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Persistent organochlorine residues and toxic evaluation of polychlorinated biphenyls in sharks from the Mediterranean Sea (Italy)

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Persistent organochlorines such as polychlorinated biphenyls including coplanar congeners, DDT compounds and HCB were measured in different tissues (muscle, liver and eggs) of two Mediterranean shark species: namely *Centrophorus granulosus* and *Squalus blainvillei*. The concentrations of organochlorines in the tissues and organs of both species were in the order DDTs > PCBs > HCB. The highest values of DDTs, PCBs and HCB were found in liver followed by eggs and muscle. Among DDTs the compound found in greatest concentration was *p,p′*-DDE. The PCB profiles were dominated by congeners 138, 153, and 180. The isomers with higher TEQs values were non- and mono-*ortho* congeners than di-*ortho* ones in muscle, liver and eggs of both species. Among the non-*ortho*, PCB 126 was the major contributing individual to the total TEQs in both species.

Keywords: shark; Mediterranean Sea; DDTs; PCBs; coplanar congeners; TEQ.

Introduction

Polychlorinated biphenyls (PCBs), DDT compounds (DDTs) and hexachlorobenzene (HCB) are of great concern due to their highly bioaccumulative nature and negative effects on many life forms. They have high lipophilicity and are resistant to degradation and therefore tend to concentrate in terrestrial and aquatic organisms. Particular attention has been paid to understand the fate of these pollutants in the higher trophic organisms, as they amplify through the food chain. In this regard, marine mammals are the focus of attention due to the occurrence of extraordinarily high concentrations of organochlorine residues in their bodies and because they are among the most vulnerable organisms to the long-term toxic effects of persistent organochlorines like PCBs (Cummins, 1988; Tanabe, 1988). Comparatively little is known about organochlorine contaminants in elasmobranch species, although also they are predators at the top of the marine ecosystem. Characteristically, sharks are long lived with comparatively slow rates of growth. Their longevity and slow growth rates considered in conjunction with their high trophic position probably contribute significantly to the accumulation of high concentrations of pollutants (Lyle, 1984). Gulper shark (*Centrophorus granulosus*) and longnose spurdog (*Squalus blainvillei*) are very common in the Mediterranean Sea at depths between 350 and 500 m. They prey on hakes, lantern fish and other deepwater bony fish, also squids. They are both ovoviviparous and moderately sized, in particular, the maximum length for gulper shark is until 120 cm, while for longnose spurdog is 110 cm (Fiches FAO, 1987).

In the present study, isomer-specific concentrations of PCBs including di-, mono-, and non-*ortho* coplanar congeners, DDT compounds and HCB concentrations were determined in muscle, liver and eggs of two different species of sharks, *C. granulosus* and *S. blainvillei*, to establish their degree of contamination. Moreover, to evaluate the toxic potentials of PCB residues in these organisms, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs) of coplanar congeners were estimated according to the toxic equivalent factors (TEFs) for fish proposed by van den Berg *et al.* (1998).

Materials and Methods

25 *C. granulosus* (length: 80–90 cm aver. 86 cm, weight: 4472–4861 g, aver. 4696 ± 139.7) and 20 *S. blainvillei* (length: 60–74 cm aver. 68 weight: 898–2375 g, aver. 1710 ± 529.1) were caught in the South Adriatic

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Sea between June and August 1999. From each specimen were taken muscle, liver and eggs and kept in a deep freeze at 20°C until chemical analysis. To determine chlorobiphenyl (PCBs = sum of 17 congeners), DDT compounds (DDTs = p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDD) and HCB concentrations the following method was used. Aliquots (2–10 g) of the homogenized samples were ground with anhydrous sodium sulphate in a mortar. The mixture was extracted with petroleum ether according to Erney's procedure (Erney, 1983). The extracts were then concentrated and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot (about 200 mg) of the remaining extract was dissolved in hexane (5 ml) and mixed with H_2SO_4 conc. for the cleanup, following the procedure described by Murphy (1972). After centrifugation, the hexane solution was concentrated (about 1 ml) and transferred on a glass column (i.d. 5 mm) filled with 1 g of florisil (activated at 120°C for 16 h) for the separation of PCBs from other organochlorine compounds. The first fraction eluted with hexane (12 ml), contained PCBs and some DDTs, whereas the second fraction, eluted with 10 ml of 15% ethylether in hexane, contained the remaining DDTs and other organochlorine compounds. An aliquot of initial fraction was run on a column (i.d. 5 mm) packed with 125 mg of activated carbon (C. Erba, Milano, Italy) for the separation of non-*ortho* PCB congeners, 3,3′,4,4′-T_4 CB, (IUPAC 77), 3,3′,4,4′,5-P_5 CB (IUPAC 126), and 3,3′,4,4′,5,5′-H_6 CB (IUPAC 169) from other PCBs following the method reported by Tanabe *et al*. (1987). Analyses were made on a Carlo Erba HR gas chromatograph 8000 Top with automatic injection system and with an electron capture detector ECD-400, Ni^{63} (temperature: 310°C). The GC was connected to an IBM PS/2 55SX PC equipped with Chrom-Card version 1.2 software program for integration purposes (C. Erba). For all the analyses a fused-silica capillary column SPB-608 Supelco (length = 30 mt, inside diameter 0.25 mm and film thickness 0.25 μm) was used. Helium at a flow rate of 1 ml/min was used as the gas carrier, nitrogen as make-up gas 60 ml/min. Temperature was programmed according to the following sequence: injection at 50°C. Oven steady for the first min and then an increased from 50°C to 180°C at a rate of 15°C/min. Oven maintained at steady temperature for 1 min and then increased from 180 to 220°C at a rate of 4°C/min; oven maintained at steady temperature for 20 min and then increased from 220 to 275°C at a rate of 5°C/min; from this point until the end of the analytical run, the column remained isothermal at a temperature of 275°C. The individual PCB congeners were 8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209 numbering system (Ballschmiter and Zell, 1980) determined against the corresponding individual standards obtained from ULTRA Scientific (chemical purity 99%). The identity of the DDT group compounds was confirmed by an alkali conversion to their respective olefins and reanalysis by GLC. Analytical data as for DDT group compounds were obtained by a comparison between sample peak area and external standard peak areas (POC mixture bought from Supelco). Recoveries were determined by adding known amounts of PCBs, DDTs and HCB standards (at three levels of concentrations) to empty samples before extraction (method of additions). The recoveries were within 80–110%. The limits of quantification were from 0.1 to 0.4 ng/g on a wet wt basis for the PCB congeners, DDTs and HCB. Quantification was done within the linear range of the detector. Non-detected constituents were assigned a value of zero. Residues in 10% of the samples were confirmed by gas–liquid chromatography–mass spectrometry (Fisons MD 800). Concentrations of PCBs, DDTs and HCB are presented as ng/g on a wet weight basis.

Results and Discussions

Concentrations of total PCBs, DDTs and HCB (ng/g wet weight) in muscle, liver and eggs of sharks of both species are presented in Table 1. The concentrations of

TABLE 1

Concentrations of total polychlorinated biphenyls, DDT compounds and HCB (ng/g wet wt) in different organs and tissues of sharks.

Species	Tissues	Lipid (%)	PCBs	DDTs	HCB
C. granulosus	Muscle	0.13–0.26 0.18 ± 0.06	18–42 28.3 ± 11.3	28–73 49.3 ± 12.6	3–6 3.5 ± 2.1
	Liver	72.0–83.0 77.50 ± 7.78	1188–2570 1741.7 ± 531	3347–6036 4481 ± 961	16–26 20.8 ± 4.2
	Eggs	19.9–20.8 20.35 ± 0.64	324–573 416 ± 85.5	840–1311 1018 ± 168	5-15 9.5 ± 2.6
S. blainvillei	Muscle	0.30–1.00 0.48 ± 0.28	2–20 10.8 ± 6.6	7–38 16.8 ± 9.2	2–5 2.5 ± 1.1
	Liver	42.7–71.0 56.23 ± 14.19	364–1885 958 ± 658	1257–2443 1625 ± 439	6–19 12.3 ± 4.4
	Eggs	11.7–14.4 13.05 ± 1.91	159–276 214 ± 40.7	170–318 238 ± 53	3–6 2.8 ± 1.8

organochlorines in tissues and organs were in the order DDTs > PCBs > HCB. In both species the highest values of DDTs, PCBs and HCB were found in liver, followed by eggs and muscle. DDTs were the dominant contaminants with concentrations ranging from 1257 to 6036 ng/g wet wt in liver, from 170 to 1311 ng/g wet wt in eggs and from 7 to 73 ng/g in muscle of both species. Among DDT metabolites, p,p'-DDE was dominant in muscle of both species, representing 100% of the total DDT burdens; in the liver of *C. granulosus* and *S. blainvillei*, p,p'-DDE was present with percentages of 68.7% and 83.8%, respectively, followed by p,p'-DDD (5.5–15.3%), o,p'-DDT (4.6–8.2%), p,p'-DDT (2.6–5%) and o,p'-DDD (2.7–3.4%). In eggs of *C. granulosus* and *S. blainvillei*, p,p'-DDE constituted 55% and 83.2%, respectively, while the percentage composition of the other metabolites was in the following order: o,p'-DDT (5.2–19.2%) > p,p'-DDD (4.4–14.6%) > o,p'-DDD (4.3–5.9%) > p,p'- DDT (3.0–5.2%). The ratio of p,p'-DDE to total DDT is cited as an indicator of whether new sources of DDT are entering an ecosystem. Aguilar (1984) suggested that a ratio greater than 0.6 implies the system is relatively stable with no new inputs. Ratio in shark livers, equal to 0.7 in *C. granulosus* and 0.8 in *S. blainvillei*, was above this threshold indicating no new inputs of DDT into the ecosystem.

Residue levels of PCBs in liver of both species were 364–2570 ng/g wet weight, in eggs the concentrations ranged from 189 to 573 ng/g wet wt, and in muscle from 2 to 42 ng/g wet weight. Although many reports exist on organochlorine compound concentrations in marine organisms, the data available on sharks are rather scanty (Corsolini *et al*., 1995; Serrano *et al*., 1997, 2000). Corsolini *et al*. (1995) reported in the adipose tissue of two shark species (*Prionacee glauca* and *Alopias vulpinis*) taken from the eastern coast of Italy, PCB concentrations ranging from 70 to 4000 ng/g wet wt. A large variety of PCB levels were found by Serrano *et al*. (1997, 2000) in the liver of eight shark species living in the northwest African Atlantic Ocean. The mean values found for these eight shark species ranged from 39 to 4723 ng/g wet wt and for few species (i.g. *D. licha* and *D. histricosa*) were of a similar order of magnitude to those found by the present authors. As regards the levels of organochlorine compounds in the eggs no comparison was possible because there are no papers concerning this topic in sharks to our knowledge. However, the relatively high levels in the eggs of both species can suggest that organochlorine compounds were conveyed from the mother to the eggs. On the other hand, the transfer of maternal persistent organic pollutant load to the offspring or eggs has been observed in other megafaunal taxa either marine or terrestrial, as marine turtles (Mckenzie *et al*., 1999), cetaceans (Tanabe *et al*., 1982), and birds (Bogan and Newton, 1977). The exposure to these contaminants during early developmental stages can induce adverse effects. Because no toxicological approach has been carried out at this regard in sharks, data from other species have been considered. Several studies have estimated that the reproductive success of terns begins to decrease if their eggs contain more than 4 μg/g of DDE (Custer *et al*., 1983). Nisbet and Reynold (1984) suggested that the critical DDE level is equal to 3 μg/g in tern eggs. Other studies estimated this critical threshold for the eggs of night heron either at 8 μg/g (Blus, 1984) or at 3 μg/g (Fasola *et al*., 1987). Hoffman *et al*. (1993) reported that 4.7 μg/g of PCBs in eggs is associated with 85% hatching success in a population of common terns, and 33% embryo mortality was exhibited, in chicken eggs at doses of 5 μg/g (Brunstrom, 1991). PCB and DDE values found in our samples of eggs did not reach so high levels however, this does not necessarily imply lack of deleterious effects for sharks because the threshold concentration at which detrimental effects might occur, is species dependent.

In both species concentrations of HCB in liver, eggs and muscle ranged from 6 to 26, 3 to 15, and 3 to 6 ng/g wet wt, respectively.

Between the two species of sharks analysed concentrations of DDTs, PCBs and HCB in liver and eggs, were greater in *C. granulosus* than in *S. blainvillei* ($p < 0.05$), while HCB levels of the same order of magnitude were observed in the muscle of both. Small sharks in the Mediterranean are residential and hence exposed to the same PCBs and DDTs sources (Corsolini *et al*., 1995); therefore, the differences in load of contaminants found between the two species could be due to several causes, such as differences in their habitat, diet, lipid content and weight. Differences in organochlorine compound concentrations as a function of the depth, sex, and diet were found in different shark species by Serrano *et al*. (1997, 2000). In the present case, the differences between the two species may be mainly due to the different sizes, rather than to the habitat and the diet considering that they are similar. For both species the maximum size is 110 cm *ca*, (Fiches FAO, 1987); on this basis it is likely to be presumed that *C. granulosus* samples, having a length (length 88–96 cm, aver. 92 cm) very close to the maximum size, are older with respect to *S. blainvillei* samples (length 60–74 cm, aver. 67.6 cm), and hence they have accumulated pollutants for a longer time.

Hexachlorobiphenyl congeners 153 and 138 were the most prevalent congeners collectively accounting for 54 and 57% of the total PCB concentrations in the liver of *C. granulosus* and *S. blainvillei*, respectively. Other chlorobiphenyls found in major amounts in shark livers include congeners 101, 105, 118 and 180, with a contribution of 33.9% in *C. granulosus* and of 32.4% in *S. blainvillei*. The other PCB congeners contributed between 10.6% and 12%. PCB 169 was detected in the liver samples of both species up to traces, while PCB 52 was below the threshold of detection only in *S. blainvillei* liver. In eggs, PCBs 153 and 138 were the dominant congeners too, together accounted for 53% of the total

PCB load in both species. Chlorobiphenyls 101, 105, 118 and 180 collectively contributed between 32.7% and 34.6% in both species, while the others had a low contribution between 12.4% and 14.3%. PCB 52 was not found in the eggs of *S. blainvillei* and PCB 169 was present at levels of traces in all the egg samples. In the muscle of both species, hexachlorobiphenyl 153 and 138 and PCB 180 were the prevalent congeners accounting for more than 80%. Other congeners, including those with less than 4 or more than 9 chlorine atoms (8, 20, 28, 35, and 209) were below the detection limits for all samples. A comparable pattern was found in other marine organisms from other areas of the Mediterranean Sea, i.e. *Mullus barbatus*, *Mugil cephalus*, *Dicentrarcus labrax* (Pastor *et al*., 1996) and in dolphins *Stenella coeruleoalba* (Kannan *et al*., 1993), *Tursiops truncatus* (Storelli and Marcotrigiano, 2000), and *Grampus griseus* (Storelli and Marcotrigiano, 2000) from Northeastern Spain and Eastern Italian coasts, respectively. Likewise, PCBs 153, 138, 180 were the dominant congeners also in marine turtles *Caretta caretta* from the Adriatic Sea (Storelli and Marcotrigiano, 2000). This indicates that the biota in Italian coastal waters are exposed predominantly to higher chlorinated PCB formulations. Also sediments from the Adriatic Sea contained a high proportion of more chlorinated PCBs with the pattern resembling Aroclor 1260 formulation (Galassi *et al*., 1993). The richness of a PCB isomer in a given organism is the result of the composition of PCBs present in the environment modulated by the decomposition processes that occur in the organism. Biotransformation of PCBs in animals can be explained as the ratio of a PCB concentration congener to that of a more persistent congener. PCB-153, extremely persistent, is a good indicator of biological alteration of PCBs (Tanabe and Tatsukawa, 1983; Barrie *et al*., 1992; Kannan *et al*., 1995). This ratio in the liver of sharks showed (Fig. 1) that the metabolic efficiencies between the two species studied were similar. In fact, both species were capable of metabolizing the lower chlorinated PCBs, in accordance with the results of Serrano *et al*. (1997, 2000), while PCBs 118, 138 and 180 were the most persistent in both species. On the other hand, studies on the structure–activity relationship suggest that the biotransformation of PCB congeners depends on the presence/absence of H atoms in the molecule and their positions (Boon *et al*., 1989; Kannan *et al*., 1995); on this basis PCBs 118, 138 and 180 belong to the group of difficult to metabolize congeners because PCB180 does not possess adjacent H atoms neither *meta-para* nor *ortho-meta* positions, while PCBs 118 and 138 only possess adjacent H atoms in *ortho-meta* positions.

The toxic equivalent factors (TEFs) developed by van den Berg *et al*. (1998) for fish were used to calculate the 2,3,7,8-TCDD toxic equivalent (TEQs) values of mono- and non-*ortho* PCBs in muscle, liver and eggs of sharks (Table 2), an important tool to estimate the risk for the organisms. The mean total 2,3,7,8-TCDD equivalents of six coplanar PCBs in *C. granulosus* and *S. blainvillei* was 197 and 166 pg/g in liver and 100 and 45 pg/g in eggs, respectively. In muscle tissue of *C. granulosus* species the TEQs values were 5 pg/g, while in *S. acanthias* were negligible. The isomers with higher TEQs values were non-*ortho* congeners than mono-*ortho* ones in liver and eggs of both species. Among non-*ortho* the PCB 126 was the major contributing individual of the total TEQs in both species, while the mono-*ortho* contributed to the toxicity with values of the same order of magnitude.

The fate and effects of these pollutants are largely governed by the presence of various xenobiotic metabolizing enzymes in the organisms. Among them, the cytochrome P 450 mono-oxygenase system and conjugating enzymes are the two most important multi-enzymatic systems involved in the metabolism and detoxication of xenobiotic compounds (García *et al*., 2000). Because these enzymes are mainly localized in liver, an organ where contaminants tend to concentrate and undergo biotransformation, it seemed interesting to compare the relative contributions by non- and mono-*ortho* polychlorinated biphenyls only to 2,3,7,8-TCDD toxic equivalents in liver of the sharks to those in the same organ of other marine organisms (Fig. 2). As shown in Fig. 2, the mono-*ortho* congeners contributed with a high percentage in dolphins, while in the case of pygmy sperm whale, non-*ortho* congeners were the major contributors of the TEQs. The pattern observed in the liver of sharks was rather different from that reported in dolphins but exhibited some similarity to that of pigmy sperm whale. Nevertheless the discrepancies between the pattern of TEQ composition of our samples and those of other species could be attributed to species-specific metabolism. For example, biochemical and immunochemical analyses in cetaceans have revealed a high activity of enzymes responsible for the biotrans-

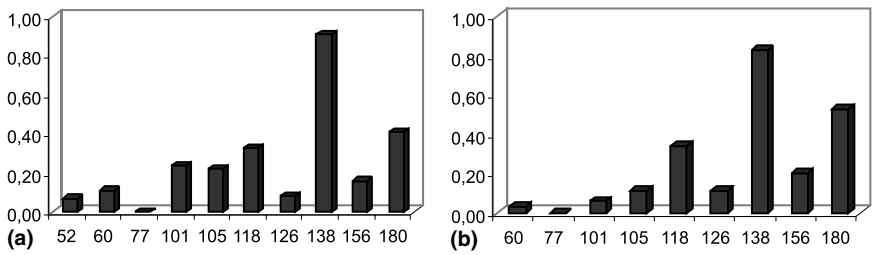

Fig. 1 X/153 ratios in liver of *C. granulosus* (a) and *S. blainvillei* (b).

TABLE 2

Mean concentrations of mono- and non-*ortho* coplanar PCBs (ng/g wet weight) and their 2,3,7,8-TCDD toxic equivalents (TEQs pg/g wet weight) in muscle, liver and eggs of *C. granulosus* and *S. blainvillei*.

C. granulosus		Muscle		Liver		Eggs	
Mono-*ortho*	TEF	Conc.	TEQs	Conc.	TEQs	Conc.	TEQs
118	0.000005	3.5	0.02	165.8	0.83	38.8	0.20
105	0.000005	1.5	–	109.5	0.55	23.6	0.12
156	0.000005	ND	–	78.3	0.39	18.8	0.10
Total		5	0.02	353.6	1.77	81.2	0.42
Non-*ortho*							
77	0.0001	0.4	0.04	0.8	0.08	0.4	0.04
126	0.005	1.0	5	38.8	195	20.2	100
169	0.00005	T.	–	T.	–	T.	–
Total		1.4	5	39.6	196	20.6	100
Total		6.4	5.1	393.2	197	101.8	100
S. blainvillei							
Mono-*ortho*	TEF	Conc.	TEQs	Conc.	TEQs	Conc.	TEQs
118	0.000005	1.5	–	103.3	0.50	21.3	0.10
105	0.000005	ND	–	35.1	0.18	10.2	0.05
156	0.000005	1.5	–	62.2	0.31	14.2	0.07
Total		3.0	–	200.6	0.99	45.7	0.22
Non-*ortho*							
77	0.0001	0.5	0.05	0.7	0.07	0.4	0.04
126	0.005	1.0	0.07	32.7	165	9.0	45
169	0.00005	T.	–	T.	–	T.	–
Total		1.5	0.12	33.4	165.1	9.4	45
Total		4.5	0.12	234	166	55.1	45.3

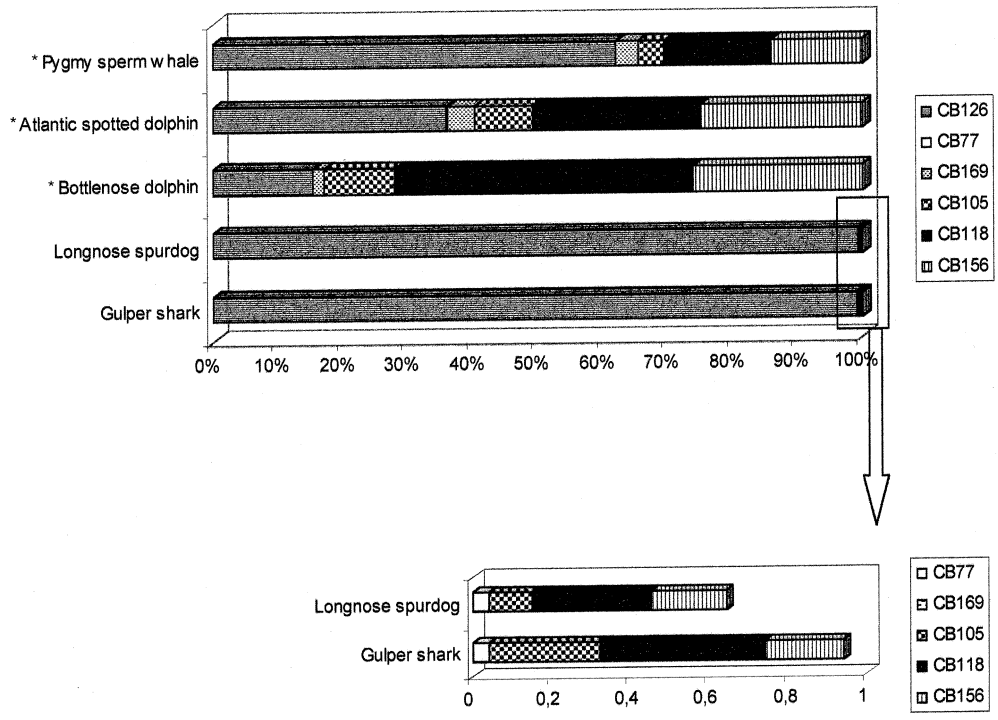

Fig. 2 Comparison of relative contributions of non- and mono-*ortho* polychlorinated biphenyls (PCBs) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) in shark and other high trophic animals. (*) Data cited from Watanabe *et al.* (2000).

formation of non-*ortho* congeners 77, 126 and 169, and a scarce activity of enzymes that have a potency to degrade mono-*ortho* congeners 105, 118 and 156 (Tanabe *et al.*, 1988; Watanabe *et al.*, 1989). Much smaller enzyme activities to metabolize PCBs were also observed in Dall's porpoise than in large seal (Tanabe *et al.*,

1988). In our samples the non-*ortho* PCB concentration patterns (126 > 77 > 169) because divergent from those of non-*ortho* coplanar PCBs characteristic of commercial mixture (77 > 126 > 169) and typical of healthy populations (Watanabe *et al.*, 2000) may suggest a certain detoxifying activity even if, the depletion of PCB 77 can be more likely attributed to its higher hydrophilic properties resulting in its easier degradation. Generally, to indicate whether there has been an enzyme induction following exposition to high levels of PCBs PCB77/PCB169 and PCB126/PCB169 concentration ratios were used. In our case, the presence of PCB 169 at levels of traces made it impossible to draw any definitive conclusions on this. However, considering that the major contributors to total TEQ are non-*ortho* chlorine substituted PCBs, responsible for toxic syndromes such as hepatic damage, reproductive abnormalities and immunotoxicity, implication for the disturbances in normal physiologic processes could occur in these species.

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