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Tissue-Related Polychlorinated Biphenyls Accumulation in Mediterranean Cetaceans: Assessment of Toxicological Status

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Polychlorinated biphenyls (PCBs) have been synthesized for use as technical mixtures in a multitude of industrial applications such as impregnating wood, paper and fabric, and as engine oil additives, capacitor fluids, transformer coolants, paint and sealant plasticizers. These compounds are lipophilic in nature and biomagnify through food chains, so bioaccumulation in the lipid fraction of tissues of long living species that occupy a top trophic position might be of special concern. Cetaceans, in particular, being typical end-points in the marine food chain, and possessing a limited capacity to metabolize these toxic contaminants (Tanabe et al., 1988; Tanabe, 2002), have a high accumulation potential and, as a consequence, are most vulnerable to the long-term toxic effects of such chemicals.

Cetacean populations living in the Mediterranean basin are especially exposed to this type of pollution because of the semi-enclosed nature of this sea, surrounded by highly industrialized countries. The morbillivirus infection of Mediterranean dolphins during the last decades has been linked to the presence of elevated PCB concentrations in the corpses of dead animals (Kannan et al., 1993; Aguilar and Borrell, 1994). Known organochlorine-mediated toxic effects in marine mammals include immune system depression and thereby increase in susceptibly to microbial and parasitic infections, as well as reproductive impairment, alteration of growth and skeletal deformities (Addison, 1989; Helle et al., 1990; Zakharov et al., 1997).

In this context, the need for a continuous monitoring of organochlorine load in these species is obvious. Such an observation is reinforced by the proposal that, globally, PCB concentrations in marine biota will continue increasing in forthcoming decades since only a small fraction of the total amount released has reached the oceans (Tanabe, 1988; Reijnders, 1996). Reijnders (1996) estimated that only about 1% of the PCB produced had reached the ocean in the mid-1990s, and Tateya et al. (1988) suggested that levels of PCBs in marine mammals would peak between 2000 and 2030. Taking into account all these facts, this study presents the results from analyses of PCBs and their distribution in liver, kidney, lung, and tissue muscle of 12 specimens of Mediterranean bottlenose dolphins (*Tursiops truncatus*). Furthermore, toxic equivalent concentrations (TEQs) based on available toxic equivalent factors (Van den Berg et al., 1998) of a number of congeners are calculated and their relative contribution to the total toxic burden in the analyzed tissues is presented.

Materials and Methods

Within an investigation on stranded dolphins between 2001–2002, 12 specimens of bottlenose dolphins (*Tursiops truncatus*) (length: 160–308 cm; sex: male) were collected in different coastal areas of the Adriatic and Ionian Sea (Fig. 1). Organ and tissue samples (liver, kidney, lung, and muscle tissue) were collected during autopsy. After collection, the samples were packed in aluminum foil and stored at –20°C until analyzed. Prior to analysis, samples

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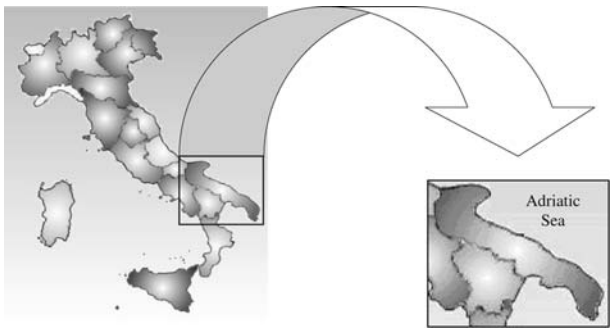

Fig. 1 Stranding locations of cetaceans

were homogenized in a teflon Ultra-Turrax homogenizer. The analytical method to determine polychlorinated biphenyl (PCBs = 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209) concentrations has been previously described (Storelli et al., 2003). Briefly, aliquots (2-3 g) of the homogenized samples were ground with anhydrous sodium sulphate in a mortar. The mixture was extracted with petroleum ether (Erney, 1983) and the extracts were cleaned up following the procedure described by Murphy (1972). 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, the method reported by Tanabe et al. (1987) was used. Analyses were done with a Carlo Erba HR gas chromatograph (GC) 8000 Top with automatic injection system, and with an electron capture detector ECD-400, Ni^{63} (temperature: 330°C). The GC was connected to a PC–Pentium III IBM equipped with Chrom-Card version 1.2 software for integration purposes (C. Erba). For all the analyses a fused-silica capillary column DB-5 Supelco (length = 30 mt, inside diameter 0.25 mm and film thickness 0.25 μm) was used. Hydrogen at a flow rate of 1 ml/min was used as gas carrier, and nitrogen as make-up gas, 60 ml/min. The individual PCB congeners were determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%). The reference material employed was CRM 349 for PCBs (cod liver oil). The recovery for each PCB (28, 52, 101, 118, 153, 180, and 138) quantified in the certified material ranged from 91% to 102%. The recoveries for the other PCB congeners, varying between 90% and 110%, were determined adding known amounts of PCB standards (at three levels of concentrations) to samples before extraction (method of additions). Residues in 100% of the samples were confirmed by gas-liquid chromatography-mass spectrometry (Fisons MD 800). Non-parametric statistics (Mann-Witney *U* test) was used for testing differences in concentrations among tissues and organs. PCB concentrations, as means of duplicate measurements, are presented in μg g^{-1} on a lipid weight basis.

Table 1 Concentrations of total PCBs (μg g^{-1} lipid wt) in tissues and organs of bottlenose dolphins

	Muscle	Liver	Kidney	Lung
Minimum	2.47	9.57	4.42	1.02
Maximum	70.06	120.43	108.39	95.16
Arithmetic mean	19.86	44.73	39.38	16.10
Standard deviation	19.01	33.59	36.51	25.73

Results and Discussion

Concentrations of PCBs in the tissues of the bottlenose dolphins are presented in Table 1. Concentrations of PCBs in liver (9.57-120.43 μg g^{-1}, mean 44.73 μg g^{-1}) and in kidney (4.42-108.39 μg g^{-1}, mean 39.38 μg g^{-1}) were comparable ($p > 0.05$), but significantly higher ($p < 0.001$) than those observed in muscle tissue (2.47-70.06 μg g^{-1}, mean 19.86 μg g^{-1}) and lung (1.02-95.16 μg g^{-1}, mean 16.10 μg g^{-1}), which presented levels of similar magnitude ($p > 0.05$). This tissue distribution is comparable to that obtained by the majority of authors on various types of marine mammals (Marsili and Focardi 1997; Wafo et al., 2005), and can be linked to differential lipid composition, blood flow rate and metabolic capacities among various tissues (Jenssen et al., 1996). However, independent of the factors controlling accumulation of these contaminants, the PCB concentrations measured here were higher than those found in other studies (Weisbrod et al., 2001, Karuppiah et al., 2005).

PCB profiles were similar in all tissues and organs and were dominated by the higher chlorinated homologues. Hexachlorobiphenyls accounted for 55.7–60.6% of total PCBs, followed by pentachlorobiphenyls making up 16.2–21.4%, by heptachlorobiphenyl PCB 180 accounting for 12.1–14.6%, and by tetrachlorobiphenyls constituting from 5.7–7.8% of the total residue. Decachlorobiphenyl PCB 209 and trichlorobiphenyls accounted only for 1.1–2.8% and 1.0–1.3% of total PCBs, respectively (Fig. 2). Major PCBs in all tissues were congeners 138 and 153, which collectively accounted for 52.0–56.2% of the total PCB concentrations, followed by PCB 180, which constituted 12.1–14.6%. Other chlorobiphenyls found in moderate amounts included congeners 101, 105 and 118 (14.8–18.7%), while the remaining congeners collectively (PCB 20, 28, 35, 52, 60, 77, 126, 156, 169 and 209) showed the lowest percentages (16.2–18.3%). Comparable patterns were found in dolphins either from the Mediterranean Sea, i.e., *Tursiops truncatus* (Storelli and Marcotrigiano, 2000; Storelli and Marcotrigiano, 2003; Wafo et al., 2005), *Stenella coeruleoalba* (Kannan et al., 1993; Wafo et al., 2005) and *Grampus griseus* (Storelli and Marcotrigiano, 2000a), or from other seas, i.e., *Lagenorhynchus acutus* (Weisbrod et al., 2001) and *Stenella frontalis* (Watanabe et al., 2000).

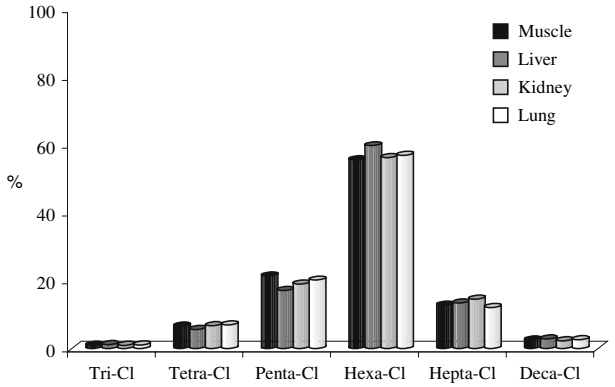

Fig. 2 Percentage of total concentration of each class of PCB isomer in tissues and organs of bottlenose dolphins

Many studies have shown that marine mammals have the capacity to concentrate organochlorinated compounds, but have a weak potential to decompose them, especially with regard to the strongly chlorinated congeners (Tanabe, 2002). The ratio of a PCB congener concentration to that of a more persistent congener, PCB 153, gives a index of biological alteration of PCBs (Tanabe and Tatsukawa, 1983; Kannan et al., 1995). The examination of this ratio in bottlenose dolphin liver, an organ known to be a good indicator of the accumulation and elimination processes for these compounds showed a depletion in the lower chlorinated congeners and a high persistency of PCBs 118, 138 and 180, which have structures that are difficult to metabolize.

Individual PCB congeners differ greatly in their toxic potency: non-*ortho* PCBs (PCB 77, 126, 169) are more toxic than mono-*ortho* ones (PCB 105, 118, 156). The highest concentrations of non-*ortho* ΣPCBs were found in the liver (1.78 μg g^{-1}) and kidney (1.55 μg g^{-1}), whilst muscle tissue (0.85 μg g^{-1}) and lung (0.62 μg g^{-1}) showed the lowest levels. PCB 126 with concentrations between 0.41–1.12 μg g^{-1}, constituted the predominant compound of three non-*ortho* coplanar congeners, while the other two individual congeners varied in their abundance between the different tissues. In particular, in liver and kidney, congener PCB 169 was higher than PCB 77. In contrast, in the muscle tissue and lung, PCB 77 was higher than PCB 169. Also, the levels of mono-*ortho* PCBs weree highest in liver (6.68 μg g^{-1}), followed by kidney (5.59 μg g^{-1}), muscle tissue (3.01 μg g^{-1}) and lung (2.42 μg g^{-1}). PCB 118 was found at concentrations of 1.15 to 3.01 μg g^{-1} and thus constituted the major congener among three mono-*ortho* PCBs, while the other two congeners showed levels quite comparable in all organs and tissues examined. The observed variation in the accumulation patterns of these PCB congeners between tissues could be due to structure-related interactions, although the mechanisms that control accumulation patterns are not known. However in all tissues examined, the concentration pattern of non-*ortho* coplanar congeners was different with respect to the pattern typical of commercial mixtures (PCB 77 > PCB 126 > PCB 169), which is, generally, observed in unexposed individuals (Watanabe et al., 2000).

Non- and mono-*ortho* PCBs have been considered in order to estimate the toxicity potential (TEQs) of PCB exposure, using the toxic equivalent factors (TEFs) for mammals developed by Van den Berg et al., (1998). TEQ values are expressed as both concentrations on a wet weight basis (pg/g) and lipid weight basis (ng/g) in order to compare values with other studies that present data in both formats (Table 2). The mean total 2,3,7,8-TCDD equiva-

Table 2 Mean concentrations of toxic equivalents (TEQs) of PCBs in the liver of different marine organisms

Species	Non-*ortho* congeners			Mono-*ortho* congeners			Total Coplanar PCBs	Reference
	PCB 77	PCB 126	PCB 169	PCB 105	PCB 118	PCB 156		
Tursiops truncatus								
TEQ (pg g^{-1} wet wt)	0.52	1920.00	84.00	3.38	5.53	16.90	2030.33	This study
TEQ (ng g^{-1} lipid wt)	0.03	104.30	4.57	0.18	0.30	0.92	110.30	
Tursiops truncatus								
TEQ (pg g^{-1} wet wt)	0.17	100.00	11.00	72.00	300.00	170.00	653.17	Watanabe et al., 2000
Stenella frontalis								
TEQ (pg g^{-1} wet wt)	0.04	36.00	4.30	8.80	26.00	25.00	100.14	Watanabe et al., 2000
Sphyrna zygaena								
TEQ (pg g^{-1} wet wt)	3.30	4825.00	ND	2.00	8.31	5.25	4843.80	Storelli et al., 2003
Prionace glauca								
TEQ (pg g^{-1} lipid wt)	0.001	0.27	ND	0.42	1.15	0.67	2.51	Storelli et al., 2005
Xiphias gladius								
TEQ (pg g^{-1} lipid wt)	—	—	—	—	—	—	8.83	Storelli and Marcotrigiano, 2006

lents of six congeners were 2030.3 pg g^{-1} w.w. (110.30 ng g^{-1} l.w.) in liver, 5172.3 pg g^{-1} w.w. (114.26 ng g^{-1} l.w.) in kidney, 910.6 pg g^{-1} w.w. (56.20 ng g^{-1} l.w.) in muscle tissue and 655.8 pg g^{-1} w.w. (41.54 ng g^{-1} l.w.) in lung. Calculations of TEQs showed that the pentachlorinated non-ortho PCB 126 contributed most to the total toxicity (94.6–97.6%), followed by PCB 156 (0.12–0.83%) and PCB 118 (0.23–0.27%), while the other congeners contributed in very low percentages. A comparison of the TEQ value in liver of these animals to those in the same organ of other dolphins (Table 2), i.e., *Stenella frontalis* and *Tursiops truncatus* from the Atlantic ocean (Watanabe et al., 2000), and of other large predators from Mediterranean Sea, i.e., *Xiphias gladius* (Storelli and Marcotrigiano 2006) and *Prionace glauca* (Storelli et al., 2005), showed that the concentrations reported here were much higher, but comparable to those reported for *Sphyrna zygaena* liver from Mediterranean Sea (Storelli et al., 2003).

The present study demonstrated that relatively high levels of PCBs were accumulated in dolphins from Mediterranean Sea and the accumulation patterns of PCB congeners were generally homogeneus across the tissues. In contrast the abundance of non-*ortho* coplanar congeners was influenced by tissue type. Furthermore, the 2,3,7,8-TCDD toxic equivalents estimated for these dolphins were high, with a maximum contribution from non-*ortho* congeners. This might mean that Mediterranean dolphins are in a state of toxicological hazard. Further studies in a larger series of cetaceans would assist in evaluating the role of these pollutants in the marine environment.

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