

PRELIMINARY SCREENING OF PERFLUORINATED AND ORGANOCHLORINE COMPOUNDS IN ANTARCTIC FUR SEAL (*ARCTOCEPHALUS GAZELLA*) PUPS FROM LIVINGSTONE ISLAND, ANTARCTICA

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Introduction

Perfluorinated compounds have distribution patterns similar to those of many halogenated POPs, with regard to global distribution and bioaccumulation. Detection of perfluorooctane sulfonate (PFOS) and related fluorinated compounds in remote areas is somehow unexpected because of their low volatility making their long range transport (LRT) improbable.^{1,2} Some Authors have hypothesized that the global distribution of PFOS may occur via airborne neutral compounds that yield the free acid upon degradation.³

Muscle and liver samples of Antarctic fur seals (*Arctocephalus gazella*) pups were analysed for perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB), in order to assess their contaminant levels and patterns. POPs are transferred from mother to offspring through lactation. Marine mammal milk is very rich in lipids. The lipid content of Antarctic fur seals milk can be up to 39.8%.⁴ Although there might be some *in utero* contaminant transfer from mother to foetus, a transfer through lactation is considered as quantitatively far more important in mammals.⁵

Materials and methods

Collection of Samples. Antarctic fur seal pups were found dead at Livingstone Island, Antarctica; samples of muscle and liver were collected between January and February 2004 (n = 20, weight = 5±1 kg, length = 72.5±4.8 cm, age = 1 day to 2 months). Samples were taken from the carcasses at the time of necropsy, wrapped in polyethylene bags and stored at -20°C until analysis.

Analyses of chlorinated compounds. PCB congeners and pesticides were analyzed following the method described elsewhere, with some modifications.^{6,7} HCB, *p,p'*-DDE, PCBs, were identified and quantified using a gas chromatograph (Perkin Elmer mod. Autosystem) equipped with ⁶³Ni electron capture detector (GC-ECD; capillary column coated with DB-5 (Supelco Inc.)). Blanks were analyzed throughout the analytical procedure. Recoveries and detection limits were previously described and validated.⁶ PCB congeners are represented by their IUPAC numbers throughout the text. Σ PCBs were calculated as the sum of the principal congeners identified (44 congeners). Results are given on a wet weight basis (wet wt).

Analyses of perfluorinated compounds. Perfluorinated compounds were analyzed following the method described elsewhere, with some modifications.^{8,9} Concentrations of PFOS and PFOA in liver and muscle of seals were measured using high performance liquid chromatography (HPLC) with electrospray tandem mass spectrometry.⁸ For quantitative determination, the HPLC system was interfaced to a Finnigan LTQ (Thermo Electron Corporation, San Jose, CA) linear ion trap mass spectrometer operated in the electrospray negative mode. Instrumental parameters were optimized to transmit the [M-H]⁻ ion for all analytes (PFOS and PFOA) resulting in the following working conditions for ESI-MS/MS: primary and product ions monitored for PFOS and PFOA determinations were 498.8 > 368.9 and 412.8 > 168.8, 218.8, respectively. The limit of quantitation (LOQ) is 10 ng/g wet wt for PFOS and 12 ng/g wet wt for PFOA.

Result and discussion

Perfluorinated Compounds. PFOS and PFOA concentrations in juvenile samples of Antarctic fur seal were below LOQ or not detected (Table 1). Therefore, exposure of Antarctic fur seal to fluorinated organic chemicals is expected to be minimal. These results suggest that the accumulation features of PFOS and PFOA may be different from those observed for PCBs and other chlorinated pesticides, although more analyses are needed to confirm this hypothesis. PFOS was not measured above the limit of quantitation of 35 ng/g in Weddell seal

(*Leptonychotes weddelli*) liver⁹. PFOA was not observed in any of the harbour seal (*Phoca vitulina*) samples of the Dutch Wadden Sea.¹⁰ Bossi *et al.*¹¹ detected 52–67 ng/g wet wt of PFOS in hepatic tissue of ringed seal (*Phoca isipida*) from East Greenland, below 10.1 ng/g wet wt (LOQ) in specimens from central West Greenland and 27 ng/g wet wt in those from Northwest Greenland. Mean concentration found in liver of ringed seals from the Baltic Sea was 460 ng/g wet wt.¹² More recently, Martin *et al.*¹³ found mean PFOS concentrations of 16 and 19 ng/g wet wt in livers of ringed seals from Holman Island and Grise Fiord (Canadian Arctic), respectively; in the same study, the concentration of PFOA was < 2 ng/g.

Chlorinated Compounds. Σ PCBs, *p,p'*-DDE and HCB average concentrations were 33±22, 4.1±3.7 and 0.1±0.1 ng/g wet wt, respectively, in the muscle, and 59±43, 7.4±8.4 and 2.1±3.9 ng/g wet wt, respectively, in the liver; concentrations were always higher in liver than in muscle samples (Table 1). PCB concentrations were one order of magnitude higher than HCB and *p,p'*-DDE and the pattern was Σ PCB > *p,p'*-DDE > HCB, as found in adult Weddell seals (*Leptonychotes weddelli*) from the Ross Sea.^{14,15,16}

PCB fingerprints showed similar percentage values in both tissue (Figure 1). In fact the POP concentrations in organs and tissues depends also on their lipid contents, as reported in many other cases.^{14,15} Fingerprints showed a predominance of low-chlorinated PCB; a relatively larger proportion of less chlorinated congeners was reported in samples from high latitude regions, such as the Southern Ocean, when compared to those from mid and low latitudes.¹⁶ The most abundant PCB congeners were the IUPAC numbers 183 > 134 > 146 > 153 > 138 > 118 in the muscle samples and 183 > 134 > 153 > 138 > 118 > 146 in the liver samples. The presence of these high chlorinated congeners (the most persistent and widespread), may be also associated with local sources (waste burning and dumping sites) from the Antarctic scientific stations, as described by Larsson *et al.* and Montone *et al.*^{20,21}

Hexa- and hepta-CBs were the most abundant class of isomers in both the tissues (Figure 2). Concentrations of hexa-CBs varied from 17.2 ng/g in the muscle to 26.7 ng/g in the liver of seal pups. These values were slightly different respect to those reported by Hidaka *et al.*¹⁸ in Weddell seal newborn male, in which predominant compounds were penta- and hexa-CBs, and similar to those found in adult Weddell seals from the Ross Sea, where hexa- and hepta-CBs made up most of the PCB residue.^{18,22} Various authors reported that while some POP concentrations are decreasing in industrialized regions and the Arctic, they are continuing to increase in the Antarctic region due to the global redistribution and recent uses in the Southern hemisphere.^{23,24,25} Our results seem to confirm this trend; in fact they are higher than those reported in a previous study in Antarctic fur seals and Weddell seals from King George Island.²⁶

p,p'-DDE shows higher bioconcentration potential compared to HCB. HCB and *p,p'*-DDE values were slightly higher than those reported by Corsolini *et al.*¹⁴ in liver of adult Weddell seals from the Ross Sea, Antarctica (0.1 ng/g wet wt of HCB and 1.2 ng/g wet wt of *p,p'*-DDE). *p,p'*-DDE concentrations were similar to those detected by Vetter *et al.*²⁶ in bubbler of adult Weddell seals from King George Island (8.8 ng/g wet wt of *p,p'*-DDE). Considering the fact that the sample analysed in this study were taken from seal pups, it could be supposed a pesticide higher concentration in adult southern fur seals.²⁶ In contrast to other chlorinated POPs, HCB shows similar concentrations in seals collected along a north-south latitudinal gradient.¹⁶ Comparable levels of HCB in both the hemispheres might be explained by its high LRT potency. Due to its widespread distribution, HCB is dispersed uniformly over the oceans and it falls out in cold regions, where it bioaccumulates in marine organisms.²⁷ Calamari *et al.*²⁸ examined worldwide HCB distribution using plants, and they suggested that the global HCB distribution was apparently temperature-dependent and that HCB was detected mainly in samples from cold regions.

The release of organochlorines (mainly PCBs) is still continuing in the Southern hemisphere and they are transported to the Antarctic Ocean²⁹ where they bioaccumulate in organisms. The presence of low-chlorinated congeners in Antarctic environments³⁰ and organisms^{14,15} has been already reported, since it is peculiar of the polar regions. Their presence in newborn pups and two months old reflects both the environment contamination and the evidence of contaminant transplacental and lactation transfers. When blubber lipids are mobilized for milk production, contaminants are co-transported with the lipids to blood and milk. However, due to differences in affinity of individual contaminants for the relatively non-polar lipids in blubber and the more polar lipids in blood and milk, a selective transfer occurs, regulated by the degree of the contaminant lipid affinity and its molecular weight.³¹ Penta-CBs (mainly congeners nos. 99, 101 and 118) exhibit a clear selective transfer from mothers to their milk. Selective excretion in milk can most likely be attributed to the lipophilic nature (expressed as log K_{ow}) of some contaminants, as reported by Wolkers *et al.*³²

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Table 1: HCB, *p,p'*-DDE, Σ PCBs average concentrations ($x \pm SD$), minimum and maximum values (min-max) and concentrations of perfluorinated compounds in seal pup muscle (m) and liver (l) (ng/g wet wt; nd = not detected).

	n	HCB		<i>p,p'</i> DDE		Σ PCBs		PFOS	PFOA
		$x \pm SD$	min-max	$x \pm SD$	min-max	$x \pm SD$	min-max	range	range
m	20	0.1 ± 0.1	0.001- 0.5	4.1 ± 3.7	0.4- 15.5	33 ± 22	7.5- 86	nd-10	nd-<12
l	20	2.1 ± 3.9	0.001- 15.2	7.4 ± 8.4	0.012- 31	59 ± 43	1.7- 151	nd-<10	nd-<12

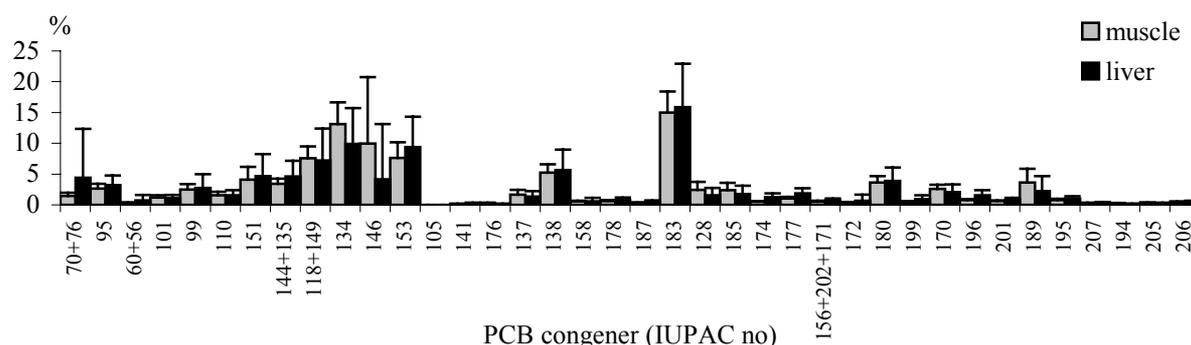


Figure 1: PCB fingerprint of seal pup liver and muscle.

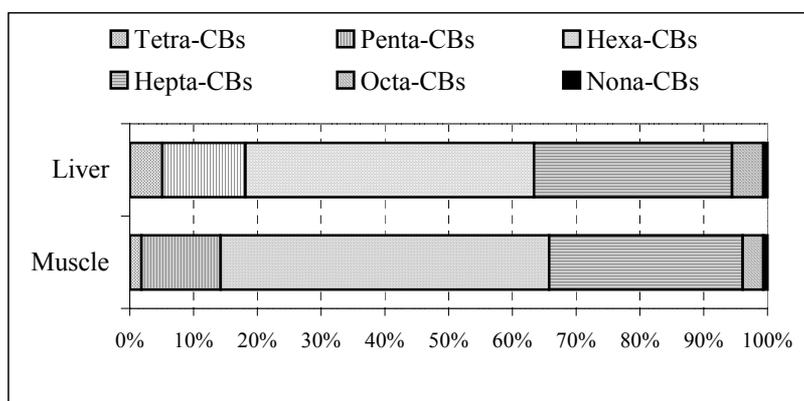


Figure 2: PCB class of isomer profile in liver and muscle samples of seal pups.

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