ENANTIOMERIC SEPARATION OF CHIRAL PCBs IN EGGS OF PREDATORY BIRDS FROM DOÑANA NATIONAL PARK (SPAIN) BY USING MULTIDIMENSIONAL GAS CHROMATOGRAPHY TECHNIQUES.

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Introduction

Due to the environmental persistence and toxicity of PCBs there has been a great deal of research to understand the enantiomeric distribution of chiral PCBs in various environmental matrices. There are 78 chiral PCBs, containing three or four chlorine atoms in the *ortho*-positions, which display axial chirality in their non-planar conformations [1], but only 19 congeners exist as stable enantiomers at ambient temperature due to restricted rotation about the central C-C bond of biphenyl. Among these 19 PCB congeners at least 12 (PCB # 84, 88, 91, 95, 131, 132, 136, 149, 171, 174, 183 and 196) are present in commercial PCB mixtures [2]. Most of these compounds are introduced into the environment as racemates, but their uptake and metabolism by organism may be enantiomer-selective [3, 4]. This fact makes that their determination could be useful in different research topics, mainly in the environmental field.

The Doñana National Park (DNP) 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 birds. Because of these characteristics they are very useful as monitoring species in the terrestrial food chain.

There are a few studies related with the enantiomeric enrichment of chiral PCBs in top predatory animal species from aquatic ecosystems [4, 5], and there is a lack of information about chiral PCBs and their enantiomeric ratio in top predatory terrestrial animals. In previous papers [6-8] we reported the enantiomeric enrichment of chiral PCBs in sharks (*C. coelolepis*) from the Northwestern Atlantic Ocean, cetaceans (*Stenella coeruleoalba, Tursiops truncatus, Grampus griseus, Balaenoptera physalus Globicephala melaena)* from the Mediterranean sea, and otter samples from DNP (Spain).

This paper reports results concerning the enantiomeric ratios of chiral PCBs in top predatory birds from terrestrial ecosystems of DNP. Nine chiral PCBs (84, 91, 95, 132, 136, 149, 174 and 176) found in infertile eggs of three species of the avian Falconiforme order, red kite (*Milvus milvus*), booted eagle (*Hieraetus pennatus*) and buzzard (*Buteo buteo*) collected at DNP (Spain), between 1999 and 2001, were separated into their atropisomers by using multidimensional gas chromatography (heart cutting) technique with two chiral GC columns.

Material and Methods

Sampling

Seventeen infertile eggs of predatory birds (red kite, booted eagle and buzzard) were collected at Doñana National Park (DNP) between 1999 and 2001. Egg samples were lyophilized and stored at 20 º C until analysis.

Extraction and clean-up

Extraction was carried out by matrix solid phase dispersion as previously described in detail elsewhere [9]. Lyophilized egg sample was homogenized with 1:1 (w/w) silica gel:anhydrous sodium sulfate powder. The mixture was ground to become a fine powder, loaded into a column 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 sulfuric acid (44%) and silica modified with KOH. The final fractionation between non-*ortho*- PCBs, PCDD/Fs, and the bulk of PCBs (where the chiral PCBs are obtained) was achieved using Supelclean^{TM} ENVI^{TM}-Carb SPE cartridges as described elsewhere [10].

Enantioseparation of chiral PCB by MDGC heart cutting technique

Gas chromatographic separation of the nine chiral PCBs in their atropisomers was carried out on a MDGC system equipped with two independently heatable ovens, ^{63}Ni-ECD detectors and split/splitless injectors (Varian Iberica). Column switching was achieved with a pneumatically controlled three-T-pieces valve (DEANS switching system) inside the first GC oven. The MDGC control software automatically performed the heart cutting. A DB-5 fused silica capillary column (30 m x 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 oven temperature program was: 80ºC (1min), 30ºC/min →185ºC (3min), 1.9ºC/min →234ºC (25min), 2ºC/min → 270ºC. Two chiral GC columns (30 m x 0.25 mm i.d., 0.25 μm film thickness) were used as main column in the second GC oven:

i) Permethyl 2,3,6-tri-*O*-methyl β –CD on a polysiloxane backbone (Chirasil-Dex; Chrompack): 100ºC (1min), 5ºC/min → 160ºC (30min), 1ºC/min → 170ºC (10min), 1ºC/min → 180ºC.
ii) 2,3 dimethyl-6-tert-butyldimethylsilylated- β–CD on 15 % phenyl methylpolysiloxane (SE-176, BGB) 100ºC(1min), 5ºC/min → 150ºC (30min), 0,5ºC/min → 160ºC (15min), 0,5ºC/min → 200ºC

Results and Discussions

The enantiomeric excess (ee) of the 9 chiral PCBs obtained in the samples studied revealed that PCB 91 (ee = 0.7-40 %), PCB 132 (ee = 9.5-73 %) and PCB 136 (28-91 %) exhibited an enantiomeric excess (ee) of the second eluted enantiomer in most of the cases. While PCB 84 (ee =5.2-77 %), PCB 95 (3.2-90 %), PCB 149 (6.3-30 %) and PCB 176 (0.63-100 %) showed an ee of the first eluted enantiomer in almost all the samples studied. In the case of PCB 135 (8.4-62 %) and PCB 174 (1.6-60 %), the relative abundance of the two atropisomers depends on the species (Table 1 and Figure 1). Some slight differences were found among the species studied, red kite species showed the highest enantioselective degradation of the chiral PCBs studied, being the enantiomeric enrichment of PCBs 95, 135 and 136 higher than 33 %. The enantiomeric enrichment

of the chiral PCBs in booted eagle was also quite high, but in a lower percentage than red kite, and PCB 174 was racemic or nearly racemic. Buzzard species showed the slightest enantioselective degradation, and only 136 exhibited enantiomer enrichment higher than 30 % in all studied exemplars (Figure 1).

Predatory birds look to be more able to enantioselective degrade chiral PCBs than top predatory animals living in aquatic ecosystems (dolphins and whales from the Mediterranean sea, sharks from the Atlantic Ocean and otters from DNP (Table1). Meanwhile PCB 95, 135, 136 and 176 showed a high enantioselective degradation in predatory birds (in some cases they have an ee of 100%), they are racemic or nearly racemic in the aquatic predatory species. PCB 132 and 174 showed enantioselective degradation in all species presented in Table 1. As it was already pointed out in similar works, the differences observed in the enantiomeric enrichment of the chiral PCBs investigated among species could not be explained by the relationship between structure and metabolism. All of them belong to the readily metabolizable PCBs (they have neighboring hydrogen atoms in both ortho/meta and meta/para, and, what it is more important, the chemical structure of both atropisomers are identical). Thus, the differences found in the metabolic degradation pathway between atropisomers of chiral PCBs and among species could be better explained by enantioselective character of the enzymatic biodegradation process than on the base of chiral PCB structure.

Table 1. Enantiomeric enrichment (ee, %) of chiral PCBs in top predatory animals from aquatic and terrestrial ecosystems.

PCBs	**Otter fat and liver [6] (DNP)**	**Shark liver [7] (SW Atlantic Ocean)**	**Cetaceans liver [8] (Mediterranean Sea)**	**Predatory birds egg (DNP)**
84	ND	--	32*	5.2-77
91	racemic	--	86*	0.7-40
95	racemic	racemic	almost racemic	3.2-90
132	5.3-72	6-14	1.0-44	9.5-73
135	0.09-100	--	almost racemic	4.8-62
136	racemic	--	almost racemic	28-91
149	racemic	racemic	4.7-31	6.3-30
174	14.2-85	--	6.2-21	1.6-60
176	4.2-100	--	almost racemic	0.63-100

* It was detected in only one sample

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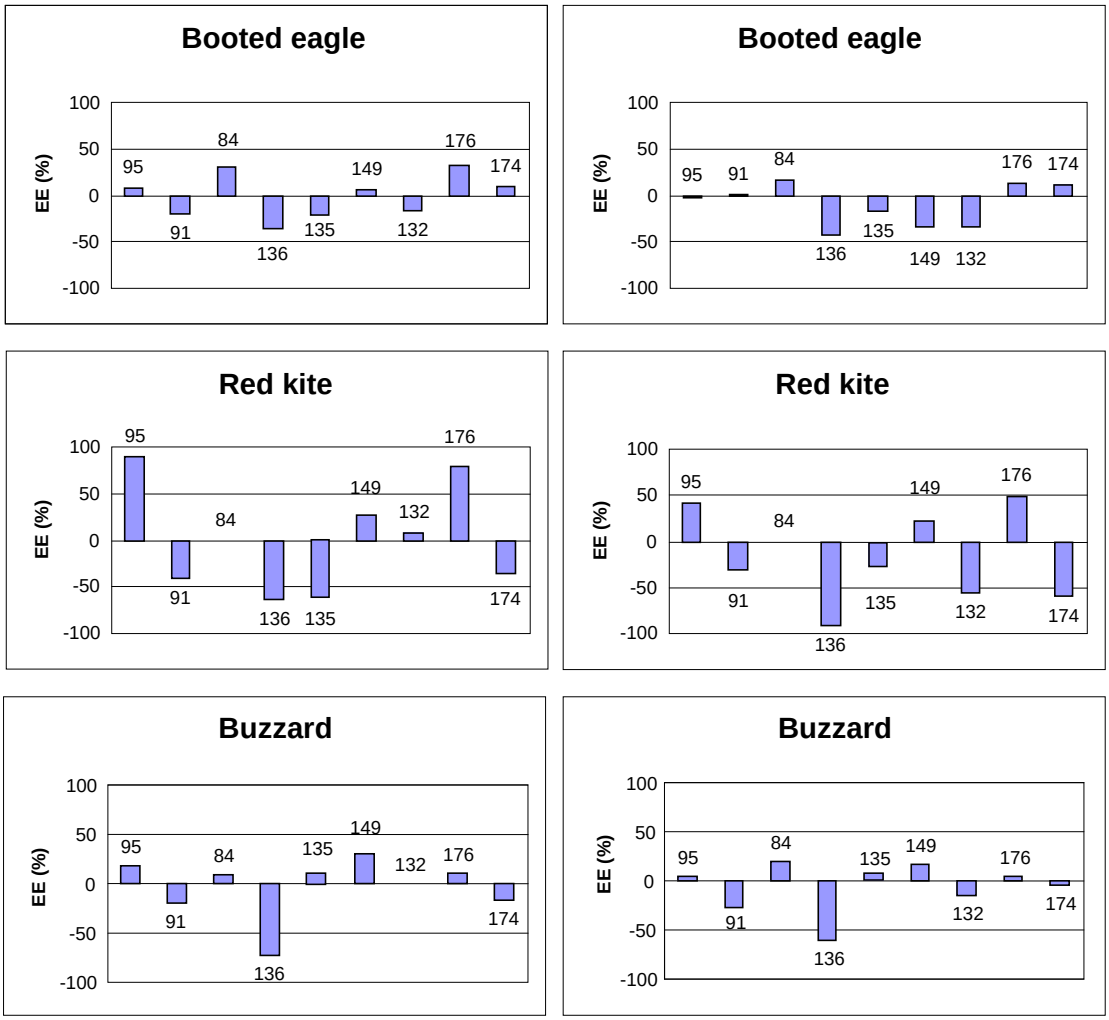

Figure 1: Enantiomer enrichment of chiral PCBs in individual egg samples of predatory birds especies. Ee was positive (+) when the first eluted enantiomer was predominant and negative (-) when the second eluted enantiomer was predominant.