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Mercury, Cadmium, Selenium, and Seven Other Elements in the Muscle, Renal, and Hepatic Tissue of Harbor Seals (*Phoca vitulina*) from Newfoundland and Labrador, Canada

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In 1997 the Canadian government passed the Canada Oceans Act. Since that time the department of Fisheries and Oceans has moved towards developing an Oceans Action Plan that will provide a framework for implementing coastal integrated management plans, marine protected areas, large ocean management areas, and marine ecosystem health initiatives. In 2001, a comparative project evaluating the harbour seal as an indicator of marine ecosystem health in Placentia Bay and surrounding waters of Newfoundland and Labrador was initiated. The objectives of the project were to: a) determine contaminant profiles for harbour seals in Placentia Bay and the South Coast of Newfoundland, b) collect the necessary harbour seal ecological data (including diet, reproductive status, and age) to comprehensively interpret contaminant results from a marine ecosystem health perspective and evaluate whether harbour seals are an effective ecosystem health indicator in coastal Newfoundland waters, and c) document the current and historical distribution, habitat use, and relative abundance of harbour seals in key areas of Placentia Bay and surrounding areas. The overall goal of this comprehensive project was to monitor longer-term cumulative effects of coastal developments, in particular those relating to Newfoundland and Labrador's offshore oil production.

This paper presents results pertaining to objective (a) above, and provides contaminant data from harbour seals collected from 5 sites around Newfoundland and Labrador. The primary objectives of this study were to; 1. determine the mean concentration of a suite of trace elements in the seal's muscle, renal, and hepatic tissue; 2. test the hypothesis that Hg and Cd were bioaccumulating in seal tissue and; 3. determine if sampling site was a significant factor in predicting the concentration of Hg and Cd in the various tissue types.

MATERIALS AND METHODS

Samples of tissue (muscle, liver, and kidney) were obtained from 66 seals taken at 5 sites: Labrador (site 212; Sandy Island n=6), the south coast of Newfoundland (site 304; Placentia Bay n=27, site 301; Burgeo/Rose Blanche n=3), the west coast of Newfoundland (site 402; St. Pauls n=25), and the east coast of the Avalon Peninsula (site 335; Chance Cove n=5;) (Figure 1). Lengths of seals were

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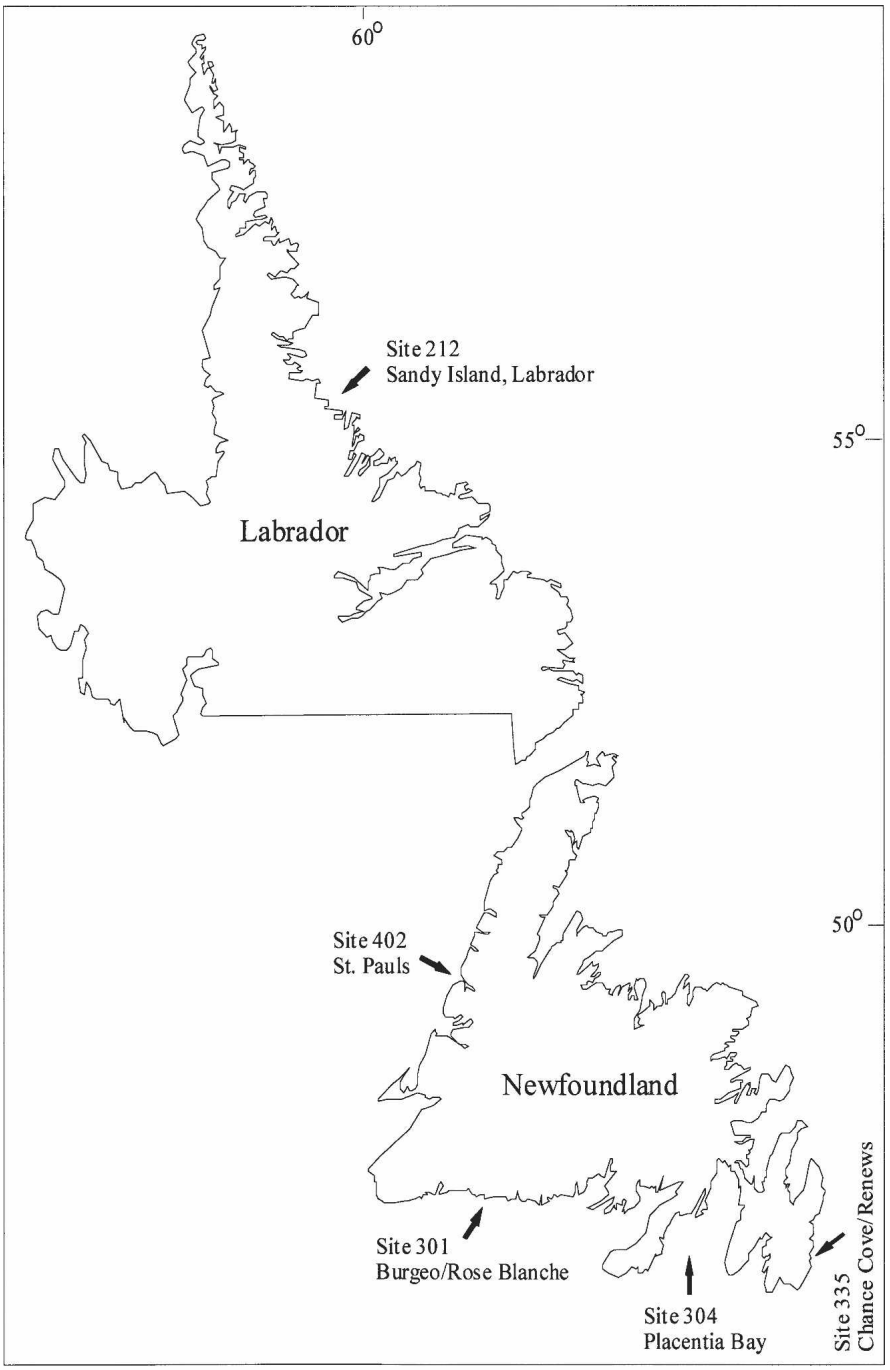

Figure 1. Coastal outline of Newfoundland and Labrador indicating location of harbour seal sampling sites.

recorded and tissue samples were shipped to the lab frozen and remained frozen at -20°C until processed. Between 8 to 13 g of frozen tissue were lyophilized then re-weighed to obtain a dry weight. Samples were ground using an agate mortar and pestle. Ground samples were stored in plastic vials until sub-samples were taken for digestion. All reagents used in the digestion of the tissue samples were purchased as either certified trace clean or ultra-pure.

For Hg analyses approximately 5ml of concentrated nitric acid, 0.5 ml of concentrated hydrochloric acid, and a ^{201}Hg spike were added to either 0.1 grams of liver or kidney or 0.3 grams of muscle tissue. The tissue, acid, and spike mixture was held at room temperature for 2 hours then the temperature of the mixture was raised to 60°C and the solution covered and left to reflux at that temperature overnight. The next day an additional 2 ml of concentrated nitric acid was added to the sample and the mixture allowed to stand at 60°C for 2 hours. Three milliliters of hydrogen peroxide was then added and the mixture remained at 60°C for a further 2 hours. Samples were cooled and diluted to 15 ml with 18 MΩ water. Samples were centrifuged at 3000 rpm for 15 min., decanted and further diluted to a volume of 40ml with 18 MΩ water. Next 3ml of BrCl was added to the samples to oxidize all forms of Hg to Hg^{2+}. After 12 hours if BrCl was still present in the sample, as evidenced by the appearance of a light yellow colour, then all Hg present was assumed to have been oxidized. If the solution was clear after 12 hours then additional BrCl was added and the sample was left to react for a further 12 hours. This was repeated until there was evidence of un-reacted BrCl in the sample. The un-reacted BrCl was neutralized by the addition of 1ml of hydroxylamine hydrochloride and diluted to a final volume of 50 ml with 18MΩ water. Aliquots were decanted into 15ml polyethylene tubes for analysis.

Mercury concentrations were determined by isotope dilution inductively coupled plasma-mass spectrometry (ICP-MS). Analyses were carried out on a PE Sciex 6100 ICP-MS. Diluted samples were mixed on line with sodium borohydride to reduce the Hg^{2+} to Hg^{0}. The Hg vapour was separated in a liquid-gas separator and the Hg gas swept into the ICP-MS by a continuous flow of Ar. This produced a steady Hg signal from which the average count rate for ^{201}Hg and ^{202}Hg was measured. The total Hg concentration was determined using the equation of Smith (1993).

For trace metal analyses approximately 0.3 g of tissue was mixed with 5 ml of concentrated nitric acid and heated to 60°C for 2 hours. The temperature was then raised to 110°C and the sampled digested for a further 4 hours. An additional 3ml of nitric acid was added and the temperature reduced to 60°C. The sample was covered and allowed to reflux overnight. The next day the temperature was increased to 110°C and the volume reduced to approximately 1ml. Samples were then diluted to a final volume of 50ml with 18 MΩ water. Aliquots were decanted into 15ml polyethylene tube for analysis.

Table 1. Average coefficients of variation (%) for each element in each tissue type.

	Element									
Tissue Type	P	Mg	Mn	Co	Cu	Zn	As	Se	Cd	Hg
Hepatic	12.1	7.9	9.3	17.7	4.5	10.0	9.6	10.3	6.6	12.0
Renal	10.3	4.7	8.8	21.8	3.8	6.2	10.8	14.8	1.1	10.3
Muscle	14.7	8.1	6.6	9.5	12.1	9.7	10.5	14.1	12.2	15.0

Trace elements were also determined using a PE Sciex 6100 ICP-MS. However, the samples for trace element analyses were introduced to the plasma as a liquid. The instrument was operated in peak hop mode and concentrations were determined by comparison to a calibration curve. The calibration curve was generated by the analysis of a certified multi-element standard.

Analytical quality was monitored by the analyses of replicate samples in duplicate and triplicate. The average coefficient of variation for the repeated analysis in each tissue type is given in Table 1. Accuracy was determined by the analyses of three certified reference materials: DORM-2 (dogfish muscle); DOLT-2 (dogfish liver); and NIST 2976 (mussel tissue) (Table 2)

An analysis of variance using the Systat statistical software program was carried out within each tissue type to determine if there were any significant differences among sites. The concentration of each element was treated as a dependant variable and site was the independent variable. Length was used as a covariate.

RESULTS AND DISCUSSION

Mean trace element concentrations for muscle, liver, and kidney samples are given in Tables 3, 4, and 5. Hepatic tissue had the highest average concentrations of P, Mn, Cu, Zn, As, Se, and total Hg at all sites. Renal tissue had the highest concentrations of Co and Cd, while muscle tissue contained the highest concentration of Mg. Within tissue types there were significant differences among sites ($p<0.05$) and significant correlations with seal length ($p<0.05$) (Tables 3, 4, and 5). In muscle tissue concentrations of Mn and Cu decreased with length whereas there was evidence of bioaccumulation of Co, As, Se, Cd, and Hg in hepatic tissue. For example there was a significant ($p<0.05$) log-linear relationship between total Hg in the hepatic tissue and seal length (Fig 2). As well, there was a very strong and significant ($p<0.05$) correlation between hepatic Hg and Se (Fig 3).

Cadmium concentrations in renal tissue were also related to the size of the animal (Fig 4). Furthermore, for renal Cd, the sites fell into two distinct groups. Samples from the South and East Coast (sites 301, 304, 335) have a more rapid increase in Cd concentrations with length compared to samples from St. Pauls (site 402) on the West Coast of the island and the Labrador site (site 212) (Fig 4).

Table 2. Comparison between mean concentrations (ug/g wet wt.) obtained in this study and the certified values for three certified reference materials; DORM-2, DOLT-2, and NIST 2976.

Elem.	DORM 2	Cert. Value	DOLT 2	Cert. Value	NIST 2976	Cert. Value
P	NA	NA	NA	NA	8900 (2100)	8300 (NA)
Mg	NA	NA	NA	NA	4500 (500)	5300 (500)
Mn	2.5 (0.2)	3.66 (0.34)	5.9 (0.5)	6.88 (0.56)	40.8 (5)	33 (2)
Co	0.12 (0.01)	0.182 (0.031)	0.20 (0.02)	0.24 (0.05)	0.62 (0.04)	0.61 (0.02)
Cu	1.8 (0.1)	2.34 (0.16)	24.3 (1.2)	25.8 (1.10)	3.7 (0.3)	4.02 (0.33)
Zn	26.3 (2.9)	25.6 (2.3)	83 (7)	85.8 (2.50)	119.9 (10)	137 (13)
As	18.7 (1.2)	18 (1.1)	14.9 (1.1)	16.6 (1.10)	13.7 (1.3)	13.3 (1.8)
Se	1.7 (0.2)	1.4 (0.09)	6.9 (0.4)	6.06 (0.49)	2.5 (0.5)	1.8 (0.15)
Cd	0.04 (0.01)	0.043 (0.008)	18.4 (0.9)	20.8 (0.50)	0.77 (0.07)	0.82 (0.16)
Hg	4.05 (0.49)	4.64 (0.26)	2.18 (0.16)	2.14 (0.28)	NA	61 (3.1)

Errors, in parenthesis, are expressed as 95% confidence intervals. NA = not available.

The mean within site concentrations of trace elements, and the range among sites around Newfoundland and Labrador (Table 3) agree well with the concentrations found in harbour seals from Alaska (Miles et al 1992), Germany (Drescher et al. 1977), and in northern pinnipeds in general (Fant et al. 2001; Julshamn and Grahl-Nielsen 2000; Yeats et al. 1999; Wagemann et al. 1998). Relative differences among tissue types are also in agreement with the results of the studies mentioned above and with a study of harp seals sampled from Newfoundland waters (Botta et al. 1983). The lower Hg liver concentrations reported by Botta et al (1983) (mean value of 2.33 ug/g compared with our study's mean of 17 ug/g) may be a reflection of differences in the average length of the animals and not a reflection of a change in exposure.

As mentioned, the correlation of total Hg in hepatic tissue with length (Fig 2) is an indication of the bioaccumulative nature of Hg. However, Wageman et al. (1998) suggested that the Se-Hg correlation (Fig 3) was an indication of the presence of tiemannite, a mineral that may result in the detoxification of Hg. On a

Table 3. Average seal length (cm) and elemental concentration (ug/g wet wt) in muscle tissue of harbour seals.

Site	212	301	304	335	402
n	6	2	27	5	25
length	114	101	124	124	136
P	2510[a]	3589	3242[b]	3413[b]	2808[a]
Mg	249	264	258	268	247
Mn*	0.17	0.16	0.18	0.14	0.13
Co	0.004	0.005	0.004	0.004	0.004
Cu*	1.89	1.28	1.49	1.46	1.42
Zn	24.13	22.77	21.40	16.89	19.77
As	0.11	0.14	0.16	0.08	0.14
Se	0.51	0.50	0.56	0.40	0.61
Cd*	0.003	-0.003	0.032	0.009	0.006
Total Hg*	0.35	0.41	0.61	0.38	0.75

For each element an asterisk indicates a significant correlation with length ($p<0.05$), and lower case letters indicate significant differences among sites. Each different letter set is a significant difference ($p<0.05$) between sites.

molar basis (Fig 5) there appears to be an excess of Se (molar ratio < 1) in smaller and presumably younger seals. Outridge et al. (2000) suggested that the less than 1:1 molar ratio between Hg and Se in smaller, younger, belugas may indicate a greater ability of the liver to detoxify Hg. Therefore, older animals may lose their ability to remove MeHg from other organs as they age which could have health implications for older animals.

The bioaccumulation of Cd in renal tissue was site dependent (Fig 4). The slope of the regression line through the data from St Pauls was significantly different from the Placentia Bay regression ($p<0.05$). The data from the Labrador sample fell along the St. Pauls regression line whereas the data from Chance Cove aligned with the Placentia Bay data. This suggests that there are different sources of Cd to seals from different areas of the province. This difference may be as simple as different food sources. There is no known source of cadmium in Placentia Bay, but the bay has been, and continues to be, the site of heavy industry (e.g. ship yard, oil transshipment terminal, US Air Force base, etc.).

However, studies that sampled blue mussels (*Mytilus edulis*) from Placentia Bay did not find elevated concentrations of Cd in their tissues (Veinott and Banoub 2004; Kennedy and Benson 1993). The small number of seals and limited lengths of the Burgeo/Rose Blanche sample made it difficult to assign the Cd data to either the Placentia Bay group or the St. Pauls group. Burgeo/Rose Blanche is an interesting site because it lies to the west of the entrance to Placentia Bay and general ocean currents flow west out of Placentia Bay and along the south coast of

Table 4. Average seal length (cm) elemental concentration (ug/g wet wt.) in hepatic tissue of harbour seals.

Site	212	301	304	335	402
n	6	3	27	5	25
length	114	97	124	124	136
P	3110	4028	3757	4134	3636
Mg	205	192	188	204	197
Mn	4.16	3.02	4.30	4.01	4.64
Co*	0.015	0.017	0.014	0.014	0.023
Cu	11.46	11.51	17.44	18.96	18.72
Zn*	34.68^{b}	28.47^{a}	49.25^{b}	40.38^{b}	42.74^{b}
As*	0.21	0.38	0.73	0.29	0.95
Se*	5.47	2.78	14.68	5.34	19.76
Cd*	0.146	0.178	9.595	1.604	0.629
Total Hg*	9.20	1.75	30.52	7.41	39.44

For each element an asterisk indicates a significant correlation with length ($p<0.05$), and lower case letters indicate significant differences among sites. Each different letter set is a significant difference ($p<0.05$) between sites.

Table 5. Average seal length (cm) and elemental concentration (μg/g wet wt.) in renal tissue of harbour seals.

Site	212	301	304	335	402
n	6	3	27	5	25
length	114	97	122	124	136
P	2465	3507	3148	3538	2651
Mg	147	142	145	138	134
Mn	0.86	0.87	0.94	0.93	0.96
Co*	0.016	0.025	0.017	0.019	0.024
Cu	6.86^{b}	7.12^{b}	6.80^{b}	9.94^{a}	5.42^{a}
Zn	18.15	20.48	26.83^{b}	24.90^{b}	19.87^{a}
As*	0.13^{b}	0.35^{a}	0.36^{a}	0.20^{b}	0.39^{a}
Se*	2.11^{a}	3.55^{b}	$3.62^{b\,d}$	3.84^{b}	$4.97^{b\,c}$
Cd*	0.341	0.251	20.252	6.816	1.563
Total Hg*	1.09	1.84	2.33	2.71	2.39

For each element an asterisk indicates a significant correlation with length ($p<0.05$), and lower case letters indicate significant differences among sites. Each different letter set is a significant difference ($p<0.05$) between sites.

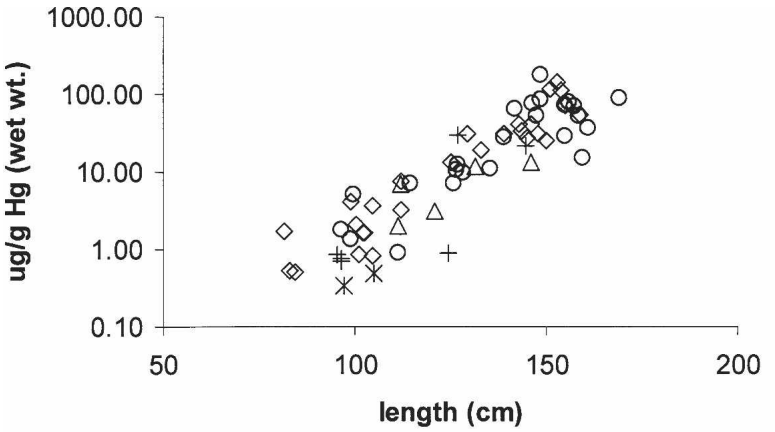

Figure 2. Relationship between seal length and total Hg in hepatic tissue. Each symbol represents a different sampling site. Labrador (+), Rose Blanche/Burgeo (∗), Placentia Bay (◊), Chance Cove/Renews (Δ), St. Pauls (○)

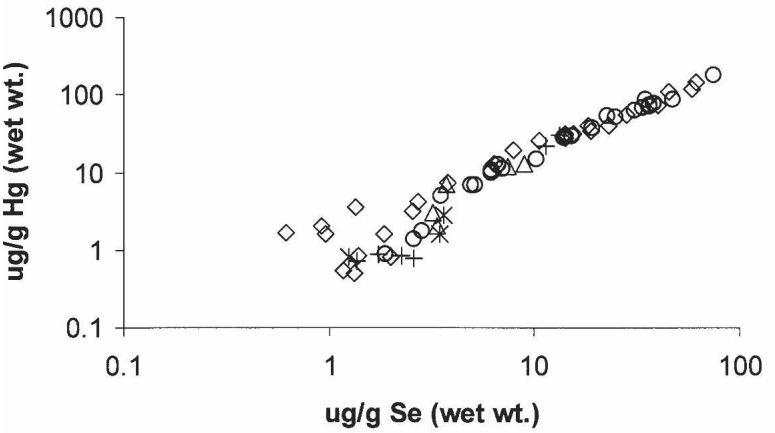

Figure 3. Relationship between the concentrations of Se and total Hg in the hepatic tissue of individual seals. Labrador (+), Rose Blanche/Burgeo (∗), Placentia Bay (◊), Chance Cove/Renews (Δ), St. Pauls (○)

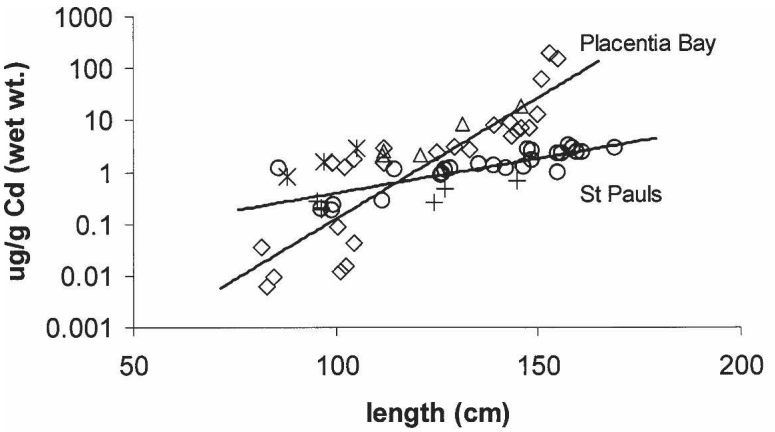

Figure 4. Relationship between seal length and cadmium concentration in renal tissue. Solid lines are least squares regression lines obtained using the Placentia Bay and St. Pauls data. Labrador (+), Rose Blanche/Burgeo (∗), Placentia Bay (◊), Chance Cove/Renews (Δ), St. Pauls (○)

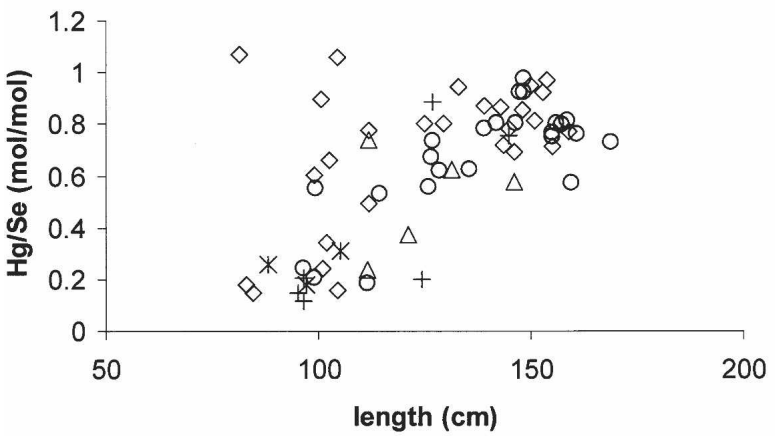

Figure 5. Relationship between seal length and the Hg/Se molar ratio in hepatic tissue. Labrador (+), Rose Blanche/Burgeo (∗), Placentia Bay (◊), Chance Cove/Renews (Δ), St. Pauls (○)

the island (Colbourne and Murphy 2004). If Placentia Bay is the source of the increased Cd, and the Cd is transported by the marine environment then increased Cd should show up in the Burgeo/Rose Blanche seal population. Additional sampling will be needed to test this hypothesis.

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