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CONCENTRATIONS AND INTERACTIONS OF SELECTED ESSENTIAL AND NON-ESSENTIAL ELEMENTS IN BOWHEAD AND BELUGA WHALES OF ARCTIC ALASKA

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ABSTRACT: In this study, we evaluated concentrations of twelve essential and non-essential elements (As, Cd, Co, Cu, Pb, Mg, Mn, Hg, Mo, Se, Ag, and Zn) in tissues of bowhead (*Balaena mysticetus*) and beluga (*Delphinapterus leucas*) whales from arctic Alaska (USA) and northwestern Canada. Tissue samples were collected between 1983 and 1997, mostly in 1995–97. The essential elements are reported to develop reference ranges for health status determination, and to help assess known or suspected interactions affecting toxicoses of cadmium (Cd) and mercury (Hg). In some tissues, Cd, Hg, and selenium (Se) were present at concentrations that have been associated with toxicoses in some domestic animals. Nevertheless, tissue levels of all elements were within ranges that have been reported previously in marine mammals. While mean Ag concentrations in beluga whale liver were relatively high (15.91 μg/g ww), Ag was not associated with hepatic Se levels or age, contrary to previous findings. Significant associations included: Cd with age, Zn, or Cu; Cu with age, Zn or Ag; and Hg with age, Se, Zn, or Cu. This study found hepatic Hg:Se molar ratios to be consistently lower than unity and different between species. Possible explanations for observed elemental correlations (i.e., interactions) and ancillary mechanisms of Cd and Hg detoxification are discussed.

Key words: *Balaena mysticetus*, Beluga whale, bowhead whale, cadmium, cetaceans, *Delphinapterus leucas*, elements, marine mammals, mercury, molar ratio, selenium, silver.

INTRODUCTION

In general, the Arctic is sparsely populated by humans and has comparatively little industry or agricultural enterprise. Consequently, detection of anthropogenic contaminants in the arctic food chain has incited public apprehension, especially among native people who consume marine mammals (Wheatley and Paradis, 1995). Of particular concern are persistent toxicants, including some metals, which have the potential to accumulate in biotic and abiotic components of the environment (Pacyna and Winchester, 1990). Cadmium (Cd) and mercury (Hg) occur naturally; however, there is no doubt that recent human activities have increased the rate at which they and other elements are mobilized to and within the biosphere (Pacyna and Keeler, 1995).

Koeman et al. (1973, 1975) examined relationships between levels of Hg and selenium (Se) in liver and brain of marine mammals off the coast of the Netherlands that were consistent with experimental monomethylmercury (MHg) toxicosis in various laboratory and domestic species. Koeman et al. (1973) indicated that prey of piscivorous marine mammals contain approximately 16-fold greater molar Se than molar Hg, and concluded that the 1:1 Hg:Se ratio in liver, kidney and brain, is established in the marine mammal (Koeman et al., 1975).

Koeman's theory (Koeman et al., 1975) of equimolar Hg:Se ratios has been corroborated by data on tissues of various marine mammal species, including ringed (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) (Smith and Armstrong, 1978); polar bears (*Ursus maritimus*) (Braune et al., 1991; Dietz et al., 1995); and harbor porpoises (*Phocoena phocoena*) (Paludan-Müller et al., 1993) with some variation from a 1:1 molar ratio noted (Wagemann et al., 1983; Hansen et al., 1990; Braune et al., 1991). Departures from uni-

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ty have been interpreted as evidence that the mechanism for Hg detoxification has been overwhelmed or is not Se-dependent. Arima and Gakura (1979) found a linear increase in muscle total Hg with age in odontocetes, in conjunction with a Hg:Se molar ratio of approximately 1.5:1, which they construed as an excess of Hg.

Zeisler et al. (1993) noted a positive correlation of Se with both silver (Ag) and total Hg (THg) in liver of beluga whales (*Delphinapterus leucas*). Becker et al. (1995) further investigated relationships among Ag, Se, and Hg in beluga whales. Hepatic total Hg concentrations were similar between Alaskan belugas and Atlantic pilot whales. In contrast, hepatic Ag concentrations in belugas were one to three orders of magnitude greater than in pilot whales (5.93–107.4 vs. 0.13–0.33 μg/g ww). Becker et al. (1995) further speculated that Ag at these concentrations might adversely affect the Se status of Alaskan belugas through competitive binding, rendering them more vulnerable to Hg toxicosis.

Major concerns regarding element levels in these species include potential toxic effects to wildlife and human consumers. We present what may be considered "normal" or reference ranges of essential elements in apparently healthy (as determined by gross and histologic examination; Woshner et al., 2000b) subsistence-harvested bowhead and beluga whales that may be used when a mineral deficiency is suspected, for comparison to other species, to identify interactions that may counter toxicoses (i.e., Se and Hg), or to establish guidelines for human consumption. A better understanding of essential elements in arctic marine mammals will help to improve health assessments and disease investigations.

The objectives of this study were to (1) evaluate concentrations of selected essential and non-essential elements in tissues of bowhead and beluga whales harvested by Alaskan Native subsistence hunters; (2) to determine whether these element levels differ with age, tissue type, gender, species, etc.; (3) evaluate elemental interactions; and (4) report "normal ranges" of essential elements.

MATERIALS AND METHODS

Sample collection and processing

From 1983 through 1997, tissues were collected from bowhead and beluga whales through the cooperation of Alaskan Eskimo (Inuit) subsistence hunters. Forty-one bowhead whales were sampled from 1983 through 1990 near Barrow (71.33°N, 156.00°W) (n = 31), Kaktovik (70.10°N, 143.23°W) (n = 3), and Wainwright (70.55°N, 159.95°W) (n = 7), Alaska. From 1995–97, 21 bowhead whales were sampled near Barrow, Alaska. Between 1992 and 1996 beluga whales were sampled in late June/early July at Pt Lay, Alaska (69.66°N, 162.83°W) (1992, n = 9; 1995, n = 10; 1996, n = 10); in spring (May, 1997) at Point Hope (68.30°N, 166.63°W), Alaska (n = 9); in August of 1996 (n = 1) and July of 1997 (n = 3) at Barrow, Alaska; summer of 1993 at the Northwest Territories (Inuvik) of Canada (68.32°N, 133.50°W) (n = 7); and in July at Kaktovik, Alaska (1997, n = 1). Tissues collected included liver, kidney, muscle, blubber and epidermis. Tissues were either frozen or chilled and subsequently processed under clean conditions following a strict protocol (Becker, 1995) to minimize contamination from handling. This included rinsing tissue with HPLC grade water (Sigma Chemical Co., St. Louis, Missouri, USA) prior to sub-sampling and cutting with titanium knives on a clean, Teflon-covered surface. Subsamples of tissues were placed in acid-washed scintillation vials (Fisher Scientific, Pittsburgh, Pennsylvania, USA) in preparation for heavy metals analysis. All tissues were frozen (−20 C) immediately after processing. Frozen samples were express delivered (24–48 hr) to College Station, Texas (USA) for archiving and analyses under appropriate National Marine Fisheries Service permits. Teeth were collected from belugas for age determination using counts of dentinal growth layer groups (GLGs), which have been estimated to deposit at a rate of two GLGs annually (Goren et al., 1987). Total GLGs were used in all analyses. Body length was used as a surrogate for age in bowhead whales (George et al., 1999).

Metals analysis

In preparation for elemental analysis, tissue samples were weighed (wet weight or ww), dried to constant weight, and digested in a wet ash procedure using concentrated (70%) nitric

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acid and microwave processing at 120 C for 60 min. All metals, except Cd and zinc (Zn), were analyzed using a Perkin-Elmer (Norwalk, Connecticut, USA) model SIMAA 6000 graphite furnace atomic absorption spectrophotometry (GFAAS) instrument, equipped with an AS-60 autosampler and Zeeman background correction (Perkin-Elmer); Cd and Zn were determined using a Perkin-Elmer instrument model 306 flame AAS. Detailed methodology followed that described by Smoley (1992) and modified by Bratton et al. (1997). For bowhead whale samples collected prior to 1995, tissues were dry-ashed at 480 F and analyzed by AAS with Smith-Hieftje background correction, using bovine liver standard #1577 (National Bureau of Standards, USA) as reference material (Bratton et al., 1993, 1997). All concentrations were reported on a ppm wet weight (ww) basis. The detection limit for all elements was approximately 0.01 μg/g (ppm).

Mercury analysis

In preparation for total Hg analysis at the Texas Veterinary Diagnostic Laboratory (Amarillo, Texas, USA) wet tissue samples were weighed and transferred to 250 ml quartz volumetric digestion tubes. Sample digestion and analysis followed Korsrud et al. (1985) with minor modifications. Briefly, 15 ml of a tertiary acid mixture, 15 N nitric acid, 36 N sulfuric acid, and 70% perchloric acid (5:1:1) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each tube. After 4 hr at room temperature, samples were heated in a five-step sequence over a period of 6 hr to a final temperature of 200 C, where it was maintained for 2 hr. After cooling, digests were transferred to volumetric flasks and brought to 25 ml with deionized water. Samples were placed in 150 ml reaction vessels, each containing 20 ml of 1 N HCl. Each analytical run included blank digests, working standards, and one standard reference material (DOLT-2; National Research Council, Ottawa, Ontario, Canada). Direct determination of total Hg (THg) concentrations was via cold vapor AAS using a Thermo-Jarrell Ash (Franklin, Massachusetts, USA) model S-11 AAS with a Thermo-Jarrell Ash AVA-440 atomic vapor accessory. Mercury vapor was generated through the automated addition of 2.5 ml of 5% stannous chloride to the sealed reaction vessel. The detection limit for THg was approximately 1 ng/g (ppb ww).

For determination of divalent Hg [Hg(II)] and monomethyl Hg (MHg), wet tissue samples were digested (Bloom, 1992), and analyzed by cold vapor atomic fluorescence spectrometry (CVAFS; Frontier Geosciences, Seattle, Washington, USA; Bloom and Crecelius, 1983; Bloom and Fitzgerald, 1988). The detection limits were 1.1 ng/g and 6.6 ng/g ww for MHg and Hg(II), respectively. The second total Hg value given in the following results was calculated by summing the MHg and Hg(II) values; SHg [SHg = sum of Hg(II) and MHg]. All Hg data were reported on a wet weight (ww) basis. Due to financial constraints and known low levels of Hg in bowhead tissues, inorganic [Hg(II)] and organic (MHg) Hg were determined only in beluga liver, kidney, muscle, and epidermis.

Statistical analysis

Student's *t*-test was used to compare elemental concentrations between genders within species. With few exceptions (i.e., beluga whale epidermal Hg(II), renal Se, and renal and hepatic As), significant differences were not observed between sexes, so data were pooled for further analyses. One-way Analysis of Variance (ANOVA) with Tukey's post-hoc test tested for differences in elemental concentrations between tissues within species, and between species within tissue, with cetaceans in this study analyzed in conjunction with ringed seals and polar bears (Woshner et al., 2001). Following Conover (1971), the values for each element were converted to ranks and Pearson correlations calculated between elemental concentrations within a tissue type, and between elemental concentrations and age indices. Linear regression was used to examine associations between selected elements within tissue and species. All statistical analyses were done using Systat (SPSS, Inc., Chicago, Illinois, USA; 1998) computer software with a *P*-value of <0.05 considered significant. For purposes of calculating summary statistics (computed only when >50% of samples had detectable levels for a given element), values below the minimum detection limit (MDL) were expressed as one-half the MDL, in accordance with accepted statistical practice (Gilbert, 1987).

RESULTS

Bowhead whales

Total body length of bowhead whales ranged from 4.0 (a fetus) to 17.7 m. Mean concentrations of elements in bowhead whale tissues are presented in Table 1. Mean concentration of Cd was highest in kidney (20.02 μg/g), with a range of 0.04 to 64.0 μg/g ww. Mean hepatic Cd was 9.63 μg/g ww and significantly lower than in kidney, while levels in muscle, blubber

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TABLE 1. Concentrations (μg/g ww) of selected elements in tissues of Alaskan bowhead whales.

	As	Cd	Co	Cu	Pb	Mg	Mn	THg	Se	Zn
Liver[a]										
Mean	0.20[1]	9.63[1]	0.02[1]	11.11[1]	0.03[1]	294.7[1]	2.27[1]	0.060[1]	1.61[1]	34.94[1]
SD[b]	0.16	10.43	0.01	21.48	0.02	46.5	0.92	0.073	0.81	16.16
n	54	55	20	55	55	20	20	55	55	55
Kidney										
Mean	0.09[1]	20.02[2]	0.02[1]	3.28[2]	0.02[1,2]	322.5[1]	0.53[2]	0.038[1,2]	1.58[1]	27.72[2]
SD	0.09	17.29	0.01	1.41	0.02	102.7	0.18	0.033	0.42	11.31
n	48	48	20	48	48	20	20	47	48	48
Muscle										
Mean	0.08[1]	0.05[3]	ND[c]	1.50[2]	0.02[1,2]	509.8[2]	0.15[2]	0.017[2,3]	0.35[3]	38.94[1]
SD	0.13	0.07		0.87	0.02	198.1	0.07	0.011	0.33	17.14
n	35	36	14	43	36	21	14	35	42	43
Blubber										
Mean	0.58[2]	0.02[3]	ND	0.60[2]	0.02[2]	49.9[3]	0.03[2]	0.002[2,3]	0.06[3]	2.58[3]
SD	0.68	0.02		0.52	0.01	22.1	0.02	0.007	0.03	2.08
n	38	34	11	41	34	11	11	30	38	34
Mean	0.08[1]	ND	ND	0.53[2]	ND	521.0[2]	0.06[2]	0.006[2,3]	0.75[2]	15.17[3]
SD	0.05			0.10		40.6	0.02	0.004	0.36	2.14
n	15	8	8	15	8	15	8	8	15	15

[a] Liver Mo and Ag were 0.38 and 0.34 μg/g ww, respectively; not detected in other tissues.
[b] Standard deviation.
[c] ND = not detected in greater than 50% of the samples.
The same superscript number ([1,2,3]) indicates mean level for that element is not significantly different among those tissue(s) as determined by one-way ANOVA with Tukey's post-hoc test.

and epidermis were significantly lower than liver and kidney Cd levels. Mean concentration of As was highest in blubber (0.58 μg/g). Levels of lead (Pb) and THg were consistently low in all tissues; the highest mean concentrations were found in liver 0.03 μg/g (Pb) and 0.06 μg/g ww (THg). Mean Cu, Mn, Ag, and Zn levels were significantly greater in liver than in kidney; and As, Co, Pb, Mg, THg, and Se were not significantly different between liver and kidney. Mean Mg concentrations were highest in muscle and epidermis.

Significant correlations ($P < 0.05$) between variables within tissue are given in Table 2. A number of elements were significantly correlated with body length, indicating that their tissue concentrations either increase or decrease with age. Hepatic Cu, Ag and Mn concentrations showed significant negative correlations with body length. Linear regression analyses of Se versus THg was conducted (Table 3) and the associated r^2 was only 0.19 (Table 3). Linear regression analysis of body length versus Se, Ag, or Cd in liver, as well as Cd in kidney, were all significant (Table 3). Among these regressions the closest associations were between body length and Cd in kidney ($r^2 = 0.40$) and liver ($r^2 = 0.38$).

Beluga whales

Mean concentrations of elements in tissues of beluga whales are presented in Table 4. Highest mean tissue concentration of As was in blubber (1.41 μg/g), which exceeded mean levels in other tissues by at least four-fold (Table 4). Mean Cd was highest in kidney (12.15 μg/g ww) followed by liver (3.81 μg/g ww) and was lowest in muscle, blubber, and epidermis. Mean Ag was highest in liver (15.91 μg/g ww). Appreciable mean Se concentrations were observed in liver (41.41 μg/g ww), kidney (6.27 μg/g ww) and epidermis (9.56 μg/g

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TABLE 2. Pearson's correlation coefficients between selected significantly correlated variables[a] ($P < 0.05$) in Alaskan bowhead whales.

	r	*P*	*n*
Liver			
Cd vs[b] Lgth[c]	0.70	<0.001	54
Cd vs As	0.32	0.019	54
Cu vs Lgth	−0.39	0.003	54
THg vs As	0.32	0.018	54
THg vs Cd	0.47	<0.001	54
Se vs Lgth	0.33	0.016	54
Se vs Cd	0.49	<0.001	54
Se vs THg	0.40	0.003	54
Ag vs Lgth	−0.65	0.002	20
Ag vs Cd	−0.60	0.005	20
Ag vs Cu	0.78	<0.001	20
Ag vs Se	−0.57	0.009	20
Zn vs As	−0.27	0.047	54
Zn vs Se	0.37	0.006	54
Blubber			
Zn vs Lgth	−0.547	0.002	30
Kidney			
Cd vs Lgth	0.74	<0.001	48
Cd vs Zn	0.32	0.026	48
THg vs Cd	0.46	0.001	47
Se vs Cu	−0.31	0.033	48
Zn vs Cu	−0.38	0.008	48
Zn vs THg	0.35	0.017	47
Zn vs Se	0.31	0.031	48
Muscle			
Cd vs Lgth	0.75	<0.001	35
THg vs Lgth	0.42	0.012	35
Se vs Cu	0.63	<0.001	42
Zn vs Cu	−0.63	<0.001	42
Zn vs Se	−0.68	<0.001	42
Epidermis			
Se vs Lgth	0.797	<0.001	15
Se vs As	0.735	0.002	15
Zn vs As	0.698	0.004	15
Zn vs Se	0.571	0.026	15

[a] Data were ranked prior to analysis.
[b] vs = versus.
[c] Lgth = body length.

ww), with Se in liver being significantly higher than in the other four tissues (Table 4). Levels of Cu, Pb, Mn, THg, Mo, Se, Ag, Hg (II), MHg, and SHg were significantly higher in liver versus kidney (Table 4). Magnesium was highest in muscle. Mean Cd and Co levels were higher in kidney than liver (Table 4).

Among beluga whale tissues, Hg concentrations were highest in liver, but varied depending upon analytical method employed. As previously noted, calculated total Hg (SHg) was determined through summation of Hg(II) and MHg. In general, SHg tended to be lower than total Hg determined directly (THg) by approximately two-fold, although not necessarily on a case-by-case basis. Mean hepatic SHg was 12.42 μg/g ww as opposed to mean THg of 23.29 μg/g ww (Table 4). This discrepancy between total Hg values attributable to analytic methodology was not nearly as pronounced in other tissues, which had lower total Hg levels, as well as (in the case of muscle and epidermis) a greater proportion of MHg. When calculated as a percentage of SHg, MHg occurred at a mean level of 14.2% in liver, 12.8% in kidney, 96.0% in muscle, and 97.1% in epidermis. In contrast, when calculated as percentages of THg, MHg was 7.4% in liver, 11.8% in kidney, 94.0% in muscle, and 113.0% in epidermis. Regardless of calculation method, it is evident that virtually all Hg in muscle and epidermis is in the methylated form while approximately 90% of mean total Hg present in liver and kidney is inorganic [Hg(II)].

In general, tissue elemental concentrations did not appear to differ significantly between males and females, but we noted a few exceptions. Mean epidermal Hg(II) was higher in males (0.03 μg/g ww) than in females (0.02 μg/g ww) but the biological significance of a 0.01 μg/g difference is difficult to interpret. Among animals from which kidney and liver were obtained, males were significantly older than females. Mean Se level in kidney was greater in males (29.72 μg/g ww) than in females (18.81 μg/g ww). Males had significantly higher concentrations of As than females in kidney (0.30 μg/g ww in males vs. 0.20 μg/g ww in females) and liver (0.16 vs. 0.12 μg/g ww in males and females, respectively).

Significant correlations between variables within tissue are given in Tables 5 and 6. A number of elements correlated

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TABLE 3. Linear regression parameters for THg versus Se (μmol/Kg[a]) in liver, and selected elements (μg/g ww[b]) versus body length (Lgth) in Alaskan bowhead whales ($P < 0.05$ only).

Tissue	Variables	n	β_1-Coef[c]	r^2	P (regression)
Liver	THg[a] vs[d] Se[a]	55	0.02*	0.190	0.001
	Se[b] vs Lgth (m)	55	0.088	0.146	0.004
	Ag[b] vs Lgth (m)	20	−0.067	0.199	0.049
	Cd[b] vs Lgth (m)	55	1.802	0.378	<0.001
Kidney	Cd[b] vs Lgth (m)	48	3.328	0.396	<0.001

[a] Expressed on a molar basis (μmol/Kg).
[b] Expressed on mass basis (ppm ww).
[c] β_1 coefficient. For THg vs Se, a * denotes that the slope was significantly ($P < 0.05$) different from one.
[d] vs = versus.

(both positively and negatively) with age (GLGs). Both organic and inorganic forms of Hg accumulated with age in all tissues except blubber. Levels of Cu declined as a function of age in liver, muscle, and epidermis. Linear regression parameters of Se versus various forms of Hg; and of Se versus the summed molar quantity of total Hg and Ag are shown in Table 6. There was a significant linear relationship between total Hg and Se (on a molar basis) in liver and kidney, with r^2 values of 0.61 and 0.55, respectively; but no such relationships between Se and Hg or Ag were evident in muscle, blubber, or epidermis. Liver and kidney were the only tissues with detectable concentrations of Ag; significant linear relationships between Se and Hg + Ag were observed, with r^2 values of 0.59 in liver and 0.55 in kidney (Table 7). Pearson's correlation coefficient for the relationship between liver Ag and Se was not statistically significant, while the correlation between THg and Se was highly significant (Table 5). Both total Hg and Se showed significant accumulation with age in the liver (Table 5).

Among linear regression analyses for selected elements versus age (GLGs) (Table 7), hepatic Se, Cd, THg, and MHg all increased significantly with age. In liver, the proportion of total mercury present as MHg (%MHg) decreased with age (Table 7). In contrast, the difference (HgD) between THg and SHg increased with age. In the kidney, THg, SHg, Hg(II), and MHg exhibited a significant positive linear relationship with age, as did Se and Cd (Table 7).

Species comparison

Bowhead whales had greater mean hepatic Cd levels than beluga whales ($P < 0.05$), while the reverse was true for Hg, Se and Ag. Zinc levels were lower in kidney of bowhead whale, while the mean Cd:Zn molar ratio was significantly greater for bowhead whale (mean ±SD Cd:Zn = 0.44 ± 0.37) as compared to the beluga whale (mean ±SD Cd:Zn = 0.19 ± 0.08). Mean Hg levels in muscle were significantly higher in beluga whale, and the mean hepatic THg:Se molar ratio was significantly greater in beluga whale as compared to bowhead whale [0.279 ± 0.169 (SD) vs. 0.016 ± 0.020 (SD), respectively].

DISCUSSION

The two cetacean species sampled for this study, bowhead and beluga whales, are important subsistence resources to Alaskan Natives, and may be good indicators of some arctic ecosystem contamination. These species are year-round inhabitants of the Arctic and Subarctic (Stewart and Stewart, 1989; Moore and Reeves, 1993; Waller, 1996), and occupy different trophic levels. Biomagnification of toxic metals in beluga whales is likely, as they consume a variety of prey, including fish [i.e., Arctic cod (*Boreogadus saida*), Arctic char

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TABLE 4. Concentrations (μg/g ww) of selected elements in Alaskan beluga whales.

	As	Cd	Co	Cu	Pb	Mg	Mn	THg	Mo	Se	Ag	Zn	HgII	MHg	SHg
Liver															
Mean	0.14[1]	3.81[1]	0.02[1]	20.91[1]	0.02[1]	261.8[1]	3.99[1]	23.29[1]	0.77[1]	41.41[1]	15.91[1]	40.36[1]	10.68[1]	1.72[1]	12.42[1]
SD[a]	0.06	1.76	0.01	23.76	0.01	69.36	0.90	25.93	0.18	32.67	10.20	10.59	10.05	0.88	10.32
n	50	50	24	50	50	24	24	24	24	50	24	50	24	24	24
Kidney															
Mean	0.27[1]	12.15[2]	0.04[2]	1.94[2]	0.01[2]	322.7[1,2]	1.51[2]	4.97[2]	0.01[2]	6.27[2]	0.06[2]	36.92[1,2]	4.02[2]	0.59[2]	4.58[2]
SD[a]	0.17	6.02	0.03	0.42	0.03	88.08	0.69	3.880	0.01	3.24	0.03	6.96	2.80	0.35	3.09
n	45	45	24	45	45	24	24	24	24	45	24	45	23	23	23
Muscle															
Mean	0.10[1]	0.02[3]	0.02[1,2]	0.92[2]	ND[b]	410.3[3]	0.44[3]	1.19[2]	ND[b]	0.28[2]	ND[b]	30.87[2]	0.05[2,3]	1.12[3]	1.16[2]
SD[a]	0.06	0.01	0.01	0.41		107.45	0.10	0.829		0.06		8.97	0.05	0.54	0.58
n	11	11	11	24	11	24	11	11	11	24	11	24	24	24	24
Blubber															
Mean	1.41[2]	ND[b]	0.01[1]	0.52[2]	ND[b]	44.91[4]	1.65[2]	0.03[2]	ND[b]	0.58[2]	ND[b]	4.02[3]	NA[c]	NA[c]	NA[c]
SD[a]	0.91		0.01	0.35		11.15	0.32	0.035		0.36		1.04	—	—	—
n	16	11	11	16	11	11	11	11	11	16	11	11	—	—	—
Epidermis															
Mean	0.30[1]	—	—	0.50[2]	—	386.4[2,3]	0.39[3]	0.60[2]	—	9.56[2]	0.01[2]	47.19[1]	0.02[2]	0.67[2,3]	0.70[2]
SD[a]	0.31			0.18		83.52	0.07	0.317		7.47	0.002	16.81	0.014	0.464	0.471
n	17	11	11	17	11	17	11	11	11	17	17	17	15	15	15

[a] SD is standard deviation.
[b] Not detected in greater than 50% of the samples.
[c] Not analyzed.
The same superscript number ([1,2,3]) indicates mean level for that element is not significantly different among those tissue(s) as determined by one-way ANOVA with Tukey's post-hoc test. Mean GLGs were 26 for liver, 25 for kidney, and 22 for muscle, blubber, and epidermis.

TABLE 5. Pearson's correlation coefficients between selected significantly correlated ($P < 0.05$) variables[a] in liver and kidney of Alaskan beluga whales.

Liver	*r*	*P*	*n*	Kidney	*r*	*P*	*n*
As vs[b] GLGs[c]	0.54	<0.001	46	Cd vs GLGs	0.61	<0.001	41
Cd vs GLGs	0.54	<0.001	46	THg vs GLGs	0.59	0.007	20
Cd vs As	0.33	0.018	50	MHg vs GLGs	0.84	<0.001	20
Cu vs GLGs	−0.58	<0.001	46	THg vs Cd	0.52	0.009	24
THg vs GLGs	0.68	0.001	20	Hg(II) vs GLGs	0.59	0.006	20
THg vs Cd	0.49	0.014	24	Hg(II) vs Cd	0.44	0.034	23
THg vs SHg	0.79	<0.001	24	Hg(II) vs THg	0.86	<0.001	23
Hg(II) vs GLGs	0.65	0.002	20	SHg vs GLGs	0.64	0.003	20
Hg(II) vs Cd	0.45	0.028	24	SHg vs Cd	0.46	0.027	23
Hg(II) vs Cu	−0.51	0.012	24	SHg vs THg	0.86	<0.001	23
Hg(II) vs THg	0.77	<0.001	24	SHg vs Hg(II)	0.99	<0.001	23
MHg vs GLGs	0.78	<0.001	20	SHg vs MHg	0.85	<0.001	23
MHg vs Cd	0.54	0.007	24	Se vs GLGs	0.60	<0.001	41
MHg vs THg	0.71	<0.001	24	Se vs Cd	0.65	<0.001	45
MHg vs Hg(II)	0.62	0.001	24	Se vs THg	0.73	<0.001	24
SHg vs GLGs	0.71	0.001	20	Se vs Hg(II)	0.67	<0.001	23
Se vs GLGs	0.75	<0.001	46	Se vs MHg	0.67	0.001	23
Se vs As	0.43	0.002	50	Se vs SHg	0.68	<0.001	23
Se vs Cd	0.62	<0.001	50	Ag vs GLGs	0.48	0.032	24
Se vs THg	0.75	<0.001	24	Ag vs THg	0.69	<0.001	24
Se vs Hg(II)	0.62	0.001	24	Ag vs Hg(II)	0.87	<0.001	23
Se vs MHg	0.65	0.001	24	Ag vs MHg	0.65	<0.001	23
Se vs SHg	0.64	0.001	24	Ag vs SHg	0.84	<0.001	23
Ag vs Cu	0.59	0.002	24	Ag vs Se	0.49	0.014	24
Zn vs Cd	0.57	<0.001	50	Zn vs Cd	0.49	0.001	45
				Zn vs Se	0.35	0.017	45

[a] Data were ranked prior to analysis.
[b] vs = versus.
[c] Growth layer groups (GLGs), GLGs/2 approximates age in years (Goren et al., 1987).

(*Salvelinus alpinus*), whitefish (*Coregonus* sp.), and Pacific herring (*Clupea harengus*)], as well as invertebrates such as shrimp (*Argus* sp., *Crangon* sp., and *Eualus* sp.), and squid (*Octopus* sp.) (Seaman et al., 1982). Seaman et al. (1982) recounted evidence that large belugas (generally older males) consumed larger fish than did the smaller whales, as the diet of adult beluga males apparently contained a substantially greater amount of larger fish species, such as cods (*Gadus* sp.) than did the diet of adult females or immature animals of either gender. Bowhead whales (*Balaena mysticetus*) feed near the base of the food chain upon small invertebrates collectively known as krill, which is comprised of a variety of euphausiid, copepod, mysid, and amphipod species (Lowry, 1993). As lower trophic level consumers, their opportunity to accumulate non-essential elements might be considered less than that of polar bears, seals, or toothed whales. However, age-dependent accumulation in particular tissues, coupled with a long bowhead whale lifespan (≥100 yr) (George et al., 1999) increase the probability that some metals amass.

While the current study relies heavily on correlation analysis, many correlations among our data have associated *P*-values of <0.01 or <0.001, which are not liable to be due to chance. Moreover, we scrutinized data for common patterns that would implicate elemental interactions across tissues and species. Also, it is important to recognize that relationships between individual elements may be due to age (thus mutually associated) or to a direct interaction.

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TABLE 6. Pearson's correlation coefficients between selected significantly correlated variables[a] ($P < 0.05$) in muscle, epidermis, and blubber of Alaskan beluga whales.

Muscle	r	P	n	Epidermis	r	P	n
Cu vs[b] GLGs[c]	−0.73	0.01	11	Cu vs GLGs	−0.59	0.032	13
Hg(II) vs GLGs	0.82	0.002	11	THg vs GLGs	0.62	0.043	11
Hg(II) vs As	0.66	0.028	11	THg vs Cu	−0.63	0.038	11
Hg(II) vs Cu	−0.64	0.001	11	Hg(II) vs GLGs	0.77	0.004	12
Hg(II) vs THg	0.65	0.032	11	Hg(II) vs As	0.52	0.045	15
MHg vs GLGs	0.73	0.011	11	Hg(II) vs THg	0.93	<0.001	11
MHg vs As	0.68	0.022	11	MHg vs GLGs	0.81	0.001	12
MHg vs Cu	−0.61	0.002	24	MHg vs Cu	−0.74	0.002	15
MHg vs THg	0.84	0.001	11	MHg vs THg	0.76	0.006	11
MHg vs Hg(II)	0.83	<0.001	24	MHg vs Hg(II)	0.60	0.018	15
SHg vs GLGs	0.73	0.011	11	SHg vs GLGs	0.81	0.001	12
SHg vs As	0.68	0.022	11	SHg vs As	0.61	0.017	17
SHg vs Cu	−0.63	0.001	24	SHg vs Cu	−0.74	0.001	15
SHg vs THg	0.84	0.001	11	SHg vs THg	0.77	0.006	11
SHg vs Hg(II)	0.84	<0.001	24	SHg vs Hg(II)	0.61	0.017	15
SHg vs MHg	1.0	<0.001	24	SHg vs MHg	1.0	<0.001	15
Se vs Cu	−0.64	0.001	24	Se vs As	−0.65	0.005	17
Se vs Hg(II)	0.49	0.015	24	Se vs THg	−0.70	0.016	11
Zn vs Cu	0.42	0.042	24	Zn vs THg	−0.71	0.015	11
Zn vs Se	−0.68	<0.001	24	Zn vs MHg	−0.60	0.019	15
				Zn vs SHg	−0.59	0.02	17
Blubber							
Se vs Cu	0.72	0.002	12				
Zn vs Cu	0.79	0.004	11				

[a] Data were ranked prior to analysis.
[b] vs = versus.
[c] Growth layer groups (GLGs), GLGs/2 approximates age in years (Goren et al., 1987).

Arsenic

Arsenic was relatively low in most tissues of both cetacean species, with highest As levels in blubber, which exceeded concentrations in liver about ten-fold. Similar findings have been reported for Canadian narwhal, in which As concentrations were also highest in blubber (Wagemann et al., 1983). The finding of highest As levels in blubber indicates that it is probably in organic form and thus lipid soluble. Bratton et al. (1997) speciated hepatic and renal As in a single bowhead whale via high-performance liquid chromatography (HPLC) and ascertained that 98% of As was in the form of arsenobetaine, which is the predominant form of As in marine animals (Neff, 1997). Several other organic As compounds have been identified in a variety of marine organisms (including marine mammals): arsenocholine, dimethylarsinic acid, methylarsonic acid and the tetramethylarsonium cation, which was detected in pinniped, but not cetacean liver (Goessler et al., 1998). These organic arsenic compounds are considered relatively non-toxic to mammals and are apparently excreted unchanged (Vahter et al., 1983; Sabbioni et al., 1991; Neff, 1997).

Previous research has found no accumulation of hepatic As with age, nor any association of As with other trace elements (Becker et al., 1997). In the present study, As in beluga liver showed a highly significant positive correlation with age, which may implicate accrual of As over time. Alternatively, higher levels of As in larger (i.e., older) as compared to smaller whales may reflect different environmental exposures, prey selection, and/or qualitative differences in blubber constituents. Larger, older whales may consume prey higher in As than that typically taken by younger, smaller animals. Or, the higher As levels in

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TABLE 7. Linear regression parameters for Se[a] versus various forms of Hg[a] or Hg + Ag[a] in tissues of Alaskan beluga whales, and selected elements (ppm ww) versus GLGs[b] (age).

Tissue	Variables	*n*	β_1-Coef[c]	r^2	*P* (regression)
Liver	Se[a] vs[d] THg[a]	24	1.90*	0.614	<0.001
	Se[a] vs THg[a] + Ag[a]	24	1.51	0.594	<0.001
	Se[a] vs Hg(II)[a]	24	2.95	0.222	0.020
	Se (ppm) vs HgD[e] (ppm)	24	0.61*	0.568	<0.001
Kidney	Se[a] vs THg[a]	24	0.90	0.525	<0.001
	Se[a] vs THg[a] + Ag[a]	24	0.89	0.551	<0.001
Liver	THg (ppm) vs GLGs[b]	20	1.34	0.353	0.006
	Se (ppm) vs GLGs	46	1.56	0.408	<0.001
	Cd (ppm) vs GLGs	46	0.07	0.279	<0.001
	MHg (ppm) vs GLGs	20	0.05	0.503	<0.001
	%MHg vs GLGs	20	−0.76	0.295	0.013
	HgD[e] vs GLGs	20	0.95	0.297	0.013
Kidney	THg (ppm) vs GLGs	20	0.20	0.354	0.006
	Se (ppm) vs GLGs	41	0.15	0.346	<0.001
	Cd (ppm) vs GLGs	41	0.27	0.346	<0.001
	HgII (ppm) vs GLGs	20	0.13	0.278	0.017
	MHg (ppm) vs GLGs	20	0.02	0.641	<0.001
	SHg (ppm) vs GLGs	20	0.15	0.331	0.008

[a] Expressed on a molar basis (μmol/Kg).
[b] Growth layer groups (GLGs), GLGs/2 approximates age in years (Goren et al., 1987).
[c] β_1 coefficient. For Se vs various forms of Hg or THg + Ag in liver and kidney, a * denotes that the slope was significantly ($P < .05$) different from one.
[d] vs = versus.
[e] Mercury level difference, HgD = total Hg (THg) − sum Hg (SHg) (all in ppm).

kidney and liver of males could result from gender-related prey preferences, blubber constituents or physiology.

Because As in these whales is probably in organic form and lipid-soluble, it can likely traverse the placenta and mammary gland, so that reproductively active females might transfer As to their offspring. While inorganic As is known to cross the placental barrier (Lindgren et al., 1984), studies on placental transfer of the organic As compounds found in seafood are lacking. However, it is not unreasonable to assume it could cross the placenta, because the predominant form of organic As in fish and crustaceans, arsenobetaine, is distributed widely throughout the body, is not metabolized, and appears to be excreted rapidly in the urine (Vahter et al., 1983). Although there were statistically significant correlations between As and various other elements, we found no consistent relationship between As and any other element across tissues or species.

Lead

Caution must be exercised in interpretation of Pb levels in animals taken using lead ammunition as was the case for beluga whales. Higher Pb levels were observed in liver as compared to kidney in both cetacean species; nevertheless, all tissues had very low Pb levels, which were well below concentrations likely to be associated with toxic effects (Puls, 1994).

Cadmium, copper, zinc

In bowhead and beluga whales mean kidney Cd concentrations were two- to four-fold higher than in liver. Cadmium in these two tissues was elevated in both species compared to what are considered normal levels in domestic species such as dogs and cattle (Puls, 1994). However, the Cd

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concentrations found in this study are well within ranges previously reported for arctic marine mammals (Hansen et al., 1990; Wagemann et al., 1996; Dietz et al., 1996, 1998). While the kidney Cd concentrations observed in this study are not unusual for some marine mammals, the threshold for Cd-induced chronic renal toxicosis is not known for these species. Among terrestrial mammals, including humans, a renal cortical concentration of 200 μg/g ww has been acceded as the critical threshold for chronic Cd toxicosis, seen clinically as a low molecular weight proteinuria (WHO, 1992). Our results, as well as most previous studies, have documented Cd concentrations in renal tissue of marine mammals from entire renicules, including medulla, and thus underestimate cortical Cd concentrations by about 25% (Dietz et al., 1998). For example, Cd has been reported at levels in excess of 50 μg/g ww in the intact bowhead renicules, which includes medullary and cortical tissue (Bratton et al., 1997). Since Cd concentrates in proximal tubular cells of the cortex, which averages 0.22 (22%) the thickness of the entire renicule (Haldiman and Tarpley, 1993), it might be assumed that cortical Cd levels are proportionately greater. Thus, Cd cortical levels may reach concentrations approximating 200 μg/g, the accepted threshold for renal toxicity (WHO, 1992). Very few studies of marine mammals have included histopathological examination of kidney in conjunction with chemical analyses. One exception was a study of ringed seals divided into three groups on the basis of kidney Cd levels. Concentrations in the five seals with the highest renal Cd burden averaged 399 μg/g ww and the five with the lowest having a range of only 1.63 to 5.19 μg/g ww. No lesions typical of chronic renal Cd toxicosis were observed among initially frozen renal tissue sections examined microscopically (Dietz et al., 1998). Histopathologic examinations of these beluga and bowhead whales in the present study would appear to support this assertion, because lesions characteristic of chronic Cd toxicosis were not observed (Woshner, 2000).

In bowhead and beluga whales, Cd accumulated with age in both liver and kidney, corroborating a well-documented phenomenon (Wagemann et al., 1983, 1989, 1990; Dietz et al., 1996; Becker et al., 1997; Krone et al., 1999); Cd also accumulated with age in muscle of bowhead whales. Cadmium demonstrated a significant positive correlation with Se in liver of both whale species, as well as in kidney of belugas. While Se has been shown to ameliorate toxic effects of Cd in laboratory animals (Ridlington and Whanger, 1981; Wahba et al., 1993; Rana and Verma, 1996), its association with Cd in the two whale species studied could have resulted from coincidental accumulation of these two elements with age. If Se were actively and proportionately assimilated as a consequence of Cd exposure in cetaceans, bowhead whales would have exhibited higher Se levels than were observed in this study. Additionally, Cd was positively correlated with Hg in liver and kidney of both species, probably as a consequence of mutual accretion with age. Because of these findings, it seems doubtful that Se plays a major role in Cd detoxification in these species; nevertheless, the possibility exists that Se may, at least to some degree, form temporary or stable complexes with Cd similar to the tiemannite (Hg-Se) complexes previously noted in liver of marine mammals (Martoja and Berry, 1980). While simple evaluation of tissue residues cannot adequately address this issue, present and future studies, especially using cell culture systems, may help elucidate the processes of Cd metabolism in marine mammal species.

Cadmium correlated positively with Zn in kidney of both species, as well as in liver of belugas. Copper also correlated positively with Ag in liver of both species. Intercorrelations among various combinations of the elements Cd, Cu, Hg, Ag and Zn were common. It has been shown repeatedly that both Cd and Hg accumulate

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with age (Norstrom et al., 1986; Braune et al., 1991; Muir et al., 1992; Wagemann et al., 1996). Cadmium binds primarily to metallothionein (MTH), an inducible, small molecular weight metal-binding protein important for both homeostasis and detoxification of various metals, especially Cd, Zn and Cu. Although we did not evaluate MTH's role in the current study, the association between Cd, Zn and Cu is most likely due to common binding with MTH, various isoforms of which are present in a wide array of tissue types. While the correlation of Cd with Hg is likely due to mutual accumulation over time, MTH is also capable of binding Hg and Ag, albeit with lesser affinity than for Cd, Zn, or Cu (Kaim and Schwederski, 1991). Various metals are capable of inducing MTH synthesis, in turn increasing the opportunity for binding of Cd and other metals to it (Piotrowski et al., 1974; Sugawara and Sugawara, 1984). Consequently, intercorrelations of the aforementioned metals could result both from mutual binding to inducible MTH, as well as coincidental accumulation with age via non-MTH processes. Previous studies in marine mammals have shown that most Hg is in the "insoluble" rather than the "soluble" cellular fraction, and thus not MTH-associated (Lee et al., 1977; Wagemann et al., 1984).

Whales in the present study had mean hepatic Cu concentrations that would be considered below the normal range for dogs or cattle (Puls, 1994). Nevertheless, levels in this study were in agreement with previously published data in cetacean species (Wagemann et al., 1996; Krone et al., 1999). The levels of essential elements, like Cu, are reported here for apparently healthy animals and may be useful in cases where a deficiency is suspected.

In belugas, the highest mean Zn concentration occurred in the epidermis. Wagemann et al. (1996) noted a similar observation in Canadian belugas and narwhals; muktuk (epidermis and blubber combined) contained approximately two to three times more Zn than muscle, liver or kidney. Wagemann et al. (1996) also found mean Zn levels to be similar across these latter three tissues within species (narwhals, belugas, and ringed seals), and Zn (and Cu) levels tended to track those of Cd. While we observed similar associations among Zn, Cd, and Cu within species, differences in mean Zn levels among tissues were more marked. This is most likely because Alaskan animals have a lower Cd burden compared to the Canadian animals, mirroring the geologic gradient of Cd, which decreases from east to west across North America (Wagemann et al., 1996).

A significant negative correlation was seen between Cu and age in liver, muscle and epidermis of belugas as well as in liver of bowheads, confirming a phenomenon previously reported in marine mammals (Wagemann et al., 1983, 1989). This decline in tissue Cu could result from loss of Cu over time, dilution of Cu levels by increased tissue mass with age, or decreased tissue level requirements for Cu (i.e., metabolic regulation).

Silver

Belugas had mean hepatic Ag levels that were markedly greater than those observed in bowhead whales (this study), ringed seals or polar bears (Woshner et al., 2001), corroborating a previously reported phenomenon for this species (Becker et al., 1995). The mean hepatic Ag concentration for beluga whales was approximately 300 times greater than levels in kidney. Other tissues of beluga (muscle, blubber and epidermis) exhibited uniformly low Ag levels, as did all tissues of bowheads. Becker et al. (1995) noted that in beluga liver, both Ag and Hg were correlated positively with Se, although Ag was more strongly correlated with Se than was Hg. In addition, there was a trend toward increasing concentrations of all three elements with age. Similar positive correlations for hepatic Ag with Se or age were not evident in the present research. In kidney of beluga and liver of bowhead whales Ag was

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positively correlated with Se, Cd, and various forms of Hg. A positive correlation between hepatic Ag and Cu was observed in both species examined and might connote binding by a common ligand.

The elevated levels of hepatic Ag in belugas remain a conundrum. This phenomenon may be a consequence of some distinctive alimentary predilection, or may reflect a novel physiologic function for this metal. The fact that hepatic Cu is low among belugas in comparison to terrestrial species suggests that Cu may be limiting in this species or not essential at the levels typically noted in terrestrial species. Since Ag is chemically similar to Cu (in terms of its valence shell configuration) and relatively benign except at high concentrations, it may function in homeostasis of Cu or other essential metals in belugas by way of MTH, but this is speculative. Australian dugongs were found to have hepatic Ag concentrations comparable to those of belugas in our research (Denton et al., 1980). This finding, in conjunction with an age-related decline in liver Cu led Denton et al. (1980) to suggest that aged dugongs may become Cu deficient, as Ag may occupy sites vacated by Cu.

Selenium and mercury

Mean Se concentrations in liver and kidney tissue of belugas were consistent with levels associated with toxicosis in domestic species such as cattle and dogs (Puls, 1994), although well within ranges previously reported for this species (Wagemann et al., 1996). In belugas, Se concentrations in liver were approximately 6-fold higher than levels in kidney. Belugas had higher Se concentrations in epidermis than bowhead whales. High concentrations of Se have been reported in epidermis of harbor porpoises from Greenlandic waters (Dietz et al., 1990; Paludan-Müller et al., 1993). Selenium in epidermis at concentrations approximately 100 times those of Hg led Paludan-Müller et al. (1993) to speculate that epidermal tissue may serve as a storage and/or excretory mechanism for Se to compensate for the Se-rich fish diet consumed by toothed cetaceans. Beluga whales molt annually, possibly reducing Se burdens through this mechanism. In bowhead whales, mean Se levels in liver and kidney were similar, and were significantly lower than in liver and kidney of belugas.

In general, Se increased with age indices in liver, kidney and epidermis of bowhead whales, as well as in liver and kidney of belugas, which echoed prior research findings in these and other marine mammal species (Dietz et al., 1996; Bratton et al., 1997), as did the close association between Hg and Se. While Hg-Se complexes have been demonstrated in liver tissue of marine mammals, the precise structure, function, and distribution of these complexes is not known (Martoja and Berry, 1980; Pelletier, 1985; Nigro and Leonzio, 1996), and presence of them in other tissues has not been addressed. In controlled experiments, Se has been proven to mitigate toxic effects of Hg (Stillings et al., 1974; Ridlington and Whanger, 1981; Cuvin-Aralar and Furness, 1991), Cd (Ridlington and Whanger, 1981; Rana and Verma, 1996) and Ag (Ridlington and Whanger, 1981; Wagner et al., 1975). It has been proposed that the ameliorative action of Se towards these three metals occurs via antioxidant activity because other antioxidants such as reduced glutathione (GSH) and vitamin E offer similar protection (Rana and Verma, 1996), or because these metals deplete the Se-dependent enzyme glutathione peroxidase (GSH-Px; Wagner et al., 1975; Sidhu et al., 1993). Evidence also exists for Se acting as an antioxidant through formation of metal selenide complexes, in particular for Ag (Aaseth et al., 1981) and Hg (Björkman et al., 1995). The strength of association between Cd and Se in the present study often rivaled that of Hg and Se. While this could be due merely to independent, concurrent increase with age, an interaction between Cd and Se cannot be ruled out.

Total Hg was present in liver and kidney of beluga at concentrations that would be

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considered high or even toxic among domestic species (Puls, 1994), but within ranges that have been reported for marine mammals (Koeman et al., 1973, 1975; Dietz et al., 1990; Braune et al., 1991; Wagemann et al., 1996). As previously mentioned, total Hg concentrations in beluga whale tissues varied depending upon analytical method employed, such that SHg was lower than THg, especially in liver. This discrepancy could be due to interlaboratory variation; alternatively, it might suggest that a substantial portion of the Hg in the organic fraction exists in a form other than MHg (Wagemann et al., 1997). The mean difference in the fraction of MHg obtained as percentage of THg versus SHg in the liver was approximately 7%. Nevertheless, whether computed as a percentage of THg or SHg, the majority of Hg in the liver (~90%) of beluga whales was inorganic [Hg(II)]. Conversely, virtually all (>90%) of the Hg in beluga muscle and epidermis was in the form of MHg.

In beluga liver, Hg(II), MHg and total Hg (either THg or SHg) increased significantly with age (GLGs). Of all forms of Hg, MHg had the lowest rate of accumulation (0.054 μg/g MHg per GLG) but the most linear relationship to age, with a regression coefficient of 0.50. While absolute concentrations of hepatic MHg increased with age, the percentage of hepatic total Hg in the form of MHg declined significantly with age. In beluga epidermis and muscle (where virtually all Hg is in the form of MHg), SHg, Hg(II) and MHg were positively correlated with age (GLGs), and there was a positive correlation between THg and age in epidermis. No significant correlation was observed between Hg and Se in epidermis, while in muscle, Se was positively correlated with Hg(II), but not with any other form of Hg. The MHg form was negatively correlated with Cu in both epidermis and muscle and with Zn in epidermis.

The 1:1 molar relationship purported to be characteristic of the association between hepatic Hg and Se in marine mammals was not borne out in the present research. Regression analyses of hepatic Se versus THg (or THg vs. Se) expressed in molar quantities for both species evaluated revealed slopes significantly different from one. While the slope for the linear regression of Se versus THg + Ag (expressed as molar quantities) did not differ significantly from one, the lack of a significant correlation between THg and Ag in liver, in conjunction with the age associations for these elements noted earlier, indicates that the accumulation of Ag is not responsible for disrupting a 1:1 Hg:Se ratio, as it does not appear to compete with Hg for Se. Various researchers have presented information supporting and refuting the consistency of a 1:1 ratio in tissues of marine mammals. Some authors have proposed a hypothesis of a threshold concentration of Se equivalent to the physiologically essential level of Se plus a reservoir of this metalloid (Hansen et al., 1990; Krone et al., 1999). These workers suggested that Hg creates a far greater binding capacity for Se, which eventuates the accumulation of the two elements in a 1:1 molar ratio fashion. If this is the case, it is possible that hepatic Hg concentrations encountered in this study did not consume the Se reservoir and thus did not stimulate markedly increased Se uptake and binding. Regardless, it is important to recognize that originally, Koeman et al. (1973, 1975) arrived at this 1:1 ratio by averaging regression slopes of Hg versus Se for 56 animals of nine different marine mammal species, as well as detecting a 1:1 Hg:Se molar ratio in brain, kidney and liver from a single seal. The tendency for Hg:Se molar concentrations to converge on a 1:1 ratio was offered as evidence that Se complexation with Hg was the probable mode of detoxification in marine mammals, occurring through an active process rather than by passive coaccumulation. While this perception was profound, subsequent researchers have suggested that deviations from this 1:1 molar status indicate abnormality, or even toxicity unaccompanied by

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detected evidence of compromised health (Arima and Gakura, 1979; Caurant et al., 1996). Findings of the present study do not counter the recognized relationship of Hg with Se, or the premise that this association could be the primary mode of Hg detoxification in marine mammals. Nevertheless, it may not be necessary to achieve a 1:1 relationship even in tissues that accumulate high levels of Hg (liver of belugas) for the animals to be protected from Hg toxicosis. The current research also suggests the existence of ancillary detoxification mechanisms for Hg in these species, especially in tissues other than liver.

Results of the present study indicate a strong need for more comprehensive research of mercury speciation, metal localization at the cellular level, coordinated assessment of health of marine mammals by gross and histological examination, and physiologic/behavioral assessments. These issues form the focus of our on-going research and present us with great challenges in understanding the toxicodisposition, interactions, essentially and potential adverse effects of these elements in arctic marine mammals.

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