ENANTIOMERIC RATIOS OF CHIRAL POLYCHLORINATED BIPHENYLS IN STRANDED CETACEANS FROM THE MEDITERRANEAN SEA.

Olga JIMENEZ[1], Begoña JIMENEZ [2], Leticia MARSILI [3], and Maria José GONZÁLEZ[2*]

[1] Depart. of Analytical Chemistry, Faculty of Sciences, University of Alcalá, 28871 Alcalá de Henares (Madrid), Spain
[2] Depart. of I.A. and Environ. Chem. Institute of Organic Chemistry (C.S.I.C.), Juan de la Cierva 3, E-28006 Madrid, Spain
[3] Depart. of Environ. Biology; University of Sienna ; Via delle Cerchia 3, I-53100 Siena, Italy

INTRODUCTION

PCBs are usually present in environmental samples as complex mixtures of about 135 PCB congeners [1, 2]. Not all the congeners have the same importance. Some of them (PCBs 28, 52, 101, 118, 138, 153 and 180 numbered according to Ballsmitter and Zell (1980), are used for biomonitoring studies due to their high concentration in technical mixtures and their recalcitrance. In recent years, attention has been focused on the toxicity of PCBs, specially on those congeners showing similar toxicity to the polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo furans (PCDFs).

Recently, attention has been focused on PCBs displaying axial chirality in their non-planar conformations. 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, 4]. The enantiomeric ratios (ratio of the first eluted enantiomer to the second) 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 level of biological activity [5].

In previous papers (6, 7, 8) we reported the enantiomeric ratios of some chiral PCBs in sharks (*C. coelolepis*) and groupers (*E. marginatus*) from the Northwestern Atlantic Ocean and dolphins (*Stenella coeruleoalba)* from the Mediterranean sea. This paper reports results concerning the enantiomeric ratios of 9 chiral PCBs (84, 91, 95, 132, 136, 149, 174 and 176) in cetacean liver samples found stranded in the western coasts of Italy since 1987 until 1992.

MATERIAL AND METHODS

Sampling

Liver samples were obtained from four species of cetaceans, *Tursiops truncatus, Balaenoptera physalus, Grampus griseus and Globicephala melaena.* All animals studied were found dead along the Italian coasts at the Tirrenian, Ligurian and Adriatic Seas in the period of 1987-1992 (Table 1). Collection and transport of the carcasses was authorised and supervised by the Centro Studi

Cetacei (Milan). Liver samples were frozen and stored at -20° C until their use. Samples for residue analysis were lyophilised in an Edwards freeze drier, and 0.5 g of sample was used for analysis.

Analytical determination

Extraction and clean up were performed as previously described in detail [9]. Basically this consisted of low-pressure chromatography on neutral and base-modified silica gel, activated carbon dispersed on glass fibers. Three fractions were eluted from the carbon column for each sample. These contained ortho-substituted PCBs, non-ortho-substituted PCBs and PCDD/Fs respectively. The first fraction containing the chiral PCBs was fractionated in a further step by HPLC on a Cosmosil-5-PYE (2-(1-pyrenyl) ethyldimethylsilylated silica gel) column, Nacalai Tesque (Kyoto, Japan) as previously described (10)

Determination of the enantiomeric ratios of atropisomeric PCBs by HRGC/ITMS (Ion Trap)

Gas chromatographic separation of the 9 atropisomeric PCB congeners in their atropisomers was carried out with the HRGC/ITMS Thermo Quest Finningan, Mod GCQ Plus system using 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 traces (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 sample peaks within 10% of the mean values obtained for the corresponding standards. The enantiomeric ratios was defined as the proportion of the peak area of the first to the second eluted atropisomer peak. The enantiomeric ratios was measured using the two characteristic ion traces (M and M+2 or M+4) obtained from each homologue family of PCBs and chromatographic window. The absolute detection limits were between 0.81 and 7.8 pg from tetra- to hepta- PCBs.

RESULTS AND DISCUSSIONS

Table 2 shows the enantiomeric ratios (ER) and the enantiomeric excess (ee) of 9 chiral PCBs found in the four species of cetaceans, *Tursiops truncatus, Balaenoptera physalus, Grampus griseus and Globicephala melaena*. PCB 84, 91 and 135 were under the detection threshold of the LRMS technique, except for bottlenose dolphin, L4 (PCB 84) and bottlenose dolphin, L5 (PCB 91 and PCB 135).
The enantiomeric ratios of the 9 chiral PCBs (84, 91, 95, 132, 135, 136, 149, 174 and 176) obtained in the samples studied (Table 2) revealed that PCB 174 was racemic or nearly racemic in almost all the investigated samples. PCB 174 revealed an enantiomeric excess of the second eluted atropisomer higher than 10 % in three liver samples, L1, L3 and L5. PCB 95 (ER = 0.86-1.01), PCB 132 (ER 0.54-0.96), PCB 135 PCB 149 (ER 0.72-0.96), and PCB 176 (ER = 0.83-0.91) revealed an ee of the second

eluted enantiomer in almost all the studied samples. PCB 84 (ER = ND-0.68), PCB 91 (ER = ND-0.13) and PCB 135 (ER = ND – 0.89), showed an ee only in one of the studied samples.
The differences observed in the enantiomeric ratios of the chiral PCBs investigated could not be explained by the relationship between structure and metabolism. All of them (PCB 84, 91, 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 84, 91, 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 structure, to explain why PCB 95 and 136 (with two neighbouring H atoms in meta/para positions) show slighter enantiomeric enrichment, than PCB 149 (with only one free meta/para position). Similar results were obtained in a previous paper (6), where we reported the enantiomeric ratios of these 9 chiral PCBs in the liver samples from dolphins (*Stenella coeruloalba)* from the Mediterranean sea. These results revealed an enantiomeric excess of PCB 95, 132, 135, 149 and 176 while PCB 136 and 174 were racemic or nearly racemic. 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].

REFERENCES

1.Larsen, B., BØwadt, S., Fachetti, S. *Int. J. Environ. Anl. Chem.* **47**, 147 (1992)
2.Ballschmiter, K., Zell, M. *Fres. Z. Anal. Chem.* **302,** 20 (1980)
3.Faller, K., Kühnerfuss, H., König, W. A., Krebber, R., Ludwig, P., *Environ. Sci. Technol.* **25**, 676 (1991)
4. Buser, H. R., Müller, M. D., Rappe, C. *Environ. Sci. Technol*. **26,** 1533 **(**1992)
5. Blanch, G. P.; Serrano, R.; Glausch, A.; Schurig, V.; Gonzalez, M. J. *J. High Resol. Chromatogr*. **19**, 392 **(1996)**
6. Reich, S., Jiménez, B., Marsili, L., Hernandez, L.M., Schurig, V., González, M.J. Environ. Sci & Technol. In press.
7. Serrano, R., Fernández, M., Rabanal, R., Hernández, L.M., González, M.J. Arch. Environ. Contam. & Toxicol. In press.
8. Rodman, L. E., Shedlofsky, S. I., Mannschreck, A., Püttmann, M., Swim, A. T., Roberson, L. W., *Biochem. Pharmacol.* **41,** 915 (1991)
9. Jiménez B., Wright C., Kelly M., Startin J. *Chemosphere* **32,** 461(1966)
10. Ramos, L., Hernández, L.M., González M.J. *Anal Chem* **71***,* 70 (1999)

Table 1. Species and characteristics of cetaceans studied

Species	Common name	Code	Lenght (cm)	Sea	Year	Sex
Grampus griseus A	Risso´s dolphin	L1	290	N.Tyrrhenian	1987	Male
Grampus griseus B	"	L2	294	Liguria	1992	Male
Tursiops truncatus C	Bottlenose dophin	L3	298	N.Tyrrhenian	1992	Male
Tursiops truncatus D	"	L4	290	N. Adriatic	1992	Female
Tursiops truncatus E	"	L5	--	N.Adriatic	1992	Male
Tursiops truncatus F	"	L6	--	--	--	--
Tursiops truncatus G	"	L7	--	N.Adriatic	1992	Male
Globicephala melaena H	Long-finned pilot whale	L8	344	Liguria	1990	Male
Balaenoptera physalus I	Fin whale	L9	19200	N. Tyrrenian	1990	Female

Table 2: Enantiomeric ratios (area of first peak/area second peak) and percentage of enantiomeric enrichment (in parenthesis) in liver of cetaceans from the Mediterranean sea determined by off line HPLC-HPGC/LRMS (IT-SIM)

Sample	PCB 84	PCB 91	PCB 95	PCB 132	PCB135	PCB 136	PCB 149	PCB 174	PCB 176
L1	ND	ND	--	0.96 (1.0)	ND	0.83 (21.7)	0.75 (27.9)	0.82 (15.5)	0.89 (13.6)
L2	ND	ND	--	0.80 (17.5)	ND	0.88 (17.0)	0.72 (30.8)	0.89 (8.2)	0.91 811.79
L3	ND	ND	0.86 (16.5)	--	ND	109 (2.8)	--	0.77 (20.6)	--
L4	0.68 (32)	ND	0.86 (16.5)	--	ND	0.97 (8.5)	0.85 (18.3)	1.06 (9.3)	ND
L5	ND	0.13 (86.3)	0.91 (11.7)	--	0.89 (16.0)	1.10 (3.8)	0.96 (7.7)	1.13 (16.59)	0.89 (13.6)
L6	ND	ND	ND	ND	ND	ND	0.75 (27.9)	ND	ND
L7	ND	ND	0.87 (15.5)	0.54 (44.3)	ND	0.95 (10.4)	0.92 (11.5)	0.91 (6.2)	0.83 (19.4)
L8	ND	ND	1.01 (1.9)	--	ND	--	0.81 (22.1)	0.89 (8.2)	ND
L9	ND	ND	0.86 (16.5)	ND	ND	0.96 (9.4)	0.79 (24.0)	0.99 82.1)	0.87 (15.5)
Standard	1.00 (0.06)	0.95 (0.05)	1.03 (0.02)	0.97 (0.03)	1.06 (0.17)	1.06 (0.13)	1.04 (0.08)	0.97 (0.03)	1.03 (0.13)