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Isomer-specific Analysis of Polychlorinated Biphenyls in Harbour Porpoise (*Phocoena phocoena*) from the Black Sea

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The highly toxic coplanar PCBs and other isomers were determined in harbour porpoises and fish (porpoise diet) collected from the Turkish coastal water of the Black Sea, during 1993. The concentrations of total PCBs were found in the range of 5.0–34 μg g^{-1} wet wt in the blubber of porpoises. Highly chlorinated members such as IUPAC Nos 138, 153 and 180 were the dominant congeners, consisting of 41% of the total PCB concentrations. The mean total 2,3,7,8-TCDD toxic equivalents (TEQs) of 13 coplanar PCBs including non-, mono- and di-*ortho* congeners were 1400 pg g^{-1} wet wt in the blubber of males and 300 pg g^{-1} wet wt in females. The IUPAC No. 118 was the most contributing congener occupying about 60% of the total TEQs. The most toxic non-*ortho* chlorine substituted coplanar PCBs such as IUPAC No. 77, 126 and 169 were minor contributors and accounted for 7.8, 4.2 and 0.7%, respectively, of the total TEQs. The activities of PB and MC-type enzymes were found to be low in Black Sea harbour porpoises, suggesting long term accumulation and possible toxic effects of PCBs in this species. © 1997 Elsevier Science Ltd

Keywords: PCBs; coplanar PCBs; Black Sea; harbour porpoise; TEQs.

Polychlorinated biphenyls (PCBs) are global pollutants of concern, in particular to marine mammals which bioaccumulate these chemicals at extraordinarily high concentrations and have the effects of mass mortalities, reproductive dysfunction etc. (Tanabe *et al.*, 1994; Colborn and Smolen, 1996). Small cetaceans possess a low metabolic capacity to degrade toxic organochlorines and thus they retain them in large quantities in their body (Tanabe *et al.*, 1988). As an example, extremely high concentrations of PCBs were found in striped dolphins from the Mediterranean Sea and these were shown to have played a pivotal role as a trigger of viral infection (Borell and Aguilar, 1992; Kannan *et al.*, 1993). PCBs have also been suspected to impair the reproductive capacity in marine mammals (Martineau *et al.*, 1987).

Among 209 PCB isomers and congeners, some members attain coplanarity and elicit toxic biological effects similar to 2,3,7,8-TCDD (Safe, 1990). The detection of toxic coplanar PCBs in cetaceans was first reported in dolphins and whales from the western North Pacific (Tanabe *et al.*, 1987a). Since this finding, many studies have indicated the occurrence of coplanar PCBs in a wide range of cetacean species and assessed toxic potential of PCBs using TEQ approach (Kuehl *et al.*, 1991, 1994; Muir *et al.*, 1992; Ford *et al.*, 1993; Kannan *et al.*, 1993; Falandysz *et al.*, 1994; Corsolini *et al.*, 1995; Jarman *et al.*, 1996). In spite of several reports on isomer-specific analysis of PCBs in cetaceans, the data for harbour porpoises (*Phocoena phocoena*) is scarce (Duinker *et al.*, 1989; Morris *et al.*, 1989; Kleivane *et al.*, 1995; Jarman *et al.*, 1996). In our earlier study, extremely high concentrations of organochlorine residues were found in harbour porpoises from the Black Sea (Tanabe *et al.*, 1997), which prompted us to assess the toxic potential of coplanar PCBs by TEQ approach in this animal. The present study examines PCB residue pattern and possible toxic effects by the isomer-specific analysis of non-, mono- and di-*ortho* congeners in the blubber of harbour porpoises from the Black Sea.

Materials and Methods

Samples

Harbour porpoises and fish samples analysed in this study were collected from Yakakent and Sinop, Turkey (Fig. 1). These animals were drowned in the turbot or sturgeon trammel nets during spring 1993. The exact cause of death is unknown. Blubber samples of 2 sexually mature female and 8 sexually mature male animals of harbour porpoise were used for analysis. Available data regarding age, growth status, sex and body length are given in Table 1. The whole body of the diet (fish) of harbour porpoise such as whiting (*Merlangius merlangus euxinus*) and European anchovy (*Engraulis encrasicolus*) was homogenized individually for analysis. The samples were stored at $-20°C$ until analysis. The age of harbour porpoises was determined by counting the growth layer groups in the dentine following the procedure of Kasuya (1976).

Chemical analysis

Blubber samples were cut into fine pieces, and about 4–5 g of the sample were taken and digested with alkaline-alcohol (1N KOH) according to Wakimoto *et al.* (1971). PCBs extracted into ethanol were transferred to 100 ml of hexane by shaking in a separatory funnel. The concentrated hexane layer was cleaned on 1.5 g of silica gel (Wako gel S-1, Wako Pure Chemical Co.) packed in a glass column (10 mm i.d.×20 cm length). PCBs were eluted with 200 ml of hexane at an elution rate of 1 drop s^{-1}. The hexane eluate was concentrated to 6 ml in a Kuderna-Danish concentrator. An aliquot of 1 ml of this extract was reserved for quantification of PCB isomers and congeners other than non-*ortho* chlorine substitution (IUPAC Nos 77, 126 and 169) after cleaning with sulphuric acid. The remaining 5 ml was passed through a glass column (5 mm i.d.) packed with 125 mg of activated carbon for the separation of

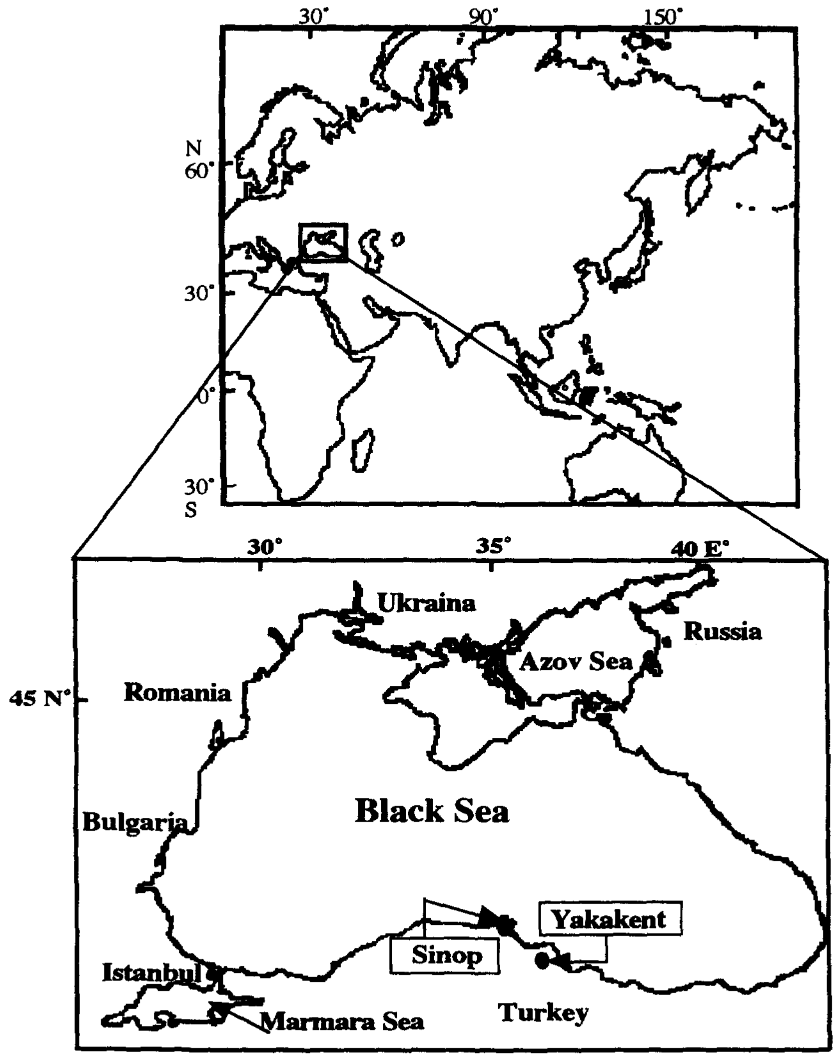

Fig. 1 A map showing sampling sites.

TABLE 1
Concentrations (μg g^{-1} wet wt) of ∑PCBs in the blubber of harbour porpoises and their diet (fish) from the Black Sea.

Sample No.	Sex	Reproductive condition	Age (GLGs)	Body length (cm)	Fat%	∑PCBs
Harbour porpoise						
1	Male	Mature	4	113.5	91	20 (22)
2	Male	Mature	6	112.0	82	21 (25)
3	Male	Mature	6	123.0	89	16 (18)
4	Male	Mature	6	125.0	82	22 (27)
5	Male	Mature	7	119.0	90	23 (26)
6	Male	Mature	8	118.5	87	34 (39)
7	Male	Mature	8	120.5	90	13 (14)
8	Male	Mature	8	121.0	80	23 (29)
9	Female	Mature	7	138.5	77	5.0 (6.4)
10	Female	Mature	10	129.5	86	5.9 (6.8)
11*	Female	Fetus		59.5	75	2.3 (3.1)
Fish			*n*		Fat%	∑PCBs
European anchovy (*Engraulis encrasicolus*)			4		6.1	0.05 (0.82)
Whiting (*Merlangius merlangus euxinus*)			2		4.1	0.14 (3.5)

Figures in parenthesis indicate the concentration on fat wt basis.
*Mother of this fetus is sample No. 9.

non-*ortho* coplanar PCBs (IUPAC Nos 77, 126, and 169) according to Tanabe *et al.* (1987b). The first fraction eluted with 20% dichloromethane in hexane (100 ml) was discarded. The second fraction eluted with benzene:ethylacetate (1:1) was collected and microconcentrated, and residues were transferred to 5 ml hexane. This was further cleaned with 5% fuming sulphuric acid in concentrated sulphuric acid.

For isomer-specific determination, gas chromatograph/mass spectrometer (Hewlett Packard 5890 GC with a 5970 mass selective detector) having an electron impact (EI) mode at 70 eV was used for the determination of PCB congeners following the methods of Tanabe *et al.* (1987c) for mono- and di-*ortho* isomers and of Tanabe *et al.* (1987b) for non-ortho coplanar PCBs. A fused silica capillary column (15 m length×0.25 mm i.d.) coated with chemically bonded DB-1 (100% dimethyl polysiloxane) and DB-1701 (14% cynopropylphenyl and 86% dimethyl polysiloxane) with film thickness of 0.225 μm were employed for the determination of PCB isomers and non-*ortho* coplanar congeners. The operating conditions were as follows: oven temperature was programmed from 70 to 260°C for DB-1 and from 160 to 260°C for DB-1701 at a rate of 2°C min^{-1}, and injector and ion-source temperatures were kept at 250 and 280°C, respectively. The carrier was helium. PCB homologues were determined by selective ion monitoring (SIM) at m/z 256–258, 290–292, 324–326, 358–360, 392–394 and 428–430 for tri-, tetra-, penta-, hexa-, hepta-, and octachlorobiphenyls, respectively. An equivalent mixture of Kanechlor 300, 400, 500, and 600 with known PCB composition and content was used as standard. For non-*ortho* coplanar PCBs, M^+ and $(M+2)^+$ cluster ions were monitored at m/z 290 and 292 for IUPAC No. 77, m/z 324 and 326 for IUPAC No. 126 and m/z 358 and 360 for IUPAC No. 169. Authentic standards of >98% purity were used for determination of these non-*ortho* coplanar PCBs. Recoveries of total PCBs and non-*ortho* chlorinated coplanar congeners (IUPAC 77, 126, 169) were examined by following the whole analytical procedure, spiking 7.5 μg and 100, 227 and 190 ng of standards, respectively, in corn oil and those values were 97–107% for non-*ortho* coplanar PCBs and 99–106% for other PCB isomers. Because of the smaller number of samples, it was not examined by a statistical method for the signifcance of obtained data in the present study.

Results and Discussion

Contamination status and composition

The residue levels of total PCBs in the Black Sea porpoises were in the range of 5.0–34 μg g^{-1} on a wet wt basis and 6.4–39 μg g^{-1} on a fat wt basis (Table 1). These levels are comparable to those in common porpoises (23–42 μg g^{-1} on a wet wt basis) from Puck Bay, Baltic Sea (Falandysz *et al.*, 1994), and slightly lower than harbour porpoises from the North Sea (47–160 μg g^{-1} fat wt) (Duinker *et al.*, 1989) and the western North Atlantic (47–79 μg g^{-1} wet wt) (Gaskin *et al.*, 1983).

Noticeable levels of PCBs in the present samples indicate the input of these contaminants from surrounding countries of the Black Sea. Some of the Eastern European countries like the former USSR produced PCBs (Sovol) for use as a dielectric fluid in the manufacture of power capacitors and transformers (Ivanov and Sandell, 1992). The usage of PCBs in the former USSR is evident from significant contamination in air, water, sediments, soil, fish and seal from the Lake Baikal region (Kucklick *et al.*, 1994; Iwata *et al.*, 1995; Nakata *et al.*, 1995). The usage pattern and major source of PCBs in other surrounding countries of the Black Sea are still unclear.

The concentrations of total PCBs were lower in mature females than in mature males (Table 1). The isomer and congener compositions of PCBs in males and females are described in Fig. 2. The highly chlorinated PCBs of IUPAC Nos 138, 153 and 180 were the dominant congeners accounting for 41% of the total PCBs. Compared to males, females revealed reduced percentage of lower chlorinated PCBs. This may be due to the lactational/gestational transfer of PCBs to the young ones (Subramanian *et al.*, 1987).

The composition of PCB isomers and congeners in female porpoise and in the fetus is shown in Fig. 3. In the fetus, the composition of tri-, tetra- and penta-chlorobiphenyls were higher than in its mother, while heptachlorobiphenyls and octachlorobiphenyls were relatively lower. This result indicates that the lower

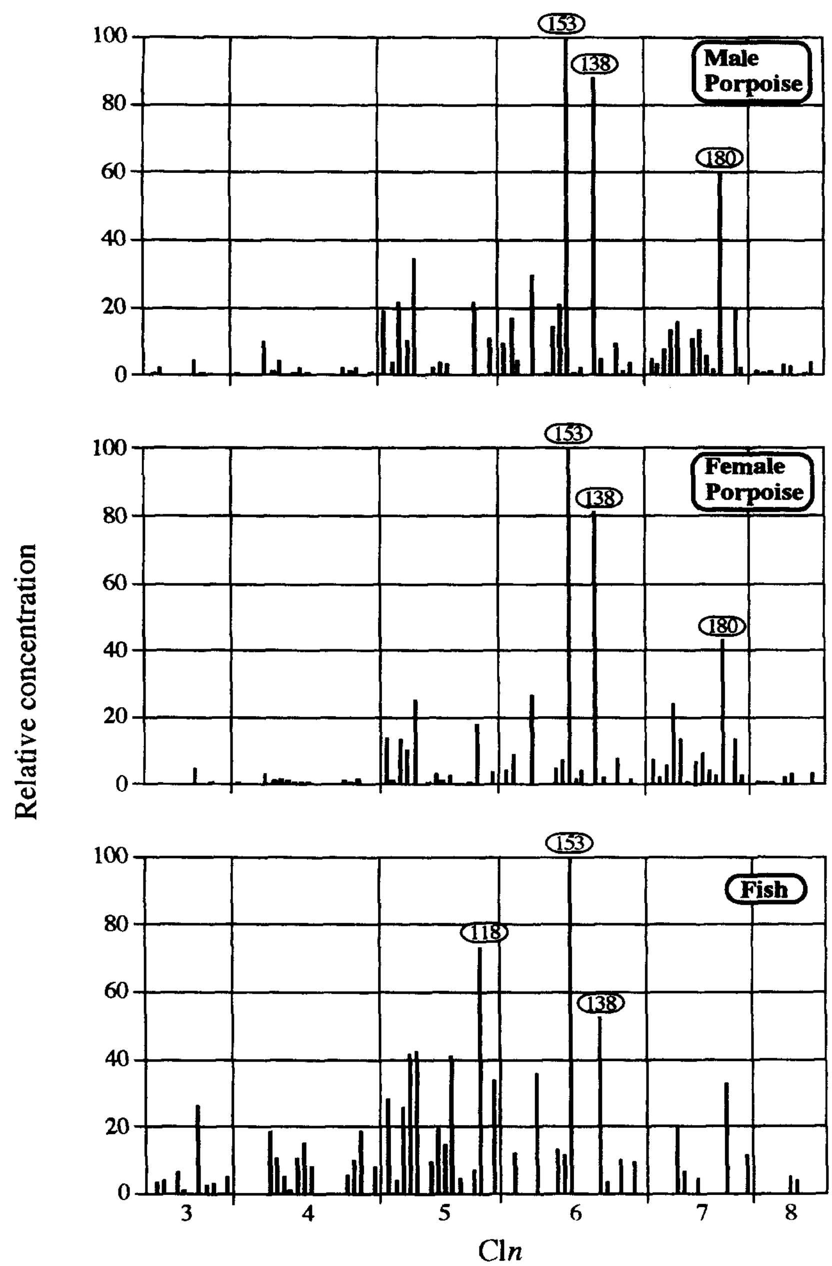

Fig. 2 PCB isomer and congener compositions in the blubber of male and female harbour porpoises and whole body of fish from the Black Sea. Vertical bars represent concentrations of individual PCB congeners relative to the most abundant congener (IUPAC No. 153), the latter being assigned to a relative concentration of 100. For further details of PCB congeners and their structures, see Tanabe *et al.* (1987c)

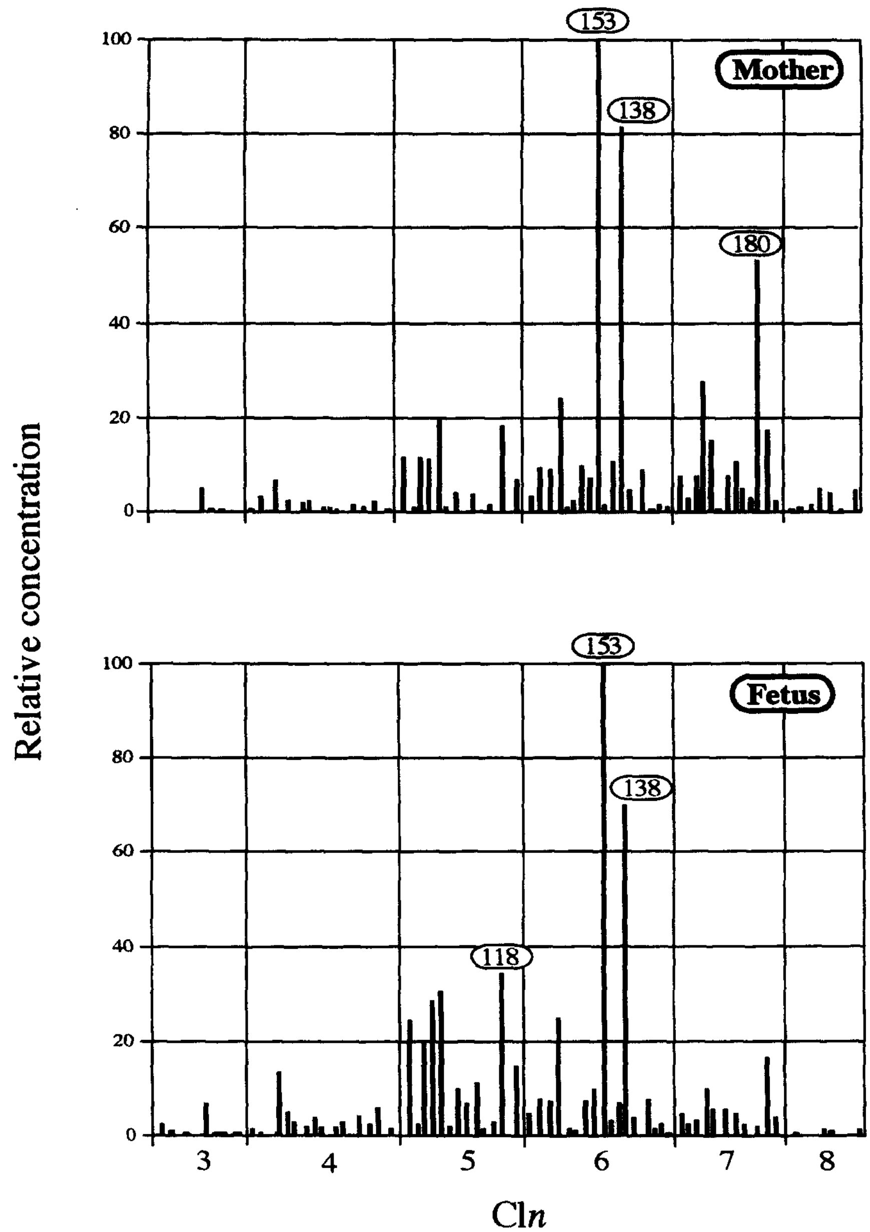

Fig. 3 PCB isomers and congeners patterns in the blubber samples of the mother and fetus of harbour porpoises from the Black Sea. For further detailed information of PCB congeners and their structures see Tanabe *et al.* (1987c).

chlorinated members are readily transferrable from mother to fetus through the placenta.

The composition of PCB isomers and fish samples is given in Fig. 2. The three predominant PCB congeners in fish were IUPAC Nos 118, 138 and 153, collectively recording 27% of the total concentration of PCBs. The composition of tri-, tetra- and pentachlorobiphenyl congeners were larger in fish than in porpoises, indicating that porpoises have the capacity to degrade lower chlorinated congeners.

Metabolic capacity

In order to elucidate the degradation of PCBs in porpoises, Metabolic Index (MI) values of PCB isomers and congeners were calculated according to Tanabe *et al.* (1988):

$MI_i = \text{Log } CR_{180}\text{-Log } C_i$

Where MI_i = Metabolic Index of PCB isomer *i*

CR_i = Concentration ratio of PCB isomer *i* in porpoise to those in fish

CR_{180} = Concentration ratio of PCB isomer *180* in porpoise to those in fish

(*i* & *180* are IUPAC No. of PCBs)

The estimated values of MI in harbour porpoises from the Black Sea and in striped dolphins from the western North Pacific (Tanabe *et al.*, 1988) are given in Fig. 4. The isomer with greater MI value indicates its

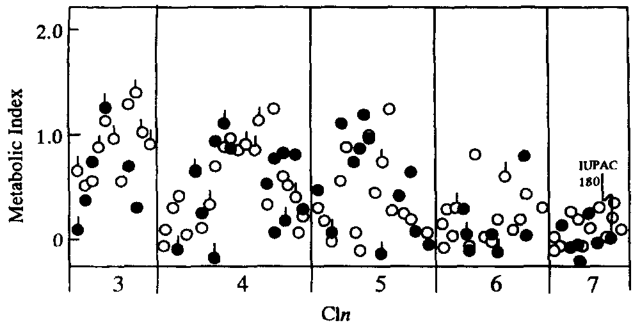

Fig. 4 Comparison of metabolic indices (MI) of PCB isomers and congeners in harbour porpoises (●) from the Black Sea with those of striped dolphin (○) from the western North Pacific. The data on striped dolphin were cited from Tanabe *et al.* (1988). For further details on metabolic index refer Tanabe *et al.* (1988). Circles with bar show the lower limits of metabolic index of PCB components which were not detected in porpoises but found in fish diet.

relatively more degradable nature in the animal body. The MI values in harbour porpoise were almost comparable with those in striped dolphins with relatively lower values for higher chlorinated members. This indicates that, similar to other cetaceans, the harbour porpoises have small capacity to decompose higher chlorinated members of PCBs and are at threat by these toxic contaminants.

Tanabe *et al.* (1988) suggested that small cetaceans lack PB (phenobarbital)-type enzymes and have lower activity of MC (3-methylcholanthrene)-type enzymes. PCB isomers and congeners having vicinal non-chlorinated *meta-para* and *ortho-para* carbons are known to be metabolized by PB-type and MC-type microsomal enzymes, respectively (Watanabe *et al.*, 1989). To estimate the PB- and MC-type enzyme activities in harbour porpoises, the MI values of IUPAC No. 52 (representative of PB-type inducer) and No. 66 (representative of MC-type inducer) were compared with those other higher animals including cetaceans (Fig. 5). Similar to other cetaceans, the MC-type enzyme activity in harbour porpoises was apparently lower than in terrestrial mammals. On the other hand, the MI value for a PCB isomer metabolized by PB-type enzyme (IUPAC No. 52) was elevated in Black Sea harbour porpoises, though it was much lower when compared with pinnipeds, birds and terrestrial mammals. The presence of PB-type enzymes in some coastal species of cetaceans has been suggested by several authors (Duinker *et al.*, 1989; Boon *et al.*, 1994; Kannan *et al.*, 1995). The different metabolic capacity found in the Black Sea harbour porpoise may impose a specific toxic interpretation. Despite the presence of slightly elevated PB-type activity the reduction of toxic contaminant accumulation in Black Sea harbour porpoises is unlikely because of the overall lower activities of both PB- and MC-type enzymes compared to terrestrial animals. Consequently, the Black Sea harbour porpoises are categorized as a group of animals having highly bloaccumulate and less biodegradable potencies to toxic contaminants.

Coplanar PCBs and their TEQs

The concentrations of toxic non-, mono-, and di-*ortho* PCB congeners in the blubber of harbour porpoises and fish samples are given in Table 2. The residue levels of most toxic non-*ortho* coplanar PCBs

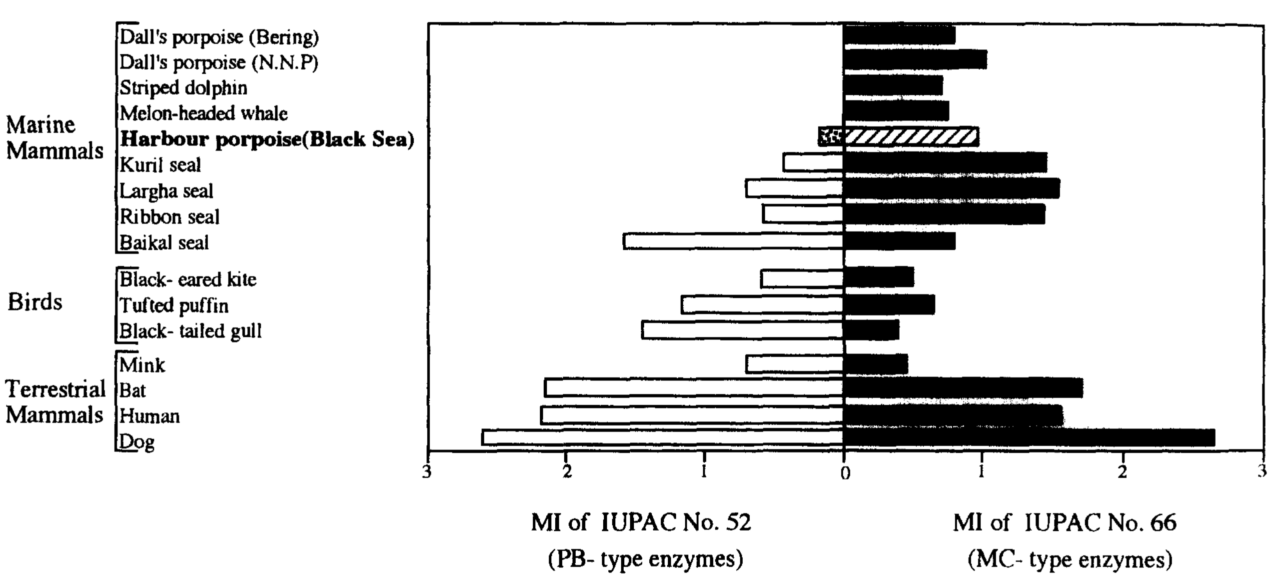

Fig. 5 Comparison of PB (phenobarbitol)- and MC (3-methylcholanthrene)-type enzyme activities in harbour porpoises from the Black Sea and other higher animals estimated by Metabolic Index of 2,2′,5,5′- and 2,3′,4,4 tetrachlorobiphenyl isomers. MI values of marine and terrestrial mammals and birds was cited from Tanabe *et al.*, 1988.

TABLE 2

Mean concentrations of di-, mono-, and non-*ortho* coplanar PCBs and their 2,3,7,8-TCDD toxic equivalents (TEQs) in the blubber of harbour porpoises and their fish diet from the Black Sea.

PCB congener (IUPAC No.)	Concentration ($ng\ g^{-1}$ wet wt)			TEF value[a]	TEQs ($pg\ g^{-1}$ wet wt)		
	Female	Male	Fish		Female	Male	Fish
Di-*ortho*							
137	60	140	0.20	0.00002	1.2	2.8	0.004
138	820	3600	0.50	0.00002	16	72	0.010
153	1000	4100	1.1	0.00002	20	82	0.022
170	130	350	0.18	0.00002	2.6	7.0	0.004
180	410	1100	0.30	0.00002	8.2	22	0.006
194	28	70	ND	0.00002	0.60	1.4	ND
Mono-*ortho*							
60	4.4	25	0.08	0.001	4.4	25	0.08
105	30	180	0.32	0.001	30	180	0.32
118	180	800	0.70	0.001	180	800	0.70
156	1.0	30	0.06	0.001	1.0	30	0.06
Non-*ortho*							
77	1.3	1.1	0.03	0.01	1.3	110	0.30
126	0.086	0.55	0.009	0.1	9.0	60	0.90
169	0.060	0.15	0.007	0.05	3.0	1.0	0.35
Total (rounded)					300	1400	2.8

[a]Toxic equivalency factor values (TEF) were obtained from Safe (1990).

were in the order of $T_4CB > P_5CB > H_6CB$ in both sexes of porpoises and in fish samples. A similar trend has been found in other marine mammals (Tanabe *et al.*, 1987a; Kannan *et al.*, 1989; Passivirta and Rantio, 1991). The concentrations of most toxic non-*ortho* coplanar congeners 3,3′,4,4′-T_4CB (IUPAC No. 77), 3,3′,4,4′,5-P_5CB (IUPAC No. 126) and 3,3′,4,4′,5,5′-H_6CB (IUPAC No. 169) ranged from 0.56–20, 0.063–0.83 and 0.054–0.33 $ng\ g^{-1}$ on a wet wt basis, respectively. The concentrations of di- and mono-*ortho* coplanar PCBs were several orders of magnitude higher than those of non-*ortho* members in porpoises and fish. In fish samples, the non-, mono-, and di-*ortho* PCB congeners were lower than in porpoises. However, similar to porpoises, T_4CB levels were apparently higher than P_5CB and H_6CB in fish. Based on this accumulation pattern, it is evident that Black Sea harbour porpoises have low degradation capacity for non-*ortho* coplanar PCBs.

The non-*ortho* colplanar PCBs (IUPAC Nos 77, 126 and 169) were also detected in the fetus. The residue levels of these compounds in the mother were 0.55, 0.06 and 0.05 $ng\ g^{-1}$ on a wet wt basis and in the fetus they were somewhat lower, in the order of 0.35, 0.05 and $<0.04\ ng\ g^{-1}$ on a wet wt basis (Table 3). This indicates that these toxic compounds are transferred from mother to fetus through the placenta. The mono- and di-*ortho* coplanar PCBs were also detected in mother and fetus of Black Sea harbour porpoises with levels higher than the non-*ortho* members.

The 2,3,7,8-TCDD toxic equivalents (TEQs) of di-, mono- and non-*ortho* coplanar PCBs were estimated in the blubber of porpoises and in the whole body of fish samples from the Black Sea. The mean total TCDD-equivalents of 13 coplanar PCBs in male and female porpoises were 1400 and 300 $pg\ g^{-1}$, respectively (Table 2). The IUPAC No. 118 was the most contributing individual occupying about 60% of the total TEQs. The isomers with higher TEQs values were non- and mono-*ortho* congeners than di-*ortho* ones both in porpoises and fish. However, in case of porpoises, some di-*ortho* members such as IUPAC Nos 138, 153 and 180 showed rather high TEQ values. The TEQs of non- and mono-*ortho* PCBs were estimated for other marine and terrestrial mammals, and compared with

TABLE 3

The concentrations of di-, mono, and non-*ortho* coplanar PCBs in the blubber of mother and fetus harbour porpoises from the Black Sea.

PCB congener (IUPAC NO.)	Concentration ($ng\ g^{-1}$ wet wt)	
	Mother	Fetus
Di-*ortho*		
137	60	30
138	600	280
153	740	310
170	130	24
180	390	70
194	34	–
Mono-*ortho*		
60	6.1	4.2
105	50	40
118	150	120
156	15	6.9
Non-*ortho*		
77	0.55	0.35
126	0.06	0.05
169	0.05	<0.04

–, No data available.

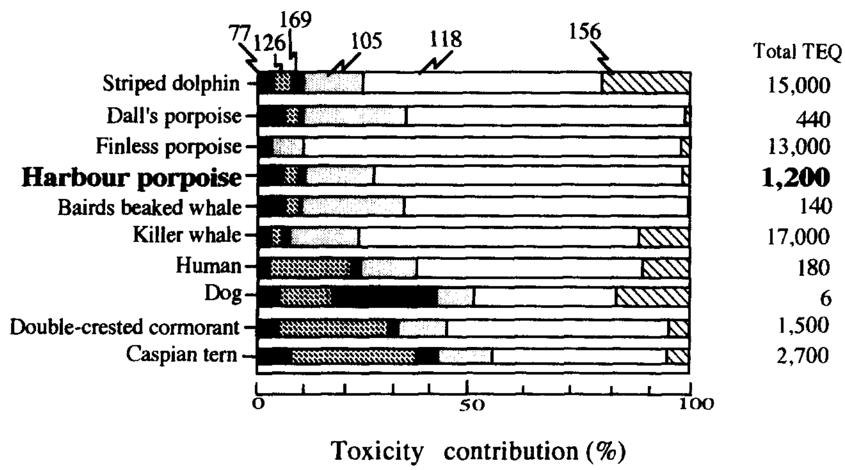

Fig. 6 Comparison of 2,3,7,8-TCDD toxic equivalents (TEQs) by non- and mono-*ortho* PCBs in male harbour porpoises to those of other male marine mammals and terrestrial higher animals. The total TEQ (in pg/g wet wt) is the sum of toxicities estimated for non- and mono-*ortho* PCB congeners only. Data on TEQs in Dall's porpoise, killer whale, Baird's beaked whale, human and dog were cited from Kannan *et al.* (1989), those of double-crested cormorants and Caspian terns from Yamashita *et al.* (1993), and those of striped dolphin from Kannan *et al.* (1993). The collected locations of animals are as follows: striped dolphin from the Mediterranean Sea, Dall's porpoise, Baird's beaked whale and killer whale from western Pacific, finless porpoise from the Seto-inland Sea, Japan, human and dog from Japan, double-crested cormorants and Caspian terns from Great Lakes, USA, and harbour porpoise from the Black Sea.

those in harbour porpoises from the Black Sea (Fig. 6). The non-*ortho* coplanar PCBs of IUPAC Nos 77, 126 and 169 have dioxin like biochemical and toxic activities, but these members were minor contributors to the total TEQs in Black Sea harbour porpoises as well as in other cetaceans. The less significance in non-*ortho* congeners in comparison with mono-*ortho* ones was specific for cetaceans, as evidenced that apparently comparable contribution to TEQs from non- and mono-*ortho* congeners were observed for terrestrial mammals (Fig. 6). Among non- and mono-*ortho* congeners, 2,3,3′,4,4′-P_5CB (IUPAC No. 105) and 2,3′,4,4′,5-P_5CB (IUPAC No. 118) seem to be highly hazardous contaminants with possible long term accumulation in Black Sea harbour porpoises.

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