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Residue levels of DDTs and toxic evaluation of polychlorinated biphenyls (PCBs) in *Scyliorhinus canicula* liver from the Mediterranean Sea (Italy)

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Organochlorine compounds, such as polychlorinated biphenyls (PCBs) and DDT compounds (DDTs), are important components of the chemical pollutants found in all parts of the marine environment. They are potentially hazardous to living systems because of their inclination to bioaccumulate in the lipid component of biological species and their resistance to degradation.

Prolonged exposure to these pollutants can interfere with normal physiology and biochemistry of the marine organisms. It is, in fact, well-known 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).

The occurrence and severity of the toxic effects depend on various factors, such as the level of pollutants in the organism, the susceptibility of the species and the duration of exposure. In the last decade, particular emphasis has been placed upon the toxicity of some PCB congeners, the so-called "coplanar" or "dioxin-like" PCBs, which may adapt a planar conformation and activate the aryl hydrocarbon (Ah) receptor. These compounds are thought to share a common mode of toxic action with dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin,TCDD), the most toxic member of the planar chlorinated hydrocarbons.

There is an extensive amount of literature reporting the levels of these contaminants in marine organisms occupying higher trophic levels, such as dolphins (Watanabe et al., 2000; Storelli and Marcotrigiano, 2000a), whales (Gauthier et al., 1998; Ross et al., 2000), and seals (Tarasova et al., 1997), whereas comparatively little data are available for elasmobranch fish (Serrano et al., 1997, 2000), especially from the Mediterranean Sea (Storelli and Marcotrigiano, 2001; Storelli et al., 2003a, 2004).

With respect to these literature findings, this study investigated the accumulation profile of individual PCB congeners and the levels of DDTs and its metabolites in the liver of a small cartilaginous fish, *Scyliorhinus canicula*, from the Mediterranean Sea. In addition, 2,3,7,8-TCDD equivalency of the mono- and non-*ortho* coplanar PCB congeners

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were estimated (TEQs), using the fish toxic equivalents factors (TEFs) of Van den Berg et al. (1998), in order to assess the relative impact of these highly toxic PCBs in species examined.

From June 2000–August 2002, 156 specimens of small spotted dogfish (*Scyliorhinus canicula*) (length: 36.5–49.0 cm, average 44.0 cm; weight: 140.0–501.6 g, average 304.2 ± 108.9) were caught in the South Adriatic Sea. Fish were gathered in 11 pools as a function of their similar size. From the specimens of each pool livers were taken and kept in a deep freeze at −20 °C until chemical analysis. To determine chlorobiphenyl (PCBs = 8, 20, 28, 35, 52, 60, 77, 101, 118, 126, 138, 153, 156, 169, 180 and 209) and DDT compound (DDTs = *p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDD) concentrations the following method was used. Aliquots (3 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 in a rotary evaporator and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot (about 100 mg) of the remaining extract was dissolved in hexane (5 ml) and mixed with concentrated H_2SO_4. For the clean-up, the procedure described by Murphy (1972) was followed. After centrifugation, the hexane solution was concentrated in a Kuderna–Danish apparatus to 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. Two fractions were collected. The first fraction was eluted with hexane (10 ml) and contained most of the PCB congeners and some DDTs. The second fraction was eluted with 10 ml of a 15% ethylether in hexane solution and contained the remaining DDTs.

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* PCBs 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: 350 °C).

The GC was connected to an PC-Pentium III IBM equipped with Chrom-Card version 1.2 software program for integration purposes (C. Erba). For all the analyses, a fused-silica capillary column DB 5 Supelco (length = 30 m, inside diameter 0.25 μm) was used. The reference material employed was BCR 349 (PCBs) and CRM 598 (DDTs) (cod liver oil). The recovery for each PCB and for organochlorine pesticides (DDT) quantified in the certified material (PCB 28, 52, 101, 118, 138, 153, 180; *p*,*p*′-DDT, *p*,*p*′-DDE, *o*,*p*′-DDD, *p*,*p*′-DDD) ranged from 91% to 102%. The recoveries for the other PCB congeners and for *o*,*p*′-DDT, varying between 92% and 100% were determined by adding known amounts of standards, at three levels of concentrations, to empty samples before extraction (method of additions). The individual PCB congeners and DDT group compounds were determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%) and Supelco, respectively. The identity of the DDT compounds was confirmed by an alkali conversion to their respective olefins and reanalysis by GLC. The limits of quantification were from 0.1 to 0.4 ng g^{-1} on a wet weight basis for the PCB congeners and DDTs. Quantification was done within the linear range of the detector. Non-detected constituents were assigned a value of zero. Residues in 100% of the samples were confirmed by gas–liquid chromatography-mass spectrometry (Fisons MD 800). Concentrations of PCBs and DDTs are presented as ng g^{-1} on lipid weight basis.

The concentrations of total PCB and DDT (ng g^{-1} lipid weight) in the liver of small spotted dogfish are presented in Tables 1 and 2. PCBs and DDTs were in similar ranges of values, varying from 500 to 2351 ng g^{-1} (average 1292 ng g^{-1}) and from 347 to 1875 ng g^{-1} (average 1171 ng g^{-1}), respectively.

Table 1
Minimum and maximum and mean concentrations (ng g^{-1} lipid weight) of individual PCB congeners in small spotted dogfish liver from the Mediterranean Sea

Congeners	Min	Max	Mean	SD
Lipid (%)	17.3	61.2	43.7	12.8
Tetrachlorobiphenyls				
PCB 52	ND	117.0	35.8	44.9
PCB 77	0.002	0.007	0.003	0.002
Pentachlorobiphenyls				
PCB 101	ND	81.0	39.0	23.7
PCB 105	ND	86.0	32.6	24.4
PCB 118	37.0	199.0	106.7	51.2
PCB 126	ND	0.049	0.018	0.019
Hexachlorobiphenyls				
PCB 138	119.0	736.0	380.9	185.2
PCB 153	160.0	937.0	495.1	243.7
PCB 156	ND	55.0	6.4	16.8
Heptachlorobiphenyls				
PCB 180	52.0	384.0	195.6	105.1
Σ PCB	500.0	2351.0	1292.1	576.8

ND = non-detected.

Table 2
Minimum and maximum and mean concentrations (ng g^{-1} lipid weight) of individual DDT compounds in small spotted dogfish liver from the Mediterranean Sea

	Min	Max	Mean	SD
p,*p*′-DDT	ND	96.0	34.5	33.3
p,*p*′-DDE	317.0	1648.0	1019.9	409.5
p,*p*′-DDD	ND	83.0	20.1	34.5
o,*p*′-DDT	ND	251.0	68.4	67.1
o,*p*′-DDD	13.0	49.0	28.1	9.3
Σ DDT	347.0	1875.0	1170.9	471.0