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Congener Specific Determination and Enantiomeric Ratios of Chiral Polychlorinated Biphenyls in Striped Dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea

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Blubber and liver samples from six striped dolphins (*Stenella coeruleoalba*) found dead in the Mediterranean sea in 1989−1990 were tested for 37 coplanar and chiral polychlorinated biphenyls (PCBs), including the enantiomeric ratios of 9 chiral PCBs. The method includes a fractionation step using HPLC (PYE column) for separating the PCBs according to the number of chlorine atoms in the ortho positions. HRGC/ECD and HRGC/LRMS with an achiral column (DB-5) were used to determine the PCB congeners. The enantiomeric ratios of nine chiral PCBs were determined by HRGC/LRMS (SIM) with a chiral column (Chirasil-Dex) and by MDGC as the confirmatory technique. The total PCB concentration (sum of 37 congeners) ranged from 7.2 to 89.6 μg/g (wet weight) and from 0.52 to 29.2 μg/g (wet weight) for blubber and liver samples, respectively. PCB profiles were dominated by congeners 138, 153, 170, and 180. The toxic equivalent values (TEQ) ranged from 0.17 to 3.93 ng/g (wet weight) and from 0.02 to 0.73 ng/g (wet weight) for blubber and liver samples, respectively. PCBs 95, 132, 135, 149, and 176 revealed an enantiomeric excess of the second eluted enantiomer in almost all of the samples, whereas PCBs 136 and 174 were racemic or almost racemic. PCBs 88 and 91 were under the detection limits of the methodology used.

Introduction

Polychlorinated biphenyl congeners (PCBs) are well-known worldwide environmental contaminants, which concentrate in living organisms, particularly in those occupying the upper trophic levels in both terrestrial and marine ecosystems (*1, 2*). In the case of marine mammals, PCBs are implicated in the decline of populations of top level predators in the marine food chain (*3*−*5*). Moreover, it has been shown that cetaceans are excellent accumulators of persistent environmental pollutants but have a poor detoxifying potential, making them very sensitive to toxic effects (*6*).

Levels of PCBs in marine mammals have been measured over almost 20 years, but values tend to be in terms of total PCBs referred to different technical PCB mixtures. The past few years, attention has been focused on a particular range of PCBs, particularly those congeners showing toxicity similar to that of the polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

Recently, attention has been focused on PCBs displaying axial chirality in their nonplanar 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 (*7, 8*). The enantiomeric ratio of chiral PCBs in animals that are in the upper trophic chain levels may give additional information on possible degradation pathways. In addition, it has been shown that PCBs 88, 139, and 197 (the racemic mixture and their respective enantiomers) have different levels of biological activity (*9*).

Thus, the determination of chiral PCB congeners' enantiomeric ratios in top predatory animal species is currently of interest to assess the risk of exposure to PCBs. There is little information about the degradation of PCB atropisomers in marine biota, probably because of the analytical difficulties in real samples of separating both atropisomers without interference. The enantiomeric enrichment of PCB 149 in marine ecosystems was first studied in blue mussels (*Mytilus edulis* L.) from the German Bight (*10*). In a previous paper (*11*) we reported the enantiomeric ratios of three chiral PCBs (PCBs 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%), whereas PCBs 95 and 149 were present in racemic or nearly racemic form.

Several studies have related mortality levels and various diseases among Mediterranean cetaceans with high detected levels of xenobiotic compounds, including PCBs (*3, 4, 12, 13*). PCBs have also recently been associated with a morbillivirus infection suffered by striped dolphin (*Stenella coeruleoalba*) in the western Mediterranean (*14*). Besides immune suppression, PCBs also induce a variety of physiological dysfunctions and pathologies that impair the reproductive capacity of marine mammals (*1, 5*). Moreover, it is well-known that some species of cetaceans are more vulnerable to lipophilic xenobiotics than terrestrial mammals (*15, 16*).

This paper reports results concerning the concentrations of 37 individual PCB congeners (18 coplanars and 19 chirals) in liver and blubber of six striped dolphins, found stranded in 1989−1990 on the western coasts of Italy during the morbillivirus infection that affected cetaceans in the Mediterranean. This is the first time that off-line HPLC (PYE)−HRGC methodology has been used (as an alternative technique to MDGC) for the unambiguous analytical determination of coplanar and chiral PCBs, including the enantiomeric ratio calculations of nine chiral PCBs in marine mammal samples.

Materials and Methods

Sampling. The six dolphins (*S. coeruleoalba*) were found dead along the Italian coast (Figure 1) in the period 1989−1990. Collection and transport of the carcasses were authorized

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FIGURE 1. Geographical location in which the striped dolphins studied were found.

TABLE 1. Details of Stranded *S. coeruleoalba* Studied

code	sex	length (cm)	weight (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	

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

Samples were frozen and stored at −20 °C until analysis. About 20 g of tissue was lyophilized in an Edwards freeze-drier. To calculate water content, a sample of ~5 g was placed in an oven at 110 °C for 24 h. The rest was kept in the freezer until analysis.

Extraction and Cleanup. Extraction and the first steps of cleanup followed a semiatumatic procedure (*17*) based on the protocol of Krokos et al. (*18*), which was an adaptation of that of Smith et al. (*19*). Basically this consisted of low-pressure chromatography on neutral and base-modified silica gel coupled to activated carbon dispersed on glass fibers. Three fractions were eluted from the carbon column for each sample. Each of them was further cleaned up using silica gel impregnated with sulfuric acid and Florisil. The three fractions contained the three non-ortho (PCBs 77, 126, and 169) PCBs (fraction A), the bulk of PCBs and lipids (fraction B), and the PCDD/Fs (fraction C), respectively. Fraction C was kept for further analysis. The lipid content in fraction B was determined gravimetrically. Prior to the initial extraction of samples, a mixture containing 10 ng of ^{13}C isotopically labeled PCBs 77, 126, and 169 as internal standard (99% of purity, Cambridge Isotope Laboratories, Cambridge, MA), was added. Samples between 0.2 and 1 g of tissue were used for analysis. One blank was carried out for every three samples.

Fractionation of Bulk of PCBs by HPLC. In a further step, HPLC on a Cosmosil-5-PYE [2-(1-pyrenyl)ethyldimethylsilylated silica gel] column, particle size = 5 μm, Nacalai Tesque (Kyoto, Japan), was used to obtain five subfractions from the bulk of PCBs (fraction B), as previously described in detail (*20*, *21*). Five fractions (I−V) were collected in which the PCB congeners were distributed according to their structure and number of ortho chlorosubstitutions. Hexane was used as mobile phase at a flow of 0.5 mL/min.

Identification and Quantification of Ortho-Substituted PCBs by HRGC/ECD. The HPLC fractions were evaporated until dry and dissolved in 40 μL of hexane containing PCB 12 as an internal surrogate standard at a concentration of 1 ng/μL. A 0.6 μL aliquot was injected in the splitless sampling mode at 240 °C in a Varian 3400 CX gas chromatograph, equipped with a Varian 8200 autosampler, a ^{63}Ni electron capture detector (^{63}Ni ECD), and the Varian Star 4.5 program. The PCB congeners were separated on a BPX5 column (60 m × 0.22 mm i.d., 0.25 μm film thickness; SGE). 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 1.9 °C/min to 234 °C, held for 65.5 min, and finally increased at 2 °C/min to 270 °C. Nitrogen was used as the carrier gas.

Identification of the individual PCB congeners was based on retention time information. For quantification purposes, calibration curves of 37 PCB congeners with a correlation coefficient of at least 0.92 were obtained. The absolute detection limit was between 0.2 and 10 pg/μL from tetra- to octachlorinated biphenyls.

Identification and Quantification of Non-Ortho-Substituted PCBs by HRGC/LRMS. Gas chromatographic separation of the three non-ortho-substituted PCB congeners (PCBs 77, 126, and 169), obtained in fraction A, was performed using a Fisons GC 8000 high-resolution gas chromatograph (Manchester, England) coupled to a low-resolution mass spectrometer (Fisons MD 800) and equipped with the Fisons Masslab program. Fraction A was evaporated until dry and diluted with 10 μL of hexane containing 10 ng of $^{13}C_{12}$ isotopically labeled PCBs 101 and 153 as recovery standards. A 2 μL aliquot was injected in the splitless sampling mode at 250 °C in a DB-5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA). The column temperature was held at 100 °C for 1 min, then programmed at 30 °C/min to 130 °C, held for 1 min, increased at 2.5 °C/min to 190 °C, held for 1.2 min, finally increased at 1.2 °C/min to 250 °C, and maintained for 20 min. Helium was used as the carrier gas (0.5 bar).

The eluate was transferred to a quadrupole mass spectrometer with electron impact ionization and subsequent ion detection. The interface temperature was 250 °C, the source temperature 200 °C, and the electron energy 70 eV. Two characteristic ions of each PCB homologue and the respective labeled internal quantification standards were monitored for each analysis.

The absolute detection limits were between 9.3 and 11 pg/μL for tetra- to octa-PCBs, and recoveries were between 95 and 110%. Quality control criteria were defined as (1) simultaneous detection of a peak for both ions by monitoring within the expected retention time window for each congener, (2) ion intensity ratio of sample peaks within 10% of the mean values for calibration standards, and (3) blank samples without chromatographic interferences.

Determination of the Enantiomeric Ratio of Atropisomeric PCBs by HRGC/LRMS. Gas chromatographic separation of the nine atropisomeric PCB congeners in their atropisomers was carried out with the Fisons HRGC/LRMS system described above. Thus, fractions I−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 ion 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 ratio was defined as the proportion of the peak area of the first to the second eluted atropisomer peak. The enantiomeric ratio was measured using the two characteristics ion traces (M and M + 2 or M + 4) obtained from each homologue family of PCBs and chromatographic window.

Confirmation of the Enantiomeric Ratio of Atropisomeric PCBs by MDGC/ECD. To confirm the observed

TABLE 2. Concentration Levels of PCB Congeners (Nanograms per Gram of Wet Weight) in Blubber and Liver of Striped Dolphins (*S. coeruleoalba*) from the Mediterranean Sea[a]

	dolphin A		dolphin B		dolphin C		dolphin D	dolphin E	dolphin F		
PCB	liver (S1)	blubber (S2)	liver (S3)	blubber (S4)	liver (S5)	blubber (S6)	liver (S7)	liver (S8)	liver (S9)	mean ± SD, liver	mean ± SD, blubber
95	17.66	27.89	3.92	30.93	135.02	681.4	7.82	3.75	74.09	40.38 ± 53.56	246.7 ± 376.4
88	0.58	ND	ND	ND	2.51	26.27	ND	ND	1.62	0.78 ± 1.05	8.76 ± 15.17
91	4.63	7.48	0.92	8.14	30.69	261.4	4.99	1.06	17.60	9.98 ± 11.8	92.4 ± 146
84+101	123.7	282.6	20.80	183.9	311.3	1123	41.34	27.54	224.3	124.8 ± 119.9	476 ± 420
136	20.53	70.85	5.71	34.25	146.7	793.1	7.78	9.79	87.41	46.33 ± 58.16	63.2 ± 25.6
110	21.92	19.83	30.28	29.58	18.36	229.3	5.84	8.46	17.45	8.95 ± 19.8	93 ± 118
77	0.05	0.42	0.08	0.62	0.71	1.06	NQ	0.15	0.10	0.22 ± 0.28	0.70 ± 0.0.33
135	30.54	27.53	0.51	10.59	253.9	1112	2.17	2.23	139.4	61.48 ± (104.1)	390 ± 625
144	16.52	62.37	4.40	18.67	119.02	593.8	4.17	8.58	81.07	38.96 ± 49.02	225 ± 320
149	260.7	337.8	16.99	360.1	1002	2145	68.84	38.72	454.0	306.9 ± 379.4	949 ± 1035
139	13.16	36.55	3.97	183.7	80.78	627.1	41.77	5.82	36.24	23.32 ± 30.98	300 ± 337
123	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
118	100.1	285.7	25.53	366.4	1015	4126	56.11	67.54	814.3	346.7 ± 445.4	1594 ± 2193
131	14.53	164.1	4.41	77.79	209.5	1632	4.36	4.15	221.3	76.38 ± 107.8	624.6 ± 873.4
114	3.60	10.32	1.02	7.04	11.30	45.79	2.03	2.23	12.54	5.50 ± 5.06	21.05 ± 21.5
153	509.8	815.7	45.90	1035	1970	6176	147.9	129.7	1012	636 ± 745.7	2675 ± 3033
132	51.86	72.74	2.23	64.23	582.4	2496	6.21	7.98	271.4	154.2 ± 233.7	877.6 ± 1401
105	37.41	121.1	9.36	6.98	283.3	2604	24.10	21.79	215.6	98.61 ± 119.16	910.7 ± 1467
176	10.16	ND	ND	18.89	116.9	506.5	0.921	ND	61.08	42.27 ± 54.43	262.7 ± 344.8
138	590.9	1282	80.70	505.8	5236	5076	107.60	258.9	2551	1471 ± 2067	2287 ± 2445
178	58.80	18.47	1.48	96.91	450.7	1806	6.86	6.15	266.9	131.8 ± 186.4	640.4 ± 1010
129	ND	ND	ND	1.753	ND	1.647	ND	ND	ND	ND	1.70 ± 0.07
175	13.18	4.72	ND	18.44	88.10	300.3	1.44	1.27	53.04	31.41 ± 38.15	107.8 ±166.8
126	0.13	NQ	NQ	0.20	0.17	0.38	NQ	NQ	NQ	0.15 ± 0.03	0.20 ± 0.002
183	138.7	141.8	8.21	234.2	1125	2849	18.43	26.37	631.8	324.8 ± 458.8	1074 ± 1536
167	15.67	ND	3.44	17.27	124.1	720	ND	3.27	79.5	45.21 ± 54.27	368.5 ± 496.8
174	81.36	91.49	1.84	97.54	790.9	3075	14.37	16.05	347.9	208.8 ± 313.8	1088 ± 1720
171	42.50	21.32	0.67	67.02	381.5	1753	5.01	2.75	182.3	102.5 ± 153.4	613.9 ± 987.0
156	34.41	42.62	8.55	134.1	302.9	1480	2.94	20.44	178.7	91.33 ± 122.7	552.4 ± 805
157	10.71	N.D.	N.D.	25.57	112.4	519	ND	2.55	69.8	48.89 ± 51.89	272.3 ± 348.8
197	5.71	N.D	N.D.	18.76	39.02	122.3	ND	ND	25.06	2.27 ± 16.73	70.55 ± 73.23
180	1023	2129	137.1	3734	7760	18879	149.4	396.9	5108	2429 ± 3229	8247 ± 9242
169	NQ	NQ	NQ	0.17	0.21	0.19	NQ	NQ	0.05	0.13 ± 0.11	0.18 ± 0.01
170	340.8	825.8	53.84	1359	4440	20016	136.7	156.8	2238	1227 ± 1780	7400 ± 10928
196	107.5	66.15	10.98	271.3	609	2133	19.52	18.9	387.5	192.4 ± 249.7	823.7 ± 1139
194	163.2	263.8	33.62	859.5	1470	5647	59.37	55.37	796.1	429.7 ± 587.0	2300 ± 2921
ΣPCBs	3865	7230	516.5	10009	29223	89558	909	1305	16658	8746 ± 11749	35599 ± 47750
Σ TEQs	0.08	0.17	0.02	0.32	0.73	3.93	0.03	0.04	0.51	0.235 ± 0.30	1.47 ± 2.13
% lipids	16	41.5	12.5	48.5	17	36	18.7	12.1	14	15 ± 2.6	42.0 ± 6.2
Σ TEQs[b]	0.50	0.41	0.16	0.66	4.29	10.9	0.16	0.33	3.64	1.51 ± 1.91	3.99 ± 5.98

[a] ND, nondetected (signal/noise > 3); NQ, nonquantified (S/N > 5). [b] Nanograms per gram of fat weight.

enantiomeric ratios, multidimensional gas chromatographic analysis (MDGC) of the bulk of PCBs (fraction B) 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 ionization detector (FID), and a ^{63}Ni-ECD.

For preseparation, 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 an 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 heated at 20 °C/min 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. 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 (10 m × 0.25 mm i.d., 0.25 μm film thickness). The temperature program depended on the PCB congener analyzed. The temperature was maintained at 100 °C until the transfer was made and then increased at 10 °C/min to 140 °C for PCB 95, to 155 °C for PCB 132, to 130 °C for PCB 135, to 125 °C for PCB 136, to 135 °C for PCB 149, to 145 °C for PCB 174, and to 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 Discussion

Total PCBs. Total PCB concentrations in dolphin (liver and blubber) samples, showing all congeners analyzed together, are presented in Table 2. As can be seen from the data, the greatest PCB concentration was found in blubber. Concentrations were in the 7.2−89.6 μg/g range (wet weight), with a mean of 35.6 μg/g ± 47.7 [standard deviation (SD)]. Concentration in liver was in the range 0.52−29.2 μg/g (wet weight) with a mean of 8.74 μg/g ± 11.7 (SD). Thus, the total PCB concentration found in blubber was ∼4 times higher than that found in liver tissue. These results agree with other studies conducted in different marine mammals tissues (*22*). PCB concentrations were lower but comparable to those observed for a healthy dolphin population on the Catalonian coast (Mediterranean sea) in 1990, with mean values of 314 μg/g (fat basis) in blubber (*23*). However, they were much

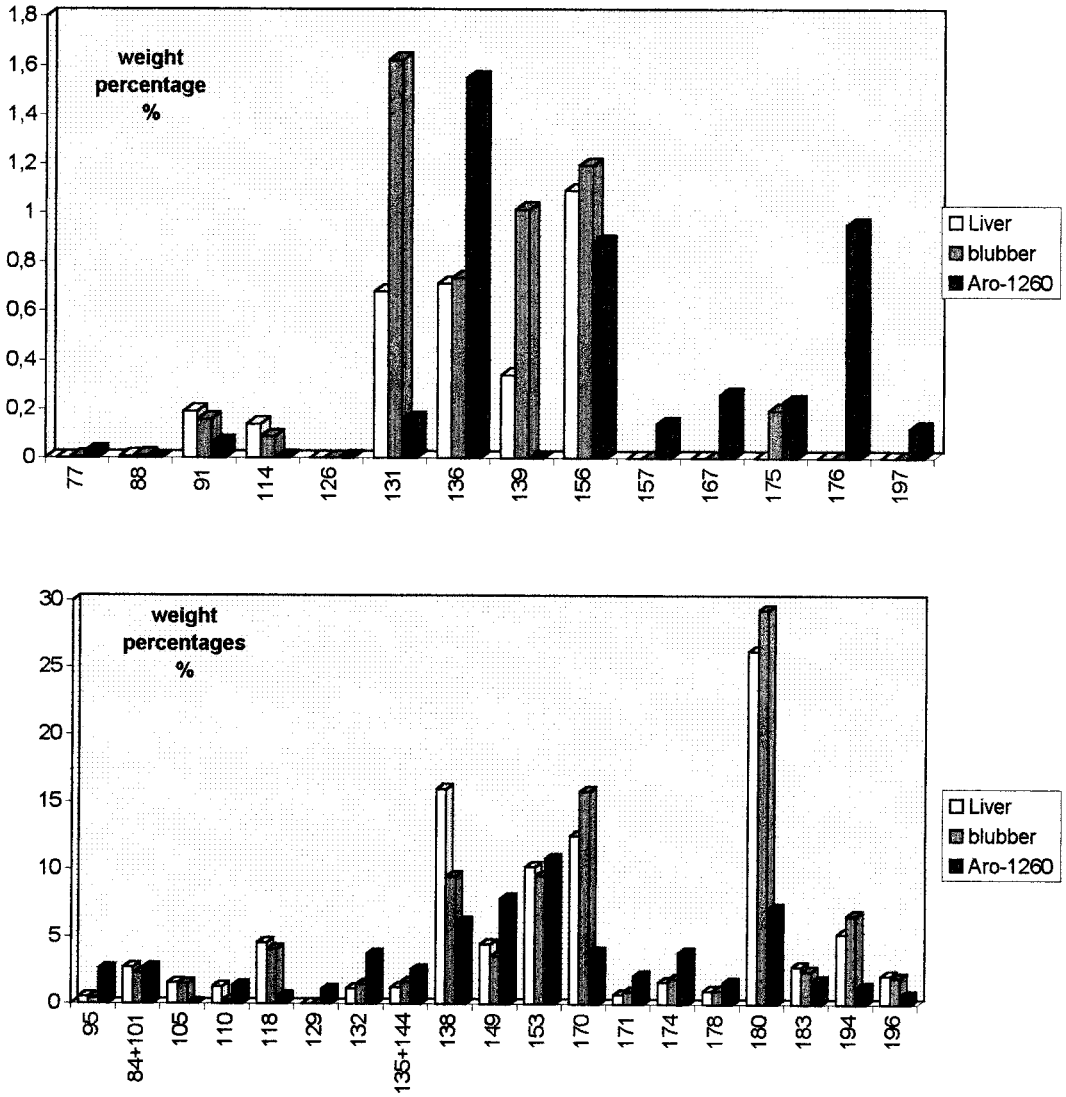

FIGURE 2. Comparison of the percentages of each PCB referred to the total found in the liver and blubber samples of the dolphins investigated with those found in Aroclor 1260.

lower than those noticed for striped dolphins, affected by epizoosis, also collected on the Catalonian coast in 1990, with mean values of 846 μg/g (fat basis) in blubber (*14*, *24*). The presence of elevated concentrations of PCBs in the corpses of diseased marine mammals washed up on coasts at various places around the world has implicated these pollutants as a cause of immune suppression (*25*−*27*). It has been suggested that a concentration of > 50 μg/g of wet weight basis of total PCB in blubber may present a health risk to cetaceans (*28*). One of the dolphins in this study (89.5 μg/g of wet weight) can be categorized as "at risk" on the basis of the total PCB levels found and the others as close to "at risk".

Concentration values of total PCBs found in the blubber of dolphins from different parts of the Mediterranean, such as the Ligurian and N. Tyrrenian Seas (*13*), the coast of Catalonia (*23*), and the French coast (*12*), are similar to our values. They are consistently higher than those found in the open sea, such as dolphins from Japan (*29*), the western Pacific (*30*), the South Atlantic (*31*), Wales (*23*), the East Pacific (*32*), and the United States (Atlantic) (*33*), for which values ranged from 6 to 59 μg/g (fat weight) in blubber tissues.

Relative Quantities of Non-Ortho-Substituted Coplanar PCBs. A study of the relative quantities of each of the non-ortho-substituted PCB congeners revealed PCB 77 to be the major congener in all of the samples analyzed, except for sample 1 (dolphin A) in which PCB 126 predominated. The three non-ortho congeners were detected in all samples; PCB 77 was quantified in eight samples (ranging from 0.05 to 1.06 ng/g), PCB 126 in four samples (0.13−0.38 ng/g), and PCB 169 in four samples (0.053−0.21 ng/g). PCB 77 was the most abundant, followed by PCBs 126 and 169 (PCB 77 > PCB126 ≈ PCB 169) in the majority of the samples (seven of nine). However, the pattern found in sample 1 (dolphin A) was different. Thus, in S1 PCB 126 was the predominant congener followed by PCBs 77 and 169, whereas in sample 6 (dolphin C) PCB 77 was the most abundant followed by PCBs 169 and 126. In general, PCB 77 was also the most abundant non-ortho PCB in the literature on marine mammals. There are some differences in the relative abundance of the other two non-ortho congeners. Thus, PCB 77 > PCB 126 > PCB 169 was found in the bottlenose dolphin, gray seal, and ringed seal from the Baltic Sea (*34*−*36*). Nevertheless, other marine mammals have patterns that differ significantly. Thus, in Danish harbor seals (*37*) and guillemot eggs from Greenland and Iceland (*38*) the concentrations decreased in the order PCB 126 ≫ PCB 77 = PCB 169, whereas in unspecified types of dolphin (*39*) the concentrations were PCB169 > PCB 77 > PCB 126. Striped dolphins from other studies with very high levels of PCBs showed a non-ortho coplanar pattern PCB 77 > PCB 169 > PCB 126 as did one specimen in this study, whereas dolphins with lower relative levels of PCBs

(<250 μg/g of wet weight) showed the coplanar pattern PCB 77 > PCB 126 >PCB 169, as did the majority of dolphin samples here. PCBs 77 and 126 are relatively more biodegradable than PCB 169 and are metabolized to a certain extent by microsomal enzymes (*37*, *40*). The fact that among the three non-ortho coplanar congeners the concentration of the more biodegradable PCB (PCB 77) continued to be present at the highest levels can be explained by the fact that its concentration is 3 or 4 orders of magnitude greater than those of the other two in commercial preparations Therefore, a higher exposure than elimination rate may be a plausible explanation for the proportion of PCB 77 being highest.

PCB Congener Distribution Pattern. The results shown in Table 2 indicate that PCBs 138, 153, 170, and 180 were the major contributing congeners, each of them making up >7% of total PCBs investigated. PCBs 84+101, 105, 118, 131, 132, 136, 149, 156, 174, 178, 183, 194, and 196 each contributed between 1 and 7%. The non-ortho PCBs 77, 126, and 169, the mono-ortho PCB congeners 114, 157, and 167, and PCBs 91, 95, 110, 135, 139, 144, 171, 175, 176, and 197 had a lower contribution (<1%). PCB 123 was not detected in any investigated sample, PCB 88 was detected in two dolphins (two livers and one blubber), and PCB 129 was detected only in the blubber of two dolphins.

PCBs 138, 153, 170, and 180 accounted for ~60% of the total congener concentration. These congeners share the characteristic of having chlorine atoms in positions 2, 4, and 5 in one (PCBs 138 and 170) or both rings (PCBs 153 and 180) (Table 2). Di-ortho PCB congeners with chlorine substitution at para and meta positions in both rings are hard to metabolize (*41*, *42*). Some studies have reported that these PCBs are nondegradable in invertebrates and fish (*43*−*45*) and in vertebrates and the adipose tissue of sea birds (*46*). Other studies reported that in marine systems, the proportions of these congeners increased slightly with increasing trophic levels (*47*, *48*).

Bioconcentration of PCBs by aquatic organisms is correlated to lipophilicity, degree of chlorination and stereochemistry (*44*). PCB congeners without meta/para vicinal H atoms are persistent and difficult to metabolize. This characteristic is essential for the accumulation of PCBs as congeners with both meta/para and ortho/meta vicinal H atoms, which are easily metabolizable (*44*, *46*, *50*, *51*).

Figure 2 shows the percentages of each PCB found in the liver and blubber of the dolphins investigated compared with the percentages found in Aroclor 1260 (*52*−*54*). Compared to Aroclor 1260, congeners 77, 95, 110, 129, 132, 135+144, 136, 149, 157, 167, 171, 174, 175, 176, and 197 were less abundant and congeners 91, 105, 114, 118, 131, 138, 139, 156, 170, 180, 183, 194, and 196 were more abundant. Abundance of congeners 88, 84+101, 126, 153, 178, and 169 was similar to that in Aroclor 1260, and PCB 123 was detected in neither the samples nor Aroclor 1260. Most of the less abundant ones have vicinal H atoms in para/meta positions (PCBs 95, 110, 129, 132, 135, 136, 149, 174, and 176) (Table 2), and some of them have additional vicinal H atoms at ortho/meta or para/meta positions (PCBs 95, 110, 129, and 132). The majority of the most abundant congeners belong to the group of persistent PCBs. All of them, except PCBs 91, 114, and 131, have the 2, 4, 5; 2, 3, 4; 2, 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 (*55*, *56*).

As reported in other studies (*57*, *58*) investigating other marine mammals, the distribution patterns of PCBs in liver and blubber tissues from some dolphins were very similar. The concentrations of each congener in each organ are different and depend primarily on the lipid levels in that tissue, but their PCB patterns remain more or less constant.

TABLE 3. Structures and Characteristics of PCB Congeners Studied

IUPAC no.	PCB structure	no. of *o*-chlorine atoms	neighboring H atoms
95	236-25	3	2 meta-para
88	2346-2	3	1 ortho-meta, 1 meta−para
91	236-24	3	1 ortho-meta, 1 meta−para
84	236-23	3	1 ortho-meta, 2 meta−para
101	245-25	2	1 meta−para
136	236-236	4	2 meta−para
110	236-34	2	1 ortho-meta, 1 meta−para
77	34-34	0	2 ortho-meta
135	235-236	3	1 meta−para
144	2346-25	3	1 meta−para
149	236-245	3	1 meta−para
139	2346-24	3	1 ortho-meta
123	35-24	1	1 orhto-meta
118	245-34	1	1 ortho-meta
131	2346-23	3	1 ortho-meta,1 meta−para
114	2345-4	1	2 ortho-meta
153	245-245	2	none
132	234-236	3	1 ortho-meta, 1 meta−para
105	234-34	1	2 ortho-meta
176	2346-236	4	1 meta−para
138	234-245	2	1 ortho-meta
178	2356-235	3	none
129	2345-23	2	1 ortho-meta, 1 meta−para
175	2346-235	3	none
126	345-34	0	1 ortho-meta
183	2346-245	3	none
167	245-345	1	none
174	2345-236	3	1 meta−para
171	2346-234	3	1 ortho-meta
156	2345-34	1	1 ortho-meta
157	234-345	1	1 ortho-meta
197	2346-2346	4	none
180	2345-245	2	none
169	345-345	0	none
170	2345-234	2	1 ortho-meta
196	2345-2346	3	none
194	2345-2345	2	none

2,3,7,8-TCDD Toxic Equivalents of Coplanar PCBs. The 2,3,7,8-TCDD toxic equivalents factors (TEFs) recommended by the World Health Organization (*59*) were used for hazard and risk assessment in this study. They are usually 5−10 times lower than those obtained using the TEFs developed by Safe (*40*), which have been used in the hazard risk assessment studies conducted up until 1994. The mean 2,3,7,8-TCDD equivalents (TEQs) found in the study samples were 1.47 ng/g (wet weight) for blubber and 0.24 ng/g (wet weight) for liver samples (Table 2).

The contribution to TEQs of the most toxic PCB congeners (77, 126, and 169) were not very highly retained, together accounting for <1% of total TEQs. The mono-ortho PCB congeners 105, 118, 156, and 157 and the di-ortho congeners 170 and 180 contributed to the maximum toxicities in the studied dolphins. Together they account for ~80% of the total TEQs calculated. These results agree with those obtained by other authors (*24*, *60*, *61*) when the contribution to TEQs of non- and mono-ortho PCBs were calculated for marine and terrestrial mammals. The contribution of mono-ortho PCB congeners in marine mammals is consistently higher than that of non-ortho PCB congeners, whereas in the case of terrestrial mammals, the toxic equivalents of non-ortho PCB congeners are comparable to those of mono-ortho PCB congeners (*60*, *61*, *62*).

Enantiomeric Ratios of Chiral PCBs. Table 4 shows the enantiomeric ratios (ER) and the enantiomeric excess (ee) of nine chiral PCBs in nine striped dolphin samples determined by off-line HPLC (PYE)−HRGC/LRMS technique as

TABLE 4. Enantiomeric Ratios (Area of First Peak/Area of Second Peak) and Percentage of Enantiomeric Enrichment (in Parentheses) in Liver and Blubber of Six Striped Dolphins (*S. coeruleoalba*) from the Mediterranean Sea Determined by HPLC−HRGC/LRMS (SIM) and MDGC/ECD[a]

sample	technique	PCB 84	CB 91	CB 95	CB132	CB135	CB136	CB149	CB174	CB176
S1 (liver)	HPLC−HRGC	ND	ND	0.71 (16.9)	0.81 (10.5)	0.63 (22.7)	1.05 (2.4)	0.8 (11.1)	0.92 (4.2)	0.85 (10.5)
S2 (blubber)	HPLC−HRGC	ND	ND	NQ	NQ	ND	ND	0.76 (13.6)	NQ	ND
S3 (liver)	HPLC−HRGC	ND	ND	0.85 (8.7)	0.55 (29.0)	0.73 (15.6)	0.89 (5.8)	0.58 (26.6)	0.77 (13)	0.92 (4.2)
S4 (blubber)	HPLC−HRGC	ND	ND	ND	ND	NQ	NQ	0.66 (20.5)	NQ	ND
S5 (liver)	HPLC−HRGC	NQ	ND	1.01(0.5)	0.65(21.2)	0.67(19.8)	1.04 (1.9)	0.88 (6.4)	1.00 (0)	0.72 (16.3)
S6 (blubber)	HPLC−HRGC	NQ	NQ	1.07 (3.9)	0.65 (21.2)	0.76 (13.6)	1.07 (3.4)	0.91 (4.7)	1.16 (7.4)	0.74 (14.9)
	MDGC			1.02 (0.9)	0.76 (13.6)	0.79 (11.7)	1.17 (7.83)	0.93 (3.6)	1.16 (7.4)	1.04 (1.9)
S7 (liver)	HPLC−HRGC	ND	ND	0.71 (16.9)	0.92 (4.2)	0.71 (17)	0.96 (2.0)	0.63 (22.7)	0.58 (26.6)	ND
S8 (liver)	HPLC−HRGC	ND	ND	ND	NQ	ND	1.01 (0.5)	0.68 (19)	NQ	ND
S9 (liver)	HPLC−HRGC	ND	NQ	0.84 (8.7)	0.67 (19.8)	0.64 (22)	0.84 (8.7)	0.67 (19.8)	0.81 (10.5)	0.78 (12.4)
	MDGC			0.82 (9.8)	0.79 (11.7)	0.78 (12.3)	0.87 (6.9)	0.81 (10.5)	0.87 (6.9)	1.06 (2.9)
standard 1	HPLC−HRGC	1.00 ± 0.03	0.94 ± 0.01	1.05 ± 0.04	0.97 ± 0.1	1.00 ± 0.01	0.97 ± 0.02	1.00 ± 0.03	1.02 ± 0.09	0.95 ± 0.05
standard 2	MDGC			0.97 (1.5)	0.98 (1.0)	1.01 (0.5)	1.00	0.99 (0.5)	1.09 (4.3)	0.96 (2.04)

[a] ND, nondetected (signal/noise > 3); NQ, nonquantified (signal/noise > 5).

well as the results obtained in two of them (S6 and S9) using MDGC/ECD as a confirmatory technique. PCBs 84 and 91 were under the detection threshold of the LRMS technique, which was lower than that of the ECD detection system used to calculate its concentration levels. The results obtained using both techniques are similar in all cases, except that of PCB 176. The atropisomers of PCB 176 are highly retained (t_R = 120 min), and they are not completely separated under the chromatographic conditions used in the off-line HPLC/LRMS system.

The enantiomeric ratios (ratio of the first eluted enantiomer to the second) of the nine chiral PCBs (84, 91, 95, 132, 135, 136, 149, 174, and 176) obtained in the samples studied (Table 4) revealed that PCBs 136 and 174 were racemic or nearly racemic in almost all of the investigated samples. PCB 136 revealed an ee of the second eluted atropisomer >5% in two liver samples (5.8 and 8.7% for S3 and S9, respectively). PCB 174 revealed an ee >5% in three of them (S3, S7, and S9). PCB 95 (ER = 0.71−1.07), PCB 132 (ER = 0.55−0.92), PCB135 (ER = 0.63−0.76), PCB 149 (ER = 0.58−0.91), and PCB 176 (ER = 0.72−0.91) revealed an ee of the second eluted enantiomer in almost all of the studied samples (Table 4). A lower ee was found for PCB 95 in samples S5 and S6, for PCB 132 in sample S7, for PCB 149 in sample S6, and for PCB 176 in sample S3.

In the case of PCB 176 the results obtained by using the two analytical techniques [HPLC (PYE)/HRGC/LRMS and MDGC/ECD] are different. The ER values obtained with the former for S6 and S9 samples were 0.74 and 0.78, respectively, whereas the ER values using the latter technique were 1.04 and 1.06, respectively. As was previously explained, this is due to the low chromatographic resolution between both atropisomers when HRGC/LRMS (SIM) was used. Although the ee values found in PCB 176 from the analyzed samples could indicate the existence of a difference between the metabolism of its atropisomers, because of the reasons given above, it is not possible to confirm this.

There does not appear to be any relationship between the quantity of PCBs accumulated in the liver and the increase or decrease in enantiomeric enrichment. Thus, S3, which showed the lowest PCB levels, also showed the highest levels of enantiomeric enrichment in some of the PCBs investigated. The calculated ER values for PCBs 132, 135, and 149 were 0.55, 0.73, and 0.58, respectively, with ee values of the second eluted atropisomer of 29, 15.6, and 26.6%, respectively. On the other hand, sample S5, which showed higher PCB concentration values, presents the chiral PCBs mentioned with lower enantiomeric excess.

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 (PCBs 95, 132, 135, 136, 149, 174, and 176) belong to the readily metabolizable PCBs. They have neighboring hydrogen atoms in both ortho/meta and meta/para positions (PCB 132), in two meta/para positions (PCBs 95 and 136), or in one meta/para position (PCBs 135, 149, 174, and 176), as has already been discussed above. It is therefore not possible, on the basis of structure, to explain why PCB 95 (with two neighboring 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 (*10*). It must be pointed out that only six dolphins have been analyzed, and more information is needed to draw more reliable conclusions.

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