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Methylmercury and total mercury in tissues of arctic marine mammals

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Abstract

Concentrations of methylmercury, total mercury and selenium in marine mammal tissues were determined in liver, muscle, skin (muktuk) and blubber of belugas, ringed seals and narwhal, using atomic absorption and capillary gas chromatography with ECD detection. Mean MeHg levels in the types of tissues analysed, except blubber, generally exceeded the Canadian Federal Consumption Guideline for mercury in fish (0.5 μg/g wet wt.). A spatial trend of higher MeHg levels in western compared to eastern Arctic belugas and ringed seals was found which followed a similar trend observed for total mercury. Factors which could explain this trend are discussed. Robust linear regression of MeHg on total Hg and MeHg on age of animals was performed and a strong correlation between the two variables was found in each case. The ratio of MeHg to total mercury as indicated by the regression coefficients was close to one for muscle and skin (muktuk) while for liver it was < 1. The mean percentage of MeHg in the liver of marine mammals was 3–12% of the total Hg in this tissue depending on species and location. It is postulated that the formation and deposition of mercuric selenide in the liver is part of the demethylation process in this tissue. This is based on the relatively low fraction of MeHg in the liver not withstanding the fact that the predominant form of mercury taken up via food is MeHg. The long half-life for total mercury and the relatively short half-life for MeHg in this organ are in accord with this postulate as is the 1:1 stoichiometric relationship between mercury and selenium in the liver.

Keywords: Methylmercury; Marine mammals; Arctic belugas; Ringed seals

1. Introduction

Extensive information on total mercury concentrations in tissues of Arctic marine mammals is now available (Wagemann and Muir, 1984; Zeisler et al., 1993; Skaare et al., 1994; Becker et al., 1995; Mackey et al., 1995; Dietz et al., 1996; Wagemann et al., 1996). In many instances the total mercury concentration significantly exceeds the Canadian Federal Guideline for mercury in fish for human consumption (0.50 μg/g wet weight). Considering that in the Arctic the diet of Indigenous people consists partly of marine mam-

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mal tissues, and that methylmercury (MeHg) is more toxic than most inorganic mercury compounds, the importance of measuring MeHg in marine mammal tissues is obvious. Some MeHg data (Julshamn et al., 1987; Wagemann, 1994; Donais et al., 1996) and 'organic' mercury data are currently available for Arctic marine mammals (Dietz et al., 1990), pilot whales (Julshamn et al., 1987; Schintu et al., 1992) and Atlantic fin whales (Sanpera et al., 1993). This information, although valuable is not extensive geographically or temporally.

The objectives of this study were: (1) to determine whether or not a spatial trend, higher in the western than the eastern Canadian Arctic, exists for MeHg in marine mammals as it does for total mercury (Wagemann et al., 1996); (2) to provide a sufficiently broad MeHg data base for use in estimating risk from dietary intake of MeHg via the consumption of beluga (*Delphinapterus leucas*), ringed seal (*Phoca hispida*) and narwhal (*Monodon monoceros*) tissues; and (3) to test circumstantially the hypothesis that an end-product of demethylation of MeHg may be mercuric selenide.

2. Materials and methods

Samples were obtained between 1987 and 1994 from six sites in the western Arctic (Sachs Harbor, Holman, Paulatuk, East White Fish, Shingle Point, Hendrickson Island) and 11 in the eastern Arctic (Repulse Bay, Sanikiluaq, Iqaluit, Resolute Bay, Coral Harbor, Pond Inlet, Broughton Island, Grise Fiord, Clyde River, Arviat, Umiujaq). Muscle, liver, skin and blubber tissues of beluga whales (*D. leucas*) and narwhal (*M. monoceros*) as well as muscle and liver of ringed seals (*P. hispida*) were analysed for total mercury and MeHg. Liver tissues were also analysed for selenium.

Cold-vapour atomic absorption spectroscopy (CVAAS) was used to measure total mercury in liver, muscle, skin and blubber and MeHg in muscle and skin. This method was less time consuming than the GC-ECD method for MeHg and was applicable for muscle and skin since nearly all the mercury in these tissues was shown to be MeHg (Wagemann et al., 1997). For liver, the GC-ECD method was used for MeHg determinations since only a small fraction of the total mercury in this tissue was MeHg (Wagemann et al., 1997). MeHg was not determined in blubber because the total mercury concentration in this tissue was relatively low.

2.1. Total mercury determination by CVAAS

The tissues (approx. 0.2 g) were digested at 90°C for 2 h with a mixture of nitric and sulfuric acids (1:4 v/v) and total mercury was determined by CVAAS, using the air-segmented, flow injection method (Armstrong and Uthe, 1971). A TM 3200 (TSP Thermo Separation Products) mercury monitor was used. Data were recorded with a 'Chrom Jet Integrator' (TSP Thermo Separation Products). Aqueous working standards (1–10 μg/l) were prepared daily from a 'Baker instra-analysed' 1000 μg/ml mercury stock solution (from mercury nitrate). The detection limit for mercury in tissues by the CVAAS method under the operating conditions employed was 5 ng/g wet wt.

2.2. Total selenium determination

Essentially the semi-automatic borohydride method of Vijan and Wood (1976) was used. Tissue samples were digested with nitric, perchloric and sulfuric acids (4:1:0.5 v/v), and the resulting digest was diluted with hydrochloric acid and water to 30% hydrochloric acid. The diluted digest and reductant (2% borohydride solution) were combined at flow rates of 4 and 1 ml/min, respectively, using a Technicon pump, Model III, coupled to a Varian programmable Model 55 sampler. The hydride was decomposed in a heated quartz tube and the selenium was analysed at a wavelength of 196.1 nm using a Varian Spectra AA-20 Absorption Spectrometer.

2.3. Tissue treatment for MeHg determination

The release of MeHg from the tissues was achieved by the commonly used procedure of Uthe et al. (1972) using acidic sodium bromide and cupric sulfate. Approx. 1 g wet wt. of tissue

was homogenized (Brinkman Kinematica Homogenizer) for 60 s with acidic aqueous sodium bromide/cupric sulfate solution (5 ml of 30% NaBr in 4N H_2SO_4 and 7.5 ml of 2.5% $CuSO_4$ in 4N H_2SO_4) in a 50 ml Teflon centrifuge tube.

2.4. MeHg determination in muscle and muktuk

To the acidic aqueous tissue homogenate, methylene chloride-hexane, 5–10 ml (3:2 vol. ratio) was added, the mixture was vortexed for 60 s and centrifuged. An aliquot (0.5–1 ml) of organic phase was heated at 60°C until the solvent evaporated, and then digested and analysed as for total mercury using the CVAAS method.

2.5. MeHg determination in liver

To the acidic, aqueous tissue homogenate, 10 ml of toluene was added, the mixture vortexed for 60 s and then centrifuged. A fraction (2–3 ml) of the toluene phase was withdrawn, aqueous thiosulfate solution (TS) was added (3–4 ml, 0.005 N $Na_2S_2O_3$), the mixture was vortexed for 45 s and centrifuged at 3000 rev./min for 3–5 min (or until separation was achieved). The addition of ethanol to the TS solution was omitted from the procedure as this resulted in better phase separation. To the separated thiosulfate solution, 1–2 ml, KI was added (0.5 ml, 3M aqueous). This solution was then extracted with toluene by vortexing the mixture for 60 s and centrifuging at 3000 rev./min for 60 s. The separated toluene extract was dried over anhydrous sodium sulfate and injected (1 μl) into the GC column for MeHg analysis.

Various operational parameters for the chromatographic procedure were partly adopted from published reports (Bulska et al., 1992; Lorenzo et al., 1993; Alli et al., 1994) with some modifications, such as a shorter capillary column, a different stationary phase, and a different temperature program. A Varian model 3400 gas chromatograph with a ^{63}Ni electron capture detector (ECD), temperature programmable injector (SPI), and a 5 m, SPB-5 mega-bore column (0.53 mm ID) with a bonded film (5 μm) of polysiloxane (94% dimethyl, 5% diphenyl, 1% vinyl) was used. The carrier gas was helium (12 ml/min), the make-up gas nitrogen (28 ml/min). The temperature of the column and injector was maintained at 50°C for 1 min, programmed to increase to 240°C (at 20°C/min), and maintained at this temperature for 15 min before the cycle was repeated. The detector was maintained at 300°C at all times. Working MeHg standards made from CH_3HgI (5–100 ng/ml Hg in toluene) for GC analysis were prepared daily from a toluene stock solution (1 μg/ml Hg). Within the concentration range of these working standards, the ECD response was linear. The absolute detection limit for CH_3HgI by GC-ECD was 2 pg Hg (based on $3 \times$ S.D. of blank analyses), or 10–80 ng/g Hg wet wt. (depending on the dilution factor) in terms of the procedure used.

2.6. Statistics

Means were compared (at $\alpha = 0.05$) using Scheffe's multiple means comparison test (Scheffe, 1959). This test is fairly robust toward the non-normal distribution of the population and does not require equal sample size. To determine the association between age of animals and MeHg, total mercury and percent MeHg, robust, linear regression (Andrews' sine) was used (Andrews, 1974) which minimizes regression errors by reiterative analysis and weights data points accordingly, thus minimizing or eliminating the influence of extreme values. Regression slopes were tested for parallelism (at $\alpha = 0.05$) using the large-number z-test (Kleinbaum and Kupper, 1978). Regressions of MeHg on total Hg were forced through the origin, since a positive intercept on the MeHg axis at zero total Hg concentration did not make physical sense. It was ascertained through repeated chemical analyses using certified reference materials that these relatively small intercepts did not represent an instrumental or analytical bias. If this had been the case, then a non-forced regression would have been appropriate. Closer examination revealed that the intercepts were in essence an artifact produced by

Table 1
Means ± S.D. (arithmetic), *n*, ranges of methylmercury (MeHg) and total mercury concentrations (μg/g Hg wet wt.) and percentages of MeHg to total mercury in tissues of belugas, narwhal and ringed seals from the western and eastern Canadian Arctic.

Area/species/ year of sampling	Muscle		Liver		Skin		Blubber
	MeHg μg/g Hg	Hg total μg/g Hg	MeHg μg/g Hg	Hg total μg/g Hg	MeHg μg/g Hg	Hg total μg/g Hg	Hg total μg/g Hg
Western Arctic Belugas, 1993–1994	(97%)		(5.9%)		(91%)		
Mean ± S.D.	1.32 ± 0.66	1.33 ± 0.66	1.87 ± 1.15	27.06 ± 24.67	0.69 ± 0.37	0.78 ± 0.41	0.103 ± 0.005
n	75	75	77	77	63	65	60
Range	0.35–3.16	0.41–3.44	0.11–6.13	0.31–116.55	0.14–1.71	0.19–1.93	0.19–0.02
Eastern Arctic Belugas, 1993–1994	(93%)		(11.7%)		(89%)		
Mean ± S.D.	0.96 ± 0.39	1.04 ± 0.43	1.39 ± 0.54	10.19 ± 8.00	0.53 ± 0.19	0.59 ± 0.22	0.07 ± 0.05
n	74	74	73	73	45	45	62
Range	0.44–2.36	0.44–2.77	0.44–3.06	1.24–38.56	0.31–1.07	0.32–1.37	0.19–0.01
Eastern Arctic Narwhal, 1992–1994	(96%)		(9.0%)		(92%)		
Mean ± S.D.	0.97 ± 0.33	1.03 ± 0.37	1.03 ± 0.42	10.77 ± 8.02	0.54 ± 0.19	0.59 ± 0.04	0.04 ± 0.03
n	56	56	55	55	48	48	45
Range	0.39–1.66	0.41–1.94	0.20–2.43	0.32–37.21	0.15–1.19	0.16–1.27	0.13–0.003
Western Arctic Ringed seals, 1987–1994	(94%)		(2.7%)				
Mean ± S.D.	0.37 ± 0.31	0.41 ± 0.37	1.03 ± 0.88	28.64 ± 29.31	n/a	n/a	n/a
n	39	39	66	66			
Range	0.11–1.49	0.1–1.58	0.19–4.08	0.54–137.17			
Eastern Arctic Ringed seals, 1992–1994	(92%)		(2.7%)				
Mean ± S.D.	0.43 ± 0.31	0.46 ± 0.33	0.61 ± 0.54	18.99 ± 26.32	n/a	n/a	n/a
n	100	100	146	144			
Range	0.05–1.80	0.05–1.93	0.04–4.02	0.09–149.5			

n/a, not analyzed.

the non-linear relationship of the two variables close to the origin, i.e. at low total Hg and MeHg concentrations. However, a non-linear fit (power curve) did not improve the overall fit relative to a linear fit, and a linear fit forced through the origin was chosen. Curve fits were performed with the computer program Table 1 Curve™, Vers. 4, Jandel Scientific, 2591 Kerner Blvd. San Rafael, CA 94901, USA. Statistical analyses were performed with the computer program NCSS (Number Cruncher Statistical Systems) Ver. 6.0, Kaysville, Utah, USA.

3. Results

3.1. Belugas

The tissue samples were collected (1992–94) at different sites in the western and eastern Arctic at locations to the west and east of longitude 105°W, respectively. No significant differences were found in the mean MeHg concentrations in tissues for sampling sites within the western and eastern Arctic, respectively. However, the overall MeHg means for the western and eastern Arctic,

Table 2
Outcome of comparison[1] of mean mercury concentration between the western and eastern Arctic

Species	Liver		Muscle		Muktuk	
	MeHg	Hg_{total}	MeHg	Hg_{total}	MeHg	Hg_{total}
Beluga	D[2]	ND[3]	D	D	D	D
Ringed seal	D	D	ND	ND	n/a[4]	n/a

[1]Using Scheffe's means comparison test (Scheffe, 1959).
[2]D, significant difference (at $\alpha = 0.05$).
[3]ND, no significant difference (at $\alpha = 0.05$).
[4]n/a, skin samples were either not available or were not analysed.

respectively (Table 1), when subjected to a similar comparison (multiple means comparison test, Scheffe, 1959) were found to be significantly different at $\alpha = 0.05$, for liver, muscle and skin (Table 2). The spatial trends for MeHg in muscle, skin and liver of belugas followed that for total mercury reported elsewhere (Wagemann et al., 1996).

Robust linear regressions of MeHg in muscle, skin and liver on age of animals showed that the two variables were significantly correlated in each tissue (Table 3). The regression slopes of MeHg on age were significantly different for the western

Table 3
Mean age, and robust regression parameters (slope, intercept, R^2, n) of methylmercury (MeHg) on age in muscle, liver and skin of belugas, and ringed seals from the western and eastern Arctic, and MeHg in tissues of narwhal on length of animals

Area/year of sampling	Age years	Muscle	Liver	Skin
Belugas — western Arctic 1993 / 94				
Slope	19.3	0.0554, $P < 0.0001$	0.0548, $P = 0.0018$	0.03406, $P < 0.0001$
Intercept		0.2402, $P = 0.1412$	0.7505, $P = 0.0286$	−0.0352, $P = 0.6836$
R^2		0.3939	0.1357	0.5171
n		69	69	58
Belugas — eastern Arctic 1993 / 94				
Slope	13.5	0.0117, $P = 0.0532$	0.0311, $P = 0.0008$	0.0149, $P = 0.0002$
Intercept		0.664, $P < 0.0001$	0.865, $P < 0.0001$	0.2836, $P < 0.0001$
R^2		0.0516	0.146	0.2866
n		73	73	45
Narwhal — eastern Arctic 1992 / 94	–	Length	Length	Length
Slope		0.0029, $P = 0.0004$	−0.000621, $P = 0.3005$	0.00124, $P = 0.0335$
Intercept		−0.312, $P = 0.30016$	1.356, $P < 0.0001$	0.0152, $P < 0.9499$
R^2		0.431	0.0486	0.267
n		25	24	17
Ringed seal — western Arctic 1987 / 94				
Slope	7.4	0.0105, $P < 0.0001$	0.0603, $P < 0.0001$	
Intercept		0.1207, $P < 0.0001$	0.156, $P = 0.0163$	n/a
R^2		0.509	0.599	
n		32	57	
Ringed seal — eastern Arctic 1992 / 94				
Slope	6.1	0.00334, $P = 0.4046$	0.0483, $P = 0.00173$	
Intercept		0.409, $P < 0.0001$	0.421, $P = 0.0003$	n/a
R^2		0.0249	0.411	
n		30	21	

Table 4
Outcome of test[1] for parallelism of two regression slopes of mercury on age of animals between the western and eastern Arctic

Species	Liver		Muscle		Muktuk	
	MeHg	Hg_{total}	MeHg	Hg_{total}	MeHg	Hg_{total}
Beluga	ND[3]	D[2]	D	D	D	D
Ringed seal	ND	ND	ND	ND	n/a[4]	n/a

[1]Using the large-number *Z*-test (Kleinbaum and Kupper, 1978).
[2]D, significant difference (at $\alpha = 0.05$).
[3]ND, no significant difference (at $\alpha = 0.05$).
[4]n/a, skin samples were either not available or were not analysed.

and eastern Arctic for muscle and skin (Table 4), indicating that the difference in the MeHg means for the western and eastern Arctic were not entirely due to age differences of animals despite a preponderance of older animals in the group from the western Arctic.

Regression slopes of MeHg in the liver on age were all positive (Table 3) reflecting, either a decreasing elimination rate of MeHg with age, possibly because of a decreasing demethylating efficiency of MeHg with age, or increasing uptake of MeHg with age. Regression slopes of percent MeHg on age were negative (not shown), most probably because of the increased accumulation of Hg with age (mostly HgSe). At low concentrations of total mercury < 10 $\mu g/g$ (i.e. in young animals) the MeHg percentage was much higher than the mean for the sample. In the limited domain near the origin, a curvilinear regression (power curve) of MeHg on total Hg seemed appropriate. Nevertheless, a linear function through the origin was fitted since this gave a better overall fit in terms of R^2.

Only total mercury was measured in blubber. The mean total mercury concentrations, 0.103 and 0.07 $\mu g/g$, in the western and eastern Arctic, respectively, were significantly different and followed the same spatial trend of higher total mercury concentrations in liver and muscle of western Arctic marine mammals as reported elsewhere (Wagemann et al., 1996).

With few exceptions, MeHg in muscle of belugas from all locations was > 0.5 $\mu g/g$ wet wt., exceeding the Federal Guideline for human consumption of fish. Average (arithmetic) MeHg concentrations in muscle tissue from approx. 75 animals from the western and a similar number from the eastern Canadian Arctic were 1.32 and 0.96 $\mu g/g$ Hg, wet wt., respectively (Table 1). On average, 97 and 93% of the total mercury in muscle in the western and eastern Arctic, respectively was MeHg. In the skin (muktuk), mean MeHg concentrations were 0.69 and 0.53 $\mu g/g$ Hg wet wt., in the western and eastern Arctic, respectively. MeHg comprised 91 and 89% of the total mercury in the western and eastern Arctic, respectively in this tissue.

Average MeHg concentrations in the liver were 1.87 and 1.39 $\mu g/g$ Hg wet wt., in western and eastern Arctic belugas, respectively, only slightly higher than in muscle, notwithstanding the much higher total mercury concentrations in the liver (27.1 and 10.2 $\mu g/g$ wet wt., in the western and eastern Arctic, respectively). On average, 6 and 12% of the total mercury in the liver of belugas was MeHg in the western and eastern Arctic, respectively. Since MeHg and total mercury were strongly correlated (Table 5), the average percentages of MeHg were obtained by regression analysis (regression slope) of MeHg on total mercury (Wagemann et al., 1997). The percent MeHg distribution is clearly positively skewed (Fig. 1). The mean percentages of MeHg obtained by regression analysis, by taking the ratio of the average MeHg over the total Hg, and by averaging the individual ratios are indicated by arrows above the curves in Fig. 1 for belugas, narwhal and ringed seals. The mean obtained by averaging the individual ratios was consistently farthest from the mode.

Linear regression of total selenium on total mercury was significant and gave, in terms of atomic concentrations, a regression slope of approximately one (Fig. 2), indicating that the two elements are stoichiometrically associated in this tissue in a 1:1 ratio as in HgSe. A hypothetical diagram (Fig. 3) shows HgSe as an end-product of demethylation.

Table 5
Parameters for robust linear regression (forced through the origin) of methylmercury on total mercury in muscle, liver and skin of belugas, narwhal and ringed seals from the western and eastern Arctic

Area/year of sampling	Muscle	Liver	Skin
Belugas — western Arctic 1993 / 94			
Slope	0.9685, $P < 0.0001$	0.0590, $P < 0.0001$	0.9069, $P < 0.0001$
R^2	0.9962	0.8708	0.9949
n	73	76	61
Belugas — eastern Arctic 1993 / 94			
Slope	0.9293, $P < 0.0001$	0.1168, $P < 0.0001$	0.8946, $P < 0.0001$
R^2	0.9969	0.8692	0.9942
n	74	73	45
Narwhal — eastern Arctic 1992 / 94			
Slope	0.9567, $P < 0.0001$	0.0902, $P < 0.0001$	0.9185, $P < 0.0001$
R^2	0.9970	0.8891	0.9977
n	56	55	48
Ringed seal — western Arctic 1987 / 94			
Slope	0.9357, $P < 0.0001$	0.0273, $P < 0.0001$	
R^2	0.9978	0.8537	n/a
n	38	66	
Ringed seal — eastern Arctic 1992 / 94			
Slope	0.9193, $P < 0.0001$	0.0265, $P < 0.0001$	
R^2	0.9972	0.8455	n/a
n	98	137	

3.2. Narwhal

Mean MeHg concentrations in narwhal muscle, liver and skin from all sampling sites in the eastern Arctic were compared using Sheffe's multiple means comparison test. The data were combined since no significant differences were found among sampling sites. Narwhal are not found in the western Arctic and a west–east comparison for these animals was obviously not possible.

The average MeHg concentration in muscle of 56 narwhal was 0.97 μg/g Hg wet wt. (Table 1), significantly exceeding the Federal Guideline for mercury in fish. In the liver it was similar (1.03 μg/g Hg wet wt.), notwithstanding the much higher total mercury concentration (10.8 μg/g Hg wet wt.) in this tissue. On average, MeHg was approx. 9% of the total mercury in this tissue. MeHg and total mercury concentrations were strongly correlated, and regression analysis was used to obtain this average percentage (Table 5).

The average concentration of MeHg in the skin of narwhal (0.54 μg/g Hg wet wt.) was nearly identical to that in the skin of eastern Arctic belugas (0.53 μg/g Hg wet wt.). As in muscle, mercury in the skin was approx. 92% in the form of MeHg.

The average concentration of total mercury in blubber was only 0.04 μg/g and therefore MeHg was not measured in this tissue.

Since an accurate and efficient method for determining the age of narwhal is still lacking, MeHg concentrations in the different tissues were regressed on length of animals (Table 3). A significant correlation between MeHg and length was found for muscle and skin but not for liver.

Total selenium and total mercury were also significantly correlated in narwhal liver, with a regression slope of approximately one, on an atomic concentration basis (Fig. 2).

3.3. Ringed seals

The mean MeHg concentration in the liver of

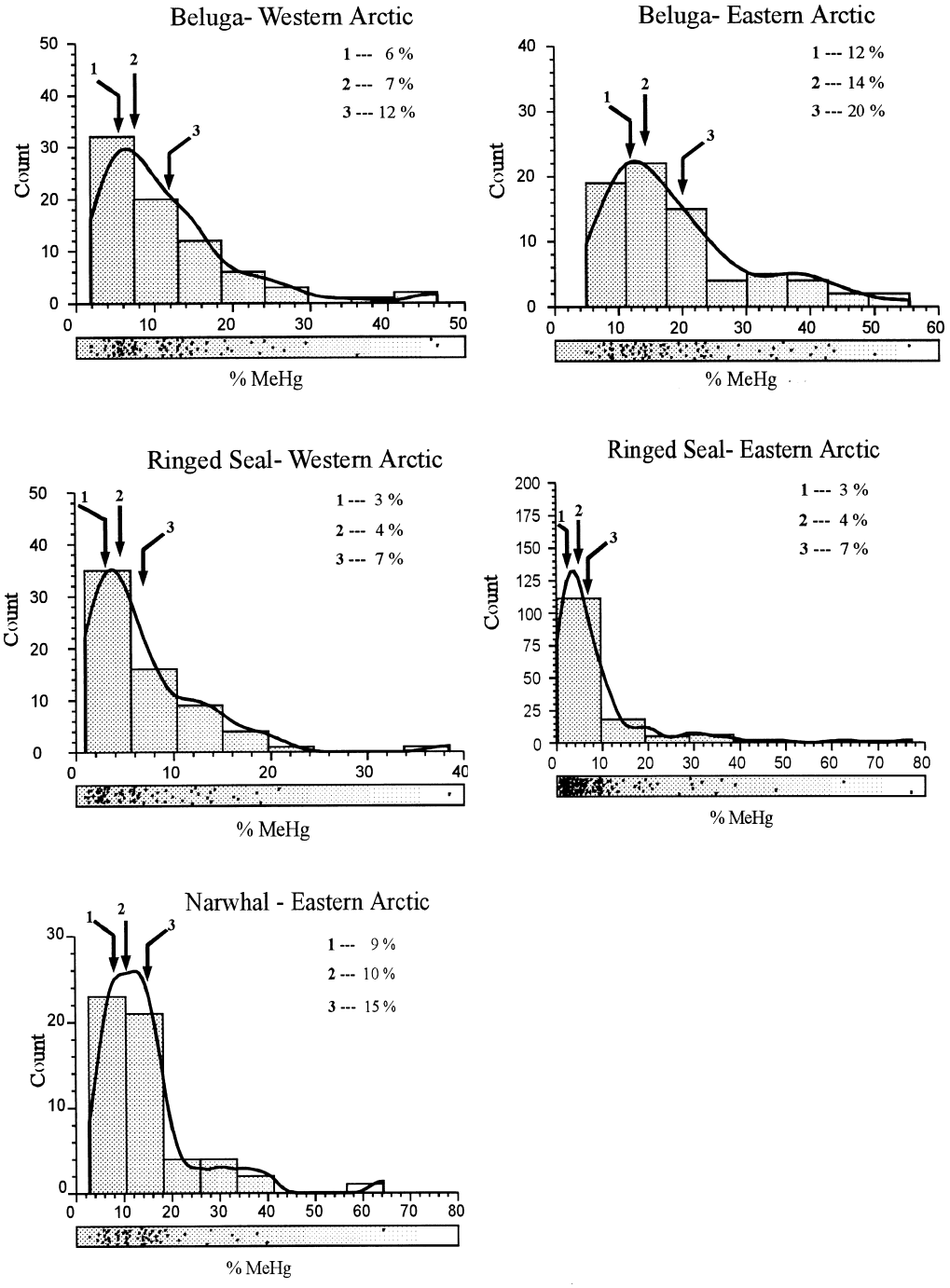

Fig. 1. Frequency distribution of percentage of MeHg in liver of marine mammals from the Canadian Arctic. The arrows above each distribution graph indicate the position of the different percentage means: 1 = by regression analysis; 2 = the ratio of the mean MeHg over the mean total Hg; 3 = the average of the individual MeHg/total Hg ratios.

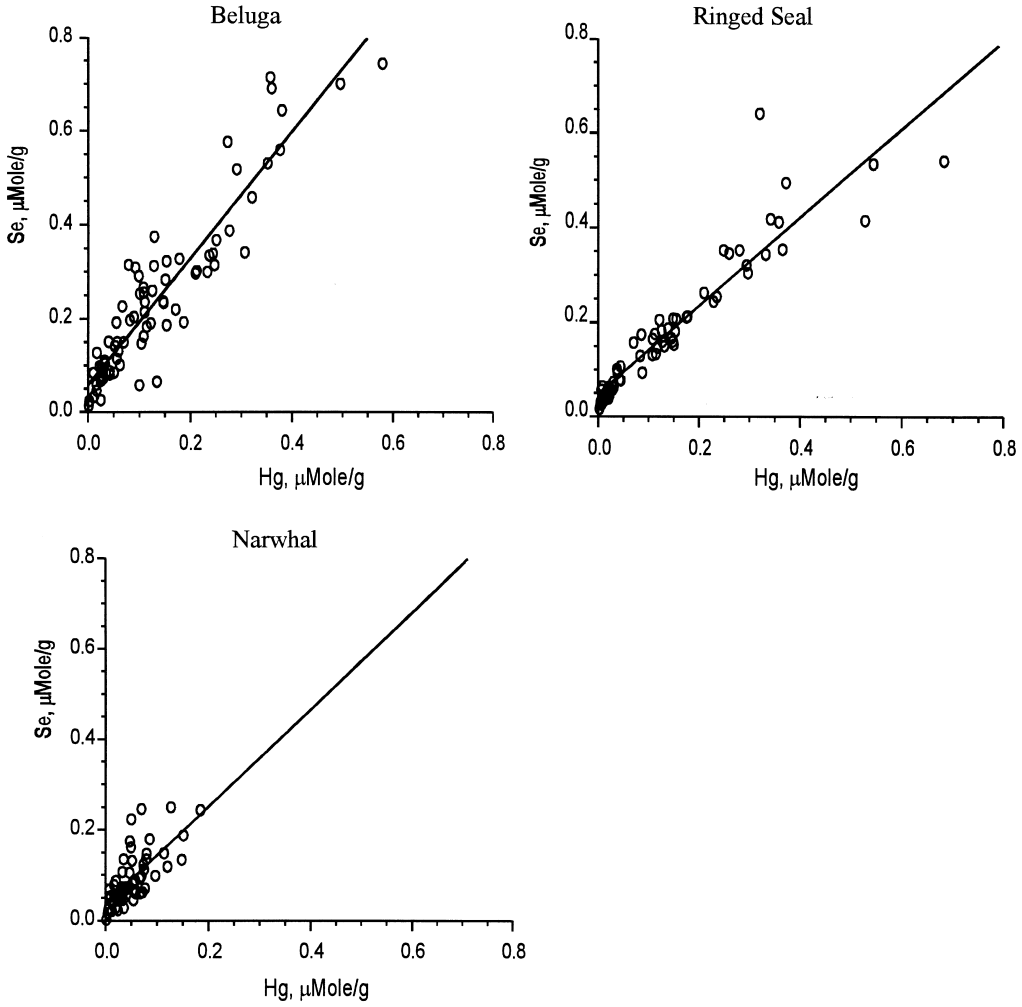

Fig. 2. Robust linear regression of total Se on total Hg (atomic concentrations) in liver of belugas, narwhal and ringed seals from the Canadian Arctic.

ringed seals was significantly higher ($\alpha = 0.05$) in the western (1.03 μg/g) than the eastern (0.61 μg/g) Arctic. There was no significant difference in the mean ages of the two groups of ringed seals and the mean concentration difference between the western and eastern Arctic was therefore not attributable to age differences between the two groups. There was a significant correlation between age and MeHg in liver, but the regression slopes of MeHg on age of animals were not significantly different for the western and eastern Arctic for any of the three tissues. The regression slope for MeHg on age was positive for western and eastern Arctic ringed seals, possibly because the uptake of MeHg increased with age, or MeHg was less efficiently demethylated or otherwise eliminated with age (Table 3). On the other hand, the regression slope of percentage MeHg on age was negative due to the increase in the total mercury burden, probably HgSe, in the liver with age.

The average MeHg concentration in the liver of ringed seals, 2.7% of the total mercury in western and eastern Arctic, was lower than in belugas and narwhal. Since MeHg and total mercury were strongly correlated, the average per-

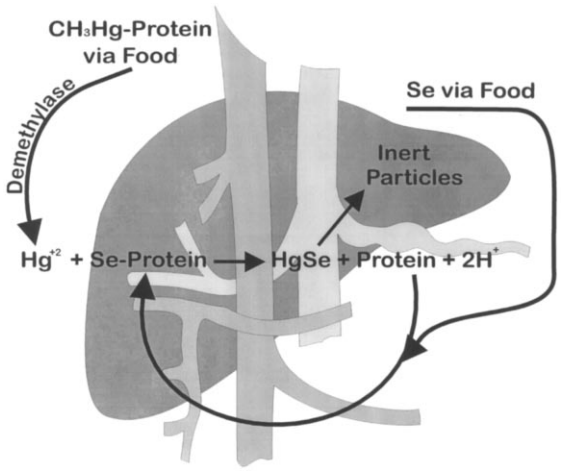

Fig. 3. Hypothetical demethylation mechanism with consequent formation of inert mercuric selenide particles in the liver.

centages were obtained by regression analysis of MeHg on total mercury (Table 5).

The mean MeHg concentrations in muscle at 0.37 and 0.43 μg/g Hg wet wt., in western and eastern Arctic seals, respectively, were clearly not significantly different, and below the Federal Consumption Guideline concentration for mercury in fish (0.5 μg/g wet wt.). Mercury was mostly in the form of MeHg (94 and 92% of the total mercury in this tissue in the western and eastern Arctic, respectively). There was a significant correlation of MeHg in this tissue with age in the western but not in the eastern Arctic.

As was the case for belugas and narwhal, total selenium and total mercury concentrations were also significantly correlated in ringed seal liver (Table 6), and the regression slope was also positive and approximately one on an atomic concentration basis, Fig. 2.

Table 6
Robust linear regression parameters for total selenium on total mercury (μg-atomic/g) in liver of marine mammals from the Canadian Arctic

Animal species and area	Robust regression parameters
Beluga Western Arctic	Slope = 1.281, $P < 0.0001$ Intercept = 0.052, $P < 0.0001$ $R^2 = 0.936$
Beluga Eastern Arctic	Slope = 1.053, $P < 0.0001$ Intercept = 0.025, $P < 0.0001$ $R^2 = 0.724$
Ringed Seal Western Arctic	Slope = 0.967, $P < 0.0001$ Intercept = 0.035, $P < 0.0001$ $R^2 = 0.974$
Ringed Seal Eastern Arctic	Slope = 0.812, $P < 0.0001$ Intercept = 0.043, $P < 0.0001$ $R^2 = 0.872$
Narwhal Eastern Arctic	Slope = 1.038, $P < 0.0001$ Intercept = 0.026, $P < 0.0001$ $R^2 = 0.737$

4. Discussion

A significantly higher mean concentration of total mercury in marine mammals in the western than in the eastern Arctic was first reported by Wagemann et al. (1995) and attributed partly to geological differences between the two regions. The question arose whether or not MeHg concentrations followed this trend. We have found that MeHg does follow this trend since the mean MeHg concentration was significantly higher in the western Arctic than in the eastern Arctic, in muscle, liver and skin of belugas and in the liver of ringed seals. Factors, such as geological background and the level of food contamination in the particular area (some of it perhaps from atmospherically-deposited Hg) must be responsible for the trend. The geology in the western Arctic is such that generally higher environmental background concentrations of mercury are present there in comparison with the eastern Arctic (Wagemann et al., 1995). This is presumably reflected in the food chain leading to marine mammals, resulting in the observed higher levels of MeHg in marine mammals in this region. Beluga muscle and muktuk unequivocally showed a spatial MeHg trend. For liver, the significantly different mean MeHg concentrations between the western and eastern Arctic can be partly explained by a mean age difference. Liver tissue may be a poor choice for determining spatial or temporal trends of MeHg because of the continuous demethylation of this compound in the liver. In all the examined tissues of belugas and ringed

seals (except in muscle of ringed seals from the eastern Arctic) MeHg was significantly, positively correlated with age (Table 3). The regression slopes (MeHg vs. age) were significantly different between the western and eastern Arctic for muscle and skin of belugas (Table 4), indicating the existence of a spatial trend notwithstanding any age differences. Ringed seals from the eastern and western Arctic were not confounded by significant age differences and the mean MeHg concentrations in liver were indicative of a spatial trend.

There is very little information in the literature on MeHg in the Arctic marine food web that could provide more insight into the observed spatial trend. Biomagnification of MeHg from lower to higher trophic levels has been reported (Korhonen et al., 1995). Belugas feed primarily on fish, and ringed seals on fish and amphipods, and the concentration of MeHg in their tissues is a reflection of the level of MeHg contamination in the regions where the animals predominantly feed. Ultimately, MeHg levels at the base of the food chain are largely determined by water chemistry which controls MeHg speciation (Mason et al., 1995). Although the animals migrate seasonally within the western and eastern Arctic, respectively, there is no significant mixing of marine mammal populations through east–west migration between the two regions (Wagemann et al., 1996) which preserved the observed concentration differences between the two populations.

Primarily MeHg, derived from muscle tissue of fish (muscle tissue makes up most of the mass of the fish) is taken up by marine mammals, but only a small fraction of the total mercury in the liver is found to be MeHg. Our results and those of others (Dietz et al., 1990; Palmisano et al., 1995) show that the mean MeHg concentration in the liver of marine mammals is usually not much higher than in muscle, seldom exceeding 2.0 $\mu g/g$ (Table 1) despite the fact that the total mercury concentration in the liver is several factors higher than in muscle. In contrast to muscle and skin where the percentage of MeHg to total mercury was between 89 and 97%, it ranged from 3 to 12% in the liver, obtained by robust, linear regression of MeHg on total Hg (Table 5). Clearly, demethylation must occur in the liver continuously, leading, we hypothesize, to the formation of inorganic mercury (Hg^{2+}) and the detoxification of the latter by reaction with selenium to form mercuric selenide (HgSe) (Fig. 3). This compound has been identified in the liver of marine mammals and humans (Martoja and Berry, 1980; Pelletier, 1985; Hansen et al., 1989) as amorphous, highly insoluble, inert particles. In support of the existence of this compound (HgSe) in the liver of the marine mammals we sampled, we have found, as have others (Koeman et al., 1973; Nielsen and Dietz, 1990; Skaare et al., 1994; Wagemann and Stewart, 1994), that total selenium and total mercury in the liver are significantly, positively correlated with a regression slope of one in terms of atomic concentrations (Table 6) indicating that these two elements are associated in a ratio of 1:1 as in HgSe. The relatively high concentration (Table 1) and long half-life of total mercury in the liver (10 years in humans; Friberg et al., 1979), the positive, high correlation between total mercury and age of animals (Wagemann et al., 1996), the strong, positive correlation between mercury and selenium with a regression slope of approximately one (Table 6), and the relatively short half-life of MeHg (20–500 days in ringed seals; Tillander et al., 1972) are consistent with a progressive accumulation of mercuric selenide (HgSe) with age, being possibly an end product of demethylation as depicted in Fig. 3.

The regression slopes of MeHg in liver on age were all positive possibly indicating a decreasing rate of demethylation with age while the regression slopes of percentage MeHg on age were negative which is consistent with the accumulation of mercury in the liver with age. The process of demethylation of MeHg and transformation into an inert form of mercury would obviously be an effective mechanism for counteracting the potentially damaging action of MeHg. Data by Hyatt et al. (1996) showed that in the brain of belugas, total mercury and selenium were also significantly correlated and that the ratio of MeHg to total mercury was relatively low, pointing to demethylation of MeHg also occurring in this tissue.

The paucity of information on MeHg in Arctic

marine mammals from other regions of the Arctic than Canadian and Greenland Arctic precludes comparison with other relevant geographic areas, and only a few comparisons can be made of MeHg levels in the tissue of marine mammals between the Canadian Arctic and other northern regions. The mean MeHg concentrations in muscle of belugas, 1.32 and 0.96 μg/g, in the western and eastern Canadian Arctic, respectively, were within the range of MeHg values reported for pilot whales, 0.06–1.72 μg/g (Julshamn et al., 1987) and were similar to organic mercury concentrations in muscle of toothed whales, 0.113–1.187 μg/g (Dietz et al., 1990). Only in the muscle of ringed seals and in the blubber of whales was the concentration of MeHg (and total mercury) below the Federal Consumption Guideline for fish. It is noteworthy that the mean total concentration in narwhal blubber sampled from 1992 to 1994 (0.04 μg/g), had not changed significantly in this tissue from the value (0.03 μg/g) found in 1978/79 (Wagemann et al., 1983) but did change over this time span in liver (Wagemann et al., 1996) where mercury preferentially accumulates.

In most of the tissues analysed the MeHg concentration exceeded the Canadian Federal Consumption Guideline for mercury in fish. These findings, combined with the great differences in toxicity among organic and inorganic mercury compounds and the fact that some mercury species can be transferred from mother to foetus (Smith and Smith, 1975; Julshamn et al., 1987; Wagemann et al., 1988) reveal the importance of differentiating mercury species in tissues used as food sources. Determination of the levels of various mercury species, such as MeHg and HgSe and not just total mercury will allow a more accurate assessment of the health risk to animals and to humans from the consumption of contaminated animal tissues.

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