0.0348	0.191	10.01	59.3	0.115
	N	8	8	8	8	8	8
Kidney	Min	0.374	1.22	1.2	0.003	1.043	0.0491
	Mean	9.28	20.3	23.8	0.295	33.4	0.154
	SD	8.42	10.8	23.7	0.297	15.08	0.0965
	Median	6.15	19.3	14.6	0.208	31.4	0.136
	Max	30.4	41.2	83.4	0.828	55.4	0.383
	N	10	10	10	10	10	10
Liver	Min	2.34	0.887	4.33	0.018	38.4	0.0576
	Mean	5.5	1.77	11.5	0.176	64.7	0.162
	SD	1.72	0.92	3.92	0.22	12.8	0.107
	Median	5.91	1.48	12.3	0.0848	64.2	0.115
	Max	7.38	3.97	16.5	0.677	78.8	0.404
	N	10	10	10	10	10	10

Table A4 contd

Tissue/metal statistics		Se	Cd	Hg	Pb	Zn	As
Adipose	Min	0.032	0.00639	0.0141	0.00191	1.15	0.0341
	Mean	0.0605	0.0159	0.0286	0.00405	2.058	0.0925
	SD	0.0323	0.0102	0.0186	0.00239	0.901	0.0447
	Median	0.0486	0.0124	0.0194	0.00357	1.74	0.0897
	Max	0.121	0.0308	0.0635	0.00508	3.81	0.166
	N	10	10	10	10	10	10
Gonad	Min	0.175	0.0477	0.0808	0.0016	9.57	0.0241
	Mean	0.268	0.104	0.141	0.00985	12.0	0.0439
	SD	0.0483	0.0512	0.0687	0.0127	5.66	0.0151
	Median	0.281	0.103	0.106	0.00388	11.9	0.0442
	Max	0.31	0.212	0.275	0.037	14.4	0.0667
	N	9	9	9	9	9	9
Hair	Min	0.502	0.0028	4.99	0.0808	104	0.0137
	Mean	0.598	2.23	7.11	0.163	130	0.0354
	SD	0.0763	0.0209	1.12	0.101	13.3	0.0285
	Median	0.588	0.0189	7.067	0.146	129	0.0288
	Max	0.696	12.1	8.022	0.414	150	0.107
	N	10	10	10	10	10	10
Plasma	Min	0.142	0.0000328	0.00647	0.000114	0.85	0.0073
	Mean	0.185	0.016	0.0561	0.0182	2.91	0.0363
	SD	0.031	0.0891	0.0877	0.0341	3.5	0.0275
	Median	0.182	0.00593	0.0154	0.00414	1.29	0.0331
	Max	0.24	0.177	0.237	0.103	6.37	0.101
	N	10	10	10	10	10	10

Appendix V – Concentration of elements in ringed seals from East Greenland

Table A5: Element concentration ($\mu\text{g/g}$) found in East Greenland ringed seals (*Phoca hispida*). (N) number of samples

Tissue/metal statistics		Se	Cd	Hg	Pb	Zn	As
Muscle	Min	0.149	0.000261	0.155	0.0000693	3.39	0.214
	Mean	0.39	0.0902	0.305	0.00811	21.7	0.537
	SD	0.105	0.142	0.131	0.018	7.0	0.187
	Median	0.388	0.0608	0.27	0.00325	22.0	0.537
	Max	0.523	0.579	0.638	0.0749	31.9	0.926
	N	16	16	16	16	16	16
Kidney	Min	1.16	0.00114	0.115	0.000871	7.33	0.517
	Mean	2.56	28.3	0.866	0.0108	38.2	1.10
	SD	0.803	28.02	0.595	0.0142	15.5	0.453
	Median	2.58	23.2	0.795	0.00548	42.9	0.989
	Max	4.33	102	2.18	0.0456	69.3	2.027
	N	16	16	16	16	16	16
Liver	Min	0.503	0.000468	0.262	0.00132	33.2	0.371
	Mean	2.79	11.7	3.78	0.0125	57.6	1.89
	SD	2.17	11.7	4.087	0.0107	23.3	0.849
	Median	2.74	12.3	2.87	0.00957	52.8	1.78
	Max	7.85	43.8	14.5	0.034	122	3.67
	N	16	16	16	16	16	16
Blubber	Min	0.0855	0.0126	0.00636	0.000505	0.829	0.932
	Mean	0.18	0.0467	0.0169	0.00324	1.62	1.57
	SD	0.0754	0.0559	0.00907	0.00529	0.701	0.548
	Median	0.156	0.0232	0.0171	0.00101	1.36	1.4
	Max	0.323	0.202	0.0385	0.0184	3.23	2.74
	N	11	11	11	11	11	11
Hair	Min	1.038	0.0028	1.29	0.0559	82.9	0.172
	Mean	1.49	1.33	3.45	0.776	125	0.663
	SD	0.326	20.07	1.79	1.19	21.9	0.801
	Median	1.53	0.836	3.33	0.204	126	0.301
	Max	2.15	7.25	7.17	3.96	157	2.29
	N	11	11	11	11	11	11
Plasma	Min	0.14	0.0000328	0.00405	0.000168	0.840	0.358
	Mean	0.476	0.0168	0.0217	0.00297	3.033	0.567
	SD	0.24	0.0415	0.0298	0.00461	4.12	0.194
	Median	0.465	0.00332	0.0104	0.000662	1.43	0.536
	Max	1.12	0.177	0.107	0.0189	15.6	0.987
	N	16	16	16	16	16	16

Appendix VI – Analysis of variance of elements in age groups of polar bears

Table 6A: Analysis of variance (ANOVA) for distribution of elements in muscle of different age groups of polar bears

		Sum of Squares	df	Mean Square	F	Sig.
Se	Between Groups	0.028	2	0.014	1.35	0.318
	Within Groups	0.072	7	0.010		
	Total	0.101	9			
Cd	Between Groups	0.000054	2	0.00003	0.551	0.600
	Within Groups	0.00034	7	0.00005		
	Total	0.00039	9			
Hg	Between Groups	0.0	2	0.000	0.00	1.00
	Within Groups	1.6×10^{-35}	7	2.29×10^{-36}		
	Total	1.6×10^{-35}	9			
Pb	Between Groups	2.52	2	1.26	1.19	0.358
	Within Groups	7.39	7	1.056		
	Total	9.908	9			
Zn	Between Groups	0.767	2	0.383	0.00538	0.995
	Within Groups	498	7	71.2		
	Total	499	9			
As	Between Groups	0.0013	2	0.0006	0.493	0.631
	Within Groups	0.0092	7	0.0013		
	Total	0.0105	9			

Table 6B: Analysis of variance (ANOVA) for distribution of elements in kidney of different age groups of polar bears

		Sum of Squares	df	Mean Square	F	Sig.
Se	Between Groups	195	2	97.6	1.55	0.278
	Within Groups	442	7	63.1		
	Total	637	9			
Cd	Between Groups	206	2	103	0.846	0.469
	Within Groups	851	7	122		
	Total	1057	9			
Hg	Between Groups	1662	2	831	1.70	0.2495
	Within Groups	3413	7	488		
	Total	5075	9			
Pb	Between Groups	0.0669	2	0.0335	0.321	0.735
	Within Groups	0.729	7	0.104		
	Total	0.796	9			
Zn	Between Groups	655	2	327	1.65	0.259
	Within Groups	1391	7	199		
	Total	2046	9			
As	Between Groups	0.0310	2	0.0155	2.048	0.199
	Within Groups	0.0529	7	0.00756		
	Total	0.0839	9			

Table 6C: Analysis of variance (ANOVA) for distribution of elements in liver of different age groups of polar bears

		Sum of Squares	df	Mean Square	F	Sig.
Se	Between Groups	6.56	2	3.28	1.15	0.371
	Within Groups	20.04	7	2.86		
	Total	26.6	9			
Cd	Between Groups	1.24	2	0.620	0.679	0.538
	Within Groups	6.39	7	0.913		
	Total	7.63	9			
Hg	Between Groups	34.9	2	17.5	1.18	0.361
	Within Groups	103	7	14.7		
	Total	138	9			
Pb	Between Groups	0.0225	2	0.0113	0.191	0.831
	Within Groups	0.413	7	0.0590		
	Total	0.436	9			
Zn	Between Groups	54.1	2	27.05	0.132	0.878
	Within Groups	1433	7	205		
	Total	1487	9			
As	Between Groups	0.0517	2	0.0259	3.57	0.0854
	Within Groups	0.0508	7	0.0073		
	Total	0.102	9			

Appendix VII – Analysis of variance of elements in muscles of polar bear

Table A7: Analysis of variance (ANOVA) for distribution of selenium, cadmium, zinc and arsenic in polar bear muscles

		Sum of Squares	df	Mean Square	F	Sig.
Selenium	Between Groups	0.00184	2	0.00092006	0.0353	0.965
	Within Groups	0.469	18	0.0261		
	Total	0.471	20			
Cadmium	Between Groups	1.14x10 ⁻⁶	1	1.14x10 ⁻⁶	0.0213	0.886
	Within Groups	0.000644	12	5.36x10 ⁻⁵		
	Total	0.000645	13			
Zinc	Between Groups	203.2	2	102	0.688	0.515
	Within Groups	2658	18	148		
	Total	2861	20			
Arsenic	Between Groups	9.45x10 ⁻⁵	2	4.73x10 ⁻⁵	0.0823	0.921
	Within Groups	0.0103	18	0.000574306		
	Total	0.0104	20			

Appendix VIII – Correlation of elements in plasma, hair, kidney and liver in polar bears

Table A8: Pearson correlation coefficients of Se, Cd and Hg in plasma (P), hair (H), kidney (K) and liver (L) in polar bears. Number of samples (N) = 10 (statistically significant values in bold).

	Se _P	Se _H	Se _K	Se _L	Cd _P	Cd _H	Cd _K	Cd _L	Hg _H	Hg _K	Hg _L
Se _P	1	0.626	0.03	-0.700*	0.878	0.096	0.187	-0.113	-0.159	0.008	-0.650*
Se _H	0.626	1	-0.007	-0.302	0.367	0.64	0.053	-0.316	0.203	-0.051	-0.246
Se _K	0.03	-0.007	1	0.129	-0.008	-0.058	0.736*	-0.505	-0.594	0.998**	0.151
Se _L	-0.700*	-0.302	0.129	1	-0.788	0.277	-0.041	0.519	0.252	0.149	0.990**
Cd _P	0.878	0.367	-0.008	-0.788	1	-0.509	-0.492	-0.336	-0.365	-0.132	-0.755
Cd _H	0.096	0.64	-0.058	0.277	-0.509	1	0.089	0.071	0.205	-0.095	0.285
Cd _K	0.187	0.053	0.736*	-0.041	-0.492	0.089	1	-0.437	-0.548	0.724*	0.041
Cd _L	-0.113	-0.316	-0.505	0.519	-0.336	0.071	-0.437	1	0.492	-0.48	0.511
Hg _H	-0.159	0.203	-0.594	0.252	-0.365	0.205	-0.548	0.492	1	-0.576	0.283
Hg _K	0.008	-0.051	0.998**	0.149	-0.132	-0.095	0.724*	-0.48	-0.576	1	0.171
Hg _L	-0.650*	-0.246	0.151	0.990**	-0.755	0.285	0.041	0.511	0.283	0.171	1

*p < 0.05; **p < 0.01; (-) negative correlation

Table B8: Pearson correlation coefficients of Pb, Zn and As in hair (H), plasma (P), kidney (K) and liver (L) in polar bears. Number of samples (N) = 10 (statistically significant values in bold).

	Pb _H	Pb _K	Pb _L	Zn _P	Zn _H	Zn _K	Zn _L	As _K	As _L
Pb _H	1	-0.221	-0.255	-0.253	-0.028	-0.192	0.083	-0.18	-0.018
Pb _K	-0.221	1	0.954**	0.09	-0.695*	0.213	-0.559	0.209	0.067
Pb _L	-0.255	0.954**	1	0.205	-0.634	0.01	-0.683*	0.112	-0.101
Zn _P	-0.253	0.09	0.205	1	-0.288	-0.231	-0.619	-0.063	-0.223
Zn _H	-0.028	-0.695*	-0.634	-0.288	1	-0.158	0.648	-0.335	0.002
Zn _K	-0.192	0.213	0.01	-0.231	-0.158	1	0.203	0.419	0.39
Zn _L	0.083	-0.559	-0.683*	-0.619	0.648	0.203	1	0.033	0.098
As _K	-0.18	0.209	0.112	-0.063	-0.335	0.419	0.033	1	0.753*
As _L	-0.018	0.067	-0.101	-0.223	0.002	0.39	0.098	0.753*	1

*p < 0.05; **p < 0.01; (-) negative correlation