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Coplanar Polychlorinated Biphenyl Congeners in Shark Livers from the North-Western African Atlantic Ocean

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Polychlorinated biphenyls have been widely used by industry throughout the world since 1930. Although their use has been banned in many countries since the late 1970s, they still represent an important class of priority pollutants due to their persistence. Most open uses of these chemicals have been severely curtailed in industrialized nations, but a considerable fraction of past productions is probably still cycling in the ecosphere.

In recent years, attention has been focused on the toxicity of PCBs, especially of those congeners showing similar toxicity as the polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDFs). It has been shown that PCB congeners' toxicity largely depends on the chlorine substitution pattern (Safe 1990). The most toxic PCB congeners are those with two para chlorines, at least two meta chlorines and 0-2 ortho chlorines. These so-called "coplanar" (non- mono- and di-ortho) PCB congeners are able to obtain planar conformation. Recently, toxic equivalence factors have been assigned to coplanar PCBs (Safe 1994). Thus determination of individual PCB congeners is important for evaluating the toxic potentials of PCB residues in , for example, wildlife (Hansen and Spliid 1993).

Sharks, as top predatory fish are good indicators of the extend of PCB contamination of aquatic marine ecosystems. This may be considered as a possible reason for decreased wildlife population (Reijnders 1986, Holden 1978). Some areas of the world have been studied in considerable detail as is the case of the Great Lakes (USA and Canada) where monitoring included most compartments of the ecosystems (Stalling et al 1985), Arctic and Antarctic oceans (Muir et al. 1992; Oheme et al. 1994). However none of them have been focused in the North African Atlantic ocean. Besides the presence of coplanar PCB congeners is a human health concern because shark form part of the Japanese and Chinese diets, and shark oil is usually used in the cosmetic industry. We present here the preliminary results of an extended study about the levels of PCB congeners including the coplanar ones in the liver of six shark species collected around the Canary Islands in the North African Atlantic Ocean and to correlate these with their sex, age, size, habitat and diet.

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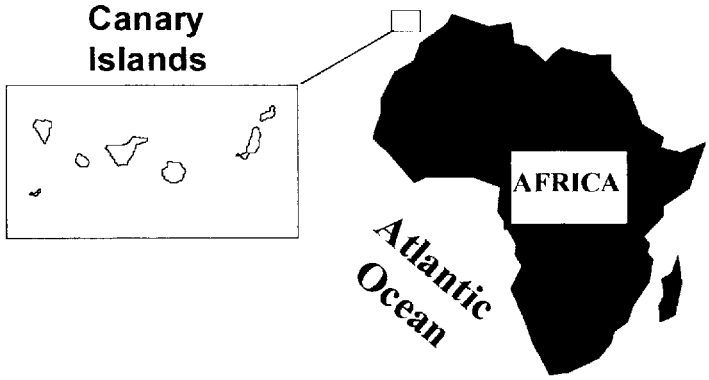

Figure 1. Geographic location of the sampling sites

Samples were collected in the surroundings of the Canary Islands (Spain) in the North African Atlantic Ocean (Figure 1) between July 93 and September 94, during spring and summer seasons, The Canary Islands are not an industrialized area and only moderately populated, with a petrol refinery located on one of the islands (Tenerife) as a possible PCB emission source. 15 samples of 6 different species of sharks with variable size, weight and location taken at several depths were investigated (table 1). All fishes were adult, females (except one male of E.princeps), and sedentary. The samples were kept frozen until they were analysed.

The samples were analysed for the following PCB congeners: # 28 (2,4,4'-TCB), # 52 (2,5,2',5'-T_4CB), #77 (3,4,3',4'-T_4CB), #101 (2,4,5,2',5'-P_5CB), #105 (2,3,4,3',4'-P_5CB), #118 (2,4,5,3',4'-P_5CB), #l26 (3,4,5,3',4'-P_5CB), #138 (2,3,4,2',4',5'-H_6CB), #l53 (2,4,5,2',4',5'-H_6CB), #156 (2,3,4,5,3',4'-H_6CB), #167 (2,4,5,3',4',5'-H_6CB), #169 (3,4,5,3',4',5'-H_6CB), #170 (2,3,4,5,2',3',4'-H_pCB), #180 (2,3,4,5,2',4',5'-H_pCB), and #194 (2,3,4,5,2',3',4',5'-O_8CB).

Silica gel 60 (70-230 mesh ASTM) was purchased from Merck (Germany). Florisil (60-100 mesh) from Floridin Co. (Berkeley Springs, West Virginia, USA). Anhydrous granulated Na_2SO_4 12-60 mesh from J.T. Baker (Chemical Co., Phillisburg, N.J., USA). Carbon Amoco (PX21) from Andersem (Development Comp., USA). The individual PCB standards, were purchased from Dr. Ehrenstorfer (Ausburg, Germany). All solvents and reagents were pesticide residue analysis degree. All the absorbents used were washed with methanol and dichloromethane before their use.

Table 1: Information on the sharks that were sed in the present study

Code	Scientific name	weight (g)	size (mm)	depth (mm)	latitude	longitude
M1	*C. coelolepis* [1]	11000	1105	2100	28°00′50" N	16°50′52" W
M2	*C. coelolepis* [1]	7500	1040	1800	27°59′55" N	16°46′26" W
M3	*C. coelolepis* [1]	9200	1060	2100	28°00′50" N	16°50′52" W
M4	*C. coelolepis* [1]	11000	1080	2100	28°00′50" N	16°50′52" W
M5	*C. coelolepis* [1]	7500	1030	1800	27°59′55" N	16°46′26" W
M6	*C. coelolepis* [1]	8450	990	1800	27°59′55" N	16°46′26" W
M7	*C. coelolepis* [1]	8600	1060	1800	28°01′07" N	16°47′27" W
M8	*C. coelolepis* [1]	9200	1060	2100	28°00′50" N	16°50′52" W
M9	*C. coelolepis* [1]	8500	1000	2100	28°00′50" N	16°50′52" W
M10	*D. histricosa* [1]	6500	1070	900	27°38′58" N	15°45′55" W
M11	*E. princeps* [2]	795	565	2100	28°00′50" N	16°50′52" W
M12	*E. princeps* [1]	1910	690	1800	25°59′57" N	16°46′09" W
M13	*D. profundorum*[1]	3000	855	900	27°38′58" N	15°45′56" W
M14	*C. cryptacanthus*[1]	6700	1000	1200	28°01′17" N	16°44′36" W
M15	*C. squamosus*[1]	10500	1220	1000	28°35′52" N	17°58′26" W

[1] female; [2] male

Extraction and cleanup followed a semiautomatic procedure based on an adaptation Smith et al. (1985) method. This comprises a low pressure chromatography system in which 0.5 g of shark liver samples, previously homogenated with a polytron apparatus and dried with anhydrous sodium sulphate, The sample was spiked with a mixture containing 20 ng of $^{13}C_{12}$ isotonically labelled PCBs 77, 126 and 169, as internal standards and placed between two layers of anhydrous sodium sulphate on the top of a multilayer column containing neutral and base-modified silicagel. This column was connected to a second one, filled up with activated carbon (Amoco-PX21) dispersed on glass fibers. Three fractions were eluted from the carbon column for each sample using cyclohexane/dichloromethane (80:20), dichloromethane/ toluene (80:20) and toluene. The first fraction containing the ortho-substituted PCB congeners, and the second fraction containing the non-ortho substituted PCB congeners were purified with different layers of silica gel (neutral, impregnated with sulphuric acid or potassium hydroxide) and Florisil. The third fraction containing PCDD/Fs was kept to further analysed.

All PCB congeners included in Fraction I were analysed by high resolution gas chromatography (HP 5890 Series II) with Electron Capture detector, using PCB#l2 and PCB#209 as internal standard using multilevel calibration method. A nonpolar fused silica capillary column DB-5 (J&W, Australia) (60 m long x 0.25 mm I. D., 0.25 µm film thickness) was temperature programmed as follows: 60°C (1 min), to 185°C at 30°C/min (hold 3 min), then to 234°C at 1.9°C/min (hold 65.5 min), then increasing to 250°C at a rate of 2°C per min. The final temperature was maintained 15 min. The absolute detection limits were between 0.2 and 10 pg/µl from tetra to octachlorobiphenyl. The confimation of the PCB congeners was performed by HRGC-LRMS/SIM.

The analysis of the non-ortho substituted coplanar PCB congeners isolated in Fraction II were performed by HRGC-LRMS (SIM) using a HP 5971A instrument

coupled to a HP 5890 series II Gas Chromatograph. Quantification was done by the 13C-labelled standards as described previously (Gonzalez et al, 1994). An 0.8 ml aliquot of the sample was injected in the splitless mode at 260°C. in an OV-1 capillary column (20 m length x 0.25 mm I.D., 0.25 µm film thickness). The column temperature was programmed from 100°C (1 min) to 130°C at 50°C/min, then to 190°C (2 min) at a rate of 4°C/min, and finally to 230°C at 2°C/min rate. The final temperature was maintained 15 min. The eluent from the column was transferred to a quadruple mass spectrometer with electron impact ionization. The interface temperature was 280°C. Two ions characteristics of each PCB homolog and on the respective labelled internal quantitation standards were monitored for each analysis. Identification of PCB congeners was based on retention time information and the comparison of the ratios of the characteristic ions with theoretical values. Factor responses of the labelled to native standard congeners were calculated using the same concentration of all of them, in the PCB sample level ranges, and in the same injection. The absolute detection limits were 5.3, 5.6 and 6.4 pg for PCB-77, 126 and 169 congeners, respectively.

RESULTS AND DISCUSSIONS

The analytical results obtained (on ng/g fresh weight basis) are reported in table 2. PCB congeners were detected in all samples analyzed, The sum of the 16 analyzed PCB congeners showed significant variations among the six shark species investigated. The *C. squamosus* species exhibited the highest values, with 2816 ng/g on a fresh weight basis, followed by *C. cryptacanthus* with 920 ng/g, *D. profundorum* with 392 ng/g, *E. princeps* (2 samples] with 238 ng/g, *C. coelolepis (* 9 samples) with 102 ± 64.1 (SD), and finally the lowest values were found in the sample from *D. histricosa.*

The differences in PCB concentrations found in the six shark species could be due to several causes, such as differences in their habitats, diet, lipid contents, reproductive strategies, age, sex, weight and sampling period. In the present case, there are no differences in their diets. The diet of Atlantic ocean sharks is dominated by teleost fishes, cephalopods, squids and crustaceans, and, to a lesser degree, marine mammals and pelagic fishes. These sharks are sedentary, and inhabit in the benthic to pelagic sea area but at different depths. The three species with higher PCB levels *(C. squamosus, C. cryptacanthus* and *D. profundorum)* lived and were caught at depths ranging between 900 and 1200 m. *E. princeps and C. coelolepis,* which had the lowest PCB levels were taken from deeper waters, below 1500 m. The differences in the depth where the sharks lived should be related with the PCB concentrations found in their livers. The decrease of organochlorine concentration with depth has been observed in other marine ecosystems by several authors that related the levels of contaminants with several factors such as temperature, salinity, sunlight, airborne deposition and water ocean fluxes (Jantunen and Bidleman 1995).

There is a relationship between the liver lipid content and the total PCB concentration found in the six investigated shark species. Thus *C. squamosus* and C. *cryptocanthus* with 90 and 85% of liver lipid content had the highest level of total

Table 2. Levels of PCB congeners (ng/g fresh weight) detected in different liver shark samples (codes in table 1).

Code	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15
28[2]*	0.26	0.66	N.C.	0.26	N.C.	0.09	2.61	0.16	0.15	0.66	0.21	3.05	5.70	5.67	42.2
52[3]	1.11	8.16	N.C.	2.6	N.C.	0.91	12.1	3.49	0.43	0.42	1.70	106	62.3	118	190.7
77[1]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.93	1.77	1.10	1.77
101[3]	13.3	37.5	13.6	15.4	37.0	4.34	51.5	7.92	5.64	4.07	0.94	64.9	81.1	133	227
105[2]	2.05	4.71	1.33	2.97	11.5	1.33	6.09	1.38	0.57	0.27	0.06	4.22	16.6	6.57	86.3
118[2]+149[†]	7.04	25.4	N.Q.	17.5	N.Q.	5.06	6.03	2.13	16.3	1.16	0.20	18.3	46.2	N.Q.	1181
126[1]	N.D.	DET.	DET.	DET.	N.D.	DET.	N.D.	N.D.	N.D.	N.D.	N.D.	0.27	0.63	0.61	0.54
138[3]	34.1	3.96	6.4	1.71	N.D.	10.9	116	16.2	0.63	5.77	1.06	93.8	63.1	228	57.2
153[3]	32.6	7.40	72.0	1.41	72.0	0.62	9.13	16.3	1.04	5.40	1.08	97.8	70.9	268	898
156[2]	3.28	1.15	N.D.	0.36	1.50	0.16	2.13	0.54	0.19	N.D.	0.40	15.1	5.30	3.55	32.7
167[2]	4.69	10.4	N.D.	N.D.	0.97	1.54	13.6	2.22	1.53	0.91	0.33	10.9	6.10	23.8	42.2
169[1]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.30	0.17	N.D.
170[3]	3.18	2.55	N.D.	0.66	N.Q.	0.19	1.88	0.56	0.25	0.55	N.D.	7.46	4.20	21.1	48.3
180[3]	27.2	2.10	1.26	22.0	48.2	6.07	2.03	14.8	5.59	3.58	0.53	48.9	30.8	112	10.4
194[3]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ΣPCBs	129	104	94.6	64.9	171	31.2	224	65.7	32.4	22.8	6.51	472	392	920	2816
% Fat	46	46	67	66	46	52	46	46	46	60	44	54	70	90	85
I-TEQ (pg/g)	4.06	7.71	1.33	4.86	1.21	1.90	7.54	1.80	2.28	0.38	0.24	6.1	118	87.9	287

*IUPAC nomenclature (Ballschmiter and Zell. 1980), [1] non-ortho PCBs, [2] mono-ortho congeners, [3] di-ortho congeners

[†] rate 118/149 = 9/1

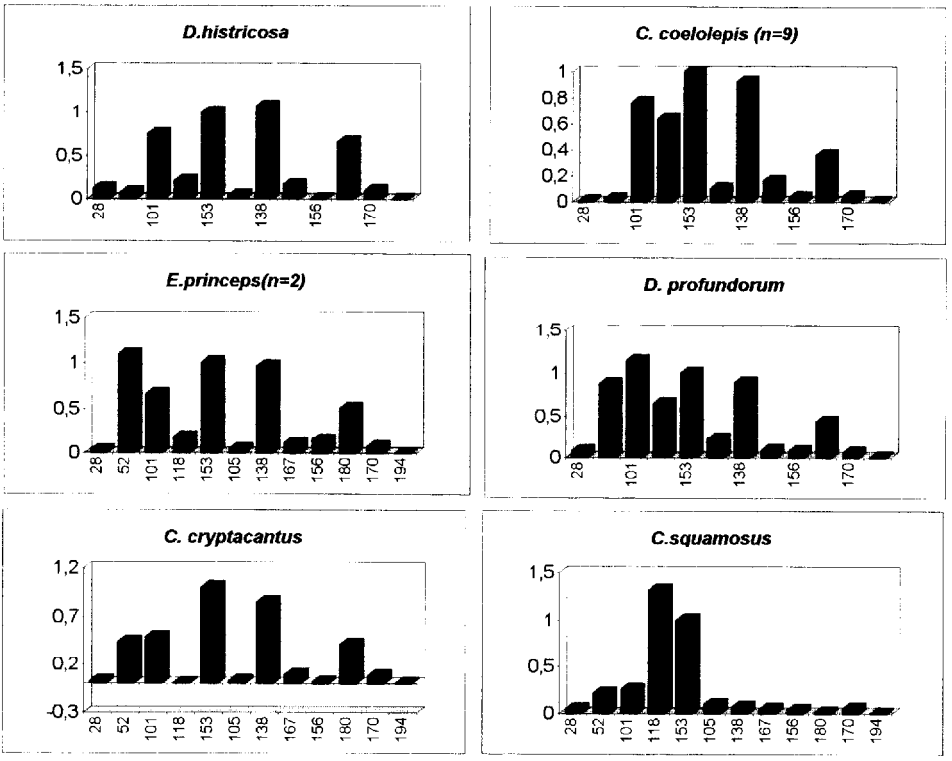

Figure 2. X/153 ration in liver of the six shark species investigated

PCBs, followed by *D. profudorum* (70% of lipids). The other three species with lower liver lipid content (< 60%) had lower PCB concentrations in their livers. No relationships were found between PCB levels in the 9 individuals of *C. coelolepis* and their specimen size.

The results shown in table 2 indicate that PCB 101, 118, 153, 138 and 180 were the major contributing congeners in five of the shark species investigated, making up more than 50/. of total PCB found, The non. ortho PCB congeners, PCB 77, 126 and 169 had a lower contribution (< 1%) , and the other 14 PCB congeners, contributed between 1 and 5%. In the case of *C. squamosus* species PCB 138 and 180 were in percentages lower than 2 % of the total PCBs. The 3 non-ortho PCB congeners did not contribute significantly to the total sum of PCBs. PCB 77 was the dominant non-ortho congener in the four shark species in which it was detected (*E. princeps, D. profundorum, C. cryptacanthus* and *C squamosus),* followed by CB 126 and CB 169 CB 169 concentration was below the detection limit levels in all the cases, except for two species (*D. profundorum* and *C. Cryptacanthus).* None of the three non-ortho coplanar congeners were detected in the *D. histricosa* species, and CB 169 was not detected in any of the 9 individual samples of *C. coelolepis* analyzed. These results agree with those reported by Ford et al, (1993) in Ringed seal samples from Canadian Arctic ocean.

A significant difference was found in the residue levels of the two *E.princeps* individuals of different sexes. The female contained higher PCB congener levels than the male. The fact of having such a limited number of samples made it impossible to elaborate any conclusion concerning the relation between PCB levels and sex.

Total 2,3,7,8-TCDD toxic equivalents (pg/g TEQs on a fresh weight basis) were calculated for PCB mixtures using the Toxic Equivalency Factors developed by Safe (1994) (Table 2). They followed similar variations to those found for total PCBs in the six shark species investigated. Thus, the *C. squamosus* species showed the highest values with 287 pg/g of TEQs (fresh weight) followed by *D. profundorum* (118 pg/g), *C. cryptocantus (88* pg/g), *E. princeps* (6.1 pg/g), *C. coelolepis* (4.84 pg/g) and finally *D. histricosa* (0.38 pg/g).

The biotransformation of PCBs in those animals is explained as the ratio of a PCB concentration congener to that of a persistent congener. For example PCB-153, which is an extremely persistent PCB and a good indicator of biological alteration of PCBs (Barrie et al. 1992). This ratio in all the six shark species is presented in figure 2. It is interesting to note that the metabolic efficiencies are different for each of the species studied. Thus, while *D. histricosa* and *C. coelolepis* are capable of metabolizing lower chlorinated PCBs, D. *profundorum* and mainly *C. squamosus* are capable of metabolizing the higher chlorinated PCBs. The most persistent PCBs are CB 101, 118, 138 and 180 for all the shark species, except for *C. squamosus* which is capable of metabolizing the CB- 180.

To our knowledge this is the first study where PCB levels were determined for individual shark species. Moreover, the absence of previous surveys in the studied area makes any comparison with similar species living in other marine environments virtually impossible. The PCB levels found in the two most polluted shark species *(C. squamosus* and *C. cryptacanthus)* showed lower but comparable pollution levels to those reported for marine species which are in similar trophic levels (whales, salmons, seals and dolphins) from Danish waters (Storr-Hansen and Spliid 1993), Arctic and Antarctic oceans (Muir et al. 1992, Oheme et al. 1994, Ford et al. 1993) and the North American Atlantic Ocean (Kuehl et al. 1991). These values were much higher than those obtained for the other four shark species found during the present investigation, which were similar to those found in mammals living around New Zealand coast in the open ocean (Hannah et al. 1993). PCB residues were found at all sampling sites tested in all species investigated, however the magnitude of residues found was variable. Two of the six shark species sampled *(C. squamosus and C. cryptacanthus)* contained residues greater than or near to 1 µg/g. The PCB residue levels found in the other four shark species investigated *(D. profundorum, E. princeps, C. coelolepis,* and *D. histricosa)* were lower. A relationship was found between liver shark PCB concentration levels and both, liver lipid content and the depth where the sharks lived.

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