

ENVIRONMENTAL LEVELS - POSTERS

STRATIFICATION OF PCBs IN THE BLUBBER OF BELUGA WHALES (*Delphinapterus leucas*) FROM THE ST. LAWRENCE ESTUARY

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Introduction

Since the early 1980's, blubber samples of dead stranded beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary have been analysed for several persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs)^{1,2,3}. However, the usefulness of these specimens to monitor organochlorine pollution in the overall population has been questioned⁴. An alternative approach has been used, where a small core of blubber is sampled from live free-ranging animals^{5,6}. There is concern, however, that the results of this technique may not be representative of the entire blubber layer in cases where only the outermost strata is sampled. This is because whale blubber is layered in terms of function and lipid composition and vertical stratification of POP concentrations has been detected in the blubber of cetaceans such as fin and sei (*Balaenoptera physalus* and *B. borealis*) whales⁷ and harbour porpoises (*Phocoena phocoena*)⁸, although not in blue and minke (*Balaenoptera musculus* and *B. acurostrata*) whales⁹. Here, we report results of PCBs measured in various strata of the blubber of dead stranded beluga whales in order to evaluate if vertical stratification of contaminants occurs in their blubber.

Materials and Methods

Samples. Blubber samples were obtained from two beluga whales, one male and one female, found dead on the shore of the St. Lawrence Estuary in 1998 (Table 1). Prior to their transportation to the laboratory for necropsy, large pieces of blubber from the dorso-lateral region were removed from the whales. These samples were wrapped in solvent rinsed aluminium foil and stored at -20°C. In the laboratory, blubber samples were sliced parallel to the skin and muscle in 1.0 cm thick strata, excluding skin underlying stratum of dense connective tissue and muscle.

Analysis of PCBs. Procedures were essentially as described by Bernt et al.¹¹. Briefly, each blubber layer sample (0.5-1 g wet wt) was chemically dried with sodium sulphate and then transferred to a glass column. A mixture of five ¹³C₁₂ PCBs was added to the column before the lipids and lipophilic compounds were extracted using dichloromethane-hexane (50:50). Lipids were removed by gel permeation chromatography and the extract was cleaned by elution through a two-layer column packed with neutral silica and alumina. The final extract was reduced in volume and spiked with an instrument performance solution containing two additional ¹³C₁₂ PCBs. Quantification of individual PCB congeners was performed using a Varian 3400CX series gas chromatograph equipped with a Varian's Saturn 4D ion trap, a

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Table 1. Characteristics of the two stranded beluga whales and the blubber samples examined

Animal				
	Code No.	DL-03-98		DL-09-98
	Sampling location			
	Region	St. Lawrence Estuary		St. Lawrence Estuary
	Sector	Rivière du Loup		Sainte-Flavie
	Collection date	1998-05-24		1998-12-04
	Age* (year)	26+		18+
	Sex	F		M
	Length (cm)	377		406
	Weight (kg)	916		1019
	Carcass condition (code ^b)	Fair (3)		Fair (3)
Blubber sample				
	Thickness (cm)	7		8
	Colour	Rose		Rose

a + sign indicates that dentine layers were difficult to read and the animal may be older

b Classification according to Geraci and Lounsbury¹⁰

Varian 1078 split/splitless programmable injector (5 µl injection volume) operated in splitless mode, and a Varian 8200CX autosampler. Chromatographic separation of the contaminants was achieved using a 30m DB-5MS column (0.25 mm ID, 0.25 µm film thickness) with helium (He) as the carrier gas. The ion source was operated in electron impact (EI) ionisation mode and the ion trap in MS/MS mode. Concentrations of PCB congeners were calculated using relative response factors (RRFs) determined from a four point calibration curve. Precision and accuracy of PCB analysis were assessed by repeated analysis of whale blubber sample, SRM1945¹².

Statistical analysis. Linear regression analysis was used to test for a significant gradient of PCB concentration as a function of blubber depth. Statistical analyses were conducted using SYSTAT for Windows (version 7.0, SPSS Inc., 1997) and statistical significance was set at 0.05.

Results and Discussion

Thirty-six individual PCB congeners were determined in blubber strata (n=5) taken from each whale. The average concentration of each PCB congener was calculated as the arithmetic mean of the 5 blubber strata. Variations in PCB concentrations in the blubber, expressed as percent deviation (%) from the average concentration, were also calculated for each congener and reported for selected congeners as a function of the blubber depth from the skin to the basal muscle (Figure 1). Two groups of congeners were apparent. The first group showed a maximum deviation from the average concentration of less than 20% (e.g. IUPAC 52 and 74) whereas the second group showed a higher maximum deviation (e.g. IUPAC 138 and 180), reaching up to 50% in a few cases (e.g. IUPAC 206). The first group was represented by about one third and one half of the congeners analysed in male and female blubber samples, respectively. Deviations from the average concentration in blubber strata were generally more significant for highly chlorinated PCB congeners. Variations in the concentrations of most PCBs in blubber strata were similar in both individuals, with the exception of a few congeners (e.g. IUPAC 156). These variations could result from either the

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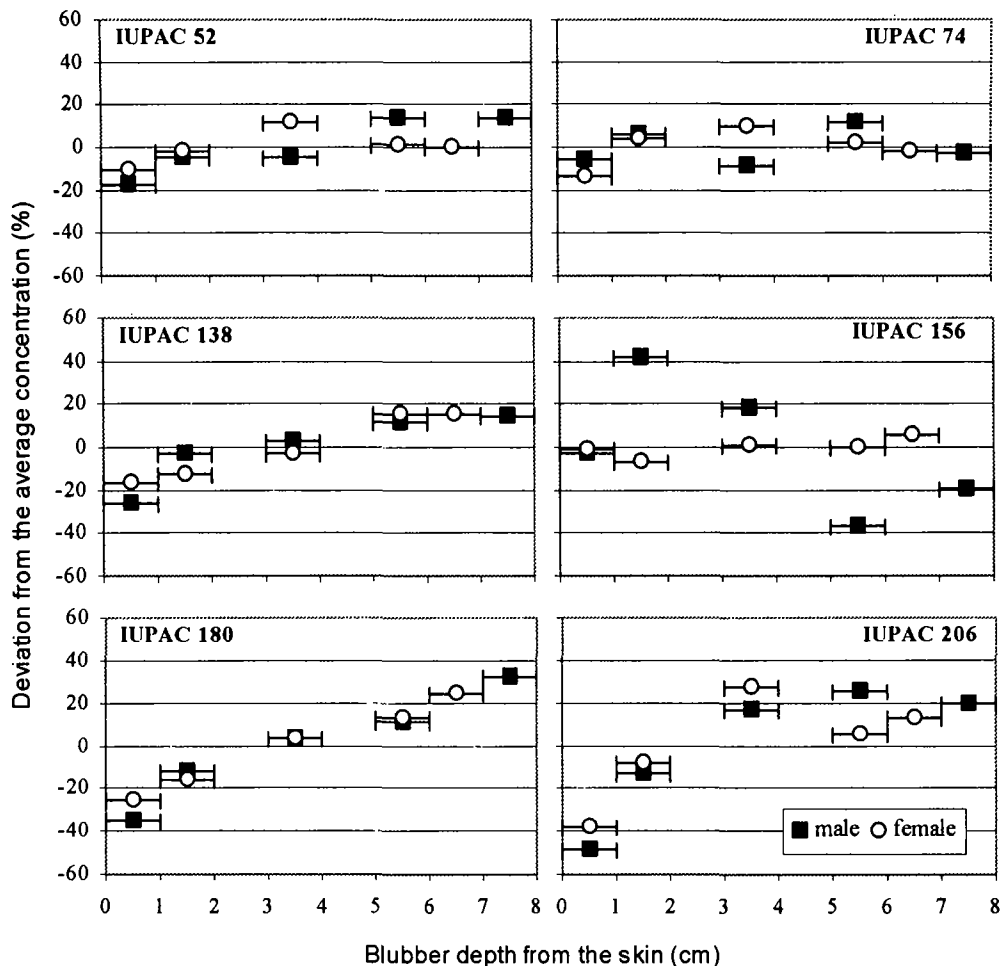


Figure 1. Variation in concentrations of selected PCB congeners in blubber strata, expressed as percent deviation (%) from the calculated average concentration, as a function of the blubber depth from the skin to the muscle for a stranded male (v) and female (O) beluga whale from the St. Lawrence Estuary.

stratification of PCBs in the blubber or from the analytical variability of PCB analysis. The analytical variability was assessed for each congener by calculating the coefficient of variation (c.v.) for the replicate analysis ($n=4$) of the whale blubber sample, SRM1945. An average c.v. of 11.8% was calculated for the SRM1945 replicate analysis whereas average c.v. for the 5 blubber strata in beluga samples were 19.4% and 15.7% for male and female, respectively. These results suggest that a certain stratification of PCB concentrations is present in beluga blubber samples.

The stratification of PCBs in beluga blubber was further investigated by assessing if there was a significant linear gradient of PCB concentrations in blubber strata. Regression analysis was used to test if the slope of the relationship between PCB concentrations in the strata and

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the blubber depth was significantly different from zero. Approximately half of the PCB congeners measured in samples from each individual showed a significant linear gradient of concentrations in the blubber, increasing from the skin to the muscle. Some examples of both non-significant (e.g. IUPAC 74) and significant (e.g. IUPAC 180) PCB concentration gradients are shown in Figure 1. For many of these congeners, differences in concentration among strata were relatively limited (c.v.< 20%), showing variability similar to that observed for long-term precision in organic contaminant analysis. However, levels of several PCB congeners, particularly the highly chlorinated congeners, were significantly lower in the strata closer to the skin than their average concentrations in the whole blubber sample.

Our results show a heterogeneous vertical distribution of PCBs in beluga blubber, which generates a limited bias towards underestimating concentrations of many congeners when only the superficial blubber strata are analysed. This bias may be reduced significantly or even eliminated by collecting deeper cores from the animal's blubber mantle. For instance, a biopsy of 5 cm in depth would permit the collection of a blubber sample from the middle strata, which contains PCB concentrations representative of the full depth of the blubber in the two animals examined. However, more belugas should be analysed to confirm whether the extent of the PCB stratification observed here is typical of other St. Lawrence beluga whales.

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References

1. Massé, R., Martineau, D., Tremblay, L and Béland, P. *Arch. Environ. Contam. Toxicol.*, **1986**, 15, 567
2. Martineau, D., Béland, P., Desjardins, C. and Lagacé A. *Arch. Environ. Contam. Toxicol.*, **1987**, 16, 137
3. Muir, D.C.G., Ford, C.A., Stewart, R.E.A., Smith, T.G., Addison, R.F., Zinck, M.E. and Béland, P.; In Smith T.G., St. Aubin, D.J. and Geraci, J.R. [eds] Advances in research on the beluga whales, *Delphinapterus leucas*. *Can. Bull. Aquat. Sci.*, **1990**, 224, 165
4. Aguilar, A. and Borrell, A.; In Fossi, C. and Leonzio C. [eds] Non-destructive biomarkers in vertebrates. Lewis Publishers, CRC Press, Boca Raton, USA, **1994**, 245
5. Taruski, A.G., Olney, C.E. and Winn, H.E.; *J. Fish. Res. Board Can.*, **1975**, 32, 2205
6. Woodley, T.H., Brown, M.W., Kraus, S.D. and Gaskin, D.E.; *Arch. Environ. Contam. Toxicol.*, **1991**, 21, 141
7. Tilbury, K.L., Stein, J.E., Meador, J.P., Krone, C.A. and Chan, S.-L.; *Chemosphere*, **1997**, 34, 2159
8. Gauthier, J.M., Metcalfe, C.D. and Sears, R.; *Mar. Environ. Res.* **1997**, 43, 157
9. Aguilar, A. and Borrell, A.; *Marine Environ. Res.*, **1991**, 31, 275
10. Geraci, J.R. and Lounsbury, V.J. Marine mammals ashore, a field guide for strandings. Texas A&M Sea Grant Publication, Galveston, USA, **1993** 305pp.
11. Bernt, K.E., Hammill, M.O., Lebeuf, M. and Kovacs, K.M.; *Sci. Total Environ* **1999**, 243/244, 243
12. National Institute of Standards and Technology. Standard reference material 1945, Organics in Whale Blubber, **1994**, 10pp.