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Coplanar Polychlorinated Biphenyl Congeners in the Liver of *Galeus melastomus* from Different Areas of the Mediterranean Sea

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The group of polychlorinated biphenyls (PCBs) comprise 209 synthetic compounds, reflecting a different chlorination with respect to the number and the position on the two coupled biphenyl rings. Several studies have shown that these compounds exhibit a broad range of toxicological responses including immunotoxicity, reproductive deficits, teratogenicity, endocrine toxicity and carcinogenity/tumor promotion (Ahlborg et al. 1994). In recent years attention has been focused on some polychlorinated biphenyls, especially on those congeners so-called "coplanar" that are the most toxic members of the PCB family.

Studies on the structure-activity relationships (Boon et al. 1989) suggested that non-*ortho* chlorine substituted PCBs, especially, 3,3',4,4'-tetra- chlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl, and 3,3',4,4',5,5'-hexachloro-biphenyl exhibit the highest toxicity, followed by the moderately toxic mono-*ortho* coplanar congeners, as they are able to adopt a planar configuration which makes them behave in a similar fashion to the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

The determination of polychlorinated biphenyls in fish species representative of different regions may provide relevant information on pollution sources, transport pathways, spatial distributions and fate in the marine environment. Moreover, the speciation of polychlorinated biphenyls in the organisms is essential for a better assessment of the toxic potential. The Toxic Equivalency Factor (TEFs) method (Ahlborg et al. 1994, Van den Berg et al. 1998) constitutes an important indirect tool to assess the toxic pollutant load and consequently the risk for exposed organisms.

Previous studies determined the presence of contaminants in *Galeus melastomus* from different areas of the Mediterranean Sea (Storelli and Marcotrigiano 2002). This study describes isomer-specific accumulation of PCBs in the liver of this species. Furthermore, 2,3,7,8-TCDD of mono- and non-*ortho* coplanar congeners were estimated (TEQs), using the toxic equivalents factors (TEFs) based on fish toxicity data according to Van den Berg et al. (1998), in order to assess the relative ecotoxicological impact of highly toxic PCBs in *Galeus melastomus*.

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MATERIALS AND METHODS

From June to September 1999, 450 specimens of *Galeus melastomus* (black-mouthed dogfish) were caught in the eastern Mediterranean Sea. Table 1 shows the provenience, the number of specimens, their range of length and weight and the number of pools. The specimens were grouped in different pools according to weight and length. The livers of the specimens constituting each pool were taken and preserved at -25 °C until analyzed.

To determine chlorobiphenyl (PCBs = sum of 17 congeners), concentrations the following method was used. Aliquots (3 g) of the homogenised 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 conc. H_2SO_4 for clean-up, following the procedure described by Murphy (1972). After centrifugation, the hexane solution was concentrated (about 1 ml) and transferred to 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 the most PCBs, whereas the second fraction, eluted with 10 ml of 15% ethylether in hexane, contained the remaining PCBs and other organochlorine compounds.

An aliquot of the 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_4CB, (IUPAC 77), 3,3',4,4', 5-P_5CB (IUPAC 126), and 3,3',4,4',5,5'-H_6CB (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).

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. The individual PCB congeners were 8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209 determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%).

Recoveries were determined by adding known amounts of PCB standards (at three concentration levels) 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 wet wt for the PCB congeners. 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 are presented as ng/g on a wet weight basis. Statistical significance was assessed

Table 1. Biometric data and specimen number of *Galeus melastomus*.

Provenience	n	n° pools	Range length (cm)	Range weight (g)
Adriatic Sea (Italy)	104	11	24.9-55.3 45.6±8.6	41.6-440.3 272.4±119.5
Adriatic Sea (Albania)	59	10	19.3-50.5 39.2±9.8	33.5-319.5 179±92.2
Ionian Sea (Italy)	165	18	12.6-52.1 33.3±12.7	6.0-395.7 140±117.2
Aegean Sea (Greece)	122	18	18.8-63.0 39.8±12.1	16.2-547.3 178.5±149.1

n = number of individual sharks.

using Student's *t*-test.

RESULTS AND DISCUSSION

The concentrations of total PCBs in the liver of *G. melastomus* are presented in Table 2. As can be seen, the concentration ranges, among the specimens from the different areas of the Mediterranean Sea, did not differ greatly, in fact no significant differences in levels of contamination were observed ($p>0.05$). Specimen weight-dependent accumulation of PCBs (Adriatic Sea-Italy: $r^2=0.77$, $p<0.0001$; Adriatic Sea-Albania: $r^2=0.79$, $p<0.0001$; Ionian Sea-Italy: $r^2=0.69$; $p<0.0001$; Aegean Sea-Greece: $r^2=0.74$, $p<0.0001$) were found.

In the specimens from the Adriatic Sea-Italy and the Aegean Sea-Greece, the polychlorinated biphenyl congener profiles showed a high proportion of more chlorinated PCBs. In the Adriatic Sea-Italy samples, the percentage composition of total PCBs included 52.4% hexa-, 18.1% penta-, 14.2% hepta-, 9.4% tri-, 3.0% tetra-, and 2.9% decachlorobiphenyls. In the sharks from the Aegean Sea-Greece, a similar percentage, with 53.5% hexa-, 18.1% penta-, 11.0% hepta-, 7.7% tri-, 5.7% deca-, and 4.0% tetrachlorobiphenyls, was observed.

The profiles relative to the specimens from the other two marine areas were differently characterized. In detail, for the samples from the Adriatic Sea-Albania, hexa- and tri- accounted for 50.0% and 19.6% of the total PCB concentrations respectively, followed by penta- and hepta, with a contribution of 16.3% and 9.1%, respectively, while both tetra- and decachlorobiphenyls showed percentages of 3.5%. In liver of the samples from the Ionian Sea-Italy, hexa- constituted 44.9%, followed by tri- 19.8%, and by pentachlorobiphenyls with 16.6%. The composition of the other isomers was in the following order: hepta- 8.9%, deca- 5.7%, and tetrachlorobiphenyls 4.0%. Among hexa-, the predominant isomer class, the congener with the highest concentration in all samples was PCB 153, followed by PCB 138 together accounting for from 38.0 % to 45.8 % of the total PCB concentrations.

Table 2. Range and mean concentrations of total PCB and mono- (ng/g wet weight) and non-*ortho* (pg/g wet weight) coplanar PCBs and their 2,3,7,8-TCDD toxic equivalents (TEQs pg/g wet weight) in liver of *G. melastomus* from different marine areas.

Provenience	Adriatic Sea (Italy)	Adriatic Sea (Albania)	Ionian Sea (Italy)	Aegean sea (Greece)
Total PCB concentrations	367-1829 1072±387	447-1690 853±471	427-1840 798±395	412-1608 793±336
Mono-*ortho* PCB concentrations				
PCB 105	10-61 35±15	10-50 23±13	11-71 24±16	11-45 25±11
PCB 118	21-165 88±40	23-138 62±42	24-157 53±38	27-117 62±30
PCB 156	ND-91 43±20	9-63 30±18	15-78 29±18	16-68 33±15
Non-*ortho* PCB concentrations				
PCB 77	T-0.7 0.5±0.1	T-0.7 0.5±0.1	T-2.1 0.8±0.4	T-0.8 0.5±0.1
PCB 126	0.6-4.4 1.8±1.0	0.6-3.1 1.5±0.9	0.6-4.8 1.6±1.2	0.8-3.7 1.8±0.9
PCB 169	0.7-10.7 3.1±2.8	0.7-3.8 1.8±1.0	0.9-5.4 2.7±1.3	1.6-5.6 3.1±1.2
Mono-*ortho* TEQ				
PCB 105	0.05-0.31 0.18	0.05-0.25 0.12	0.06-0.36 0.12	0.06-0.23 0.13
PCB 118	0.11-0.83 0.44	0.12-0.69 0.31	0.12-0.79 0.27	0.14-0.59 0.31
PCB 156	0.06-0.46 0.20	0.05-0.32 0.15	0.08-0.39 0.15	0.08-0.34 0.17
Non-*ortho* TEQ (pg/kg)				
PCB 77	T-0.07 0.05	T-0.07 0.05	T-2.10 0.08	T-0.08 0.05
PCB 126	3.0-22.0 9.0	3.0-15.5 7.5	3.0-24.0 8.0	4.0-18.5 9.0
PCB 169	0.03-0.54 0.16	0.03-0.19 0.09	0.05-0.27 0.14	0.08-0.28 0.16
Total TEQ	0.83	0.55	0.59	0.62

T= traces

In specimens from the Adriatic Sea-Italy and the Aegean Sea-Greece, other chlorobiphenyls found in major amounts, included PCB congeners 118 and 180, with a contribution of 8.2% and 9.4% and of 7.9% and 11%, respectively by site. In the samples from the other two areas, the other congener present in major amount was PCB 20, accounting for 18.6 % in specimens from the Ionian Sea-Italy and 18.2% in those from the Adriatic Sea-Albania.

In agreement with our data, several authors reported hexa-, penta-, and heptachlorobiphenyls as the dominant components in marine organisms belonging to different classes such as reptiles (Corsolini et al. 2000), mammals (Storelli and Marcotrigiano 2000), teleosts (Bayarri et al. 2001), and elasmobranch fish (Storelli and Marcotrigiano 2001) from the Adriatic Sea, suggesting that the biota in Italian Adriatic Sea waters are exposed predominantly to more chlorinated PCB formulations. Also the prevalence of congeners PCB 153 and PCB 138 encountered in the organisms in question is in accordance with that reported in literature (Bayarri et al. 2001; Storelli and Marcotrigiano 2001). On the other hand the major presence of these congeners is a direct consequence of their molecular structure. The biotransformation of PCB congeners, in fact, depends on the presence/absence of H atoms in the molecule and their positions (Boon et al. 1989); these compounds do not have adjacent H atoms in *ortho-meta* positions, and therefore belong to the group of difficult to metabolize congeners.

Of particular interest, in the sharks from the Ionian Sea-Italy and in those from the Adriatic Sea-Albania was that the polychlorinated biphenyl congener profile was rich in trichlorobiphenyls. Differences in the relative abundances of the various species in the *G. melastomus* diet (i.e. crabs, molluscs and little fish) at various localities, together with the different prey ability to absorb these contaminants, could contribute to explain this peculiar congener profile. Porte and Albaiges (1993), in a paper on polychlorinated biphenyls compounds in mollusc bivalves, crabs and fish, have reported that the congener bioaccumulation pattern varied substantially among species. Also it may be hypothesized that these marine areas were influenced by different contaminant sources, with those having higher industrial activity being richer in heavy congeners than the ones with less developed industry in which low chlorinated PCB formulations predominated.

Individual PCB congeners differ greatly in their toxic potency, with non-*ortho* congeners constituting the most toxic compounds, thereby showing effects comparable to those reported for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin through a proposed common mechanism of action (Ahlborg et al. 1994). Generally, in unexposed individuals the order of non-*ortho* PCB coplanar concentrations is PCB 77>PCB 126> PCB 169. This pattern resembling those of commercial mixtures, denotes low or non-existent detoxifying metabolic activity (Tanabe et al. 1988). In fact severe induction of drug metabolizing enzymes, due to very high PCB concentrations, results in the breakdown of the more biodegradable PCB 77 and PCB 126. In the specimens analysed in this study, the order of PCB coplanar non-*ortho* concentrations was PCB 169> PCB 126>PCB 77 (Table 2). This

pattern, together with low values for PCB77/PCB169 and PCB126/PCB169 ratios, suggested a detoxifying activity (Adriatic Sea-Italy: PCB77/PCB169=0.16, PCB126/PCB 169=0.58; Adriatic Sea-Albania: PCB77/PCB169=0.28, PCB 126/ PCB169=0.83; Ionian Sea-Italy: PCB77/PCB169=0.29, PCB126/PCB169=0.59; Aegean Sea-Greece: PCB77/PCB169=0.16, PCB126/PCB 169=0.58). In fact, low values of such ratios are assumed to induce hepatic microsomal enzymes following exposure to high levels of PCBs. For example, in the case of humans exposed to very high PCB concentrations, such as "Yusho poisoning victims", the PCB77/PCB169 and PCB126/169 ratios were 1.8 and 1.9, respectively, while in healthy people they were 3.8 and 3.9 (Tanabe et al. 1989). Also, Corsolini et al. (2000) in marine turtles reported high values of the above mentioned ratios, suggesting the absence of enzymatic activity.

Fish-specific 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin toxic equivalency factors developed by Van den Berg et al. (1998) were used to estimate the TEQs of non- and mono-*ortho* PCBs in the liver of *G. melastomus* (Table 2). The mean total 2,3,7,8-TCDD equivalents of 6 coplanar PCBs ranged from 0.55 pg/g in specimens from the Adriatic Sea-Albania to 0.83 pg/g in those from the Adriatic Sea-Italy. The isomers with the highest TEQ values were mono-*ortho* congeners, contributing >99% of the total toxicity, whereas the contribution of non-*ortho* was extraordinarily low, below 1%. In fact non-*ortho* PCB congeners represented only a small percentage (Adriatic Sea-Italy: 4.9%; Adriatic Sea-Albania: 4.1%; Ionian Sea-Italy: 6.1%; Aegean Sea-Greece: 6.6%) of the total PCB load in these fish. However, it is noteworthy that among non-*ortho* members, the coplanar PCB 126 was the major responsible for their toxicity. In the liver of *Centrophorus granulosus* and of *Squalus blainvillei* from the south Adriatic Sea, Storelli and Marcotrigiano (2001) reported mono- and non-*ortho* congeners toxic equivalent values of 197 pg/g and 166 pg/g, respectively. Serrano et al. (1997) in the liver of different species of sharks found TEQ values from 0.38 pg/g to 287 pg/g. It is apparent from the above comparisons that our TEQ values fit this general picture well.

PCB concentrations in our specimens were not elevated, but the trend of non-*ortho* congeners and the low values of PCB77/PCB169 and PCB126/PCB169 ratios, suggest that this species should be frequently monitored since the risk threshold levels for the organisms in question are unknown. The necessity of a constant control is also supported by the fact that TEQ values account for only the toxicity of the six coplanar PCB and do not consider other contaminants to which the organisms are always exposed to.

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