Enantiomeric fractions and congener specific determination of polychlorinated biphenyls in eggs of predatory birds from Doñana National Park (Spain)

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Abstract

The content of 30 polychlorinated biphenyls (PCBs) and the enantiomeric fractions of 10 chiral PCBs were determined in 17 infertile eggs from three different predatory bird species collected in Doñana National Park (DNP, Spain) in the period 1999–2000. The highest PCB concentration was found in eggs from red kites (0.52–110 μg/g on a fresh weight basis, f.w.) followed by buzzard (0.08–13 μg/g f.w.) and booted eagle (0.10–1.5 μg/g f.w.). Seventy-five percent of the red kite eggs had PCB levels above 4.7 μg/g f.w., which is associated in the literature with reproductive failure. This could be related to the fact that red kite populations have decreased by more than 50% in the last five years in DNP. PCBs # 138, 153, and 180 were the most abundant in all cases. This is the first time that atropisomers of 10 chiral PCBs (PCBs # 45, 84, 91, 95, 132, 135, 136, 149, 174, and 176) in predatory bird eggs have been performed. The enantiomeric fractions (EFs) for most PCBs investigated were non-racemic (EF $\neq$ 0.5), ranging from 0.05 to 0.95. The results suggested that predatory birds, mainly red kite species, are highly polluted by PCBs, and PCBs # 95, 132, 135, 136, and 174 strongly deviate from the racemic-mixture values.
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Keywords: Polychlorinated biphenyl; Enantioselective analysis; Predatory bird eggs; Doñana National Park

1. Introduction

Organochlorine compounds such as polychlorinated biphenyls (PCBs) are well known as toxic and persistent contaminants that accumulate in the upper trophic levels of food chains, apparently associated with the habitat and dietary habits. In recent years there has been increasing concern about these chemicals, mainly with regard to their estrogenic properties. Diminished reproductive success in numerous bird species has been associated with eggshell thinning and reduced production caused by this type of xenobiotics (Wiemeyer et al., 1984; Hoffman et al., 1998).

For years attention has mainly been focused on those PCBs presenting similar toxicity to dioxins and furans, the so-called coplanar PCBs (mono-*ortho* and non-*ortho* PCBs). They cause dioxin-like effects through binding to the aryl hydrocarbon receptor (AhR). Other PCBs (the di-*ortho* PCB congeners) cause toxicological and biochemical effects mediated by the induction of

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CYP2B. They are usually at higher concentrations than mono- and non-*ortho* PCBs in the environment and in some cases they interact with dioxins in both synergic and antagonistic reactions (Safe, 1995). Recently attention has also focused on the 19 chiral PCBs (with three or four chlorine atoms in the *ortho* positions), which display axial chirality and are stable at ambient temperature (Kaiser, 1974), due to restricted rotation about the central C–C bond of biphenyl. Of these 19 PCB congeners, at least 12 (PCB IUPAC Number (Ballschmiter and Zell, 1980) 84, 88, 91, 95, 131, 132, 136, 149, 171, 174, 183, and 196) are present in commercial PCB mixtures (Duinker et al., 1988). Most of these compounds are produced and emitted as racemates; however, their uptake and metabolism by organisms may be enantiomer-selective (Buser et al., 1992; Klobes et al., 1998), mainly in higher organisms, whose enantioselective alteration mechanisms can be very complex. Although both enantiomers have identical physical and chemical properties, some studies suggest that the biological and toxic behavior of each enantiomer is different (Rodman et al., 1991). Enantioselective analysis of PCBs would provide information about the enantioselective degradation and accumulation of chiral PCBs in humans and biota.

The Doñana National Park (DNP), located in the southwest of Spain (SW Spain 36°48′–37°20′N, 6°12′–6°40′W), is a protected nature reserve that serves as a wild life sanctuary or refuge for thousands of sedentary and migratory birds, which nest and in some cases reside there temporarily. These birds offer an abundance of suitable biotopes for many species, including predatory birds and mammals. Predatory birds are top-level predators, excellent accumulators of persistent environmental pollutants, and they are long-lived. Because of these characteristics they are very useful as monitoring species in the terrestrial and aquatic food chains. Although this remarkable ecosystem was given official protection in 1969, having survived relatively unspoiled for centuries thanks to the absence of permanent settlements, it has always suffered the impact of human activities. One of the most serious is the mining of pyrite ores in Aznalcollar, rich in heavy metals and metalloids (Hernández et al., 1999). Some of our previous results indicated that organochlorine pollution is also important in the Park (González et al., 1984; Hernández et al., 1986). Thus, in the 1980s large amounts of DDTs and PCBs were detected in different species from aquatic and terrestrial ecosystems in DNP, including predatory birds.

Studies dealing with enantioselective degradation of PCBs in the literature are scarce. Most of them concern top predatory animal species from aquatic ecosystems (Blanch et al., 1996; Klobes et al., 1998; Schwinge et al., 1999; Jiménez et al., 2000; Harju et al., 2003), but there is very little information about top predatory terrestrial animals (Ramos et al., 1996; Hoeskstra et al., 2003).

In two previous short papers (Gómara et al., 2002a; Gómara and González, 2003), we reported results for organochlorine compounds, including PCBs and the enantiomeric enrichment of nine chiral PCBs in a small number of predatory bird egg samples from DNP, Spain. The present paper is an extended study concerning the concentrations of 30 individual PCB congeners (coplanar and non-planar, including chiral PCBs) and the enantioselective enrichment of 10 chiral PCBs found in infertile eggs of predatory birds from Doñana National Park (Spain). This is the first time that enantiomeric composition of chiral PCBs in birds of prey has been reported.

2. Materials and methods

2.1. Sampling

Seventeen infertile eggs from predatory birds were collected at Doñana National Park (DNP) between 1999 and 2000. Twelve were from red kite (*Milvus milvus*), three from booted eagle (*Hieraetus pennatus*) and two from buzzard (*Buteo buteo*). All of them are protected species and red kite is endangered in DNP because they have suffered a 50% decrease during the last five years. These species present different food and migratory habits. Red kites and buzzard are sedentary in DNP while booted eagle are migratory. They essentially feed carrion and small sized mammals and birds, and also fish, lizards, amphibians and invertebrates, but in a lower extend. Besides red kites captured significantly more prey items from scrub habitats and dumping sites (Veiga and Hiraldo, 1990). Egg samples were lyophilized and the lyophilized samples were stored at 20 °C until analysis.

2.2. Extraction and clean-up

Extraction was carried out by matrix solid phase dispersion as previously described in detail elsewhere (Bordajandi et al., 2003). Lyophilized egg sample was homogenized with 1:1 (w/w) silica gel:anhydrous sodium sulfate powder. The mixture was ground to a fine powder, loaded into a column, supplemented with a mixture containing $^{13}C_{12}$ labeled PCB # 77, 81, 126, and 169, and extracted with 400 ml of 1:1 (v:v) acetone:hexane mixture. Clean-up was carried out using a multilayer column filled with neutral silica, silica modified with sulphuric acid (44%) and silica modified with KOH. The final fractionation between non-*ortho* PCBs and the bulk of PCBs (where the chiral PCBs are obtained) was achieved using Supelclean™ ENVI™-Carb SPE

cartridges as described elsewhere (Concejero et al., 2001).

2.3. Determination of ortho substituted PCBs by GC-μECD

The determination of *ortho* substituted PCBs were performed by GC-μECD (Agilent 6890 Series II, PA, California, USA) as was described elsewhere (Gómara et al., 2002b). The following PCBs were determined: PCBs # 28, 45, 52, 84, 91, 95, 101, 105, 114, 118, 123, 132, 135, 138, 149, 153, 156, 157, 167, 170, 174, 176, 180, 183, 189, and 194. The PCB congeners were separated on a DB-5 column (60 m × 0.25 mm i.d., 0.25 μm film thickness; J.W. Scientific, USA). The column temperature was maintained for 1 min at 80 °C, then programmed at a rate of 30 °C/min to 185 °C, held for 3 min, then increased at 1.5 °C/min to 230 °C, held for 15 min, finally increased at 5 °C/min to 270 °C. Nitrogen was used as the carrier gas at a constant flow of 1.5 ml/min. The absolute detection limit was between 0.004 and 0.2 pg/μl from tetra- to octa-chlorinated biphenyls.

2.4. Determination of non-ortho substituted PCBs by GC-LRMS

Gas chromatographic separation of the four non-*ortho* substituted PCB congeners (PCB # 77, 81, 126, and 169), was performed on a Varian GC (CP-3800, CA, USA) equipped with an Ion Trap Detector (ITD, Saturn 2000, Varian) in the MS/MS mode. The extract containing the non-*ortho* PCB fraction was evaporated until dry and diluted with 10 μl nonane containing 50 pg/μl of $^{13}C_{12}$ isotopically labelled PCB # 70, 111, 138, and 170 as recovery standards. A 4 μl aliquot was injected in the PTV mode (100 °C, hold for 0.2 min, and then to 300 °C at 200 °C/min; splitless time 2.0 min) in a capillary BPX-5 column (60 m × 0.25 mm i.d., 0.25 μm film thickness, SGE, Australia). The column temperature was programmed from 60 °C (3 min) to 200 °C (3 min) at a rate of 30 °C/min, then to 230 °C (15 min) at 3 °C/min and then to 270 °C (15 min) at 5 °C/min. Helium was used as the carrier gas at a constant flow rate of 1 ml/min. All the GC-ITD(MS/MS) conditions have been previously described (Gómara et al., 2002c). PCB determination by GC-ITD(MS/MS) was based on isotopic dilution. This method is based on the simultaneous detection at the corresponding retention time of two ions, in this case, of the daughter clusters (M-Cl$_2$) for each congener, the maintenance of the theoretical ratio among them within an appropriate range, and the simultaneous detection of the two selected ions of the daughter cluster for the corresponding labeled congeners. The absolute detection limits were between 0.04 and 0.2 pg/μl for tetra- to

hexa-PCBs and recoveries of $^{13}C_{12}$-PCBs # 77, 81, 126, and 169 were between 95% and 110%.

2.5. Enantioseparation of chiral PCB by MDGC heart cutting technique

Gas chromatographic separation of the 10 chiral PCBs in their atropisomers was carried out on a Multi-dimensional Gas Chromatography (MDGC) system equipped with two independently heatable ovens, ECD detectors and split/splitless injectors (Varian CP-3800, CA, USA). Column switching was achieved with a pneumatically controlled three-T-pieces valve (DEANS switching system) placed inside the first GC oven. A DB-5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used as pre-column in the first oven for pre-separation of PCBs. The transfer line was heated at 280 °C, and the DEANS valve pressure was 20 psi to transfer the selected fraction from the pre-analytical column to the main GC column. Two different β-cyclodextrin-based GC-chiral columns, Chirasil-Dex (2,3,6,-tri-*ortho*-methyl-β-cyclodextrin, 25 m × 0.25 mm i.d., 0.25 μm film thickness, Varian-Chrompack, Middelburg, The Netherlands) and BGB-SE176 (20% 2,3-dimetyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin, 30 m × 0.25 mm i.d., 0.25 μm film thickness, BGB Analytik, Adliswil, Switzerland) were used as main GC column. Injections of 1–2 μl of extract in isooctane was made in the splitless mode (1 min) at 270 °C. Nitrogen was used as carrier gas at 30 psi column head pressure. Oven temperature programs were as follows: DB-5: 80 °C (1 min), at 30 °C/min to 185 °C (3 min), at 1.9 °C/min to 234 °C (25 min), at 2 °C/min to 270 °C. Second oven: Chirasil-Dex: 100 °C (1 min), at 5 °C/min to 160 °C (30 min), at 1 °C/min to 170 °C (10 min), at 1 °C/min to 180 °C; BGB-SE176: 100 °C (1 min), at 5 °C/min to 150 °C (30 min), at 0.5 °C/min to 160 °C (15 min), at 0.5 °C/min to 200 °C. The EF determinations of PCBs # 84, 132, 135, 136, 149, 174, and 176 was carried out by using Chirasil-Dex, while for PCBs # 45, 91, and 95, BGB-SE176 was used due to its high chromatographic resolution. The results were expressed as enantiomeric excess (ee) and enantiomeric fraction (EF). The enantiomeric excess, ee (values between 0% and 100%) was defined as the excess of the predominant enantiomer expressed as a percentage. EFs (values between 0 and 1) (de Geus et al., 2000) defined as area of the (+) enantiomer divided by the sum of the areas of both enantiomers (−) and (+) for PCBs # 84, 132, 135, 136, 149, 174, and 176, and the area of the first eluting enantiomer divided by sum of the areas of both atropisomers in the case of PCBs # 45, 91, and 95. The chromatographic resolution (Rs) for Chirasil-Dex GC column was between 0.59 (PCB # 174) and 2.0 (PCB # 132) and higher than

1.5 for the three PCBs determined by BGB-SE176 GC column.

To evaluate the quality of the EF determinations, a standard mixture containing the 10 racemic PCBs was determined using both mentioned chiral GC columns. The coefficients of variation (CV) for repeatability ($n = 4$ within the same day) and reproducibility ($n = 5$ in five different days) were lower than 3%. All EFs were in acceptable average values ($n = 9$), ranging from 0.47 to 0.50.

3. Results and discussions

3.1. Total PCB concentrations

The sum of the concentration of the 30 PCB congeners found in egg samples (on a fresh weight basis, f.w.) showed significant variations among the birds species investigated (Table 1). Thus, red kites exhibited the highest values (range from 0.52 to 110 µg/g f.w.), fol-lowed by buzzards (range from 0.08 to 13 µg/g f.w.), and booted eagles (range from 0.10 to 1.5 µg/g f.w.). Except for the booted eagle, these values were significantly higher ($p < 0.05$) than in eggs from predatory bird species collected in Doñana National Park in the period 1980–1983 (González et al., 1984), where PCB levels found in eggs of five predatory bird species, including booted eagle (0.22–0.95 µg/g f.w.) and red kite (1.15–5.13 µg/g f.w.) ranged from 0.22 to 6.44 µg/g f.w. The mean PCB values found in red kites were also higher and more variable than those found in eggs from various species of predatory birds in Norway (PCB geometric mean values from 1.4 to 9.1 µg/g f.w.) (Herzke et al., 2002), peregrine falcon (*Falco peregrinus*) from central Spain (PCB concentrations ranged from 0.43 to 3.33 µg/g f.w.) (Bordajandi et al., 2001), ospreys (*Pandion haliaetus*) from the US Pacific Northwest (geometric mean from 0.1 to 4.62 µg/g f.w.) and from highly industrialized areas in the US Atlantic Northeast (geometric mean from 2.5 to 26 µg/g f.w.) (Steidl et al., 1991; Elliot et al., 2000). Seventy-five percent of red kite

Table 1
PCB congener concentrations (arithmetic mean and range), in eggs of three species of predatory birds from DNP (Spain), expressed in ng/g fresh weight basis

PCB IUPAC No.[a]	Booted eagle ($n = 3$)	Buzzard ($n = 2$)	Red kite ($n = 12$)
28	1.1 (0.3–2.2)	(0.5–11)	55 (2.2–235)
45	0.01 (0.01–0.02)	(0.01–0.5)	3.6 (0.03–12)
52	1.8 (1.1–2.3)	(1.7–86)	35 (2.2–91)
77[b]	0.2 (0.03–0.4)	(0.03–0.4)	0.1 (0.01–0.13)
81[b]	NA	NA	0.06 (ND–0.12)
84 + 101	3.1 (1–6.6)	(1.3–412)	960 (6–3298)
91	0.1 (0.06–0.2)	(0.09–5)	13 (0.7–41)
95	2.0 (0.8–3.3)	(1.2–96)	102 (4–303)
105	3.0 (0.9–6.4)	(0.8–155)	126 (4–320)
114	1.1 (0.2–2.9)	(0.2–24)	87 (1–218)
118	14 (3–37)	(2.3–449)	992 (21–3197)
123 + 149	2 (0.4–4.0)	(0.8–108)	133 (3–501)
126[b]	0.06 (0.04–0.08)	(0.08–0.09)	1.1 (0.9–3.1)
132	0.08 (ND–0.25)	(ND–0.3)	6.6 (ND–74)
135	0.39 (0.1–0.79)	(0.2–7)	24 (0.62–81)
138	61 (10–161)	(9–2017)	5783 (89–17535)
153	188 (29–505)	(24–3628)	11023 (160–33785)
156	5 (0.9–12)	(0.9–116)	608 (5–2135)
157	1 (0.2–2.4)	(0.3–22)	56 (2–136)
167	3.2 (0.7–8)	(0.7–92)	146 (4–357)
169[b]	0.01 (0.0001–0.02)	(0.0002–0.02)	0.16 (0.0002–0.32)
170	32 (7–81)	(6–711)	2407 (33–7466)
174	0.6 (0.1–1.5)	(0.2–24)	40 (0.8–98)
176	0.6 (0.2–1.4)	(0.2–12)	55 (1–168)
180	184 (31–488)	(23–3226)	10512 (128–30211)
183	16 (2.4–45)	(2–502)	1729 (16–5095)
189	2.6 (0.7–6.4)	(0.7–93)	86 (3–391)
194	32 (6.6–83)	(6–799)	1973 (32–5985)
Total PCBs	556 (102–1460)	(82–12596)	36960 (519–110316)

[a] Ballschmiter and Zell (1980).
[b] Non-*ortho* PCBs; ND = non-detected; NA = non-analyzed.

eggs and 50% of buzzard eggs analyzed were also above the 4.7 µg/g f.w. level, which has been associated with egg-shell thinning and reduced mean production in the related literature (Hoffman et al., 1993, 1998). This would probably be related to the population decrease of red kite species in DNP. The high variability of PCBs found in the three birds of prey species studied could be explained by different exposure due to migration in the case of migratory species and feeding habits in all cases.

3.2. PCB congener distribution pattern

PCBs # 138, 153, and 180 were the most abundant PCB congeners, each accounting for more than 11% of the total PCBs investigated, followed by PCBs # 118, 170, 183, and 194 (each accounting for more than 2%). Non-*ortho* PCBs # 77, 126, and 169, mono-*ortho* PCBs # 114, 123, 157, 167 and 189, and PCBs # 28, 45, 52, 91, 95, 132, 135, 174, and 176 were the smallest contributors to the total PCBs (less than 1%). PCB # 77 was the dominant non-*ortho* PCB followed by PCBs # 126 and 169, except for red kite species for which the pattern was PCB # 126 > 77 > 169. PCB # 81 was only calculated in red kite species and exhibited the lowest concentration levels of all the non-*ortho* PCBs. These findings are consistent with the data in the literature, where PCBs # 138, 153, and 180 have generally been identified as the most abundant in eggs of predatory bird species (de Voogt et al., 1990). All the most abundant congeners belong to the group of persistent PCBs, according to the chlorine substitution patterns. All of them have the 2,4,5-; 2,3,4- and 3,4-chlorine substitution in each biphenyl ring. When that pattern occurs on each ring, the molecules are persistent, and they are more persistent still when both rings have 2,4,5- or 2,3,5-chlorine substitution patterns as in the case of PCBs # 153, 180, 183, and 194. But not all the most abundant PCB congeners belong to the group of highly persistent PCBs according to data in the literature, based on the absence of vicinal H atoms in the molecule (Bush et al., 1984; McFarland and Clarke, 1989; de Voogt et al., 1990). PCBs # 153, 180, 183, and 194 have no vicinal H atoms, while PCBs # 118, 138, and 170 have vicinal H atoms in *ortho/meta* positions combined with one (PCB # 118) or two chlorine (PCBs # 138 and 170) atoms in an *ortho* position. Therefore, the results indicate that the chlorine substitution pattern might play a more important role in the biodegradation processes of PCBs in predatory birds than the presence/absence of vicinal H-atoms in *ortho/meta* and *meta/para* positions and *ortho* Cl substitution.

3.3. Enantiomeric enrichment of chiral PCBs

The enantiomeric fractions (EFs) and the enantiomeric excess (ee) of the 10 chiral PCBs (# 45, 84, 91, 95, 132, 135, 136, 149, 174, and 176) found in egg sam-

ples are shown in Table 2. The results show a high percentage of enantiomeric enrichment in almost all the detected chiral PCBs, reaching almost 90% in some samples.

Some differences were found among the species studied. Red kite species presented the highest enantiomeric excess of the chiral PCBs studied; the enantiomeric enrichment mean of PCBs # 84, 95, 132, 135, 136, and 174 was higher than 32%, while the others were between 15% and 29%. The enantiomeric enrichment of the chiral PCBs in booted eagle was also quite high, but in a lower percentage than red kite, and PCBs # 45, 91, 95, and 174 were racemic or nearly racemic. Buzzard species presented the least enantiomeric excess, and only PCB # 136 and 149 exhibited more than 30% of enantiomer enrichment in one of the studied samples.

On Chirasil-Dex GC column the optical rotation of PCBs # 84, 132, 135, 136, 149, 174 and 176 has been experimentally assigned using pure enantiomers. The (−) enantiomer elutes before the (+) enantiomer for all atropisomers, except PCBs # 135 and 174 for which elution order is reversed (Haglund and Wiberg, 1996; Harju and Haglund, 1999).

The results from the samples studied (Table 2) revealed that in PCB # 84 (EF = 0.54–0.88), PCB # 132 (EF = 0.52–0.95), and PCB # 149 (EF = 0.39–0.70) the second eluted enantiomer (+) was predominant in almost all the cases (EF > 0.5). For PCB # 135 (EF = 0.16–0.62), PCB # 136 (EF = 0.15–0.82), PCB # 174 (EF = 0.16–0.56) and PCB # 176 (EF = 0.12–0.68), the relative abundance of the two optical atropisomers depends on the bird species. These results indicate that the optical isomer (+) of three chiral PCBs was predominant in the predatory birds studied.

The knowledge of PCB atropisomers in real samples is incomplete at present, probably due to the difficulties in achieving their enantiomeric separation. In previous studies (Blanch et al., 1996; Ramos et al., 1996; Jiménez et al., 2000;) we reported the enantiomeric enrichment of nine chiral PCBs in otter (*Lutra lutra*) from DNP (Spain) and cetaceans from the Mediterranean Sea (*Grampus griseus*, *Tursiops truncates*, *Balaenoptera physalus* and *Globicephala melaena*), and PCBs # 95, 132, and 149 in sharks (*Coelolepis coelolepis*) from the SW Atlantic Ocean. Some differences and similarities were found when the results were compared. While PCB # 95, 135, and 136 showed enantiomeric excess in predatory birds (in some cases they have an ee of almost 90%), they were racemic or nearly racemic in otter, shark and cetacean species. Otter samples exhibited greater enantioselective enrichment of PCB # 176 than samples of predatory birds, while in cetacean species it was racemic. PCBs # 132 and 174 showed enantiomeric enrichment in all species, but greater in bird and otter than in cetacean species. Some authors reported (Harju et al., 2003) that PCBs # 91, 95, and 132 have greater deviations from

Table 2
Arithmetic mean values and ranges of enantiomeric fractions (EF) and enrichment (ee) of chiral PCBs in egg samples of predatory birds species from DNP (Spain)

Species	PCB IUPAC No.[a]	Enantiomeric fraction (EF)		Enantiomeric enrichment (ee)	
		Mean	Range	Mean	Range
Booted eagle ($n = 3$)	45	0.52[b]	–[b]	4.0[a]	–[b]
	84	0.62	0.59–0.65	24	17–30
	91	0.48	0.47–0.50	3.4	0.73–7.0
	95	0.50	0.48–0.51	2.2	0.78–3.2
	132	0.70	0.57– 0.87	39	13–73
	135	0.43	0.40–0.46	15	8.8–20
	136	0.39	0.36–0.64	22	9.6–28
	149	0.44	0.39–0.51	12	2.6–22
	174	0.53	0.49–0.56	7.7	1.6–12
	176	0.34	0.12–0.45	33	9.0–75
Buzzard ($n = 2$)	45		ND		ND
	84		0.54–0.60		8.4–20
	91		0.38–0.53		6.5–24
	95		0.49–0.59		0.80–18
	132		0.52–0.56		4.2–11
	135		0.54–0.57		8.4–13
	136		0.33–0.46		7.9–34
	149		0.58–0.65		16–30
	174		0.42–0.47		5.2–16
	176		0.43–0.50		0.60–14
Red kite ($n = 12$)	45	0.60[b]	–[b]	20[b]	–[b]
	84	0.88[b]	–[b]	77[b]	–[b]
	91	0.55	0.30–0.83	29	1.6–66
	95	0.75	0.53–0.95	48	6.1–90
	132	0.84	0.55–0.95	67	9.5–90
	135	0.34	0.16–0.62	32	0.79–68
	136	0.48	0.15–0.82	36	12–69
	149	0.56	0.46–0.70	15	0.32–40
	174	0.27	0.16–0.42	46	17–68
	176	0.47	0.28–0.68	18	2.9–44

[a] Ballschmiter and Zell (1980).
[b] EF and ee values correspond to $n = 1$.

racemic in Baltic grey seal samples, and others (Hoekstra et al., 2002) that PCBs # 174 and 183 exhibited the highest enantiomeric enrichment in bowhead whale species. These results suggested that PCBs # 132 and 174 strongly deviates from racemic in all cases, and PCB # 132 showed a selective metabolization of the first eluting enantiomer (−) (EF > 0.5) in almost all cases.

This is the first time that enantiomeric excess of chiral PCBs in predatory birds has been reported. It is worth mentioning that considerable deviations from racemic mixtures of the 10 chiral PCBs investigated were found in those predatory birds, mainly red kite species.

As has been pointed out in similar studies, the differences observed in the enantiomeric enrichment of the chiral PCBs investigated among species cannot be explained by the relationship between chlorine substitution and metabolism. It is well known in the related literature (Bush et al., 1984; McFarland and Clarke, 1989; de Voogt et al., 1990) that all of them are readily metabolizable PCBs (they have neighboring hydrogen atoms in both *ortho*/*meta* and *meta*/*para* positions). The EFs differences found between atropisomers of chiral PCBs and among species could be better explained by the enantioselective character of the enzymatic biodegradation process in different species (Wong et al., 2004) than by chiral PCB structure.

Additional processes that could be enantioselective for PCBs in eggs of predatory birds may also came into play such as contamination uptake during formation of the eggs within the mother (Lehmler et al., 2003), microbial degradation in infertile eggs (Herzke et al., 2002), and membrane transfer to the egg formation (Ulrich et al., 2001).

We would note that only 17 predatory bird eggs have been analyzed, and the results do not indicate which of the mentioned possibilities are involved in the processes. Some studies have found *in vivo* enantioselective biotransformation of chiral PCBs (e.g. PCBs # 91, 95, 136, 149, 174, and 176) in lower level trophic organisms in the aquatic food web (macrozooplank-ton, forage fish and lake trout) and also uptake of non-racemic residues from prey (Wong et al., 2004). It is also well known in literature that birds in general, and predatory birds in particular biotransform PCBs (Guruge and Tanabe, 1997; Drouillard et al., 2001). In any case, future research about EFs of chiral PCBs in the food web of predatory birds will be necessary to draw reliable conclusions in order to determine the extent of enantioselective PCB biotransformation.

4. Conclusions

Red kite species from DNP exhibited PCB values higher than those associated with reduction in mean production, which could be related to the decline in their populations over the last few years. Chiral PCBs, which are racemic when they enter the environment, undergo biochemical degradation or enantioselective bioaccumulation through the food web and are particularly important in top predatory animals. PCBs # 95, 132, 135, and 176 from predatory birds, mainly red kites, strongly deviate from racemic mixtures and PCB # 132 in particular shows a high capacity to be enantioselectively degrade or accumulate in the food web.

References

Ballschmiter, K., Zell, M., 1980. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Fres. Z. Anal. Chem. 302, 20–31.

Blanch, G.P., Glausch, A., Schurig, V., Serrano, R., González, M.J., 1996. Quantification and determination of enantiomeric ratios of chiral PCB 95, PCB 132, and PCB 149 in shark liver samples (*C. coelolepis*) from the Atlantic Ocean. J. High Resol. Chromatogr. 19, 392–396.

Bordajandi, L.R., Merino, R., Jiménez, B., 2001. Organochlorine exposure in peregrine falcon (*Falco peregrinus*) eggs and its avian prey (*Columba livia*). Organohalogen Compounds 52, 139–142.

Bordajandi, L.R., Gómez, G., Fernández, M.A., Abad, E., Rivera, J., González, M.J., 2003. PCDD, PCDF, PCB,

heavy metal and arsenic levels in eels and trouts from the Turia River basin (Spain). Chemosphere 53, 163–171.

Buser, H.R., Müller, M.D., Rappe, C., 1992. Enantioselective determination of chlordane components using chiral high-resolution gas chromatography–mass spectrometry with application to environmental samples. Environ. Sci. Technol. 26, 1533–1540.

Bush, B., Snow, J., Connor, S., Koblintz, R., 1984. Polychlorobiphenyl (PCB) congeners, *p,p′*-DDE and hexachlorobenzene in maternal and fetal cord blood from mothers in upstate New York. Arch. Environ. Contam. Toxicol. 13, 517–527.

Concejero, M.A., Ramos, L., Jiménez, B., Gómara, B., Abad, E., Rivera, J., González, M.J., 2001. Suitability of several carbon sorbents for the fractionation of various subgroups of toxic polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. J. Chromatogr. A 917, 227–237.

de Geus, H.J., Wester, P.G., de Boers, J., Brinkman, U.A.Th., 2000. Enantiomer fraction instead of enantiomer ratios. Chemosphere 41, 725–727.

de Voogt, P., Wells, D.E., Reutergardh, L., Brinkman, U.A.Th., 1990. Biological-activity, determination and occurrence of planar, mono-*ortho* and di-*ortho* PCBs. Int. J. Environ. Anal. Chem. 40, 1–8.

Drouillard, K.G., Kimberly, J.F., Smits, J.E., Bortolotti, G.R., Bird, D.M., Norstrom, R.J., 2001. Bioaccumulation and toxicokinetics of 42 polychlorinated biphenyl congeners in American kestrels (*Falco sparverius*). Environ. Toxicol. Chem. 20, 2514–2522.

Duinker, J.C., Schulz, D.E., Petrick, G., 1988. Multidimensional gas chromatography with electron capture detection for the determination of toxic congeners in polychlorinated biphenyl mixtures. Anal. Chem. 60, 478–482.

Elliot, J.E., Machmer, M.M., Wilson, L.K., Henny, C.J., 2000. Contaminants in ospreys from the Pacific Northwest: II Organochlorine pesticides, polychlorinated biphenyls, and mercury, 1991–1997. Arch. Environ. Contam. Toxicol. 38, 93–106.

Gómara, B., González, M.J., 2003. Enantiomeric separation of chiral PCBs in eggs of predatory birds from Doñana National Park (Spain) by using multidimensional gas chromatography techniques. Organohalogen Compounds 62, 273–276.

Gómara, B., Fernández, M.A., Baos, R., Herrero, L., Jiménez, B., Abad, E., Hiraldo, F., Ferrer, M., Rivera, J., González, M.J., 2002a. Presence of organochlorine pollutants (PCDDs, PCDFs, PCBs and DDTs) in eggs of predatory birds from Doñana National Park, Spain. Organohalogen Compounds 58, 441–444.

Gómara, B., Ramos, L., González, M.J., 2002b. Determination of polychlorinated biphenyls in small-size serum samples by solid-phase extraction followed by gas chromatography with micro-electron-capture detection. J. Chromatogr. B 766, 279–287.

Gómara, B., Fernández, M.A., González, M.J., Ramos, L., 2002c. Optimization of the ionization conditions for the trace determination of polychlorinated biphenyls with ion trap MS/MS. Organohalogen Compounds 55, 107–110.

González, M.J., Hernández, L.M., Rico, M.C., Baluja, G., 1984. Residues of organochlorine pesticides, polychlorinated

biphenyls and heavy metals in the eggs of predatory birds from Doñana National Park (Spain), 1980–1983. J. Environ. Sci. Health B 19, 759–772.

Guruge, K.S., Tanabe, S., 1997. Congener specific accumulation and toxic assessment of polychlorinated biphenyls in common cormorants, *Phalacrocorax carbo*, from Lake Biwa, Japan. Environ. Pollut. 96, 425–433.

Haglund, P., Wiberg, K., 1996. Determination of gas chromatography elution sequences of the (+)- and (−)-enantiomers of stable atropisomeric PCBs on Chirasil-Dex. J. High Resol. Chromatogr. 19, 373–376.

Harju, M.T., Haglund, P., 1999. Determination of the rotational energy barriers of atropisomeric polychlorinated biphenyls. Fres. J. Anal. Chem. 364, 219–223.

Harju, M., Bergman, A., Olsson, M., Rooss, A., Haglund, P., 2003. Determination of atropisomeric and planar polychlorinated biphenyls, their enantiomeric fractions and tissue distribution in grey seals using comprehensive 2D gas chromatography. J. Chromatogr. A 1019, 127–142.

Hernández, L.M., Rico, M.C., González, M.J., Hernán, M.A., Fernández, M.A., 1986. Presence and time trends of organochlorine pollutants and heavy metals in eggs of predatory birds of Spain. J. Field Ornithol. 57, 270–282.

Hernández, L.M., Gómara, G., Fernández, M., Jiménez, B., González, M.J., Baos, R., Hiraldo, F., Ferrer, M., Benito, V., Suñer, M.A., Devesa, V., Muñoz, O., Montoro, R., 1999. Accumulation of heavy metals and As in wetland birds in the area around Doñana National Park affected by the Aznalcollar toxic spill. Sci. Total Environ. 242, 293–308.

Herzke, D., Kallenborn, R., Nygard, T., 2002. Organochlorines in egg samples from Norwegian birds of prey: congener-, isomer- and enantiomer specific considerations. Sci. Total Environ. 291, 59–71.

Hoekstra, P.F., Wong, C.S., O'Hara, T.M., Solomon, K.R., Mabury, S.A., Muir, D.C.G., 2002. Enantiomer-specific accumulation of PCB atropisomers in the bowhead whale (*Balaena mysticetus*). Environ. Sci. Technol. 36, 1419–1425.

Hoeskstra, P.F., Braune, B.M., Wong, C.S., Williamson, M., Elkin, B., Muir, D.C.G., 2003. Profile of persistent chlorinated contaminants, including selected chiral compounds, in wolverine (*Gulo gulo*) livers from the Canadian Artic. Chemosphere 53, 551–560.

Hoffman, D.J., Smith, G.J., Rattner, B.A., 1993. Biomarkers of contaminant exposure in common terns and black-crowned night herons in the Great Lakes. Environ. Toxicol. Chem. 12, 1095–1103.

Hoffman, D.J., Melancon, M.J., Klein, P.N., Eiseman, J.D., Spann, J.W., 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels and common terns. Environ. Toxicol. Chem. 17, 747–757.

Jiménez, O., Jiménez, B., González, M.J., 2000. Isomer-specific polychlorinated biphenyls determination in cetaceans from the Mediterranean Sea: enantioselective occurrence of chiral polychlorinated biphenyls congeners. Environ. Toxicol. Chem. 19, 2653–2660.

Kaiser, K.L.E., 1974. On the optical activity of polychlorinated biphenyls. Environ. Pollut. 7, 93–105.

Klobes, U., Vetter, W., Luckas, B., Skirnisson, K., Plötz, J., 1998. Levels and enantiomeric ratios of α-HCH, oxychlordane and PCB 149 in blubber of harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) from Iceland and further species. Chemosphere 37, 2501–2512.

Lehmler, H.J., Price, D.J., Garrison, A.W., Birge, W.J., Robertson, L.W., 2003. Distribution of PCB 84 enantiomers in C57BL/6 mice. Fres. Environ. Bull. 12, 254–260.

McFarland, V.A., Clarke, J.U., 1989. Environmental occurrence, abundance and potential toxicity of polychlorinated congeners: considerations for a congener specific analysis. Environ. Health Perspect. 81, 225–239.

Ramos, L., Jiménez, B., Fernández, M., Hernández, L., González, M.J., 1996. Coplanar and chiral PCB determinations using pyrenil-silica HPLC and HRGC/ECD. Application to real samples. Organohalogen Compounds 27, 376–381.

Rodman, L.E., Shedlofsky, S.I., Mannschreck, A., Püttmann, M., Swim, A.T., Roberson, L.W., 1991. Differential potency of atropisomers of polychlorinated biphenyls on cytocrome P-450 induction and uroporphyrin accumulation in the chick embryo hepatocyte culture. Biochem. Pharmacol. 41, 915–922.

Safe, S.H., 1995. Polychlorinated biphenyls (PCBs)—environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. 24, 87–149.

Schwinge, M., Vetter, W., Luckas, B., 1999. Enantioselective determination of atropisomeric PCBs after liquid chromatographic enrichment. Organohalogen Compounds 40, 405–408.

Steidl, R.F., Griffin, C.R., Niles, L.J., 1991. Contaminant levels of osprey eggs and prey reflect regional differences in reproductive success. J. Wild Manage. 55, 601–608.

Ulrich, E.M., Willett, K.L., Caperell-Grant, A., Bigsby, R.M., Hites, R.A., 2001. Understanding enantioselective processes: a laboratory rat model for hexachlorocyclohexane accumulation. Environ. Sci. Technol. 35, 1604–1609.

Veiga, J.P., Hiraldo, F., 1990. Food habits and the survival and growth of nestling in two sympatric kites (*Milvus milvus and Milvus migrans*). Holarctic Ecol. 13, 62–71.

Wiemeyer, S.N., Lamont, T.G., Bunck, C.M., Sindelar, C.R., Gramlich, F.J., Fraser, J.D., Byrd, M.A., 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs (1969–79) and their relationships to shell thinning and reproduction. Arch. Environ. Contam. Toxicol. 13, 529–549.

Wong, C.S., Mabury, S.A., Whittle, D.M., Backus, S.M., Teixeira, C., Devault, D.S., Bronte, C.R., Muir, D.C.G., 2004. Organochlorine compounds in Lake Superior: chiral polychlorinated biphenyls and biotransformation in the aquatic food web. Environ. Sci. Technol. 38, 84–92.