ENANTIOMERIC RATIOS OF CHIRAL PCBs IN STRIPED DOLPHINS *(Stenella coeruleoalba)* FROM THE MEDITERRANEAN SEA

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INTRODUCTION

Polychlorinated biphenyl congeners (PCBs) are well-known environmental contaminants, which are spread world-wide, and are concentrated in living organisms, particularly in those that occupied the upper trophic levels in terrestrial and marine ecosystems [1, 2]. Chirality of biologically active compounds is of special importance due to the fact that most of these compounds are introduced into the environment as racemates and their uptake and metabolism by organisms may be enantiomer-selective [3]. The enantiomeric ratios (ER) of chiral PCBs in animals which are in the upper trophic chain levels may give additional information on possible degradation pathways. In addition, it has been shown that PCB 88, 139 and 197 (the racemic mixture and their respective enantiomers) have different levels of biological activity [4].

Thus, the analysis of chiral PCB congeners and the determination of their enantiomeric ratios in top predatory animal species is currently of interest in order to assess the risk of exposure to PCBs. The enantiomeric excees of PCB 149 in marine ecosystems was first studied in blue mussels (*Mytilus edulis L.)* from the German Bight [5]. In a previous paper [6] we reported the enantiomeric ratios of three chiral PCBs (PCB 95, 132 and 149) in shark liver samples (*C. coelolepis*) from the Atlantic Ocean. The investigations revealed a small enantiomeric bias of PCB 132 in most of the samples studied (ER = 0.75-0.89, ee = 6-14%), while PCB 95 and PCB 149 were present in racemic or nearly racemic form.

This paper reports results concerning the enantiomeric ratios of 9 atropisomeric PCBs (84, 91, 95, 132, 136, 149, 174 and 176), in liver and blubber of six striped dolphins, found stranded in 1989-1990 in the western coasts of Italy during the morbilli-virus infection that affected this cetaceans in the Mediterranean.

MATERIAL AND METHODS

The six dolphins (*Stenella coeruleoalba)* were found dead along the Italian coast (Lygurian and Tirrhenian seas) in the period 1989-1990. Collection and transport of the

carcasses was authorised and supervised by the Centre Studio Cetacei (Milan). Details of all animals studied are presented in Table 1.

Extraction and clean up were performed as previously described in detail [7]. Basically this consisted of low pressure chromatography on neutral and base-modified silica gel, activated carbon dispersed on glass fibres, silica gel impregnated with sulphuric acid, and Florisil. Three fractions were eluted from the carbon column for each sample. The first fraction contain the ortho-substituted PCBs (Fraction A), and the two last fractions containing the non-ortho-substituted PCBs and PCDD/Fs were kept for further analysis.

The ortho-substituted PCBs (Fraction A) were fractionated by HPLC on a Cosmosil-5-PYE column (2-(1-pyrenyl) ethyldimethylsilylated silica gel, particle size 5 µm, Nacalai Tesque (Kyoto, Japan) as previously described [8]. Six fractions (I to VI) were collected in which the PCB congeners were distributed according their structure and number of ortho chlorosubstituted. Fractions I to IV contain the chiral PCBs were used to determine their enantiomeric ratio. Fractions V and VI containing the mono and non-ortho chlorosubstituted PCBs were kept for further analysis.

Gas chromatographic separation of the 9 atropisomeric PCB was carried out with the Fissons HRGC/LRMS MD-800 system . Therefore the fractions I to IV were injected in a chirasil-Dex column (25 m × 0.25 mm i.d., 0.2 µm film thickness. The column temperature was held for 1 min at 90 °C, then programmed at 30 °C/min to 160 °C, held for 20 min, increased at 1 °C/min to 170 °C, held for 20 min, finally increased at 1 °C/min to 180 °C, and held for 80 min. Helium was used as the carrier gas (0.5 bar). Two characteristic ions (M and M+2) for each PCB homologue family were monitored using five different chromatographic windows. Identification of the PCBs was based on retention time information and the ion intensity ratio of samples peaks within 10% of the mean values obtained for the corresponding standards. The enantiomeric ratio was defined as the proportion of the peak area of the first to the second eluted atropisomer peak.

In order to confirm the observed enantiomeric ratios, multidimensional gas chromatographic analysis (MDGC) of the PCB fraction A was carried out on a Siemens Sichromat-2 MDGC (Karlsruhe, Germany) equipped with two independently heatable ovens, a pneumatically controlled six-port valve (Valco, Schenkon, Switzerland), an on-column injector, a flame ionisation detector (FID) and a ^{63}Ni-ECD [9].

For pre-separation, two achiral columns with different polarities were used: a DB-5 column (30 m × 0.22 mm i.d., 0.1 µm film thickness) and a OV-1701 column (25 m × 0.25 mm i.d., 0.25 µm film thickness). The DB-5 column was held for 3 min at 60 °C, then at 20 °C/min heated to 180 °C, held for 15 min and increased to 250 °C at 20 °C/min. The OV-1701 column was held at 60 °C for 3 min, then heated at 20 °C/min to 200 °C, held for 20 min and increased to 230 °C at 20 °C/min. For both columns hydrogen was used as the carrier gas (1 bar). Detection was carried out using an FID (250 °C).

The transfer of the respective PCB congener on the second-chiral- column was achieved by the six-port valve. Peak broadening was minimized by cooling the first part of the second column with air, precooled by liquid nitrogen. The cut fraction was separated on a Chirasil-Dex column (10m × 0.25 mm i.d., 0.25 µm film thickness). The temperature programme depended on the PCB congener analysed. The temperature was maintained at 100 °C until the transfer was made, then increased at 10 °C/min to 140 °C for PCB 95, 155 °C for PCB 132, 130 °C for PCB 135, 125 °C for PCB 136, 135 °C for PCB 149, 145 °C for PCB 174, and 130 °C for PCB 176. Hydrogen was used as carrier gas

(0.4 bar). Detection was carried out using a ^{63}Ni-ECD (250 °C, nitrogen gas used as make up).

RESULTS AND DISCUSSIONS

Table 2 shows the enantiomeric ratios (ER) and the enantiomeric excess (ee) of 9 chiral PCBs in 9 stripped dolphin samples determined by off-line HPLC (PYE)-HRGC/LRMS technique as well as the results obtained in two of them (S6 and S9) using MDGC/ECD as confirmatory technique.

The enantiomeric ratios (relation of the first eluted enantiomer to the second one) of the 9 chiral PCBs (84, 91, 95, 132, 135, 136, 149, 174 and 176) obtained in the samples studied revealed that PCBs 95 (ER= 0.71-1.07), PCB 136 (ER = 0.84-1.07) PCB 174 (ER= 0.58-1.16) and PCB 176 (ER= 0.72-0.97) were racemic or nearly racemic in almost all the investigated samples. PCB 95 revealed an enantiomeric excess (ee) of 17 % of the second eluted atropisomer in two liver samples (S1 and S7) and PCB 174 revealed an ee of 26.6 % in liver sample S7. PCB 132 (ER 0.55-0.92), PCB135 (0.63-0.76); and PCB 149 (ER 0.58-0.91) revealed and ee of the second eluted enantiomer in almost all the studied sample. No ee was found for PCB 132 in sample S7 and for PCB 149 in sample S6.

The differences observed in the enantiomeric rates of the chiral PCBs investigated could not be explained by the relationship between structure and metabolism. All of them (PCB 95, 132, 135, 136, 149, 174 and 176) belong to the readily metabolizable PCBs. They have neighbouring hydrogen atoms in both ortho/meta and meta/para positions (PCB-132), in two meta/para positions (PCB 95, 136) or in one meta/para position (PCB 135, 149, 174 and 176). It is, therefore, not possible, on the basis of its structure, to explain why PCB 95 (with two neighbouring H atoms in meta/para positions) shows only slight enantiomeric enrichment, while PCB 149 (with only one free meta/para position) exhibited higher enantiomeric enrichment. Thus, the differences found in the metabolic degradation pathway between the two atropisomers of these chiral PCBs could be better explained by the enantioselective character of the enzymatic biodegradation processes [3].

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Table 1. Details of stranded *Stenella coeruleoalba* studied.

Code	Sex	Length (cm)	Weigth (kg)	Sea	Year	Liver	Adipose
dolphin A	M	200	--	N. Tyrrhenian	1989	S1	S2
dolphin B	F	201	88	Ligurian	1990	S3	S4
dolphin C	M	174	48	Ligurian	1990	S5	S6
dolphin D	M	148	--	N. Tyrrhenian	1990	S7	
dolphin E	M	215	--	N. Tyrrhenian	1990	S8	
dolphin F	F	185	--	N. Tyrrhenian	1990	S9	

Table 4. Enantiomeric ratios (area first peak/area second peak) and percentage of enantimeric enrichment (in parenthesis) in liver and blubber of six striped dolphins (S. Coeruleoalba) from the Mediterranean sea determined by HPLC-HRGC/LRMS (SIM) and MDGC/ECD

Sample	Technique	PCB 84	PCB 91	PCB 95	PCB 132	PCB 135	PCB 136	PCB 149	PCB 174	PCB 176
S1 (liver)	HPLC-HRGC	N.D.	N.D.	0.71	0.81 (10.5)	0.63 (22.7)	1.05	0.8 (11.1)	0.92	0.85
S2 (blubber)	HPLC-HRGC	N.D.	N.D.	N.Q.	N.Q.	N.D.	N.D.	0.76 (13.6)	N.Q.	N.D.
S3 (liver)	HPLC-HRGC	N.D.	N.D.	0.85	0.55 (29)	0.73 (15.6)	0.89	0.58 (26.6)	0.77	0.92
S4 (blubber)	HPLC-HRGC	N.D.	N.D.	N.D.	N.D.	N.Q.	N.Q.	0.66(20.5)	N.Q.	N.D.
S5 (liver)	HPLC-HRGC	N.Q.	N.D.	1.01	0.65 (21.2)	0.67 (19.8)	1.04	0.88 (6.4)	1.00	0.72
S6 (blubber)	HPLC-HRGC	N.Q.	N.Q.	1.07	0.65 (21.2)	0.76(13.6)	1.07	0.91 (4.7)	1.16	0.74
	MDGC			1.02	0.76	0.79	1.17	0.93	1.16	1.04
S7 (liver)	HPLC-HRGC	N.D.	N.D.	0.71	0.92 (4.2)	0.71(17)	0.96	0.63 (22.7)	0.58	N.D.
S8 (liver)	HPLC-HRGC	N.D.	N.D.	N.D.	N.Q.	N.D.	1.01	0.68 (19)	N.Q.	N.D.
S9 (liver)	HPLC-HRGC	N.D.	N.Q.	0.84	0.67 (19.8)	0.64(22)	0.84	0.67 (19.8)	0.81	0.78
	MDGC			0.82	0.79	0.78	0.87	0.81	0.87	1.06
Standard 1	HPLC-HRGC	0.97	0.94	1.09	0.89 (5.8)	0.99 (0.5)	0.95	1.04 (2)	0.92	0.87
Standard 2	MDGC			0.97	0.98	1.01	1.00	0.99	1.09	0.96

N.D. = S/N < 3 ; N.Q. = S/N < 5; [1] The enantiomeric excess percentage values is only given for the non racemic PCBs