Poly-Brominated Diphenyl-Ethers (PBDEs) and other Persistent Organic Pollutants in Blood of Penguins from the Ross Sea (Antarctica)

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Introducton

There is continuing concern about the potential effects of Persistent Organic Pollutants (POPs) on polar environments. Most volatile compounds are expected to travel from the tropics and the other source-areas to the polar regions. The presence of PCBs at high latitudes and their decrease along the North-South latitudinal gradient has been reported decades ago[1]. Although polar regions are four times less extended than mid-to-low latitudes, icecaps act as a sink for POPs, which can reach substantial levels in such remote areas. Under cold conditions and long dark winters, POPs are less likely to degrade biologically than temperate or tropical areas. Basically, the ice entraps POPs which can then be released during melting and enter the polar food webs where they accumulate in organisms[2], eventually causing a variety of harmful effects.

Polychlorobiphenyls (PCBs), hexachlorobenzene (HCB), polybrominated diphenyl ethers (PBDEs) and dichlorodiphenyl-dichloro ethane (*pp'*-DDE) including its isomers and metabolites are known as POPs very well. POPs are particularly hazardous to wildlife not only because they are toxic but because they are persistent and distributed on global scale. Polybrominated diphenyl ethers (PBDEs) are a class of POPs used worldwide as flame retardants with an increasing trend[3] in the market demand (67.4 ktons in 2001), but with some restrictions in their usage in Europe[4]. PBDEs are hydrophobic, highly soluble in lipids, resistant to biodegradation and have similar behavior to polychlorinated biphenyls (PCBs) in aquatic and terrestrial ecosystems[5]. Their bioaccumulation and biomagnification properties, as well their global increasing presence, have already been reported by many authors[6-9]. PBDEs have been detected in remote Arctic regions[10] that seem to be their final sink. PBDEs show acute toxicity and a prolonged exposure can affect the function of thyroid and cause neurodevelopmental disorders and estrogenic and hepatic effects[11-12]. Furthermore, a synergic effect with dioxin-like compounds or other POPs cannot be excluded.

The two aims of this study are: 1) to evaluate accumulation levels and patterns of PCBs, PBDEs and chlorinated pesticides in blood samples of the Adélie penguin, *Pygoscelis adeliae,* the Emperor penguin, *Aptenodytes forsteri* and the South Polar skua, *Cataracta maccormicki* from three sites in the Ross Sea (Antarctica); 2) to assess the suitability of blood for the detection of POP residues in supposedly low contaminated organisms that live in protected/ecologically sensitive areas.

Materials and Methods

Collection of samples.

Blood samples of Adélie penguin *Pygoscelis adeliae* (n = 22) and South Polar skua *Cataracta maccormicki* (n = 7) were collected at Edmonson Point (EP; 74°19'85''S, 165°08'44''E); Emperor penguin *Aptenodytes forsteri* were bled at Cape Washington (CW; 74°43'S, 165°15'E n = 37) and Coullman Island (CI; 73°20'S, 169°20'E; n = 11) during the 2001/02 Italian expedition in Antarctica, in the framework of the National Program of Research in Antarctica (PNRA). Samples were analyzed individually and pooled.
Penguins were caught using a hand held net and after the capture, a black cap was put over the bird's head to quiet them and minimize stress. Blood samples (0.2-2 mL) were drawn from the brachial vein, beneath the ventral surface of the flipper[13], or from the intradigital vein of the foot. A special throw net was used to catch South polar skuas; they were bled from the intertarsal vein of the foot. Researchers that were familiar with methods in handling and bleeding the penguins performed all the sampling. Moreover, only a few mL of blood were collected from each animal (maximum amount: 1 mL in South Polar skua, 2 mL in small penguins, 4 mL in Emperor penguins).
Samples were kept at –30°C until analyses were performed in the laboratory.

Analytical methods for chlorinated chemicals.

PCB congeners and pesticides were analyzed following the method described elsewhere[14], with some modifications. PCB congeners were identified and quantified using a gas chromatograph (Perkin Elmer mod. Autosystem) equipped with ^{63}Ni electron capture detector (GC-ECD). A fused silica capillary column coated with DB-5 [poly(5% diphenyl/95% dimethylsiloxane), 30 m × 0.2 mm i.d.; Supelco Inc.] having a film thickness of 0.25 μm was used. PCB congeners were identified against a standard mixture of known composition and content (Supelco, certified by EPA, U.S.A.). The oven temperature was programmed from 80 to 160°C at a rate of 40°C/min and then to 170°C at 10°C/min, to 250°C at 4°C/min and then to 296°C at 8°C/min with a final hold time of 10 min. The split-splitless injector temperature was 200°C. The detector temperature was 380°C. The carrier gas was nitrogen and the scavenger was argon/methane (95/5). Recoveries of PCB congeners, HCB and *p,p'*-DDE through the analytical procedure were between 90 and 100%. Procedural blanks were analyzed through the whole analytical procedure to check for interferences. The detection limits of individual PCB congener varied depending on the sample mass, response factor and interference; generally, detection limit for individual congeners was 1 to 75 pg/g wet weight. PCB congeners are represented by their IUPAC numbers throughout the text.

nalytical methods for brominated chemicals.

PBDEs were identified and quantified following a method described elsewhere[15] with some modifications. PBDE congeners were identified and quantified by using a ThermoFinnigan PolarisQ Ion trap GC/MSn equipped with a SPB-5 capillary column (30 m × 0.25 mm i.d., 0.25

µm) from Supelco. The MS/MS condition are reported in www.Thermo.com. A Wellington Laboratories Inc. solution containing 17 PBDEs in nonane was used as calibration standard and PCB- 141 ($^{13}C_{12,}$ 99%) in isooctane, supplied from Cambridge Isotope Laboratory, was used as internal standard.
PBDE-47 and –99 were the compounds detected in the blanks. For the others compounds, the analytical detection limit was used based on the signal-to-noise ratio of 3. Detection limits, calculated as mean blank +3SD, were typically 0.8 - 1.6 pg/µl = 0.04 - 0.08 ng/g tissue.

Results and Discussion

PBDEs and PCBs: The average concentrations of PCBs and PBDEs are reported in Tables 1-2. Results obtained for Emperor penguins are reported separately for the two sampling sites, because different accumulation patterns were noticed between them. ΣPBDEs were 664±1585 pg/g wet wt in Adélie penguins, 96.74±87.29 pg/g wet wt and 36.36±16.88 pg/g wet wt in Emperor penguins from Coullman Is. and Cape Washington, respectively, and 504.48 pg/g wet wt in South Polar skua (Table 1). ΣPCB concentrations was 2.23±2.17 ng/g wet wt in Adélie penguin, 0.28±0.2 ng/g wet wt and 0.53±0.33 ng/g wet wt in Emperor penguin from CI and CW, respectively, and 3.85 ng/g wet wt in South Polar skua (Table 2). Lower chlorinated hydrocarbon concentrations were found in Emperor penguins but were higher in Adélie penguin and South polar skua. Highest levels in skua were expected because of biomagnification: they are top predators that feed on penguin eggs and chicks. Adélie and Emperor penguins occupy the same trophic level, feeding on krill, small fish and plankton[16]. The higher concentrations detected in Adélie penguin respect to the Emperor penguin might be due to a different metabolism of xenobiotics in the two species. Court *et al.*[18] reported the detoxifying activity of P450 cytochrome and in particular CYP3A (specific for PCB detoxification) measured in Adélie penguins was rather low; the activity of CYP3A enzyme was 65±22 pmol/min/mg protein in Adélie penguin, much lower than 325±240 pmol/min/mg protein measured in humans[17]. The cytochrome P4501A induction can be evaluated by measuring the 7-ethoxyresorufin-*o*-deethylase (EROD) activity. Court *et al.*[18] suggested that the low levels of EROD activity in Adélie penguin (<0.02 nmol sub/mg/prot/min) from Ross Is. (Ross Sea) indicates the lack of chemical stress by MC-type inducer contaminants, such as PCBs. Low detoxifying activity probably indicates low exposure of penguins to pollutants[18]. An EROD value of 0.15 nmol sub/mg/prot/min in South Polar skua from Ross Is., Ross Sea[18] can explain the class of isomer pattern in this species: the detoxifying activity allows the metabolism of low-chlorinated PCBs, while the high-chlorinated congeners are accumulated due to their persistency in organisms.
Emperor penguins collected at Coullman Is. showed levels two times higher respect to penguins from Cape Washington. This pattern is opposite to those found for PCBs, HCB and DDTs: concentrations in Emperor penguins from Cape Washington were always higher than those in penguins from Coullman Is. (Table 2). Such latitudinal gradient in concentrations from South to North may be attributed to the presence of scientific stations in the Southern part of the Ross Sea (Zuchelli Station - Italy, McMurdo Station - USA, Scott Base – New Zealand). However, our data are insufficient to confirm this hypothesis and further studies are needed.
PBDEs have been detected in Adélie penguin eggs by Corsolini *et al.*[19] (290 pg/g wet wt). PBDE levels in organisms from the North Sea ranged 51-59.8 ng/g lipid wt in shrimp and 40.4-119.4 ng/g lipid wt in cod muscle[6]. In general, levels in the Antarctic organisms studied here were lower than those from other areas[20-21].

Most of the PBDE residue was due to BDEs 47 and 99 in Adélie penguin (Table 1), according to results found by Corsolini *et al.*[19] in Adélie penguin eggs, fish and krill from the Ross Sea. Interestingly, the pattern differs in Emperor penguin and South Polar skua, where BDE100 was the most abundant congener, followed by BDEs 99 and 47 (Table 2). The detection of BDEs 47, 99 and 100 in Antarctic organisms confirm the global distribution of PBDEs[8].
The PBDE residue was mainly due to tetra- and penta-BDEs in penguins and skua (Figure 1); BDE7, a di-BDE, was detected in one sample of Emperor penguin (Table 1). Presence of low-brominated congeners, tri- to penta-BDEs might suggest that contamination is due to long-range transport (LRT) by air or water. Even the PCB class of isomers patterns could confirm this hypothesis: low chlorinated PCB congeners, from tri- to penta-CBs, constitute 34-62% of the PCB residue (Figure 1).

Pesticides: HCB concentrations in seabird blood samples were 0.003±0.006 ng/g wet wt in Adélie penguin, 0.002±0.002 ng/g wet wt in Emperor penguin from Cape Washington and 0.208 ng/g wet wt in skua; HCB was below the detection limit in penguins from Coullman Is (Table 1). This confirms that the polar regions are a final sink for HCB according to the cold distillation theory[22] and because of its high mobility.
Levels of ΣDDTs were 0.16±0.15 ng/g wet wt in Adélie penguin, 0.03±0.01 in Emperor penguins and 0.28 ng/g wet wt in skua (Table 1). In Adélie penguin, the DDT residue was due to the pesticide *o,p'*-DDT (15%) and to the metabolite *p,p'*-DDE (80%). *p,p'*-DDE was also the most abundant isomer in the skua (73%) and in Emperor penguin (approximately 40%). *p,p'*-DDT, the active pesticide, showed very low values in Emperor penguins from Cape Washington, 0.001±0.001 ng/g wet wt, but was undetectable in the other blood samples (Table 2). High concentrations of *p,p'*-DDE and low levels of *p,p'*-DDT indicate that low or no amounts of new *p,p'*-DDT have reached the Ross Sea recently. However, *p,p'*-DDE is the most persistent metabolite and thus its detection in samples is also due to a high degree of biomagnification in the Antarctic food web.
Despite low concentrations in our samples, penguins and other Antarctic predators should be periodically monitored since, to date, contaminant and related toxicity threshold levels have not been established for them and because contaminant input through the diet may be considerable.
Our results confirm the suitability of blood for the detection of POP residues in supposedly low contaminated organisms that live in protected/ecologically sensitive areas. We would like to highlight the importance of using this tissue. Blood has already been used for residue analyses of contaminants in animals from other areas of the world (e.g.: Gabrio *et al.*[23], Fossi *et al.*[24]) and in Antarctic seabirds by van den Brink *et* al.[25]. We suggest the use of blood for residue analyses when protected/endangered organisms and/or protected/ecologically sensitive areas are being investigated. Bleeding can be done when seabirds or other vertebrates are caught for other studies (biometric data collection, tagging, stomach flushing for diet composition, etc.); it is very important that only researchers that are familiar with methods in handling and bleeding animals perform the sampling. This would allow researchers to minimize handling and related stress while providing more information of the health and ecological status of the population.

Table 1: Concentrations (average, standard deviation, minimum and maximum values) of PBDE congeners (pg/g wet wt) in penguins and skua.

IUPAC no.	no. of Br	Adélie penguin (EP)	Emperor penguin (CI)	Emperor penguin (CW)	South polar skua (EP)
BDE3	1	nd	nd	nd	nd
BDE7	2	nd	1.64±2.85 (nd-4.93)	nd	nd
BDE15	2	nd	nd	nd	nd
BDE17	3	nd	nd	nd	nd
BDE28	3	nd	nd	nd	nd
BDE47	4	232±629 (3-2125)	21.14±17.97 (7.21-41.43)	8.64±4.22 (3.84-16.53)	133.45
BDE49	4	16±55 (nd-181)	nd	0.95±2.52 (nd-6.65)	nd
BDE66	4	nd	nd	nd	nd
BDE71	4	nd	nd	nd	nd
BDE77	4	nd	nd	nd	nd
BDE85	5	0,92±2 (nd-6)	0.10±0.18 (nd-0.31)	nd	nd
BDE99	5	293±737 (13-2503)	27.01±21.12 (10.79-49.53)	8.64±3.11 (5.35-13.91)	169.69
BDE100	5	96±116 (nd-427)	46.85±51.20 (8.84-105.07)	14.93±7.55 (8.57-30.47)	201.34
BDE119	5	19±56 (nd-188)	nd	nd	nd
BDE154	6	6±21 (nd-70)	nd	3.14±8.31 (nd-22)	nd
ΣBDEs		664±1585 (36-5424)	96.74±87.29 (32.08-196.03)	36.36±16.88 (21.37-70.53)	504.48

Table 2: Concentrations (average, standard deviation, minimum and maximum values) of ΣPCBs (sum of 55 congeners), HCB, DDT isomers and ΣDDTs (ng/g wet wt) in penguins and skua.

	Adélie penguin (EP)	*Emperor penguin (CI)*	*Emperor penguin (CW)*	*South polar skua (EP)*
Tri-CBs	0.09	0.01	0.01	0.37
Tetra-CBs	1.29	0.15	0.17	0.79
Penta-CBs	0.03	nd	0.01	0.22
Hexa-CBs	0.26	0.03	0.08	0.03
Hepta-CBs	0.30	0.03	0.14	1.32
Octa-CBs	0.27	0.07	0.13	1.10
ΣPCBs	2.23±2.17 (nd-7.54)	0.28±0.2 (0.09-0.5)	0.53±0.33 (0.06-1)	3.85
HCB	0.003±0.006 (nd-0.02)	nd	0.002±0.002 (nd-0.005)	0.08
o,p'-DDE	0.02±0.05 (nd-0.16)	0.01±0.01 (0.006-0.026)	0.005±0.004 (0.001-0.01)	0.05
p,p'-DDE	0.13±0.14 (nd-0.43)	0.010.003 (0.01-0.02)	0.01±0.01 (0.003-0.02)	0.21
p,p'-DDD	0.004±0.01 (nd0.03)	0.002±0.0001 (nd-0.005)	0.01±0.01 (nd-0.03)	0.02
o,p'-DDT	0.003±0.005 (nd-0.02)	nd	0.0001±0.0003 (nd-0.001)	nd
p,p'-DDT	nd	nd	0.001±0.001 (nd-0.003)	nd
ΣDDTs	0.16±0.15 (nd-0.43)	0.03±0.01 (0.02-0.04)	0.03±0.01 (0.01-0.04)	0.28

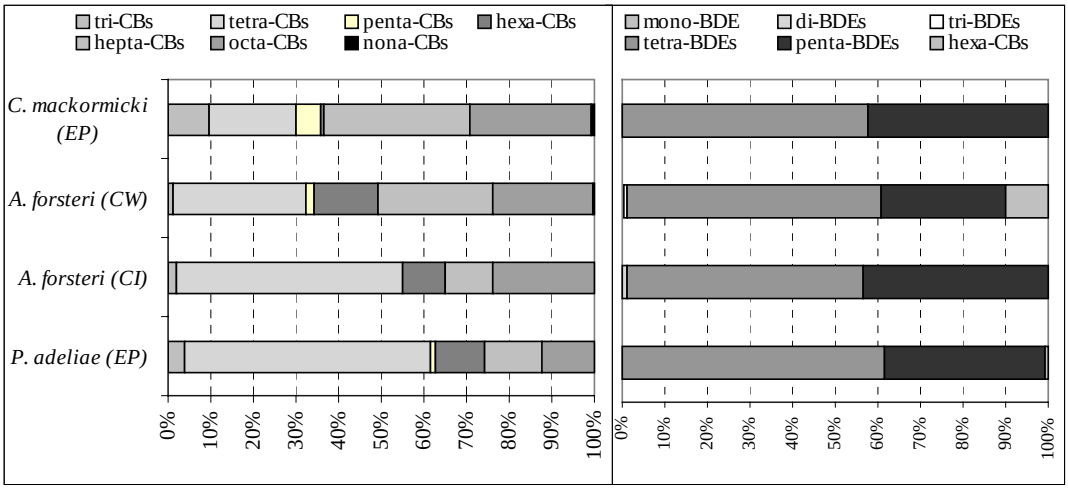

Figure 1: Percentage composition of PCB and PBDE class of isomers in penguin species and South Polar skua.

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