Cadmium, mercury, zinc and selenium in ringed seals (*Phoca hispida*) from Greenland and Svalbard

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ABSTRACT

Muscle, liver, and kidney tissue from 456 ringed seals (*Phoca hispida*) from eight areas in Greenland were analysed for cadmium, mercury, zinc and selenium. In general, cadmium concentrations were high in liver and kidney tissue, with geometric means of 7.79 and 33.5µg/g (all data on wet weight basis), respectively. Muscle levels were considerably lower, at 0.067µg/g. The concentration of mercury was relatively high in liver tissue with a geometric mean of 2.59µg/g. Muscle and kidney mercury levels were somewhat lower, with geometric means of 0.210 and 0.956µg/g, respectively.

Cadmium and mercury levels were strongly dependent upon age and sampling area, as well as the interaction combinations, indicating that the accumulation of cadmium and mercury varies with age and area. Mercury accumulated in all three tissues throughout life, whereas cadmium in liver and kidneys peaked in the age group 5-10 years old where after it dropped significantly.

Cadmium levels showed a tendency towards higher concentrations in the northern municipalities, which may be due to the higher cadmium levels in certain prey items in the northern areas. Mercury levels were higher in seals from East Greenland compared to West Greenland. Variations in feeding habits probably explain some of the differences in levels of cadmium and mercury in ringed seals from different geographical areas.

Cadmium concentrations were correlated (both pairwise and partial) in the three organs. This was true for mercury as well, whereas only half of the combinations were significant for zinc and selenium. Cadmium was strongly correlated to mercury in all three tissues and zinc only in liver and kidneys. Mercury was only correlated to selenium in liver and not to zinc.

High concentrations of cadmium were found in the bile from 58 ringed seals, and were about 10-fold higher than in muscle. The concentration of mercury in bile was relatively low, being only one third of the muscle level. The bile levels reflect that substantial amounts of especially cadmium are circulated through the bile. However, it is uncertain whether these amounts are actually excreted or reabsorbed in the intestine (enterohepatic circulation).

INTRODUCTION

Ringed seals (*Phoca hispida*) have been the most numerous and most commonly hunted marine mammal species by the Inuit population in Greenland and other Arctic areas for centuries (Reeves *et al.* this volume, Teilmann and Kapel this volume). Ringed seals are abundant throughout the year in most of Greenland's coastal waters. Annual catches of between 70,000 to 100,000 have been reported from Greenland during the last decades (Teilmann and Kapel this volume).

During recent years there has been an increasing interest in documenting and understanding food chain accumulation and geographical and temporal trends of contaminants levels. Some of these issues have recently been elucidated for the Greenland and the Arctic marine ecosystems (e.g. Dietz *et al.* 1996, 1997, in press b). Heavy metals data from a number of animal groups, including fish, seabirds, walrus (*Odobenus rosmarus*), whales and polar bear (*Ursus maritimus*), have previously been documented from the Greenland marine ecosystem (e.g. Born *et al.* 1991, Nielsen and Dietz 1989, Hansen *et al.* 1990, Dietz *et al.* 1990, Paludan-Müller *et al.* 1993. Dietz *et al.* 1995, Riget *et al.* 1997a,b). Baseline data on seals from Greenland waters are included in a review of the marine ecosystem (Dietz *et al.* 1996). In the present study, however, a much more detailed presentation and data analysis is provided.

This article focuses on accumulation of cadmium, mercury, zinc and selenium in ringed seals from Greenland waters. Information is presented on tissue differences, age-related differences as well as geographical trends. A number of authors have previously documented that Arctic seals have quite high levels of cadmium and mercury, and that the cadmium levels are higher than in seals from the industrialised part of the World (e.g. Sergeant and Armstrong 1973, Smith and Armstrong 1975, 1978, Johansen *et al.* 1980, Wagemann *et al.* 1996).

The physiological interactions between the contaminant levels are illustrated by statistical comparisons. In addition to muscle, liver and kidney samples, the contaminant level in bile was examined in some of the animals. The purpose was to provide information on the entero-hepathic pathway and the excretory abilities of heavy metals.

MATERIALS AND METHODS

Sampling

Tissue samples were collected from 456 ringed seals from different areas of Greenland and Svalbard (Fig. 1, Tables 1-3). The Greenland samples were collected from the traditional hunt, mainly during May and June. Sampling took place during 1978-1985 in the municipality of Uummannaq, during 1983-1985 in Danmarkshavn, during 1984 in Avanersuaq, during 1985 in Upernavik and Kong Oscars Fjord and during 1986 in Ittoqqortoormiit and Nanortalik. Samples from Svalbard were obtained from a scientific sampling program in 1986.

Scientists, students, laboratory workers and local inhabitants collected the samples. The samples were stored in polyethylene plastic bags. Prior to sampling, the animals were measured and information on date, location and sex was recorded. The lower jaw was collected for later extraction of canine teeth for age estimation.

Samples were kept at outdoor temperatures (-5 to -30°C) until stored in a freezer. The tissues were kept during storage in Greenland, shipment from Greenland and storage in Copenhagen at c. -20°C.

Biological and chemical analysis

Ages were estimated by counting annual layers in the cementum of the canine tooth as described by Dietz *et al.* (1991).

Kidney samples included both medulla and cortex tissue, as the kidneys of seals is divided into numerous lobules or renules (Harrison and King 1965). No particular tissue preference was addressed in the liver, since metals are homogeneously distributed within this tissue (Nielsen and Dietz 1990).

Most of the metal analyses were performed at the laboratory of the Department of Arctic Environment (DAE) in Copenhagen, Denmark. The exceptions are the seals from Uummannaq (1978-1985) which were analysed by Centre of

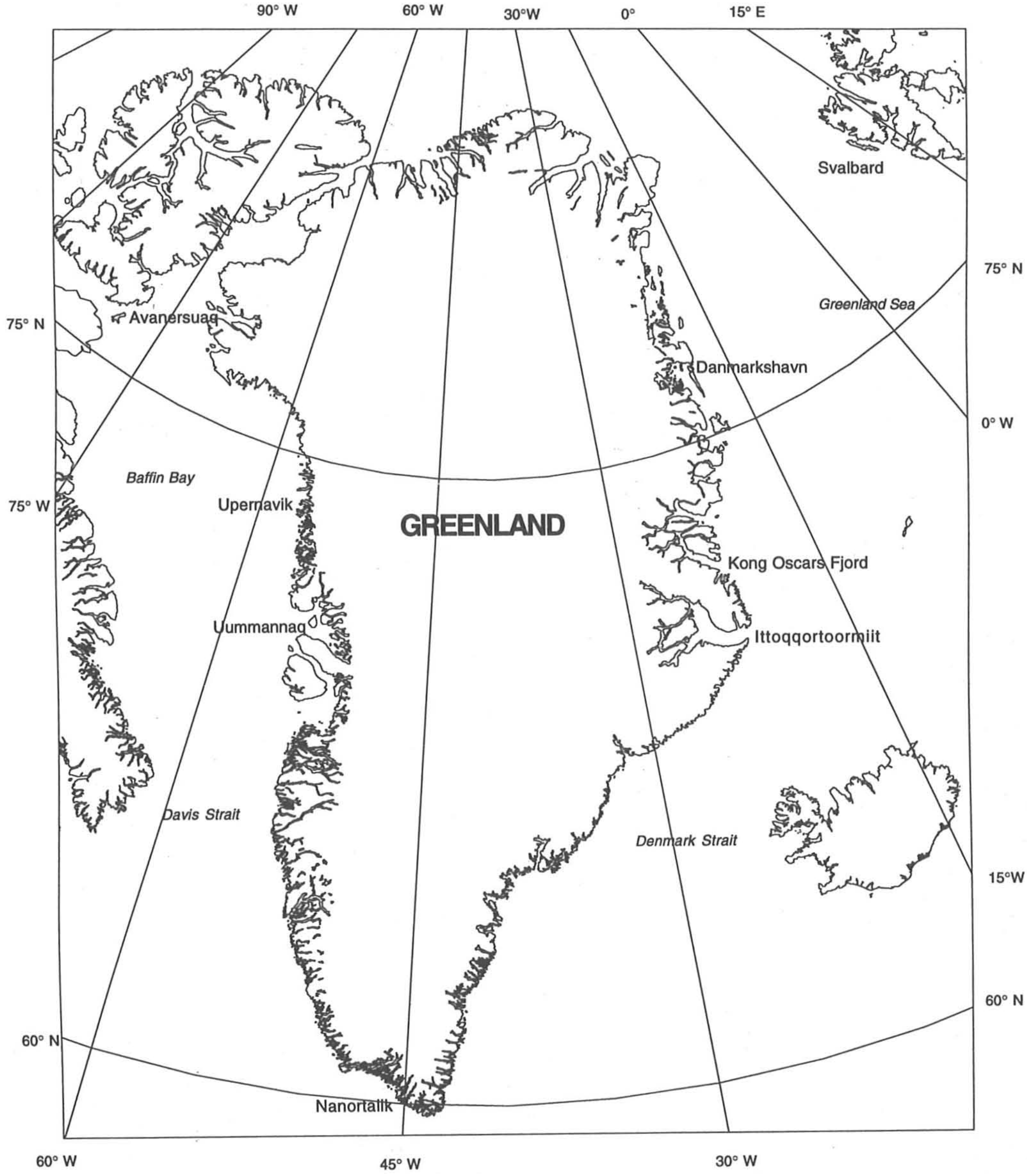


Fig. 1
Map showing localities and municipalities in Greenland mentioned in the text.

Industrial Research (CIR) in Oslo, Norway, and those from Avanersuaq (1984) which were analysed at the Institute for Environmental and Occupational Medicine (IEOM) at the University of Århus, Denmark.

DAE and IEOM used more or less the same procedure for analyses. Samples were dissolved in nitric acid using Teflon bombs (Berghof). At DAE all cadmium samples were screened by flame Atomic Absorption Spectroscopy (AAS, Perkin-Elmer 3030), however, the graphite furnace technique (Perkin-Elmer 3030 with Zeeman background correction) was used for the final analysis of samples with concentrations less than 2.5µg/g wet weight. This latter technique was also used for selenium analyses. Mercury analyses were performed by hydride generation and the amalgam technique as previously described by e.g. Dietz *et al.* (1990, 1995, 1996). At IEOM, cadmium was analysed using graphite furnace AAS. Mercury was analysed by hydride generation and amalgam technique after the samples were burned in quartz tubes in an oxygen rich atmosphere and the mercury was collected in potassium permanganate. At CIR, samples were analysed by flame AAS (Perkin Elmer 303, 460 or 503). Low metal

Table 1. Cadmium concentrations (µg/g ww) in ringed seals from Greenland and Svalbard. Number of observations (N), geometric mean (GM) and 95% confidence intervals (95% CI) in different age groups are shown. Only municipalities with N ≥3 are presented, though all data are included in 'All'.

Age group years	Mean age years	Municipality	Muscle			Liver			Kidney		
			N	GM	95% CI	N	GM	95% CI	N	GM	95% CI
All	5.4	All	455	0.067	0.060-0.075	454	7.79	7.00-8.67	456	33.5	30.5-36.6
0	0.0	All	18	0.046	0.027-0.081	19	3.24	1.82-5.79	20	15.3	8.28-28.2
1	1.0	All	123	0.034	0.028-0.040	123	5.48	4.56-6.58	122	25.5	21.6-30.1
	1.0	Avanersuaq	14	0.035	0.017-0.074	14	2.84	1.11-7.28	13	16.9	6.76-42.5
	1.0	Upernavik	12	0.200	0.119-0.337	12	15.3	11.2-21.0	12	73.4	56.0-96.2
	1.0	Uummannaq	75	0.029	0.025-0.035	75	6.09	5.11-7.26	75	27.6	23.7-32.1
	1.0	Nanortalik	10	0.010	<0.015-0.013	10	1.91	1.26-2.90	10	8.93	6.41-12.4
	1.0	Ittoqqortoormiit	9	0.035	0.028-0.044	9	7.55	5.08-11.2	9	25.6	16.3-40.5
2-4	2.8	All	155	0.067	0.056-0.081	154	9.40	7.83-11.3	155	40.7	35.5-46.7
	3.0	Avanersuaq	22	0.206	0.137-0.308	22	13.5	5.98-30.5	22	72.9	46.1-115
	3.1	Upernavik	21	0.295	0.175-0.497	21	22.3	18.3-27.2	21	71.7	58.6-87.7
	2.5	Uummannaq	79	0.036	0.030-0.042	79	6.69	4.46-8.20	79	32.9	28.1-38.5
	3.0	Ittoqqortoormiit	24	0.070	0.055-0.089	24	13.9	10.5-18.4	24	46.7	37.9-57.4
	3.1	Svalbard	7	0.021	<0.015-0.058	7	2.94	0.998-8.65	7	10.9	3.01-39.4
5-10	6.8	All	86	0.119	0.094-0.151	86	13.4	10.8-16.7	88	48.3	39.4-59.2
	7.4	Avanersuaq	12	0.311	0.218-0.444	11	36.8	25.1-53.6	12	111	70.5-175
	6.1	Upernavik	10	0.446	0.213-0.933	10	36.4	24.9-53.0	10	108	75.0-157
	6.5	Uummannaq	16	0.046	0.023-0.092	16	5.68	2.84-11.4	16	35.3	18.0-69.2
	6.7	Ittoqqortoormiit	20	0.113	0.077-0.167	22	15.2	12.2-19.0	22	42.3	31.6-56.6
	7.0	Kong Oscars Fjord	5	0.073	0.034-0.154	5	10.8	4.94-23.7	5	51.0	21.8-119
	7.2	Danmarkshavn	6	0.063	0.030-0.132	6	8.58	4.21-17.5	6	22.1	9.33-52.4
	6.9	Svalbard	17	0.102	0.082-0.127	16	9.11	6.10-13.6	17	34.2	22.2-53.0
11-15	13.1	All	25	0.130	0.077-0.218	25	8.86	5.61-14.0	25	32.4	20.6-50.9
>15	20.8	All	48	0.112	0.079-0.160	47	5.18	3.55-7.57	46	25.2	18.3-34.7
	23.2	Avanersuaq	4	0.296	0.094-0.938	4	12.3	1.74-86.5	3	58.1	5.35-631
	19.7	Uummannaq	3	0.024	<0.015-0.857	3	4.27	0.863-21.2	3	33.3	5.92-187
	21.6	Ittoqqortoormiit	7	0.184	0.129-0.263	7	12.1	6.17-23.7	6	65.5	44.2-97.1
	20.0	Kong Oscars Fjord	11	0.089	0.054-0.146	11	3.41	1.16-10.0	11	20.6	11.2-37.7
	18.5	Danmarkshavn	10	0.197	0.076-0.509	9	4.79	2.44-9.42	10	22.1	12.0-40.7
	22.8	Svalbard	12	0.065	0.027-0.158	12	3.42	1.41-8.32	12	14.6	6.07-35.1

Table 2. Mercury concentrations (mg/g ww) in ringed seals from Greenland and Svalbard. Number of observations (N), geometric mean (GM) and 95% confidence intervals (95% CI) in different age groups are shown. Only municipalities with N≥3 are presented, though all data are included in 'All'.

Age group years	Mean age years	Municipality	Muscle			Liver			Kidney		
			N	GM	95% CI	N	GM	95% CI	N	GM	95% CI
All	7.5/7.6	All	295	0.210	0.188-0.236	248	2.59	2.17-3.09	246	0.956	0.859-1.06
0	0.0	All	10	0.064	0.028-0.146	10	0.617	0.406-0.940	10	0.648	0.432-0.975
1	1.0	All	56	0.122	0.101-0.148	56	0.810	0.676-0.971	55	0.545	0.472-0.63
	1.0	Avanersuaq	14	0.152	0.088-0.262	14	0.621	0.379-1.02	13	0.561	0.355-0.877
	1.0	Upernavik	10	0.182	0.132-0.252	10	0.868	0.690-1.09	10	0.616	0.468-0.811
	1.0	Uummannaq	10	0.068	0.055-0.084	10	0.514	0.383-0.689	10	0.368	0.294-0.460
	1.0	Nanortalik	10	0.084	0.064-0.109	10	0.948	0.754-1.19	10	0.464	0.404-0.534
	1.0	Ittoqqortoormiit	9	0.174	0.128-0.235	9	1.46	1.13-1.89	9	0.797	0.605-1.05
2-4	3.0	All	53	0.211	0.172-0.259	43	1.99	1.61-2.45	44	0.868	0.729-1.03
	3.0/2.8	Avanersuaq	21	0.225	0.145-0.349	12	1.58	1.29-1.94	12	0.813	0.682-0.971
	3.0	Ittoqqortoormiit	24	0.251	0.215-0.294	24	2.78	2.16-3.59	24	1.05	0.899-1.24
	3.0	Svalbard	6	0.094	0.052-0.169	6	1.01	0.499-2.03	6	0.353	0.246-0.506
5-10	6.8	All	76	0.217	0.188-0.281	75	3.13	2.33-4.22	75	1.02	0.828-1.26
	7.4	Avanersuaq	12	0.241	0.086-0.679	12	2.59	1.56-4.33	11	0.966	0.655-1.43
	6.1	Upernavik	10	0.215	0.154-0.301	10	3.46	1.91-6.22	10	1.10	0.897-1.35
	6.9	Uummannaq	7	0.191	0.097-0.375	7	0.878	0.460-1.68	7	0.920	0.490-1.73
	6.7	Ittoqqortoormiit	22	0.292	0.257-0.332	22	5.89	4.72-7.35	22	1.57	1.21-2.05
	7.3	Kong Oscars Fjord	4	0.684	0.191-2.45	4	30.0	6.75-134	4	4.65	1.75-12.3
	7.2	Danmarkshavn	6	0.553	0.352-0.867	6	12.5	6.15-25.4	6	2.01	0.895-4.51
	6.7	Svalbard	14	0.070	0.054-0.090	14	0.691	0.422-1.13	15	0.288	0.225-0.369
11-15	13.1	All	18	0.305	0.172-0.542	18	8.60	4.44-16.7	18	1.43	0.925-2.20
>15	20.9	All	47	0.388	0.316-0.478	46	8.56	5.41-13.6	44	1.76	1.36-2.27
	23.3	Avanersuaq	4	0.416	0.175-0.991	4	1.47	0.575-3.77	3	1.03	0.166-6.37
	19.7	Uummannaq	3	0.266	0.043-1.65	3	1.27	0.141-11.5	2	2.37	0.515-10.9
	21.6	Ittoqqortoormiit	7	0.323	0.235-0.445	7	14.4	6.05-34.4	6	2.74	1.75-4.25
	20.0	Kong Oscars Fjord	11	0.691	0.505-0.946	10	24.9	7.85-79.1	11	3.64	2.72-4.86
	18.5	Danmarkshavn	10	0.684	0.506-0.927	10	20.3	9.42-43.9	10	2.51	2.09-3.01
	23.2	Svalbard	11	0.174	0.148-0.206	11	3.97	1.88-8.40	11	0.557	0.436-0.711

Table 3. Zinc and selenium (mg/g ww) in ringed seals from Greenland. Number of observations (N), geometric mean (GM) and 95% confidence intervals (95% CI) in different age groups are shown.

Age group years	Mean age years	Muscle			Liver			Kidney		
		N	GM	95% CI	N	GM	95% CI	N	GM	95% CI
Zinc										
All	5.4	438	23.1	22.5-23.7	435	46.2	45.3-47.1	435	46.3	45.0-47.8
0	0.0	20	26.2	23.9-28,6	20	41.4	38.2-45.0	20	43.6	38.1-50.0
1	1.0	121	22.8	21.7-23.8	121	43.6	42.4-45.0	120	43.4	41.0-45.8
2-4	2.8	144	22.5	21.6-23.4	143	47.7	46.1-49.3	144	46.3	44.1-48.8
5-10	6.8	82	23.3	21.8-24.9	80	49.3	47.0-51.8	82	51.7	48.4-55.2
11-15	13.0	24	23.4	20.8-26.2	24	47.7	44.6-51.1	24	47.5	41.4-54.5
>15	20.8	47	24.4	21.7-27.4	47	44.6	41.2-48.3	45	46.0	41.3-51.3
Selenium										
All	8.2/6.6/7.1	87	0.243	0.217-0.272	118	1.95	1.61-2.37	64	2.54	2.31-2.80
0	0.0	9	0.265	0.186-0.378	9	1.12	0.804-1.55	3	2.12	0.817-5.52
1	1.0	21	0.217	0.179-0.262	41	1.15	1.00-1.33	19	2.81	2.24-3.51
2-4	3.2	12	0.367	0.293-0.459	11	1.95	1.32-2.88	12	3.03	1.44-3.77
5-10	7.5/6.8/7.3	14	0.271	0.207-0.355	30	2.36	1.62-3.46	12	2.61	2.07-3.28
11-15	12.9/12.0/12.7	9	0.139	0.095-0.204	5	4.44	0.834-23.7	7	2.16	1.82-2.57
>15	19.7/19.9	22	0.244	0.187-0.320	22	4.19	2.12-8.30	11	1.99	1.63-2.43

concentrations were extracted using Ammonium pyrrolidinedithiocarbamate (APDC) and Methyl isobutyl ketone (MIBK) (Johansen *et al.* 1991).

Quality assurance

The analytical quality was checked by repeating analyses and by the frequent use of various reference standards; in particular Tort-1 (lobster hepatopancreas), supplied by the National Research Council of Canada (Marine Analytical Chemistry Standards Program), and the dried tuna internal standard of the National Food Agency of Denmark. The DAE laboratory participates in the international intercalibration exercises conducted by the International Council for the Exploration of the Sea (ICES), and by the Department of Fisheries and Oceans (DFO), Winnipeg, Canada.

Detection limits and conversion factors

For laboratory analyses the detection limits for cadmium, mercury and selenium, were 0.015, 0.005 and 0.20µg/g wet weight respectively. All concentrations are reported as µg/g wet weight (ww). For conversion into µg/g dry weight, the factors 3.37 (muscle), 3.38 (liver), and 4.47 (kidneys) were estimated. These values were computed from the means of dry weight percentages routinely recorded in the laboratory.

Statistical analysis

For statistical treatment, values below the detection limit were recorded as being half the detection limit value prior to logarithmic transformation. The assumptions of normal distribution and homogeneity of variance were met after logarithmic transformation of the data. StatView 4.5 (Abacus Concepts Inc.) for Mac-

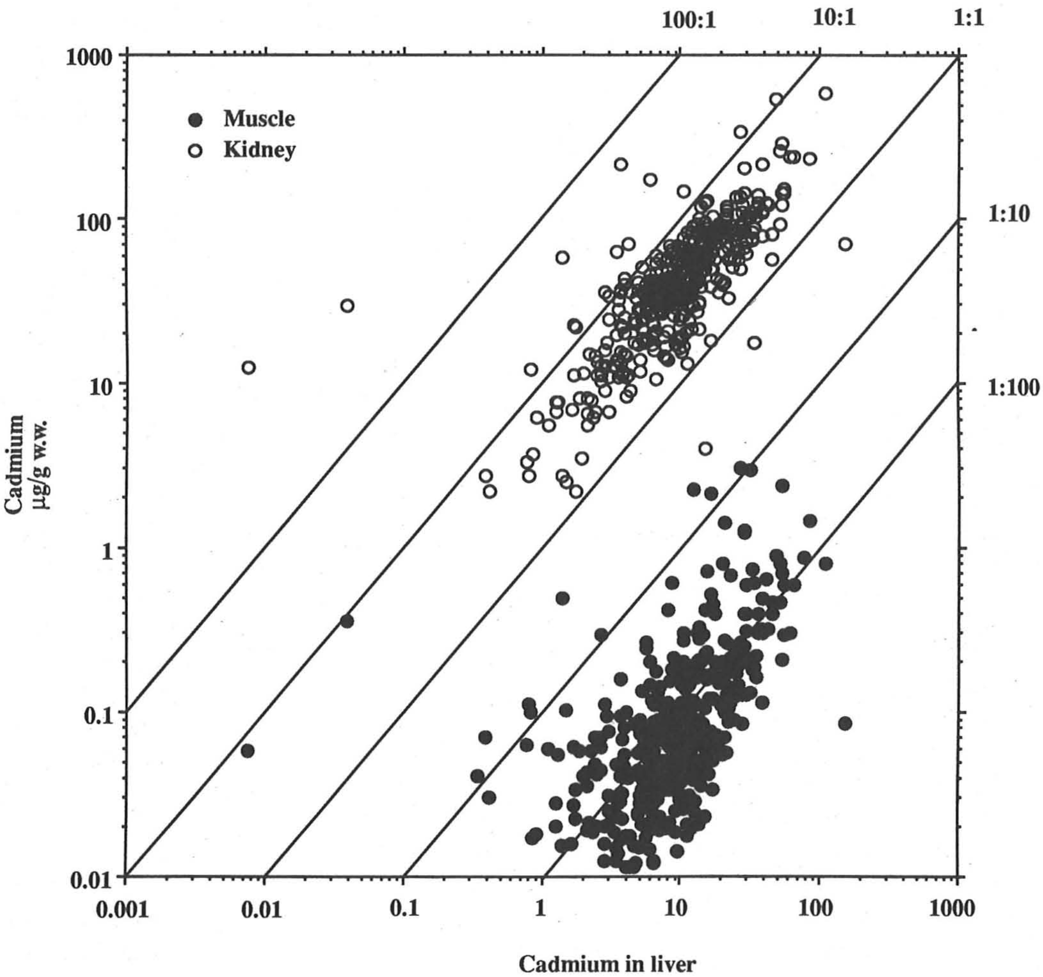


Fig. 2 Cadmium concentration in the liver versus concentrations in the muscle and kidneys of ringed seals from Greenland. Lines indicate the decal ratios.

intosh was used to perform parametric correlation analysis and t-tests on the transformed data. Analysis of variance (ANOVA) and comparison among group means (Tukey) were performed on transformed data using SuperANOVA 1.11 (Abacus Concepts Inc.) for Macintosh, supplemented with tables from Zar (1984). Two factor ANOVA was used to test for the element concentrations dependency of location and age in all tissues. One-way ANOVAs were performed to further examine the differences in cadmium and mercury levels between the areas of comparable age groups. Age-accumulation factors in ringed seals were computed from least square estimates of age class effect in two-way analyses of variance of log-concentration, with sampling area as the other factor. The differences between the least square estimates for one-year-olds compared with each of the three other age groups were tested for statistical differences using a t-test.

RESULTS

Cadmium and mercury data in ringed seal from the municipalities sampled are presented for the following age groups: one year, 2-4 years, 5-10 years old and > 15 years, in order to elucidate geographical differences (Tables 1 and 2). Data from the remaining age groups (new-born and 11-15 years old) and all the data for zinc and selenium are presented without geographical subdivision, as the sample size is too small to allow meaningful comparisons. In the case of zinc the metal is believed to be regulated by the organism, and hence is unlikely to show geographical differences (Table 3). Few significant differences were detected between sexes, and among those that were significant no consistencies were found. Hence, gender was omitted as a covariate in the statistical analysis and data presentations.

Tissue differences

Ringed seals accumulate most cadmium in the kidneys (GM = 33.5µg/g ww), with the level being 4.3 and c. 500 times higher than in the liver (GM = 7.79 µg/g ww) and muscle (GM = 0.067µg/g ww) respectively (Tables 1 and 4). The kidney/liver cadmium ratio is independent of concentration level, whereas liver/muscle ratio decreases as a function of concentration (Fig. 2). However, individual variation may be considerable.

In general, the differences in tissue concentrations of mercury are less pronounced than for cadmium. The liver of ringed seal is the major organ for deposition of mercury, the average level (GM = 2.59µg/g ww) being about 2.5 and 12 times higher than in kidney (GM = 0.956µg/g ww) and muscle tissue (GM = 0.210µg/g ww), respectively (Table 2 and 4). The ratio between mercury in muscle and kidneys versus liver increases with increasing liver concentration (Fig. 3).

The zinc concentrations in liver and kidneys of ringed seal are almost identical (GM = 46µg/g ww) and about twice the concentration found in muscle (see Table 3).

The concentration of selenium in ringed seals is about the same in liver and kidneys (GM = 2.54 and 1.95µg/g ww, respectively) and about 10 times higher than in muscle tissue (see Table 3).

Geographical comparisons among different ringed seal age groups

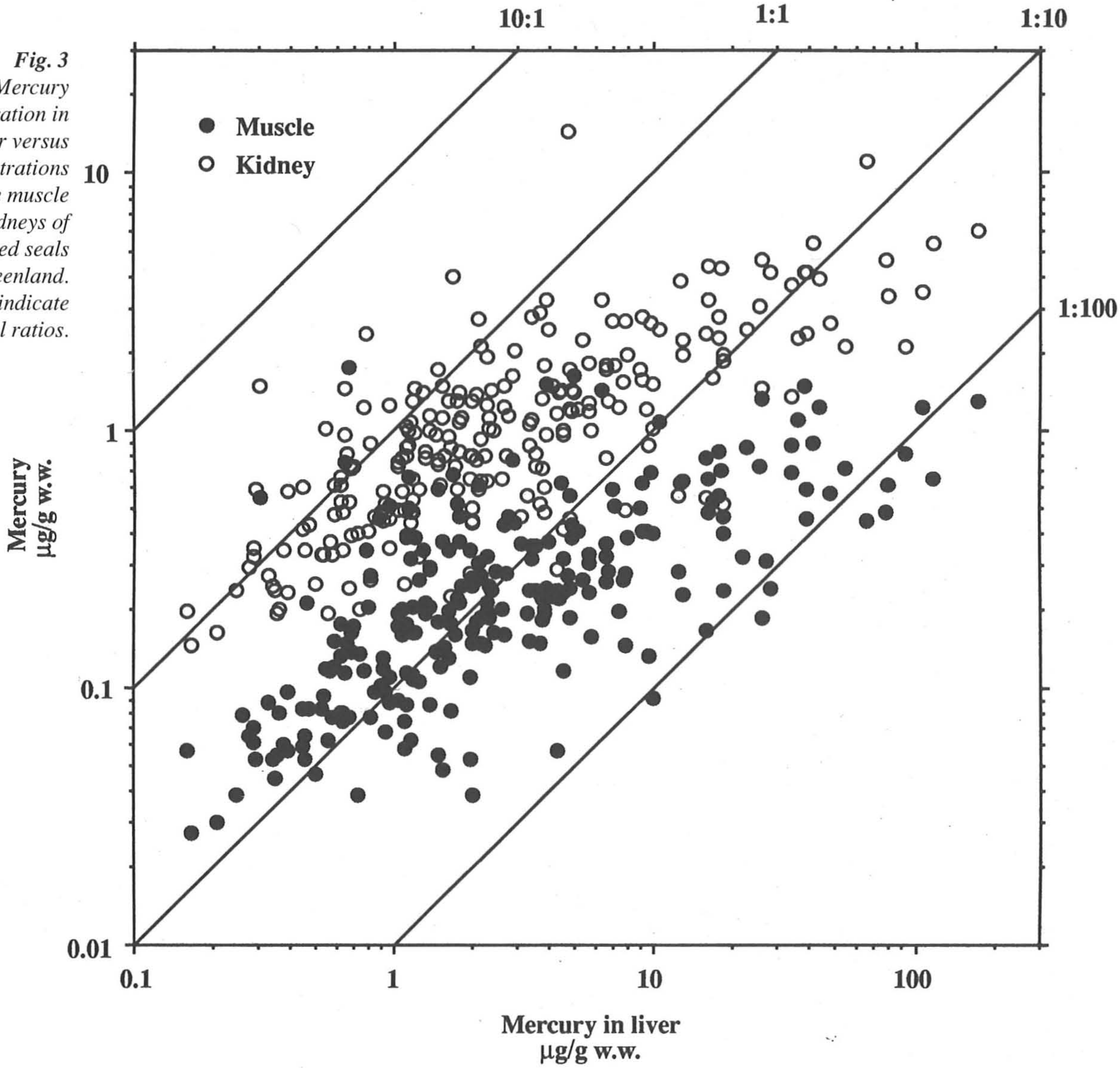
Cadmium

There is considerable variation in the cadmium level between geographical areas, and also between individuals, as reflected by the wide 95% confidence intervals (Table 1). In one-year-old seals, cadmium concentration in muscle varies by a factor of up to 20 between the geographical areas, and a factor of 8 in liver and kidneys. In 5-10 year-old and older seals, the levels vary by factors of 4-10 (Table 1).

Cadmium level is strongly dependent on age and municipality, and there is a significant interactive effect between these two variables (Table 5). Thus, the accumulation of cadmium varies with age from one area to another. With some exceptions, multiple comparisons show a tendency towards higher cadmium levels in the northern municipalities for seals of the same age groups (Table 6).

Among one-year old ringed seals, the cadmium levels in muscle and kidneys were significantly lower in Nanortalik compared with municipalities in West Greenland (Uummannaq, Uperna-

Fig. 3
Mercury concentration in the liver versus concentrations in the muscle and kidneys of ringed seals from Greenland. Lines indicate the decal ratios.



vik and Avanersuaq). A similar pattern was found in liver tissue from most of the West Greenland municipalities, although for seals from Avanersuaq the levels were not significantly higher than in seals from Nanortalik. Seals from Nanortalik also had lower cadmium concentrations than seals from Ittoqqortoormiit; the only East Greenland municipality from which this age group was analysed. Cadmium levels in ringed seals from Avanersuaq were significantly lower than in seals from Upernavik and Uummannaq, except for muscle, which was not significantly different from the concentration found in seals from Uummannaq. However, the Avanersuaq data are highly variable, as indicated by the unusually large 95% confidence intervals (Table 1). Among the one-year-old ringed seals, the cadmium concentration in general was highest in Upernavik compared with the other areas.

In 2-4 and 5-10 year-old ringed seals, the cadmium concentrations were significantly higher in seals from Upernavik and Avanersuaq compared with seals from Uummannaq, East Greenland and Svalbard. The only exceptions were the levels in liver and kidney in 2-4 and 5-10 year-old seals from Ittoqqortoormiit, where cadmium concentrations were of a similar level. Only minor differences were observed between areas on the Greenland East Coast (Ittoqqortoormiit, Danmarkshavn and Kong Oscars

Table 4. Ratios (calculated from geometric means) between concentrations of cadmium and mercury in muscle, liver and kidney for different age groups of ringed seals.

Cadmium	Kidney/Liver		Kidney/Muscle		Liver/Muscle	
Age group	N	Ratio	N	Ratio	N	Ratio
0	19	5.14	18	377	18	71.9
1	122	4.55	122	761	123	163.0
2-4	154	4.38	155	604	154	139.0
5-10	85	3.54	85	404	83	111.0
11-15	25	3.65	25	249	25	68.2
>15	45	5.24	46	240	47	50.3
All	450	4.31	451	507	450	117.0
Mercury	**Liver/Kidney**		**Liver/Muscle**		**Kidney/Muscle**	
Age group	N	Ratio	N	Ratio	N	Ratio
0	10	0.952	10	9.68	10	10.20
1	55	1.52	56	6.62	55	4.42
2-4	43	2.39	43	8.45	44	3.72
5-10	73	2.99	74	14.20	74	4.79
11-15	18	6.03	17	25.80	17	4.44
>15	43	5.39	46	22.40	44	4.40
All	242	2.75	246	12.20	244	4.54

Fjord). The cadmium concentrations in tissues from ringed seals collected at Svalbard were not significantly different from the concentrations in 5-10 year-old seals from East Greenland. However, for seals aged between 2-4 years, tissue cadmium levels were lowest in seals from Svalbard.

Among old seals (>15 years), cadmium levels were found to be highest in ringed seals from Avanersuaq, although not significantly higher than in seals from Danmarkshavn and Ittoqqortoormiit for example. The cadmium level in muscle tissue was more than 10 times higher in ringed seals from Avanersuaq compared with Uummannaq, and three and two times higher in liver and kidney tissues, respectively. Seals from Kong Oscars Fjord in East Greenland, and to some extent from Danmarkshavn, had higher cadmium concentrations compared with seals from the more southern municipality of Ittoqqortoormiit. In seals >15 years from Svalbard, the cadmium concentrations in all three tissues were among the lowest, as also found for the 2-4 year-old seals.

Mercury

The differences in mercury levels among the different areas of Greenland are moderate for most age groups and tissue combinations. In tissues from one-year old seals, the geometric means varied by no more than a factor of three (Table 2). A similar variation was found in kidney samples from adult and old seals. However, the mean concentrations in the liver varied by a factor as large as 20 among the different areas.

A two-way ANOVA showed that the mercury concentration was strongly dependent on location and age (Table 5). Multiple comparisons (Fishers PLSD-test) showed that mercury levels were generally higher in ringed seals from East Greenland compared with municipalities in West Greenland (Table 6). The tissue levels in one-year-old ringed seals from Ittoqqortoormiit were in several cases significantly higher than in seals from Avanersuaq, Uummannaq and Nanortalik. However, the mercury levels recorded in Ittoqqortoormiit were similar to these levels in seals from Upernavik. The concentrations in adults (5-10 years) and old ringed

Table 5. Two-way ANOVA between municipality (Mun) and age for ringed seals from Greenland.

Muscle	N	Mun	Age	Mun*Age
Cadmium	454	***	***	***
Mercury	295	***	***	***
Zinc	438	-	-	*
Selenium	87	*	***	**
Liver	N	Mun	Age	Mun*Age
Cadmium	54	***	***	***
Mercury	48	***	***	***
Zinc	35	***	-	**
Selenium	18	***	***	*
Kidney	N	Mun	Age	Mun*Age
Cadmium	456	***	***	***
Mercury	246	***	**	***
Zinc	435	***	-	-
Selenium	64	**	**	*

* $0.01 < P < 0.05$
** $0.001 < P < 0.1$
*** $P < 0.001$

seals (>15 years) from East Greenland (Kong Oscars Fjord, Danmarkshavn and Ittoqqortoormiit) were higher than in ringed seals from West Greenland in all cases, with the exception of muscle concentrations in seals from Avanersuaq, which were higher than in seals from Ittoqqortoormiit. In most cases, the differences were significant and most pronounced for seals from Danmarkshavn and Kong Oscars Fjord.

The variation in mercury level was only moderate from north to south. The mercury levels in liver and kidney in one-year-old seals from Nanortalik for example, were similar to the levels in Avanersuaq and Upernavik, whereas the levels in muscle were lower. The lowest mercury level was found in seals from Uummannaq. There were no significant differences between adult seals from Avanersuaq and Upernavik, whereas Uummannaq was significantly lower in two out of three comparisons. In old seals no clear difference was found between seals from Avanersuaq and Uummannaq. On the east coast, the highest concentrations were found in seals from Kong Oscars Fjord, followed by slightly lower levels in the more northern region around Danmarkshavn. The lowest levels were observed in the more southern municipality of Ittoqqortoormiit, being significantly different in only some of the cases (Table 6).

The mercury concentrations in ringed seals from Svalbard were low, and in most cases significantly lower than in seals from East and West Greenland.

Zinc
The zinc levels ranged from 33.1µg/g ww in ringed seals from Nanortalik, to 54.7µg/g ww in those from Upernavik. A two-way ANOVA showed that zinc concentrations in liver and kidneys may differ between municipalities (Table 5). Age, however, was of no importance in any of the tissues. Multiple comparisons revealed only few significant differences between areas. The only significant difference in zinc levels was found in one-year-old seals from Nanortalik which had significantly lower kidney tissue levels than in any of the other subgroups (Upernavik, Avanersuaq, Ittoqqortoormiit and Uummannaq).

Selenium
For selenium the same significant differences were found between age and municipalities as observed for cadmium and mercury. Due to the regulative properties of the micronutrient selenium and the smaller number of samples (N from 64 to 118), the comparisons were significant at a lower level than for cadmium and mercury.

Accumulation with age
In order to extract a general pattern, age accumulation factors were calculated between seals aged one year and the older age groups (Table 7). The increase of cadmium in ringed seals from one year to 2-4 years is moderate (1.42-1.60), whereas, the increase to 5-10 years is

Table 6. Geographic comparison for cadmium and mercury (geometric means) in ringed seals from Greenland and Svalbard. Continuous underlining indicates areas that are not significantly different ($P > 0.05$) by multiple comparisons Fishers PLSD-test. The following municipalities and areas were tested: Avanersuaq (Ava), Upernavik (Upv), Uumannaq (Uum), Nanortalik (Nan), Ittoqqortotmiit (Itt), Kong Oscars Fjord (Kof), Danmarkshavn (Dmh) and Svalbard (Sva).

CADMIUM

MUSCLE

1 year old						
Upv	Ava	Itt	Uum	Nan		
(12)	(14)	(9)	(75)	(10)		
0.200	0.035	0.035	0.029	0.010		
2-4 years old						
Upv	Ava	Itt	Uum	Sva		
(21)	(22)	(24)	(79)	(7)		
0.295	0.206	0.070	0.036	0.021		
5-10 years old						
Upv	Ava	Itt	Sva	Kof	Dmh	Uum
(10)	(12)	(20)	(17)	(5)	(6)	(16)
0.446	0.311	0.113	0.102	0.073	0.063	0.046
> 15 years old						
Ava	Dmh	Itt	Kof	Sva	Uum	
(4)	(10)	(7)	(11)	(12)	(3)	
0.296	0.197	0.184	0.089	0.065	0.024	

LIVER

1 year old							
Upv	Itt	Uum	Ava	Nan			
(12)	(9)	(75)	(14)	(10)			
15.3	7.55	6.09	2.84	1.91			
2-4 years old							
Upv	Itt	Ava	Uum	Sva			
(21)	(24)	(22)	(79)	(7)			
22.3	13.9	13.5	6.69	2.94			
5-10 years old							
Ava	Upv	Itt	Kof	Sva	Dmh	Uum	
(11)	(10)	(22)	(5)	(16)	(6)	(16)	
36.8	36.4	15.2	10.8	9.11	8.58	5.68	
> 15 years old							
Ava	Itt	Dmh	Uum	Sva	Kof		
(4)	(7)	(9)	(3)	(12)	(11)		
12.3	12.1	4.97	4.27	3.42	3.41		

KIDNEY

1 year old						
Upv	Uum	Itt	Ava	Nan		
(12)	(75)	(9)	(13)	(10)		
73.4	27.6	25.6	16.9	8.93		
2-4 years old						
Ava	Upv	Itt	Uum	Sva		
(22)	(21)	(24)	(79)	(7)		
72.9	71.7	46.7	32.9	10.9		
5-10 years old						
Ava	Upv	Itt	Kof	Sva	Dmh	Uum
(12)	(10)	(22)	(5)	(17)	(6)	(16)
111	108	51.0	42.3	35.3	34.2	22.1
> 15 years old						
Itt	Ava	Uum	Dmh	Kof	Sva	
(6)	(3)	(3)	(10)	(11)	(12)	
65.5	58.1	33.3	22.1	20.6	14.6	

Table 6 (continued)

MUSCLE — LIVER — KIDNEY

MERCURY

1 year old

MUSCLE	Upv	Itt	Ava	Nan	Uum
	(10)	(9)	(14)	(10)	(10)
	0.182	0.174	0.152	0.084	0.068

LIVER	Itt	Nan	Upv	Ava	Uum
	(9)	(10)	(10)	(14)	(10)
	1.46	0.948	0.868	0.621	0.514

KIDNEY	Itt	Upv	Ava	Nan	Uum
	(9)	(10)	(13)	(10)	(10)
	0.797	0.616	0.561	0.464	0.368

2-4 years old

MUSCLE	Itt	Ava	Sva
	(24)	(21)	(6)
	0.251	0.225	0.094

LIVER	Itt	Ava	Sva
	(24)	(12)	(6)
	2.78	1.58	1.01

KIDNEY	Itt	Ava	Sva
	(24)	(12)	(6)
	1.05	0.813	0.353

5-10 years old

MUSCLE	Kof	Dmh	Itt	Ava	Upv	Uum	Sva
	(4)	(6)	(22)	(12)	(10)	(7)	(14)
	0.684	0.553	0.292	0.241	0.215	0.191	0.070

LIVER	Kof	Dmh	Itt	Upv	Ava	Uum	Sva
	(4)	(6)	(22)	(10)	(12)	(7)	(14)
	30.0	12.5	5.89	3.46	2.59	0.878	0.691

KIDNEY	Kof	Dmh	Itt	Upv	Ava	Uum	Sva
	(4)	(6)	(22)	(10)	(11)	(7)	(15)
	4.65	2.01	1.57	1.10	0.966	0.920	0.288

> 15 years old

MUSCLE	Kof	Dmh	Ava	Itt	Uum	Sva
	(11)	(10)	(4)	(7)	(3)	(11)
	0.691	0.684	0.416	0.323	0.266	0.174

LIVER	Kof	Dmh	Itt	Sva	Ava	Uum
	(10)	(10)	(7)	(11)	(4)	(3)
	24.9	20.3	14.4	3.97	1.47	1.27

KIDNEY	Kof	Itt	Dmh	Uum	Ava	Sva
	(11)	(6)	(10)	(2)	(3)	(11)
	3.64	2.74	2.51	2.37	1.03	0.557

higher (1.99-2.65). All increases are significant relative to one-year-old seals (Table 7). A further increase in the cadmium level takes place in muscle tissue of seals >15 years (3.02), whereas a decrease occurs in liver and kidney tissue. Cadmium concentrations in the liver drop to about half the level in the 5-10 year-old seals, and approach the levels found in one-year-old seals (Fig. 4). For cadmium, the kidney/liver ratio remains relatively constant throughout the animal's lifetime (Table 4). The ratio of kidneys and liver to muscle concentrations are lowest for yearling seals, highest in one-year-old seals, and hereafter the ratio shows a decline to approximately one third in the oldest age group. A rough extrapolation of cadmium levels between liver and kidneys can therefore be performed independent of age, whereas this is not the case for muscle tissue.

The concentration of mercury in ringed seals increases in all tissues from one-year olds to older seals (Tables 2 and 7). Accumulation is most pronounced in the liver, where it more than triples from one-year old seals to 5-10 year-old seals, and almost doubles by the time seals are >15 years old. In general, the age accumulation factors for mercury in liver are higher than for cadmium. The levels increase by 1.57-1.99 times from one year to 2-4 years, and are even more pronounced for 5-10 year-olds (1.99-2.65) and >15 year-olds (2.85-6.94). Thus, mercury accumulates throughout the entire lifetime of ringed seals in all three tissues examined (Fig. 5). Calculations of the ratios relative to age revealed an increase in the liver-kidney ratio throughout the life, increasing by almost 6 times in the oldest seals compared with the youngest seals. The liver-muscle ratio increased by more than 3 times the level in one-year-old seals. The ratio between the mercury concentrations in kidneys and muscle are more or less constant for age groups older than yearlings, whereas the ratio is twice as high for

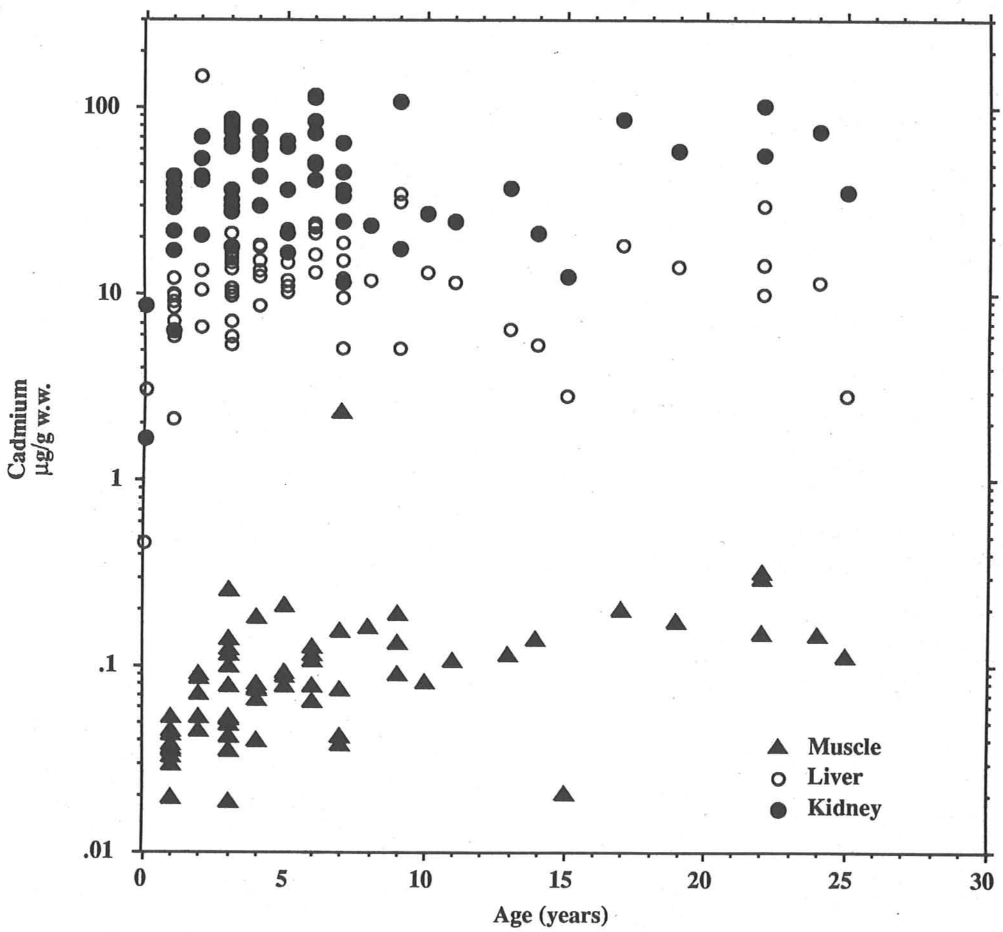
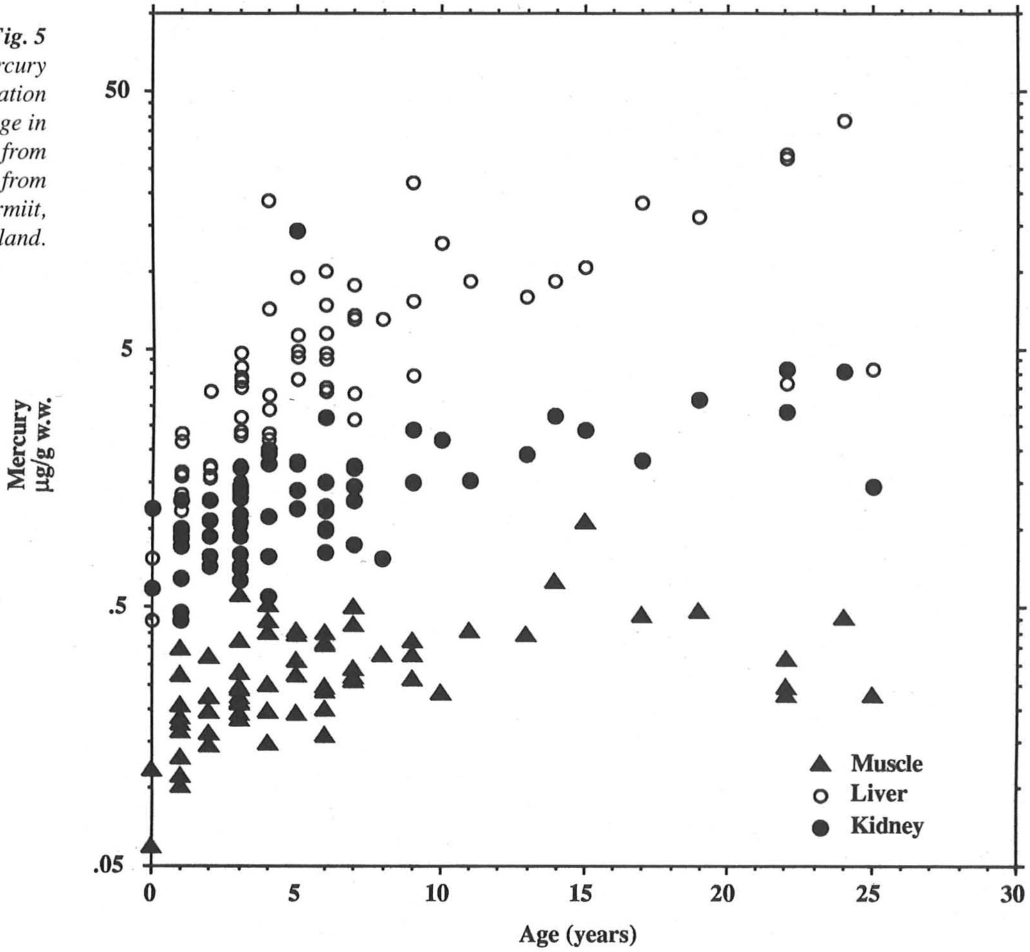


Fig. 4
Cadmium concentration versus age in tissues from ringed seals from Ittoqqortoormiit, Greenland.

Fig. 5
Mercury concentration versus age in tissues from ringed seals from Ittoqqortoormiit, Greenland.



yearling ringed seals (Table 4). Mercury therefore increases relatively more by age in liver compared to both kidney and muscle.

The calculation of age accumulation factors for zinc underlines that the concentration of this essential metal only changes slightly with age in ringed seals. There are no significant differences in zinc concentrations in muscle, while the concentrations in liver and kidneys show a slight increase with time, some being significant. Zinc levels in liver and kidneys in 2-4 year-old and 5-10 year-old seals are slightly higher (1.02-1.17) than in one-year-old seals (Table 7). A minor decrease in seals older than 15 years (1.02-1.14) can be detected.

No distinct age accumulation was found for selenium in muscle and kidney tissue. However, the increase in liver (1.7) was significant for ringed seals older than 5 years of age (Table 7).

Inter tissue correlations
Cadmium concentrations in muscle, liver and kidneys were found to be strongly pairwise correlated (Table 8; Fig. 2). The variation in the tissue ratios for different age groups was presented in the section on 'Tissue differences'.

When three (or more) variables have a significant pairwise correlation it may be that one of the three relations is indirect and only significant because of the correlation of the two variables to the third variable. Partial correlation coefficients correct for such interactions of three or more variables by mathematically holding the third variable constant (see e.g. Sokal and Rohlf 1981).

For partial correlations the correlation coefficients were not as strong, but had a similar level of significance. Although cadmium shows a decrease in liver and kidneys in the oldest age

Table 7. Age accumulation factors in ringed seal. Computed from least square estimates of age class effect in two-way analyses of variance of log-concentration with sampling area as the other factor. N = number of observations included in the analysis of variance. The asterisks indicate differences between the least square estimates for one years old compared with each of the three other age groups.

Element	Tissue	Total N	From age 1 year to 2-4 years	From age 1 year 5-10 years	From age 1 year to >15 years
Cadmium	Muscle	412	1.60 /***	2.65 /***	3.02 /***
	Liver	410	1.45 /**	2.27 /***	1.21
	Kidney	411	1.42 /***	1.99 /***	1.45 /*
Mercury	Muscle	232	1.58 /**	1.81 /***	2.85 /***
	Liver	220	1.99 /***	3.49 /***	6.94 /***
	Kidney	218	1.57 /***	2.03 /***	2.96 /***
Zinc	Muscle	394	0.97	0.94	0.96
	Liver	391	1.06 /**	1.08 /*	1.02
	Kidney	391	1.02	1.17 /***	1.14 /*
Selenium	Muscle	69	1.78 /**	1.21	1.06
	Liver	104	1.64	1.71 /**	1.70 /*
	Kidney	54	1.27	1.14	0.91

* $0.01 \le P < 0.05$, ** $0.001 \le P < 0.01$, *** $P < 0.001$

group (> 15 years) the decline is small. Furthermore, the relatively large number of analyses from the younger age groups dictates the strong correlation between these two tissue groups and muscle tissue.

There is also a strong correlation in mercury concentrations between the analysed tissues (Table 8), even though animals with a high mercury burden store relatively more mercury in liver compared to muscle and kidney tissue

Table 8. Ringed seal inter tissue correlation coefficients (pairwise and partial) regarding muscle, liver and kidney. N indicated in parenthesis.

	Inter-tissue correlation		
	Muscle-Liver	Muscle-Kidney	Liver-Kidney
Cadmium(446)			
Pairwise	0.625/***	0.707/***	0.755/***
Partial	0.221/***	0.316/***	0.679/***
Mercury (238)			
Pairwise	0.678/***	0.651/***	0.809/***
Partial	0.312/***	0.405/***	0.529/***
Zinc (426)			
Pairwise	0.122/*	-0.068/-	0.484/***
Partial	0.177/***	-0.147/-	0.497/***
Selenium (52)			
Pairwise	0.161/-	0.595/***	0.222/-
Partial	0.037/-	0.581/***	0.159/-

* $0.01 \le P < 0.05$, ** $0.001 \le P < 0.01$, *** $P < 0.001$

(Fig. 3). The correlation coefficients for partial correlation revealed the same pattern as for cadmium.

The zinc concentrations in liver and kidneys were also strongly correlated, as was the concentration of selenium in muscle and kidneys (Table 8). Zinc was the only metal for which an increase was found in the correlation coefficient when considering partial correlation. The significance of the muscle-liver relation also improved when considering the partial correlation coefficient.

Inter element correlations

There was a strong pairwise correlation between cadmium and zinc in kidneys and liver and a less pronounced correlation in muscle (Table 9). The molar concentrations of zinc versus cadmium in kidneys seem to reach a ratio close to 1:1. This ratio is reached when the cadmium concentration is larger than 1,000nmol/g (112µg/g ww) in the kidneys. At lower cadmium levels, zinc concentrations may be up to 20 times higher than cadmium on a molar basis (Fig. 6).

Mercury was strongly correlated to selenium in the liver. The ratio is close to 1:1, where the mercury concentrations increase above 40 nmol/g, corresponding to about 18µg/g ww (Fig. 7). Cadmium and mercury were pairwise correlated in all tissues, whereas cadmium and selenium, as well as mercury and zinc were only correlated in liver and kidneys (Table 9).

The partial correlation coefficients confirm the strong pairwise correlation between cadmium and zinc in liver and kidneys and between mercury and selenium in liver (Table 9). However, the apparent pairwise correlation between cadmium and selenium in the liver, and between mercury and zinc in the liver and kidneys, are excluded by the partial correlation. Furthermore, the correlation coefficients for cadmium-selenium and mercury-zinc in liver are nega-

Table 9. Number of observations (N), pairwise and partial intra tissue correlation coefficients (CC) between element concentrations (pairwise and partial) and significance levels (SL) for ringed seals from Greenland.

	Muscle	Liver	Kidney
	N / CC / SL	N / CC / SL	N /CC / SL
Cadmium-Zinc			
Pairwise all	434/0.140 /**	433/0.554/***	435/0.695/***
Partial	84/0.081/-	103/0.617/***	52/0.535/***
Cadmium-Selenium			
Pairwise all	87/0.002/-	116/0.242/**	64/0.356/**
Partial	84/-0.007/-	103/-0.182/-	52/0.317/*
Cadmium-Mercury			
Pairwise all	258/0.298/***	246/0.185/**	246/0.187/**
Partial	84/0.285/**	103/0.275/**	52/0.277/*
Mercury-Selenium			
Pairwise all	84/0.032/-	105/0.905/***	52/-0.130/-
Partial	84/0.028/-	103/0.905/***	52/-0.261/-
Mercury-Zinc			
Pairwise all	239/-0.008/-	226/0.156/*	226/0.287/***
Partial	84/-0.038/-	103/-0.061/-	52/0.067/-

* $0.01 \leq P < 0.05$, ** $0.001 \leq P < 0.01$, *** $P < 0.001$

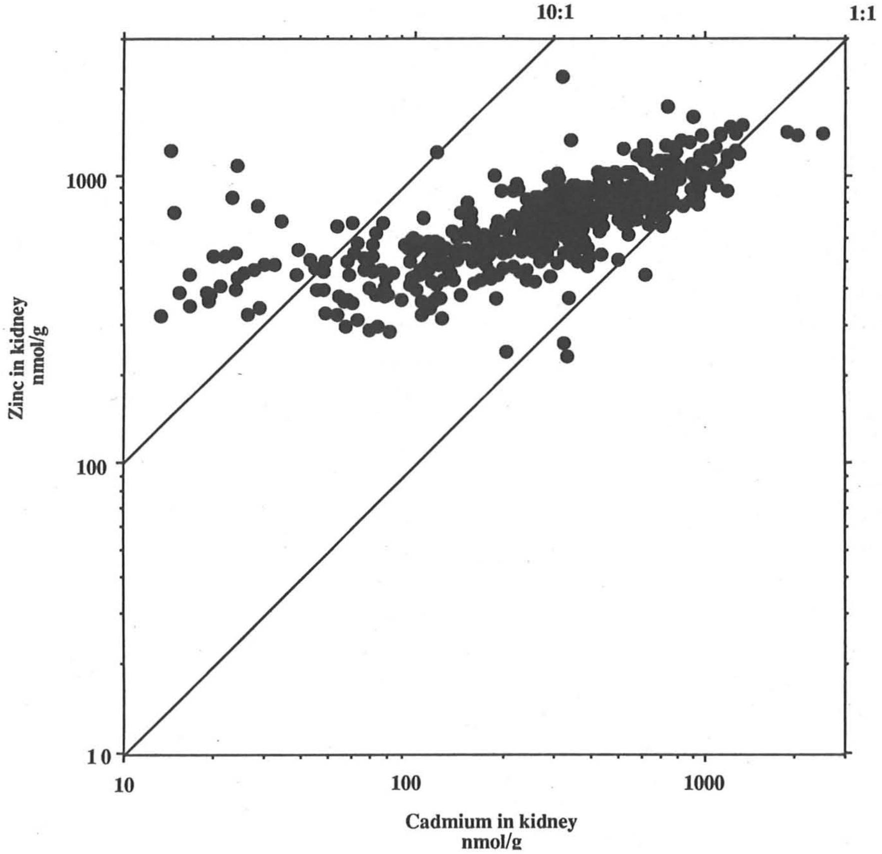


Fig. 6
Molar cadmium concentrations versus molar zinc concentrations in kidneys of ringed seals. Lines indicate the decal ratios.

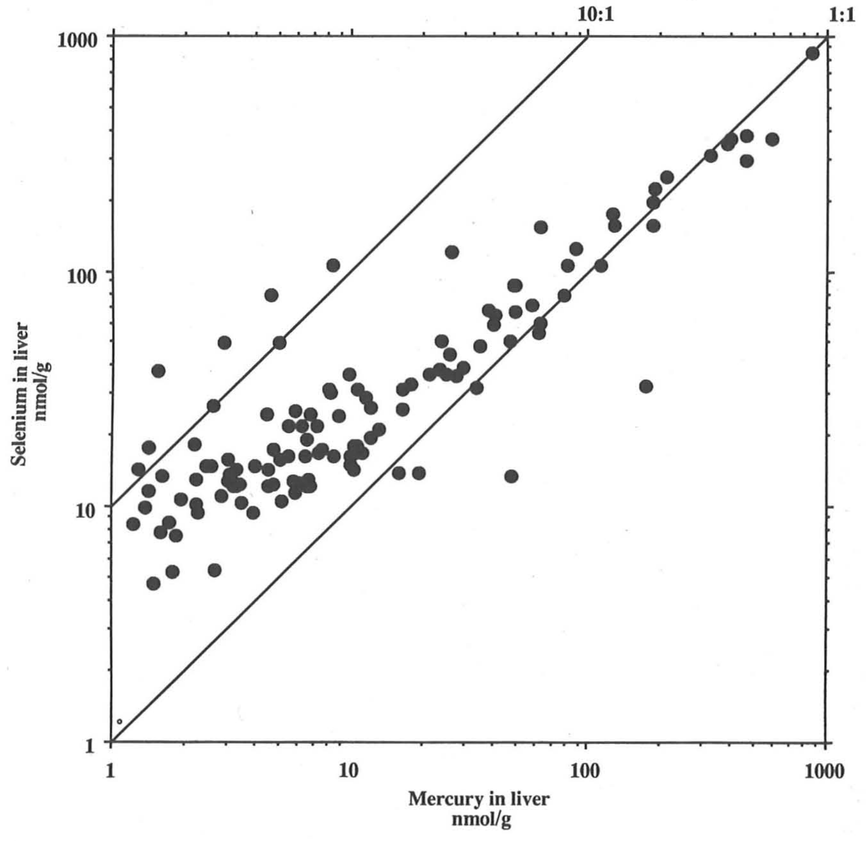


Fig. 7
Molar mercury concentrations versus molar selenium concentrations in liver of ringed seals. Lines indicate the decal ratios.

Table 10. Element concentrations (µg/g ww) in bile from ringed seals with corresponding data for the concentration in liver, kidney and muscle.

Matrix:	Bile			Liver			Kidney			Muscle		
Element:	N	GM	95% CI	N	GM	95% CI	N	GM	95% CI	N	GM	95% CI
Cadmium	58	2.57	1.85-3.55	57	17.2	13.4-22.1	58	55.3	43.2-70.8	56	0.234	0.175-0.311
Mercury	29	0.103	0.066-0.161	29	3.86	2.02-7.38	29	1.25	0.927-1.68	29	0.283	0.213-0.377
Zinc	57	6.30	5.43-7.31	57	51.3	48.5-54.2	57	53.3	49.4-57.7	57	23.8	22.6-25.0
Selenium	21	0.353	0.275-0.453	19	1.25	1.06-1.48						

tive. Thus, all the relations apart from cadmium-zinc, mercury-selenium and cadmium-mercury are secondary.

Heavy metals and selenium levels in the bile

The concentration of cadmium in bile from ringed seals was relatively high (GM = 2.57µg/g ww; Table 10). The bile level was about 15% of the level in the liver, ranging from 1.5-70%. Cadmium in the bile is 5% of the level in the kidneys, whereas it is about 10 times higher than in muscle. Inter tissue correlation (pairwise) shows cadmium in bile to be strongly correlated to the level in liver, kidneys and muscle (Table 11 and Fig. 8). However, partial correlation shows that cadmium in bile was only strongly correlated to cadmium in liver, and weakly correlated to cadmium in muscle (Table 11). A highly significant correlation was found between cadmium and zinc in bile, while cadmium was not correlated to mercury or selenium (results not tabled).

The concentration of mercury in bile from ringed seals (GM about 0.1µg/g ww) was relatively low compared to the other examined tissues. The bile contains about 2.7% of the mercury concentration in liver (ranging from 0.3% to 30%), 8% of the kidney concentration, and about 36% of the muscle concentration.

A strong pairwise correlation was found between mercury in bile and in the other tissues (Table 11 and Fig. 9), but the partial correlation only showed a significant correlation between bile and kidneys. Mercury in bile was not correlated to any of the other elements analysed.

DISCUSSION

Tissue, sex and age differences

The tissue differences and lack of differences between sexes found in the present study are similar to other studies of Arctic marine mammals, and marine mammals in general (e.g. Law 1996, Dietz *et al.* 1996, 1997, in press b, Wagemann *et al.* 1996, Riget *et al.* 1997a). A number of monitoring programs (e.g. Arctic Monitoring and Assessment Programme) have generally chosen to focus on element levels in only one tissue to reduce costs, and a single tissue is often used to elucidate temporal and geo-

Table 11. Inter tissue correlation's (pairwise and partial) between element concentration in bile and the other tissues from ringed seal. CC = correlation coefficients, SL= significance levels. Number of observations in parentheses.

	Inter-tissue correlations		
	Bile-Liver CC/SL	Bile-Kidney CC/SL	Bile-Muscle CC/SL
Cadmium (56)	0.820/*** 0.548/***	0.728/*** -0.113/-	0.575/*** 0.279/*
Mercury (29)	0.743/*** 0.141/-	0.842*** 0.523/**	0.716/*** 0.104/-
Zinc (57)	0.371/** 0.272/*	0.264/* -0.028/-	0.019/- 0.024/-
Selenium (19)	-0.481/Neg.*	-	-

* $0.01 \leq P < 0.05$, ** $0.001 \leq P < 0.01$, *** $P < 0.001$

graphical trends. However, these trends may not always be consistent among different tissues (e.g. Dietz *et al.* 1996). Assessment of human intake of marine mammal tissue and toxicological evaluations require a number of tissues, since many different tissues are consumed, and metal levels evidently vary between organs. A rough extrapolation from one tissue group to another can be conducted, although considerable variation is observed among individuals, metals and age. Similar variability has been documented for ratios of cadmium concentrations in human kidney cortex and liver for individuals of different ages (Kjellström 1979).

Geographical differences within the different age groups of ringed seals

Geographical differences in heavy metal levels in animal tissues have been discussed by a number of authors in order to evaluate the exposure of these toxic compounds, both to biota and humans in different regions. It is of course important that the groups to be evaluated are comparable. Factors such as age and sex are in general eliminated through selection of comparable groups, or through normalisation of data. Other factors, such as differences in food selection, temperature, local geology and anthropogenic input have also been proposed as factors affecting the contaminant levels, but such variables are seldom considered and quantified (e.g. Dietz *et al.* 1996, in press b, Wagemann *et al.* 1996). Seasonal and annual differences have been documented for other species, and some caution should be taken in making rigid conclusions in relation to geographical comparisons or temporal trends, as the biological variation may be substantial (e.g. Bignert *et al.* 1993, 1994, Olsson 1995). In addition, a number of conditions may contribute to individual variation, such as migration patterns for non-stationary species. A number of variables are of importance for stationary species of the lower trophic levels, including local geographical trends, mineralisation rate, wind and current directions, and precipitation (Olsson 1995). Although information on all the variables mentioned above was not available, geographical comparisons were conducted in order to see whether a pattern was apparent in seals from Greenland waters.

Geographical differences in cadmium of ringed seals within Greenland and its possible derivation from food

Prey selection and the cadmium content of the prey only partly explain the observed geographical differences in cadmium levels in Greenland ringed seals. The low concentrations found in seals from Nanortalik is in accordance with the relatively low concentrations present in their major food items (capelin, *Mallotus villosus*)

Fig. 8
Cadmium concentration in bile versus concentration in muscle, liver and kidneys in ringed seals. Lines indicate the decal ratios.

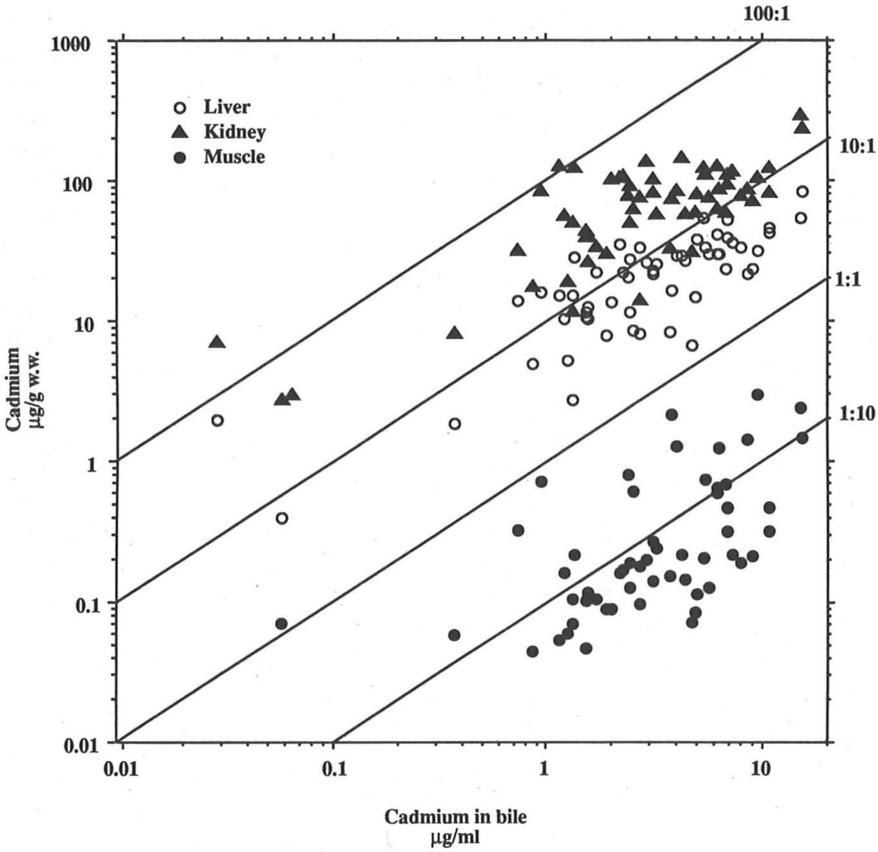
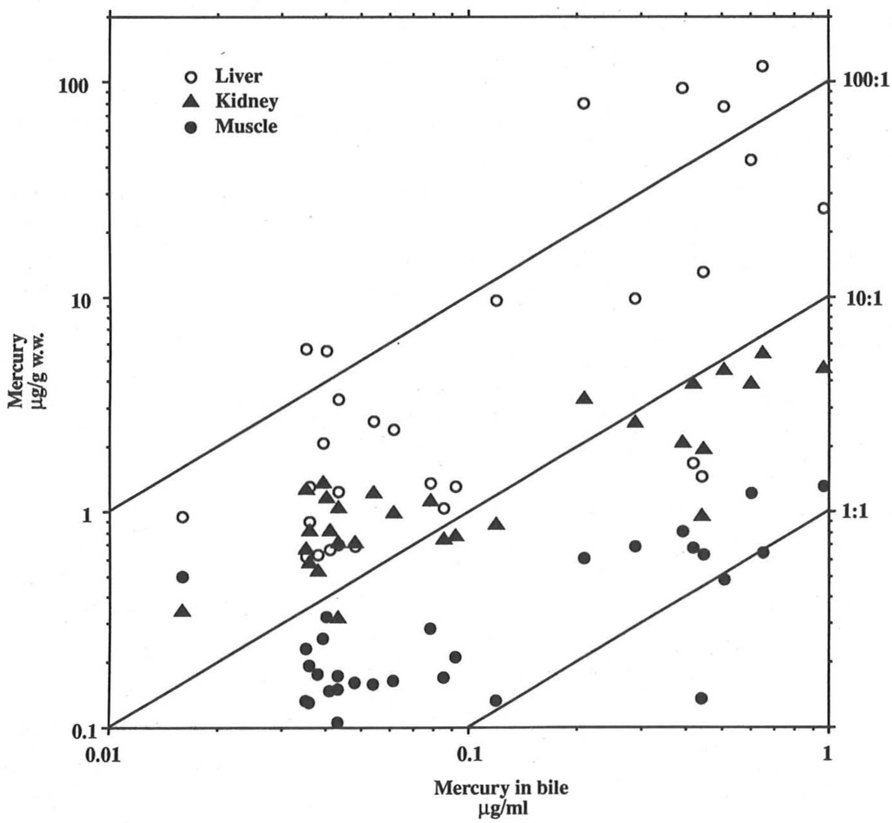


Fig. 9
Mercury concentration in bile versus concentration in muscle, liver and kidneys in ringed seals from Greenland. Lines indicate the decal ratios.



see Siegstad *et al.* (this volume) for details on ringed seal prey) in this area (Riget *et al.* 1997b). The high cadmium levels in adults and old seals from Avanersuaq correspond well with the high levels that are found in their major food items, polar cod (*Boreogadus saida*). However, the low levels observed in one-year-old seals cannot be explained. No clear differences in food preference have been found between different ringed seal age groups from Avanersuaq (Siegstad *et al.* this volume).

The low cadmium levels in seals from Uummannaq correspond well with the low levels found in Greenland halibut (*Reinhardtius hippoglossoides*) and in *Euphausiacea* (Dietz *et al.* 1996, Riget 1997b). However, higher levels were expected considering the large intake of *Parathemisto libellula* with a relatively high cadmium content (e.g. Macdonald and Sprague 1988, Dietz *et al.* 1996, Zauke *et al.* 1996, Ritterhoff and Zauke 1997). It is not clear why ringed seals from Upernavik have such a high cadmium content when their principal food item, Arctic cod (*Arctogadus glacialis*), has a rather low cadmium content.

Knowledge of seasonal or year to year differences in ringed seal diet is limited. Such dietary differences may explain the apparent lack of correlation between metal levels in some seals and their prey. The samples from Avanersuaq, Upernavik and Nanortalik were collected during a one-month sampling period, whereas the samples from Uummannaq were collected over a longer period (October-June) during which major fluctuations in prey selection of ringed seals occured (Siegstad *et al.* this volume). *Parathemisto* was the dominant prey in October, but it was not consumed at all in June. The opposite was the case for *Euphausiacea*, while *Liparis* spp. was the only prey item consumed from October to February. A similar fluctuation in prey selection by seals from other areas could influence estimates of total cadmium intake considerably. Another uncertainty may arise from annual variations, which may appear in both the feeding habits of the seals and the cadmium content of their prey.

The results show to some extent higher cadmium concentrations in seals from more northerly areas, which may be caused by a generally higher cadmium level in prey items. This is supported by the low levels observed in one-year-old seals from Nanortalik compared with all the other subgroups of one-year-old seals, and by the high levels in adult and old seals from Avanersuaq and Upernavik. Species such as *P. libellula*, polar cod, shorthorn sculpin (*Myoxocephalus scorpius*), as well as several seabird species, have also been documented to have higher cadmium concentrations in Northwest Greenland compared to Southwest Greenland (Nielsen and Dietz 1989, Dietz *et al.* 1996, Riget *et al.* 1997b).

Data on feedings habits in East Greenland are only available from Kong Oscars Fjord (Siegstad *et al.* this volume). Adult ringed seals from Kong Oscars Fjord have lower cadmium levels than seals from e.g. Avanersuaq and Upernavik in all analysed tissues. The proportion of crustaceans in Kong Oscars Fjord is, however, intermediate to these two areas and fish constitute the bulk of the diet (c. 90% of the mass of the diet) in all three areas.

Finally, it is worth noting that the variation in cadmium levels in seals cannot be explained by differences in geological background levels, as has been suggested for Canadian ringed seals (Wagemann *et al.* 1996). Analysis of sediment samples taken in zones of different geological origin along the coast of Greenland show only small and insignificant differences between areas (Loring and Asmund 1996). It can therefore be concluded that the geographic variation in cadmium levels among seals from different areas reflects differences in prey selection. However, no straightforward correlation between the concentrations in the seals and their prey selection could be demonstrated.

Cadmium in ringed seals from Greenland compared to other Arctic areas

The average cadmium concentration in ringed seals from Greenland was about the same as in ringed seals from the eastern Canadian Arctic. Wagemann *et al.* (1996) were not able to detect any significant differences between sites in eastern and western Arctic. However, the levels in ringed seals from the eastern Arctic were reported to be twice as high as in the western

Canadian Arctic. Ringed seals from Eureka on Ellesmere Island, had even lower levels than seals from the western Canadian Arctic, and seals from Svalbard were also reported to have low cadmium concentrations (Carlberg and Bøler 1985, Skaare 1994, Wagemann *et al.* 1996, this study). The decline in cadmium concentrations from eastern to western Canada, and the low cadmium levels from Svalbard, have also been observed for other marine mammal species such as beluga whales (*Delphinapterus leucas*) and polar bears (Norstrom *et al.* 1986, Braune *et al.* 1991, Dietz *et al.* 1995, Wagemann *et al.* 1996). Wagemann *et al.* (1996) attributed the geographical trend within Canada to different natural background levels, dictated by geological differences. However, this could not be demonstrated within Greenland (Loring and Asmund 1996). A more likely explanation is that diet may differ regionally. If, for example, crustaceans constitute a significant part of the diet, the cadmium intake will be higher than if fish is the main prey; which on the other hand would result in a higher mercury intake. The similarities between geographical trends in belugas and ringed seals may be explained by similar prey preference, whereas the correlation between polar bear and ringed seal metal burdens are most likely a result of polar bear predation on ringed seals (e.g. Stirling and Archibald 1977, Smith 1980, Wagemann *et al.* 1996).

Cadmium in seals from North European waters

Cadmium levels in seals from West Greenland were much higher than the levels found in ringed seals from the Gulf of Bothnia. Levels in muscle were approximately 15 times higher (mean values), and liver and kidney levels were 16-202 and 24-93 times higher, depending on areas and age groups compared (Dietz 1981, Frank *et al.* 1992). High cadmium levels in the Arctic seals (ringed seals and bearded seals, *Erignathus barbatus*) have also been found in other studies (e.g. Sergeant and Armstrong 1973, Smith and Armstrong 1975, 1978, Wagemann *et al.* 1996). Johansen *et al.* (1980) also concluded that cadmium levels were higher in Arctic seals. Studies of harp seals (*Phoca groenlandica*) from five locations in Arctic Canada (Ronald *et al.* 1984) showed similar cadmium concentrations as observed in ringed seals in the present study. The levels in grey seals (*Halichoerus grypus*) from the Gulf of Bothnia and harbour seals (*Phoca vitulina*) from Skagerrak and British waters correspond to the levels in the ringed seal from the Gulf of Bothnia (Helle 1981, Botta *et al.* 1983, Perttilä *et al.* 1986). An increase towards the north has also been demonstrated in harbour porpoises (*Phocoena phocoena*), with cadmium levels being about 10 times higher in harbour porpoise from Arctic waters compared to more boreal parts of the North Atlantic (Paludan-Müller *et al.* 1993).

The prey of Arctic ringed seal, such as crustaceans, including *Parathemisto*, and Arctic cod, contain high cadmium concentrations (e.g. Macdonald and Sprague 1988, Petri and Zauke 1993, Dietz *et al.* 1996, Zauke *et al.* 1996, Riget *et al.* 1997a,b, Ritterhoff and Zauke 1997). The consistently high levels in marine mammals from Arctic waters can therefore be explained by the importance of these prey items in the ringed seal diet.

The high cadmium levels in fish and crustaceans from the Arctic may be explained by lower growth rates in Arctic waters, which could lead to increased cadmium accumulation. It is well known that the growth rate of fish decreases with falling temperature, whereby a relatively higher proportion of food intake is utilised for basic metabolism and activity.

Geographical differences in mercury of ringed seals within Greenland

Ringed seals from East Greenland generally have a higher mercury burden than those from West Greenland. Mercury levels in Danmarkshavn, Kong Oscars Fjord and to some extent Ittoqqortoormiit, exceed the levels in Avanersuaq, Upernavik, Uummannaq and Nanortalik. The high mercury levels in adult ringed seals from Kong Oscars Fjord can only partly be explained the feeding habits observed by Siegstad *et al.* (this volume). Arctic cod has been shown to contain higher mercury burdens than e.g. polar cod, whereas the opposite is the case for cadmium (Dietz *et al.* 1996). Arctic cod constitutes as much as 60% of the seal's diet in Kong Oscars Fjord, which is, much more than in

Avanersuaq and Uummannaq, where polar cod is more important. Seals from Upernavik, however, shows a similar proportion of Arctic cod without similarly high mercury levels.

Studies of feeding habits offer insight to the last meal of the seal whereas metal burdens are accumulated over years. The opposite cadmium-mercury trend observed between East and West Greenland, could indicate a difference in the ratios between crustacean and fish or polar cod and Arctic cod, that is not revealed by the studies of feeding habits.

Analyses of sediments collected along the coasts of East and West Greenland indicate that mercury levels are about twice as high on the east coast, probably due to influence from the volcanic activity around Iceland (Loring and Asmund 1996). To what extend geology influence the metal content in biota is uncertain and it cannot alone explain the large differences observed in ringed seal tissue between areas in Greenland.

The mercury data from municipalities in West and East Greenland indicate no tendency towards higher levels in the northern areas, in contrast to what was found for cadmium. In West Greenland, the most pronounced differences are the low levels in seals from Uummannaq and the high levels in seals from Upernavik. The low levels in seals from Uummannaq may be attributable to the high intake of *Parathemisto* and *Euphausiacea*, which have low mercury content (Dietz *et al.* 1996). In Upernavik, the high levels observed might be explained by the intake of Arctic cod, which has a much higher content of mercury than other fish species from Greenland waters (Dietz *et al.* 1996, Riget *et al.* 1997b).

Mercury in seals from other parts of the Arctic

Data from seals sampled in Alaskan waters indicate high mercury concentrations (1.52 - 3.52µg/g ww) in one and two-year- old ringed seals (Zeisler *et al.* 1993). These levels correspond well with the concentrations reported from the western Canadian Arctic and in East Greenland. In the eastern Canadian Arctic, West Greenland, Svalbard and northern Norway the levels are approximately 3 times lower (Smith and Armstrong 1975, 1978, Johansen *et al.* 1980, Carlberg and Bøler 1985, Wagemann 1989, Skaare 1994, Wagemann *et al.* 1996).

Smith and Armstrong (1978) found no significant differences between mercury concentrations in liver or muscle tissue in areas as widely separated as Holman, in the western Canadian Arctic, and Pond Inlet, on northern Baffin Island. However, Eaton and Farant (1982) pointed out that, based on the results from Smith and Armstrong (1978), the age accumulation of mercury in liver of ringed seals increased from east to west. The increasing trend of mercury in ringed seal liver from eastern to western Canada is supported by a recent study by Wagemann *et al.* (1996), but no similar trend was found for kidneys and muscle. Juvenile ringed seals from Jarfjord in Norway, had lower mercury concentrations in liver and kidneys than ringed seals from Canadian and Greenland waters (Skaare 1994, Wagemann *et al.* 1996).

The high mercury levels in the western part compared to the eastern part of the Canadian Arctic have also been documented in beluga whale tissue (Wagemann *et al.* 1991, 1996). Comparisons of mercury accumulation rates in liver (data were not given for muscle and kidneys) of belugas from western and eastern Canada showed that the rate was more than three times higher in western Canada (Wagemann *et al.* 1996). Similarly, polar bears from western Arctic Canada accumulated mercury in liver tissue (and hair) to a greater extent than polar bears from eastern Arctic Canada. They in turn accumulated more mercury than bears from Hudson Bay, Greenland and Svalbard (Eaton and Farant 1982, Norstrom *et al.* 1986, Renzoni and Norstrom 1990, Born *et al.* 1991, Braune *et al.* 1991, Norheim *et al.* 1992, Dietz *et al.* 1995). This decrease from west to east is, however, inconsistent with the high mercury levels in ringed seals from East Greenland. Norstrom *et al.* (1986) suggested that the differences between western Arctic Canada and eastern Arctic Canada were most likely caused by higher mercury levels in the ringed seal food chain, resulting from higher natural levels in the sediments (and consequently in the lower food chain) of the Melville

Island area. The evidence presented above indicates a geographic trend in mercury levels in ringed seal liver, but these differences are not apparent in kidneys and muscle tissue.

Mercury in seals from Northern European waters

The levels of mercury in ringed seals from the Gulf of Bothnia and the Gulf of Finland exceed even the highest values reported for the Arctic (Helle 1981, Perttilä *et al.* 1986, Frank *et al.* 1992). Studies involving grey seals and harbour seals indicate that levels in seals from northwestern European waters are higher than the Arctic (Frank *et al.* 1992, Law *et al.* 1991). This is further supported by a comparison of harbour porpoises from the two areas (Paludan-Müller *et al.* 1993). In seals found dead off the coast of the Netherlands, the mercury concentration in liver was extremely high, 257-326µg/g ww (Koeman *et al.* 1975), most likely due to anthropogenic sources.

Age-accumulation of cadmium and mercury

Our data showed that the cadmium concentrations in ringed seals reached maximum levels in mid-life. Cadmium in muscle seemed to maintain a constant level, while there was a significant decline in liver and kidney tissue in older seals. A similar tendency has been found in cetaceans. In belugas from West Greenland there was a 20% and 50% reduction in liver and kidney levels, respectively, from adults to old individuals. In narwhals (*Monodon monoceros*) and harbour porpoises, cadmium levels in kidneys drop by 20 % with age (Hansen *et al.* 1990, Paludan-Müller *et al.* 1993). In minke whales (*Balaonoptera acutorostrata*) from the Antarctic, liver concentrations were reported to reach a maximum level in 20-year-old animals, declining to approximately two-thirds in animals aged 25-40 years (Honda *et al.* 1987).

On the basis of this information, some caution should be taken when interpreting age accumulation observations (depicted as simple linear regressions) of cadmium in liver and kidneys presented in several studies of marine mammals (Tohyama *et al.* 1986, Falconer *et al.* 1983, André *et al.* 1990, Wagemann *et al.* 1991). The significance of regression or correlation analysis is strongly dependent on the age composition of samples.

The decrease in cadmium in liver and kidneys in old animals may be related to several factors. A lower food intake due to declining growth and activity with age may be part of the explanation (Honda *et al.* 1987). Also a change in diet from crustaceans to fish, as seen in several ringed seal populations (cf. Siegstad *et al.* this volume), is expected to cause a decline in cadmium intake. An increase in urinary excretion may also be an important factor. In humans, age conditioned physiological changes in the kidneys give rise to renal cadmium excretion exceeding uptake around the age of 55 years (Nordberg and Kjellström 1979). The biological half time of cadmium in kidneys has been reported to decrease when renal tubular dysfunction occurs as a result of increased urinary excretion (WHO 1992).

The concentration of mercury in ringed seals seems to increase throughout life, especially in the liver. This is in accordance with several studies of cetaceans, (e.g. Wagemann *et al.* 1983, 1996, Wagemann 1989, André *et al.* 1990, Hansen *et al.* 1990, Paludan-Müller *et al.* 1993, Wagemann *et al.* 1996), although in some of the studies, a correlation was not found for all tissues. The high accumulation in the liver is probably related to the fact that the liver is the major organ responsible for demethylating methyl-mercury and storage of inorganic mercury. The ratio of inorganic to organic mercury suggests that ringed seals tolerate a certain level of organic mercury. When that is exceeded they demethylate and store most of the mercury in the liver, and to some extent in the kidneys (Freeman *et al.* 1975, Himeno *et al.* 1989, Dietz *et al.* 1990).

Age-accumulation of zinc and selenium

The results for zinc and selenium in ringed seals are similar to those reported from other studies of marine mammals (e.g. Law 1996). Zinc is an essential element, and the body is capable of regulating the physiologically required levels (Dietz *et al.* 1995). Mammals also appear to be capable of maintaining selenium homeostasis at low levels of exposure (Dietz *et al.* in press b). Further discussion of the levels of these metals is included in the section concerning the inter-element correlation between cadmium and mercury.

Inter-tissue correlations - liver as an indicator tissue

The strong inter-tissue correlation found for both cadmium and mercury is in accordance with several other studies of marine mammals in the Arctic (e.g. Hansen *et al.* 1990, Dietz *et al.* 1995). Even though a correlation is clear, tissue ratios may change. The ratio between cadmium in kidneys and liver seems to be relatively constant. The ratio of these tissues relative to muscle will, however, vary with age and concentration level. A similar variability has been documented in human tissue, where the cadmium ratio between kidney cortex and liver was dependent upon the age group studied (Kjellström 1979). There was a strong inter-tissue correlation in the levels of mercury found between tissues in ringed seals. However, the mercury concentrations in muscle and kidneys seem to reach a plateau at about 1µg/g and 5µg/g ww, respectively, irrespective of the levels in the liver, which may rise to more than 100µg/g. A similar pattern was found in other studies of marine mammals from the Arctic (Hansen *et al.* 1990, Paludan-Müller *et al.* 1993). Therefore the ratio between mercury levels in muscle and kidneys versus liver increases with increasing concentration and age. Calculations of the ratios relative to age revealed that the liver-kidney ratio of mercury concentration increases throughout life, increasing by almost 6 times between the youngest and the oldest age groups. The liver-muscle ratio more than triples with age. The ratio between the mercury concentrations in kidneys and muscle is more or less constant for all age groups older than yearlings.

Inter-element correlations

A strong correlation between cadmium and zinc levels in liver and kidney tissue has been found in several studies of marine mammals. This correlation is due to the binding of cadmium to zinc-metallothionein, thereby inducing synthesis of new zinc-metallothionein (Tohyama *et al.* 1986, Wagemann and Hobden 1986, Bremner 1987).

The strong correlation between mercury and selenium has also been demonstrated in several other studies of marine mammals (Koeman *et al.* 1973, 1975, Hansen *et al.* 1990, Paludan-Müller *et al.* 1993, Dietz *et al.* 1995). The observed 1:1 molar ratio between selenium and mercury in liver suggests that these metals act as antagonists for each other, and are detoxified as inert mercuric selenide (HgSe, or tiemannite, André *et al.* 1990).

The finding that the pairwise correlation coefficient between cadmium and selenium in ringed seal liver becomes negative when using partial correlation indicates that metabolism of cadmium in the liver does not involve selenium. This observation is supported by studies of polar bears, where the use of partial correlation also strongly reduced the correlation coefficient (Dietz *et al.* 1995). This raises some uncertainty in relation to earlier results, including some of our own work (e.g. Wagemann *et al.* 1983, Hansen *et al.* 1990). The pairwise correlations found are probably related to the fact that both elements are strongly correlated to mercury; cadmium through age-dependent accumulation and selenium through the binding to mercury.

The partial correlation coefficient for cadmium and selenium in the kidneys, on the other hand, is only slightly reduced compared to the pairwise correlation coefficient. This indicates some interaction with selenium during cadmium metabolism in the kidneys, although the cadmium-zinc interaction is the most significant. A strong reduction in the correlation coefficient was also found for cadmium and selenium in polar bear kidneys using partial correlation (Dietz *et al.* 1995).

The large reduction in the mercury-zinc correlation coefficient between ringed seal liver and kidneys when using partial correlation indicates that zinc is not involved in mercury metabolism. A similar result was found with polar bears (Dietz *et al.* 1995). Again, this suggests that pairwise correlation's observed earlier should be taken with caution (e.g. Honda *et al.* 1987, Muir *et al.* 1988, Hansen *et al.* 1990).

Heavy metals and selenium levels in the bile

The relatively high cadmium concentrations in the bile of ringed seals, being more than 10 times higher than in muscle, indicates a high daily uptake of cadmium from food. While this uptake is reflected by the high concentration in the bile, it is uncertain whether the cadmium is actually excreted or reabsorbed from the intestine (enterohepatic circulation).

The strong pairwise correlations between cadmium in the bile and the other tissues suggest that cadmium in the bile is not only a result of daily uptake, but is also related to the total cadmium burden in the body. However, the strong pairwise correlation between cadmium levels in bile and kidneys is eliminated when considering partial correlation even though the kidneys are the major organs for cadmium storage. Similarly, the pairwise correlation coefficient for cadmium in muscle versus bile is reduced by partial correlation. The cadmium burden of the bile appears to be connected mainly with the level in the liver, as the partial correlation between these tissues is highly significant. These results suggest that the liver is the major organ responsible for excretion of the daily cadmium uptake from food, while the kidneys are involved in long term storage of cadmium, and excretion from this organ would rather be reflected in the urine, which was not analysed in this study. To our knowledge, no other data for heavy metals and selenium in bile from marine mammals have been published.

The results presented in this study differ in several respects from studies on humans and from some animal experiments. In humans, only about 5 to 7% of the cadmium is estimated to be absorbed in the intestine, and little cadmium is excreted again (Friberg *et al.* 1986). The biological half-life in the liver has been estimated to be between 5 and 15 years (WHO 1992, Nordberg *et al.* 1986). In other mammals, the liver half-life has been estimated to be between 10 to 50% of the lifespan (WHO 1992). The actual excretion of cadmium through the bile has been studied in rats given repeated subcutaneous injections of cadmium (Elinder and Pannone 1979). These doses resulted only in a moderate increase of cadmium in the bile (average 0.014µg/ml), compared to the almost 200 times higher level found in ringed seals in Greenland. Despite this, the liver and kidney cadmium levels obtained in rats (70µg/g and 58µg/g ww, respectively) were higher than those for ringed seals in the present study (17µg/g and 55µg/g ww, respectively).

To some extent, ringed seals and most likely other marine mammals, metabolise cadmium differently to humans and rodents, with a greater cadmium intake from food followed by high excretion via the bile. This could be an adaptation to the high cadmium exposure of marine mammals in some areas. Such an adaptation is supported by preliminary effect investigations, where kidney damage in ringed seals has not so far been demonstrated, although cadmium levels of > 200 (µg/g) were found in the kidneys (Dietz *et al.* in press a). Concentrations above this limit and probably even at lower levels are believed to cause kidney damage in humans and animals (Elinder and Järup 1996).

The strong correlation between cadmium and zinc in the bile suggests that excretion of cadmium involves zinc-metallothionein. This protein is responsible for storing cadmium in liver and kidneys in marine mammals and other mammal groups including humans (Cousins 1979, 1985, Wagemann and Hobden 1986, WHO 1992). Studies of rats have shown that cadmium in the bile is associated with a low molecular weight compound, partly characterised as Cd-glutathione, following the degradation of metallothionein in the kidneys and liver (Cherian and Vostal 1977, Nordberg *et al.* 1985). This is likely to be the same for marine mammals. Thus, the correlation between cadmium and zinc in the bile is likely to be due to the breakdown of metallothionein, followed by an excretion of both metals to the bile.

The concentration of mercury in the bile from ringed seals is about half the burden in the muscle, indicating that seals may excrete a substantial amount of mercury through the bile. Faecal excretion, however, is strongly dependent on resorption from the intestine. The large variation in the bile/liver mercury ratio implies that the excretion of mercury is related to the actual mercury intake from the diet rather than the general body burden. Calculation of partial correlation shows that mercury in bile is only correlated to kidney levels, and this probably reflects the daily uptake of mercury from the diet followed by excretion via the kidneys. This is supported by the fact that the kidneys have only limited involvement in the long time storage of mercury. Studies on humans and animal experiments also show that mercury is continuously excreted from both the kidneys and from the liver (WHO 1976). However, there was no correlation evident between mercury in bile versus liver and muscle in ringed seals, despite the fact that the liver is the major organ for mercury storage, and together with the muscle tissue, contains by far the majority of the mercury body burden.

The faecal excretion of mercury depends on the amount reabsorbed from the intestine (enterohepatic circulation), which again depends on whether mercury is in inorganic or organic form. In general, methyl mercury in mammals has been reported to be efficiently (>95%) taken up in the intestine, while only a limited amount (c. 15%) of the inorganic mercury is taken up (WHO 1990). In marine mammals, organic mercury (methyl-mercury) constitutes the majority of the mercury intake. Organic mercury in fish makes up to 80% or more of the total mercury burden (WHO 1990, Dietz unpublished data). On the one hand, this suggest that methyl-mercury constitutes the major fraction of the mercury in the bile of ringed seals, and that the majority is likely to be reabsorbed from the intestine, thereby limiting the actual excretion. On the other hand, the demethylating process in the liver might to some extent increase the inorganic fraction of mercury in the bile. Several studies have strongly indicated that methyl-mercury is demethylated in the liver of marine mammals, followed by storage of inorganic mercury in the form of a selenium holding complex (Martoja and Berry 1980, Lindh and Johansson 1987, Dietz *et al.* 1990). However, some inorganic mercury may be excreted into bile during these processes.

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