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Isomer-specific analysis and toxic potential evaluation of polychlorinated biphenyls in Antarctic fish, seabirds and Weddell seals from Terra Nova Bay (Ross Sea)

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Abstract: To provide data on the degree of contamination of the marine ecosystem isomer-specific concentrations of polychlorinated biphenyls, including planar, mono- and di-*ortho* congeners, were measured in the Weddell seal, the Adélie penguin, the south polar skua, and in two species of Antarctic fish (*Trematomus bernacchii* and *Chionodraco hamatus*) from Terra Nova Bay, Antarctica. The results show a clear relation between PCB concentrations and trophic level, in the order fish < Adélie penguin < Weddell seal. The higher values found in the skua appear to be related to its migration to more contaminated lower latitudes. The data for the various PCB congeners were used to calculate toxic potential in the different animals. The 2,3,7,8-TCDD toxic equivalents of coplanar congeners were estimated by the "toxic equivalent factors" (TEFs) approach. The highest values of TEFs were found in the south polar skua and Weddell seal. Values in the other species were considerably lower.

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Key words: organochlorines, PCB congeners, coplanar PCB, TEF, Ross Sea

Introduction

The absence of human activities protected the Antarctic ecosystem from direct pollution for many years but this has now changed. Recent studies have now detected many xenobiotics, especially substances the diffusion of which is facilitated by their persistence and mobility (Risebrough *et al.* 1976, Tanabe *et al.* 1983, Stromberg *et al.* 1990). The aim of this study was to determine isomer-specific concentrations of polychlorinated biphenyls, including planar, mono- and di-*ortho* congeners, in the Weddell seal, in two species of fish (*Chionodraco hamatus* and *Trematomus bernacchii*) and in two species of Antarctic seabirds, the Adélie penguin (*Pygoscelis adeliae*) and the south polar skua (*Catharacta maccormicki*). All samples were from Terra Nova Bay, Ross Sea near the Italian Antarctic scientific station. These species were chosen because they are at different levels in the food chain and can provide useful information on the degree of contamination of the ecosystem by these xenobiotics.

From data available for individual PCB isomers in the literature, it appears that the environmental concentration of biochemically active planar PCBs is several orders of magnitude higher than those of dioxins (Tanabe *et al.* 1987). Hence it is important to assess the environmental significance of these compounds in terms of toxic potential, especially that of planar, mono-, di- and non-*ortho* congeners.

Materials and methods

Specimens of *Trematomus bernacchii*, *Chionodraco hamatus*, *Pygoscelis adeliae*, *Catharacta maccormicki* and skin biopsies of *Leptonychotes weddellii* from Terra Nova Bay (74°41'42"S, 164°07'23"E) were analysed. The method of extraction involved digestion of samples (1g for skin biopsies, 5g for other samples) with 1N KOH-ethanol solution for 1 h followed by transfer of the extract to hexane. The concentrated hexane layer was cleaned on 1.5 g silica gel (Kieselgel 60, 0.630–0.200 mm, Merck), dried at 130°C for 3 h, and packed in a glass column (10 mm i.d. × 20 cm length). The eluate from the silica gel column was concentrated to 6 ml in a rotary evaporator. An aliquot of 3 ml of this extract was reserved for injection into GC-ECD and GC-MS for the determination of PCB isomers. The remaining 3 ml was passed through a column (5 mm i.d.) packed with 125 mg of activated carbon for the separation of non-*ortho* coplanar PCB congeners from other PCB isomers. An initial eluate of 100 ml of 20% dichloromethane in hexane contained PCBs with *ortho* chlorines, other xenobiotics and biogenic substances. A second fraction eluted with 100 ml of benzene/ethylacetate (50/50) containing non-*ortho* chlorine substituted coplanar PCBs was microconcentrated and the residues transferred to 5 ml of hexane. This hexane extract was cleaned with 5 ml of 10% fuming sulphuric acid and rinsed in distilled water. Analysis of total PCBs by gas chromatography was performed with a Perkin-Elmer model Autosystem gas chromatograph equipped with Ni^{63} electron capture detector. A SPB-5 bonded phase (0.25 μm film thickness) fused silica capillary column (30 m long, internal diam. 0.2 mm) from Supelco was used. The carrier gas was helium at 110 kPa (split ratio 25/1). The detector scavenger was argon/methane (95/5) at a flow of 30 ml min^{-1}. Oven temperature was 100°C for 10 min and was then increased by 5°C min^{-1} to 280°C. Injector temperature was 200°C. Detector temperature was 380°C. A mixture of specific isomers was used for calibration, recovery evaluation and confirmation.

GC/MS was used to quantify coplanar PCBs, using a Finnigan MAT GC/MS MAGNUM ITD System using the same gas

Table I. Average and range of PCBs (ng g^{-1} wet weight) and non-*ortho* PCB congeners (pg g^{-1} wet weight) concentrations in the different species.

Species	PCBs	33'44'-TCB IUPAC-77	33'44'5-PCB IUPAC-126	33'44'55'-HCB IUPAC-169
L. weddellii (*n*=3)	585 (406–750)	1870 (1665–2330)	390 (150–573)	964 (447–1923)
P. adeliae (*n*=6)	101 (56–188)	250 (115–442)	79 (34–112)	21 (5–48)
C. maccormicki (*n*=4)	1162 (885–1676)	1641 (1220–2300)	293 (54–552)	560 (134–877)
P. bernacchii (*n*=10)	21 (15–44)	195 (55–312)	100 (21–234)	10 (2–25)
C. hamatus (*n*=10)	36 (18–77)	28 (5–60)	6 (1–29)	1 (0.1–4)

chromatographic conditions as for GC/ECD (PCB-77 m/z 292, PCB-126 m/z 326, PCB-169 m/z 360). Recovery rates for non-ortho substituted PCBs were: PCB-77: n 6, mean 98±10%, PCB-126: n 6, mean 93±18%, PCB-169: n 6, mean 87±18%. Blanks were determined before analysis of samples and their maximum values were: PCB-77 10 pg, PCB-126 1 pg, PCB-169 0.1 pg. Absolute detection limits for GC/MS for non-ortho substituted PCBs were: PCB-77 0.1 pg, PCB-126 0.1 pg, PCB-169 0.08 pg.

The results obtained for the various PCB congeners were used to calculate toxic potential in the different animals. The 2,3,7,8-TCDD toxic equivalents of coplanar congeners were estimated by the "Toxic Equivalency Factors" (TEFs) approach, proposed by Safe (1990).

Results and discussion

PCBs and PCB congeners

PCBs were calculated as the sum of the principal congeners identified (about 50). A marked variation in PCB concentration was evident between the species with an average in liver *c.* 20–40 ng g^{-1} wet wt in fish, about 100 ng g^{-1} in the Adélie penguin and 1200 ng g^{-1} in the south polar skua. The concentration of PCBs in subcutaneous fat of the Weddell seal was 585 ng g^{-1} (Table I). These results show a clear relation between PCB concentrations and trophic level, in the order fish < Adélie penguin < Weddell seal. They are also in agreement with the proposed differences in the role of respiration in uptake and clearance of hydrophobic contaminants in aquatic (i.e., gill-breathing) and terrestrial (i.e., lung-breathing) vertebrates (Bacci 1994). The high values in the skua are almost certainly related to its migration to more contaminated lower latitudes. South polar skuas spend only about 4–5 months in Antarctica, then migrate through the Southern Ocean and are not uncommon in the northern Pacific and northern Atlantic during the southern winter (Devillers 1977, Furness & Hutton 1989).

Comparison with the literature shows that our PCB concentrations in fishes are higher than those detected by Subramanian *et al.* (1983) in *T. bernacchii* (about 0.17 ng g^{-1} whole body), and *Trematomus* sp. (from 0.08–0.77 ng g^{-1}), and similar to those detected by Focardi *et al.* (1992), even though the results are not precisely comparable because of the different methods of calculation used. In a previous study we detected 6000 ng g^{-1} on a lipid basis in the muscle of south polar skuas (Focardi *et al.* 1993) as compared to about 4000 ng g^{-1} l.b. found by Stromberg *et al.* (1990). The data for the subcutaneous fat of the Weddell seal agrees with that of Schneider *et al.* (1985) from Gould Bay (310 ng g^{-1} l.b. of PCBs); Stromberg *et al.* (1990) reported a range of PCBs values from 5 to 760 ng g^{-1}.

Since PCB concentrations in the Antarctic air and seawater are about 60–180 pg m^{-3} and 40-70 pg l^{-1} respectively (Tanabe *et al.* 1983), several orders of magnitude lower than in the organisms, we can conclude that these contaminants are subjected to bioaccumulation in the Antarctic marine food chain.

Regarding the composition of PCBs in our samples (Table II), a wide range of differences in fingerprint were observed between the various species (Fig. 1). The most abundant congeners were tetrachlorobiphenyl 22'55' (IUPAC N° 52), pentachlorobiphenyls 22'344' (IUPAC-85) and 23'44'5 (IUPAC-118), hexachlorobiphenyls 22'344'5' and 22'44'55' (IUPAC Ns. 138

Table II. IUPAC number and structure of PCB congeners.

IUPAC number	Structure number	IUPAC	Structure
Di-CB		Hexa-CB	
4	22'	128	22' 33' 44'
7	24	133	22' 33' 55'
Tri-CB		134	22' 33' 56
20	233'	137	22' 3445
24	236	138	22' 344' 5'
28	244'	141	22' 3455'
33	2' 34	151	22' 355' 6
34	2' 35	153	22' 44' 55'
Tetra-CB		156	233' 44' 5
40	22' 33'	Hepta-CB	
42	22' 34'	170	22' 33' 44' 5
44	22' 35'	172	22' 33' 455'
52	22' 55'	174	22' 33' 456'
60	2344'	176	22' 33' 466'
70	23' 4' 5	177	22' 33' 4' 56
Penta-CB		178	22' 33' 55' 6
83	22' 33' 5	180	22' 344' 55'
85	22' 344'	183	22' 344' 5' 6
99	22' 44' 5	187	22' 34' 55' 6
101	22' 455'	Octa-CB	
102	22' 456'	194	22' 33' 44' 55'
105	233' 44'	195	22' 33' 44' 56
110	233' 4' 6	196	22' 33' 44' 56'
118	23' 44' 5	201	22' 33' 4' 55' 6
		Nona-CB	
		206	22' 33' 44' 55' 6
		207	22' 33' 44' 566'

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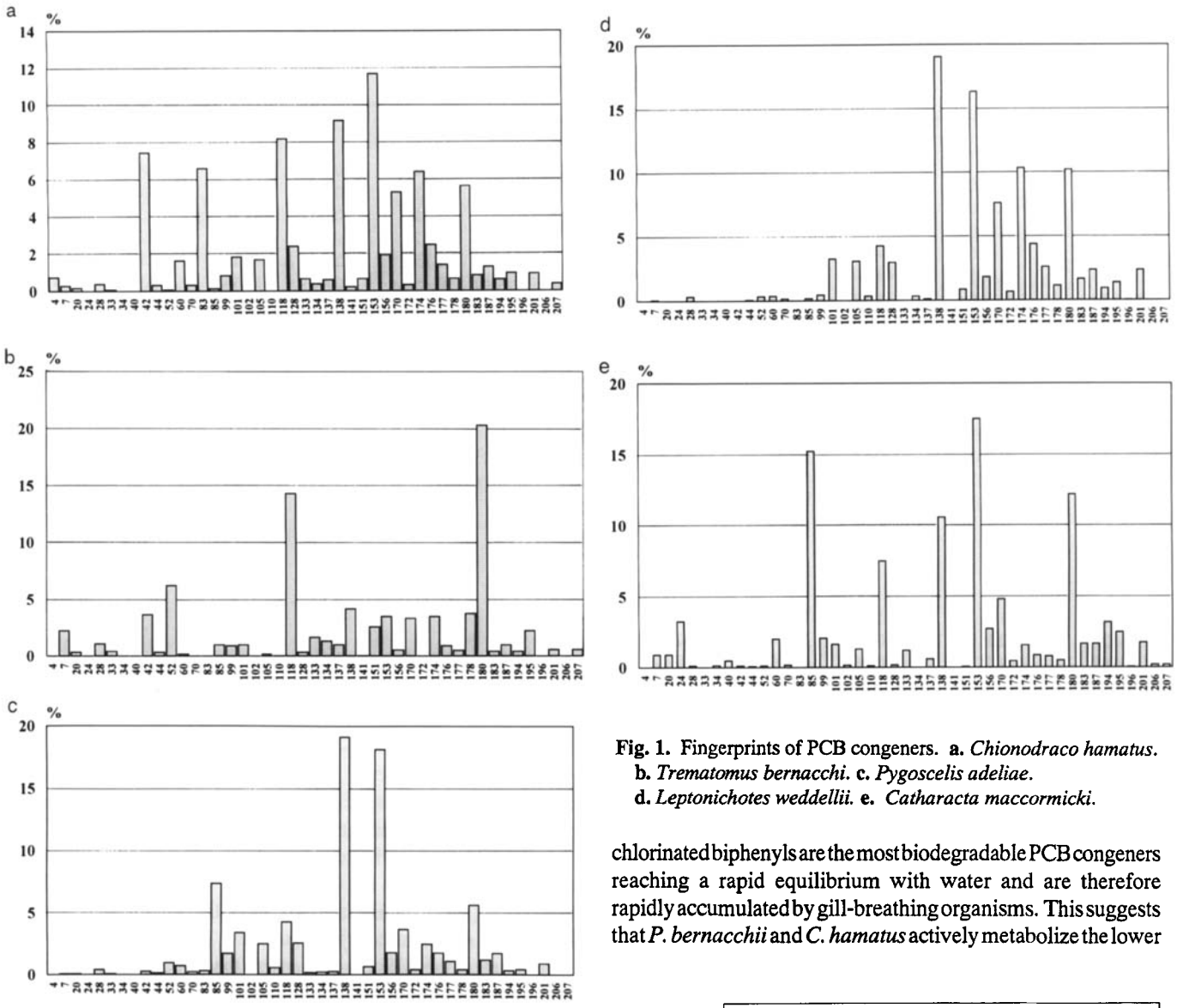

Fig. 1. Fingerprints of PCB congeners. **a.** *Chionodraco hamatus.* **b.** *Trematomus bernacchi.* **c.** *Pygoscelis adeliae.* **d.** *Leptonichotes weddellii.* **e.** *Catharacta maccormicki.*

and 153), and heptachlorobiphenyls 22'33'44'5 (IUPAC-170) and 22'3'44'55' (IUPAC-180). Risebrough *et al.* (1976), who first recorded PCBs in Antarctic birds, noted that the bulk of the residue in their samples consisted of pentachlorobiphenyls. In our samples, tetra- and pentachlorobiphenyls predominated in *T. bernacchii*, whereas hexachlorobiphenyls and heptachlorobiphenyls constituted most of the residues in birds and in Weddell seal.

Comparison of the fingerprints of Antarctic seawater and the organisms analysed (Fig. 2) shows some important differences. The fingerprints of seawater samples show a remarkable concentration of lower chlorinated biphenyls and an absence of the higher ones (Tanabe *et al.* 1983), while penta-, hexa-, hepta- and octa-chlorobyphenils are detectable only in the organisms, in which they account for a large proportion of total PCBs. In fish, the lower chlorinated biphenyls were also detected as they are taken up from both seawater and food. In fact, the lower chlorinated biphenyls are the most biodegradable PCB congeners reaching a rapid equilibrium with water and are therefore rapidly accumulated by gill-breathing organisms. This suggests that *P. bernacchii* and *C. hamatus* actively metabolize the lower

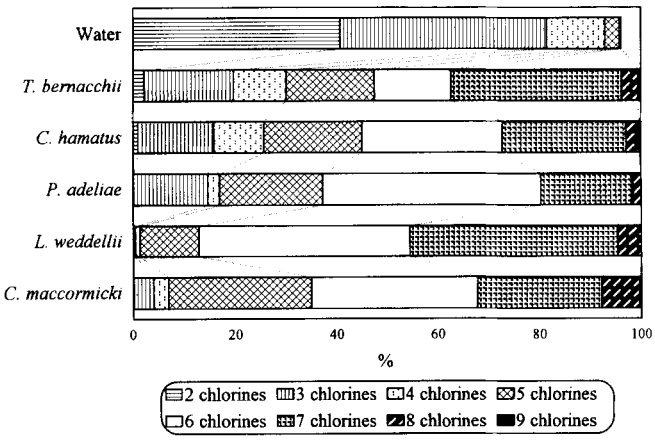

Fig. 2. Chlorobiphenyl composition (%) in water (Tanabe *et al.* 1983) and in the organisms.

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Table III. Average (ng g^{-1} wet weight) of di-, mono- and non-*ortho* PCB congeners in the species and their TEQ (2,3,7,8-tetrachlorodibenzo-*para*-dioxin equivalents) expressed in pg g^{-1} wet weight.

PCB congeners	TEF	*L. weddellii* (fat)		*P. adeliae* (liver)		*C. maccormicki* (liver)		*T. bernacchii* (liver)		*C. hamatus* (liver)	
Di-*ortho*		Conc.	TEQ	Conc.	TEQ	Conc.	TEQ	Conc.	TEQ	Conc.	TEQ
PCB-153	0.00002	78.82	1.58	15.06	0.30	191.8	3.84	0.42	0.008	3.49	0.07
PCB-137	0.00002	0.50	0.01	0.23	0.005	5.3	0.11	0.12	0.002	0.22	0.004
PCB-138	0.00002	91.95	1.84	15.88	0.32	130.6	2.61	0.50	0.01	3.33	0.07
PCB-128	0.00002	14.36	2.29	2.13	0.04	31.0	0.62	0.04	0.001	0.56	0.01
PCB-180	0.00002	49.34	0.99	4.67	0.09	117.7	2.35	2.44	0.05	2.05	0.04
PCB-170	0.00002	36.10	0.72	3.01	0.06	22.6	0.45	0.40	0.008	1.93	0.04
PCB-194	0.00002	4.50	0.09	0.35	0.007	23.1	0.46	0.05	0.001	11.58	0.23
Total		275.56	7.52	41.33	0.82	522.1	10.44	3.97	0.08	23.16	0.46
Mono-*ortho*											
PCB-60	0.001	1.78	1.78	0.60	0.60	12.1	12.1	0.02	0.02	0.59	0.59
PCB-118	0.001	20.59	20.59	3.56	3.56	85.3	85.3	1.72	1.72	2.00	2.00
PCB-105	0.001	14.81	14.81	2.08	2.08	6.5	6.5	0.02	0.02	0.61	0.61
PCB-156	0.001	8.79	8.79	1.46	1.46	49.8	49.8	0.07	0.07	0.70	0.70
Total		45.97	45.97	7.70	7.70	153.7	153.7	1.83	1.83	3.90	3.90
Non-*ortho*											
PCB-77	0.01	1.87	18.70	0.25	2.50	1.64	16.4	0.19	1.90	0.28	2.80
PCB-126	0.1	0.39	39.00	0.08	8.00	0.29	29.0	0.10	10.00	0.006	0.60
PCB-169	0.05	0.96	48.00	0.02	1.00	0.56	28.0	0.01	0.50	0.001	0.05
Total		3.22	105.70	0.35	11.50	2.49	73.4	0.30	12.40	0.29	3.45
TOTAL		324.75	159.19	49.38	20.02	678.3	237.5	6.10	14.31	27.35	7.81

chlorinated biphenyls. In birds and mammals, intake via food is the main route of contamination. This is why the Weddell seal and Adélie penguin have different PCB composition and why hexachlorobiphenyls and heptachlorobiphenyls constituted most of the residues in Weddell seal.

Coplanar PCBs and 2,3,7,8-TCDD toxic equivalents

The concentrations of three highly toxic non-*ortho* coplanar PCBs in the liver of *C. hamatus* were 28 pg g^{-1}, 6 pg g^{-1} and 1 pg g^{-1} wet wt. for IUPAC Ns. 77, 126 and 169 respectively (Table I). Higher concentrations were found in the other species, with maximum values in the Weddell seal (even higher than in the south polar skua). The pattern was $T_4CB > P_5CB > H_6CB$ in fish and birds and $T_4CB > H_6CB > P_5CB$ in the seal. According to Tanabe *et al.* (1987) normal healthy populations of marine mammals from various parts of the world generally have a non-*ortho* coplanar PCB pattern of $T_4CB > P_5CB > H_6CB$, which resembles the concentration pattern in most technical PCB formulations. Muir & Ford (1990) report that the concentration ratios of coplanar PCBs varied significantly in seal blubber from the Canadian Arctic and in harp seals. The deviation $T_4CB > H_6CB > P_5CB$ is observed in diseased/highly contaminated individuals (Corsolini *et al.* in press). Mono-*ortho* congeners (Table III) contributed 64% of the estimated TCDD-equivalents in the south polar skua and 50% in *C. hamatus*; PCB congener IUPAC N° 118 was the highest toxic contributor in both species. In the other species, non-*ortho* coplanar PCBs accounted for most of the toxicity, with IUPAC 169 as the highest contributor in the Weddell seal. This is at variance with reports in the literature that the contribution of non-*ortho* coplanar PCBs is lower than that of mono-*ortho* PCBs in marine mammals (Kannan *et al.* 1993).

In conclusion these findings confirm the information in the literature on the presence of polychlorinated biphenyls in the Antarctic ecosystem and their penetration of the food chains. Evaluation of the current toxic potential of these xenobiotics showed toxic equivalents that were generally an order of magnitude lower than those of bird and mammal populations from temperate areas (Kannan *et al.* 1989, Tillit *et al.* 1991). However it should be borne in mind that in birds a TCDD-equivalent concentration of 0.4–1 ng g^{-1} may result in severe reproductive impairment (Kubiak *et al.* 1989, Tillit *et al.* 1991). The high values found in the south polar skua are certainly correlated with its migratory habits, whereas the low ones in the other species, resident in the Antarctic Ocean, are related to the low concentrations of these contaminants in the Antarctic food chain. The study of these xenobiotics is therefore also a useful tool for evaluating feeding and migratory behaviour of species living in Antarctica.

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