

Polychlorinated biphenyls and other chlorinated organic contaminants in the tissues of Mediterranean loggerhead turtle *Caretta caretta*

M.M. Storelli, G. Barone, G.O. Marcotrigiano *

Pharmacological–Biological Department, Chemistry and Biochemistry Section, Veterinary Medicine Faculty, University of Bari–Strada Prov. le per Casamassima Km 3, 70010 Valenzano, Ba, Italy

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Abstract

Polychlorinated biphenyls including coplanar congeners and DDT compounds were measured in different organs and tissues (liver, kidney, lung and muscle tissue) of loggerhead turtles *Caretta caretta* from the eastern Mediterranean Sea. The highest levels of these compounds were found in liver (PCBs: 52.32 ng/g; DDTs: 18.27 ng/g), followed by kidney (PCBs: 19.05 ng/g; DDTs: 5.70 ng/g), lung (PCBs: 12.75 ng/g; DDTs: 3.76 ng/g) and muscle tissue (PCBs: 4.65 ng/g; DDTs: 1.45 ng/g). PCBs revealed a profile dominated by hexa-, penta- and hepta-chlorinated congeners, while among DDTs, the compound in the greatest concentration was *p,p'*-DDE, (liver: 85.2%, kidney: 93.6%, lung: 86.4%, muscle tissue: 93.2%). The estimated toxic equivalents (pg TEQs/g wet wt) of non- and mono-*ortho* PCBs were in the range of 1.54–5.86 pg TEQs/g wet wt. Non-*ortho* coplanar PCB 77 accounted for more than 90% of the total TEQs leaving to mono-*ortho* only 2.6–6.2%.

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Of the three species of marine turtles living regularly in the Mediterranean Sea, loggerhead turtle *Caretta caretta* is the most common and extends its distribution to the whole basin. The presence of this species has been documented in the western Mediterranean Sea, (Broderick et al., 2002), as well as in the northeastern (Lazar et al., 2000) and northwestern Adriatic Sea, in particular close to the Po Delta (Vallini et al., 2001). Demographic studies indicate that the loggerhead turtle population is in decline with only 2000 nesting females per year in the

Mediterranean region (Groombridge, 1990). Overexploitation of eggs and meat as a food resource (Hutchinson and Simmonds, 1991), incidental mortality relating to marine fisheries (Pinedo and Polacheck, 2004) and degradation of marine and nesting habitats (Groombridge, 1990) had a dramatic impact on marine turtle populations. Also pollution cannot be neglected as a potential threat for marine turtles, although the mortality resulting from marine pollution is one the most difficult to estimate of all human-induced sources of mortality. Among a wide variety of synthetic organic compounds, polychlorinated biphenyls (PCBs), DDT and its metabolites (DDTs) have a particular significance in the ecotoxicology because of their undesirable effects on environmental quality and animal health

* Corresponding author. Tel.: +39 80 5443865; fax: +39 80 5443863.

E-mail address: g.o.marcotrigiano@veterinaria.uniba.it (G.O. Marcotrigiano).

(Ahlborg et al., 1994). These lipophilic pollutants tend to biomagnify up the food chain and consequently, higher trophic animals in aquatic food webs have been the subject of prior concern. Several reports, in fact, exist in the literature on the levels of these contaminants in different Mediterranean megafaunal taxa such as, dolphins (Aguilar and Borrell, 1994; Corsolini et al., 1995; Storelli and Marcotrigiano, 2000a, 2003), monk seals (Georgakopoulou-Gregoriadou et al., 1995), sharks (Corsolini et al., 1995; Storelli and Marcotrigiano, 2001; Storelli et al., 2003), tunas and swordfish (Corsolini et al., 1995; Storelli and Marcotrigiano, 2006). In these organisms, exposure to such pollutants has been shown to affect a variety of biological parameters with the most prominent toxic effects being on the immune, endocrine, developmental and reproductive systems (Keller et al., 2004a,b, 2006). Although much was written to date about chlorinated hydrocarbon concentrations in large predators, few studies have been dedicated to marine turtles (Miao et al., 2001; Gardner et al., 2003; Keller et al., 2004a), especially for those from the Mediterranean Sea (Corsolini et al., 2000; Storelli and Marcotrigiano, 2000b). No information on detrimental threshold concentrations are available, and little is known about the consequences of exposure of organochlorine compounds to sea turtles. A recent study has shown that high concentrations of DDE alter aromatase activity, the key enzyme responsible for the conversion of testosterone to 17 β -estradiol (Keller and McClellan-Green, 2004). Progress towards the understanding of the possible organochlorine compound impact on turtle health might be obtained with more data on accumula-

tion and distribution of these compounds within their body. In this perspective the present study determined the concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (DDTs) in specimens of loggerhead turtles from the Adriatic Sea and evaluated the variation in accumulation levels between different tissues. In addition toxicity of mono- and non-*ortho* PCBs was estimated by the toxic equivalence approach (Van den Berg et al., 1998).

1. Materials and methods

1.1. Samples collection

Within an investigation on stranded turtles between 1999–2001, 19 specimens of loggerhead turtles (*C. caretta*) (SCL: 27–69.5 cm; mean 49.5 ± 12.6) were collected in different coastal areas of the Adriatic and Ionian Sea (Fig. 1). Organs and tissues samples (liver, kidney, lung, and tissue muscle) were collected during necropsy. After collection, the samples were packed in aluminum foil and stored at -20°C until analyzed. Prior to analysis, samples were homogenized in a Teflon Ultra-Turrax homogenizer. All specimens had recently died prior to sample collection with no evidence of tissue decomposition.

1.2. Organochlorine compound analysis

The analytical methods to determine polychlorinated biphenyl (PCBs=8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209) and DDT compounds (DDTs=*p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT,

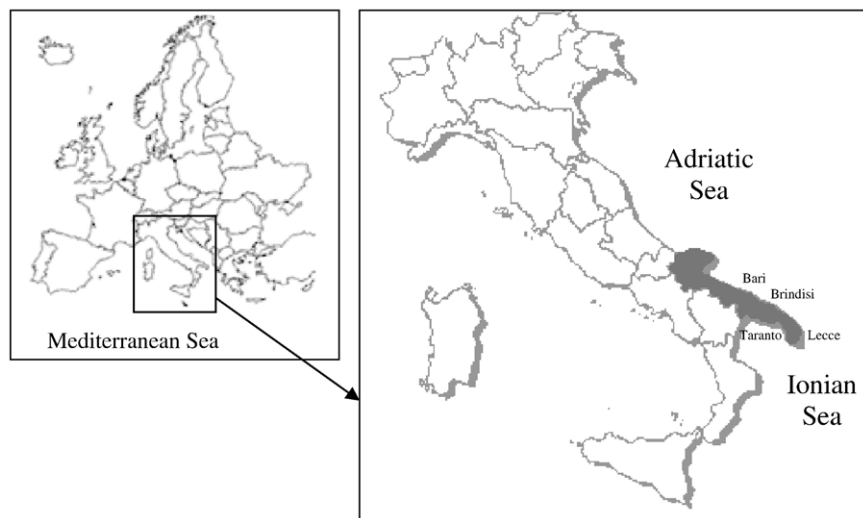


Fig. 1. Sampling area for collection of stranded loggerhead turtles (shaded zone).

p,p'-DDD, *o,p'*-DDD) concentrations have been previously described (Storelli et al., 2005). 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 cleaned up following the procedure described by Murphy (1972). For the separation of non-*ortho*-PCB congeners, 3,3',4,4'-T₄CB (IUPAC 77), 3,3',4,4',5-P₅CB (IUPAC 126), and 3,3',4,4',5,5'-H₆CB (IUPAC 169) from other PCBs, the method reported by Tanabe et al. (1987) was used.

1.3. High resolution gas chromatography-electron capture detector (HRGC-ECD) and mass spectrometric detector (MSD)

Analyses were made on a Carlo Erba HR gas chromatograph 8000 Top with automatic injection system and with an electron capture detector ECD-400, Ni⁶³ (temperature: 330 °C). The GC was connected to a PC — Pentium III IBM equipped with Chrom-Card version 1.2 software program for integration purposes (C. Erba). All analyses used a fused-silica capillary column DB-5 Supelco (length=30 m, inside diameter 0.25 mm and film thickness 0.25 µm). Hydrogen at a flow rate of 1 ml/min was used as gas carrier, nitrogen as make-up gas 60 ml/min. Temperature was programmed according to the following sequence: injection at 90 °C. The oven was held steady for the first min and then increased from 90 to 180 °C at a rate of 15 °C/min. Then the oven was maintained at steady temperature for 1 min and then increased from 180 to 220 °C at a rate of 4 °C/min; finally, the oven was maintained steady 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 determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%). The reference material employed was CRM 349 for PCBs and CRM 598 for DDTs (cod liver oil). The recovery for each PCB (28, 52, 101, 118, 153, 180, and 138) and DDT (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD) quantified in the certified material ranged from 91% to 102%. The recoveries for the other PCB congeners and *o,p'*-DDT, varying between 90% and 110%, were determined adding known amounts of PCB and *o,p'*-DDT 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). Concentrations of PCBs and DDTs,

means of duplicate measurements, are presented as ng/g on a wet wt basis.

1.4. Statistical analyses

Mann–Whitney's *U* test was conducted to verify the difference in the levels of PCBs and DDTs and to determine whether there were differences as a function of tissues. The regression analysis (Spearman's rank correlation) was used to examine the effects of lipid content on organochlorine load in tissues. The level of significance was set at $P < 0.05$.

2. Results and discussion

2.1. PCB load and comparison with other species

PCB concentrations measured in the four tissues, liver, kidney, lung and muscle tissue are reported in Table 1. Of the 17 PCB congener peaks for which analyses were conducted in this study, PCBs 118, 138, 153, 180 were detected in all samples, PCBs 52, 60, 77, 101, 105, 156 and 209 were found in 14.5%, 39.5%, 17.1%, 35.5%, 82.9%, 55.3%, 6.6% of examined samples, respectively, whereas the remaining congeners, PCBs 8, 20, 28, 35, 126 and 169, were below detection limit in all samples. As revealed by statistical analysis, PCBs were differentially distributed in the analyzed tissues with the liver having the highest concentration (average: 52.32 ng/g wet wt), followed by kidney (average: 19.05 ng/g wet wt), lung (average: 12.75 ng/g wet wt) and muscle tissue (average: 4.65 ng/g wet wt). This different distribution of contaminants was related to tissue lipid content which is commonly observed in other marine organisms (Marsili and Focardi, 1997; Gauthier et al., 1998; Storelli and Marcotrigiano, 2000a, 2001). Organochlorine levels were found to be, in fact, positively correlated with lipid content in the different tissues analyzed in the current study (liver: $r = 0.67$, $P < 0.002$; kidney: $r = 0.61$, $P < 0.006$; lung $r = 0.71$,

Table 1
PCBs and DDTs concentrations (range, arithmetic mean ± standard deviation) (ng/g wet wt) in tissues and organs of loggerhead turtles

Tissue or organ	Lipid %	PCBs	DDTs
Liver	0.5–23.3	6.85–297.49	2.32–73.61
	6.5 ± 5.7	52.32 ± 74.99	18.27 ± 18.40
Kidney	0.2–10.5	0.92–113.02	0.42–28.52
	2.7 ± 3.1	19.05 ± 27.19	5.70 ± 6.49
Lung	0.2–10.2	0.54–116.13	0.12–33.10
	1.6 ± 2.6	12.75 ± 29.66	3.76 ± 7.69
Muscle tissue	0.04–2.7	0.63–23.49	0.25–4.91
	0.6 ± 0.7	4.65 ± 5.58	1.45 ± 1.10

$P < 0.001$; muscle tissue $r = 0.64$, $P < 0.002$). Few studies have been conducted to document level of these contaminants in marine turtles from all over the world, and particularly scarce is the information for specimens from Mediterranean Sea. However, Corsolini et al. (2000) reported levels in liver and muscle of *C. caretta* stranded along the northern coasts of the Adriatic Sea which were slightly higher than those here detected. Gardner et al. (2003) reported comparable concentrations in the liver of three different species of marine turtles (*Chelonia mydas*, *Lepidokelys olivacea* and *C. caretta*) from the eastern Pacific, while Lake et al. (1994) obtained higher PCB concentrations in the liver of *Lepidokelys kempfi* (43–1950 ng/g wet wt) from Long Island (USA). Higher concentrations were found in other marine organisms from different areas of the Mediterranean Sea, i.e. dolphins, *Stenella coeruleoalba*, *Tursiops truncatus* (Wafo et al., 2005), bony fish, *Thunnus thynnus* (Corsolini et al., 1995), *Lophius budegassa* (Storelli et al., 2004b) and cartilaginous fish, *Chimaera monstrosa* (Storelli et al., 2004a).

2.2. PCB congener patterns

PCB profiles in the turtle tissues were dominated by the higher chlorinated homologues. Hexachlorobiphenyls were predominant and accounted for 65.2–67.6% of the total PCBs, followed by pentachlorobiphenyls making up 15.5–16.3%, and by heptachlorobiphenyl PCB 180 accounting for 12.9–14.4% of the total residue. Tetrachlorobiphenyls constituted from 2.4 to 5.3%, while decachlorobiphenyl PCB 209 accounted only for 0.1–1.6% of total PCBs. This isomer pattern of PCBs was similar among the different tissues, except for the presence of a greater proportion of PCBs with four chlorine atoms in muscle (Fig. 2), probably as result of the major affinity of these congeners for the lipid components of this tissue. Such a finding is in agreement with other study in marine turtles showing a higher proportion of lower-chlorinated congeners in muscle than other tissues (Corsolini et al., 2000; Gardner et al., 2003). Concerning individual congeners, the most abundant were hexachlorobiphenyls 153 and 138, collectively accounting for 62.9% to 65.2% of the total PCB concentrations, followed by PCB 180 constituting from 12.9% to 14.4%. Another chlorobiphenyl found in relatively high percentage was PCB 118 accounting for 10.4–11.2%, whereas the remaining six congeners collectively made up a small fraction of the total PCB residue (9.9–12.3%). The composition of different isomer classes, as well as, the abundance of individual PCB congeners in these analysed organisms

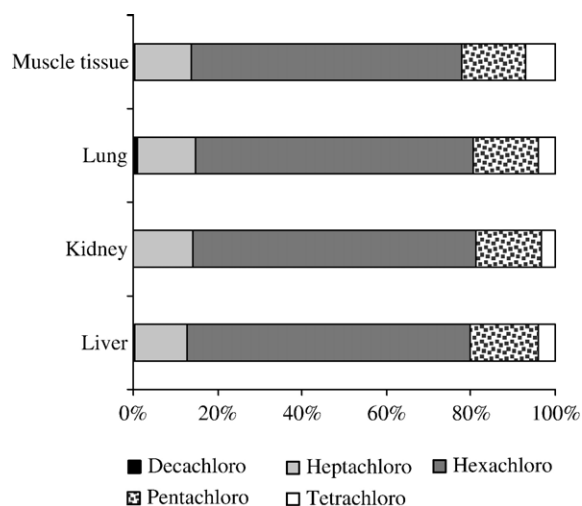


Fig. 2. Percentage composition of PCB congeners in loggerhead turtles.

reflect the patterns reported elsewhere for marine turtles. Corsolini et al. (2000) and Storelli and Marcotrigiano (2000a,b), found that higher chlorinated congeners dominated the PCB profiles in tissues of *C. caretta* stranded along the coast of the Adriatic Sea and that PCBs 153, 138, 180 and 118 were the most abundant congeners. These compounds were predominant also in different species of marine turtles from other seas, i.e. *Lepidochelys olivacea* from Pacific Ocean (Miao et al., 2001) and *Lepidochelys kempfi* from the Atlantic Ocean (Rybicki et al., 1995). Inversely, Gardner et al. (2003) in *C. mydas*, herbivorous species, from the Eastern Pacific encountered a profile dominated by lower-chlorinated congeners. Differences in PCB patterns in sea turtles may be relate to differences in the congener compositions of environmental media among regions, dietary differences or differences in the abilities of the various species and populations to metabolize PCBs (Gardner et al., 2003). An enrichment of highly chlorinated PCBs in the profile of turtles in this study may suggest that they were exposed via environmental media, like water and sediment, predominantly to more chlorinated PCB formulations. Likewise, it may be that loggerhead turtles' food consumption is of higher trophic level organisms. Loggerhead turtles are, in fact, generally carnivorous, feeding mostly on shellfish crabs jellyfish and other fish (Godley et al., 1997). The presence of a high proportion of more chlorinated PCBs with the pattern resembling Aroclor 1260 formulation in sediments from the Adriatic Sea (Galassi et al., 1993), as well as the predatory status of loggerhead sea turtles (Tomas et al., in press) seem confirm these hypotheses. Alternatively, the congener pattern observed in the

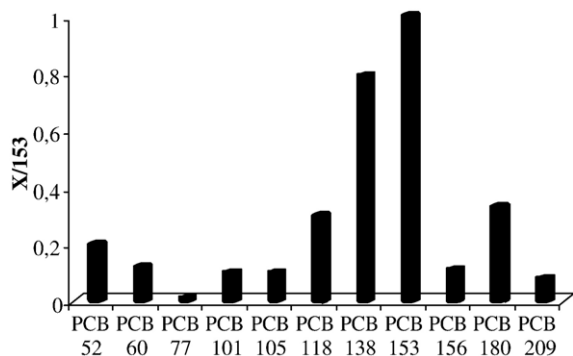


Fig. 3. PCB X/PCB 153 ratio in loggerhead turtle liver.

organisms in question may indicate that these species metabolize less-chlorinated PCB congeners efficiently, although the ratio of PCB hepatic concentration congeners to that of more persistent congener PCB 153, considered a good indicator of biological alteration of PCBs (Tanabe and Tatsukawa, 1983; Barrie et al., 1992; Kannan et al., 1995) has not shown a strong depletion in the lower chlorinated congeners (Fig. 3).

2.3. Coplanar congeners and toxic equivalents (TEQs)

The chlorobiphenyl congeners exhibiting the greatest toxicity are isostereomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and include non-*ortho* coplanar congeners 77, 126, and 169. Mono-*ortho* substituted PCB congeners, such as PCB 118, 105 and 156, also exhibit similar but weaker toxic responses (Van den Berg et al., 1998). The non-*ortho* PCB congeners 126 and 169 were not found in turtle tissues, while PCB 77 was present at levels from 0.03 ng/g in

muscle tissues to 0.11 ng/g in livers. Among the three mono-*ortho*, PCB 118 was the predominant congener, with concentrations from 0.52 to 5.67 ng/g, followed by PCB 105 and PCB 156 with levels between 0.15–1.72 ng/g and 0.10–1.26 ng/g, respectively in the four tissues. Bird toxic equivalency factors (TEFs), which are fractional potencies of individual congeners to the most toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, have been used to estimate toxic potency as dioxin toxic equivalents (TEQs) in organisms analyzed here (Van den Berg et al., 1998) (Table 2). The estimated toxic equivalents (pg TEQs/g wet wt) of non- and mono-*ortho* PCBs were in the range of 1.54–5.86 pg TEQs/g wet wt. Non-*ortho* coplanar PCB 77 accounted for more than 90% of total TEQs leaving to mono-*ortho* only 2.6–6.2%. Among the three mono-*ortho* congeners, PCB 105 and PCB 156 made the greatest contribution to total TEQ value, while PCB 118 made up a very small fraction of total toxicity. The TEQs values obtained here were higher than those in loggerhead turtles collected along the northern coast of the Adriatic Sea (Corsolini et al., 1995). Such a discrepancy between TEQs may be due to the relatively high levels of PCB 77 found in our investigation compared with that reported by Corsolini et al. (1995). However, because TEFs for reptiles are not available, the TCDD equivalent approach needs to be interpreted with some caution; therefore, the toxicological significance of such concentrations cannot be fully evaluated.

2.4. Organochlorine pesticides

Similar to the PCBs, the statistical analysis revealed that residue level of DDTs followed the order: liver

Table 2

Mean concentrations of mono- and non-*ortho* coplanar PCBs (ng/g wet wt) and their 2,3,7,8-TCDD toxic equivalents (TEQs pg/g wet wt) in loggerhead turtles

		TEFs		Liver		Kidney		Lung		Muscle tissue	
				Concentrations	TEQs	Concentrations	TEQs	Concentrations	TEQs	Concentrations	TEQs
Non-ortho											
PCB 77	0.05	0.11	5.50	0.04	2.00	0.04	2.00	0.03	1.50		
PCB 126	0.10	ND	ND	ND	ND	ND	ND	ND	ND		
PCB 169	0.001	ND	ND	ND	ND	ND	ND	ND	ND		
Total		0.11	5.50	0.04	2.00	0.04	2.00	0.03	1.50		
Mono-ortho											
PCB 105	0.0001	1.72	0.17	0.52	0.05	0.37	0.04	0.15	0.02		
PCB 118	0.00001	5.67	0.06	2.07	0.02	1.33	0.01	0.52	0.005		
PCB 156	0.0001	1.26	0.13	0.53	0.05	0.41	0.04	0.10	0.01		
Total		8.65	0.36	3.12	0.12	2.11	0.09	0.77	0.04		
Total		8.76	5.86	3.16	2.12	2.15	2.09	0.80	1.54		

ND=not detected; TEFs=Toxic Equivalent Factors (Van den Berg et al., 1998).

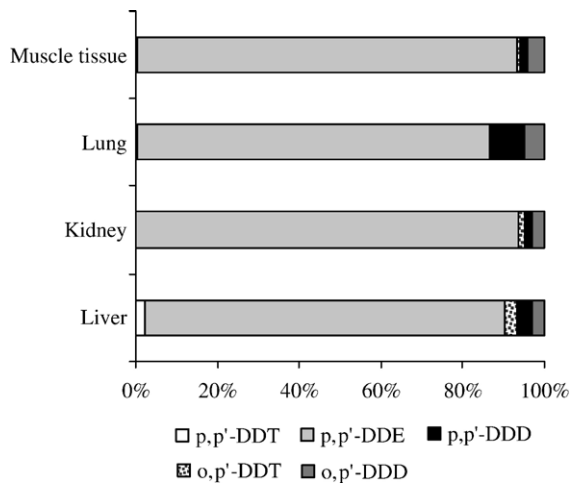


Fig. 4. Percentage composition of DDT compounds in loggerhead turtles.

(18.27 ng/g), kidney (5.70 ng/g), lung (3.76 ng/g) and muscle tissue (1.45 ng/g) (Table 1). Also for this compound class a relationship between residue levels and tissue lipid content was observed (liver: $r=0.76$, $P<0.0001$; kidney: $r=0.65$, $P<0.003$; lung $r=0.76$, $P<0.001$; muscle tissue $r=0.61$, $P<0.006$). Of the different organochlorine pesticides, *p,p'*-DDE was detected in all samples, whereas *p,p'*-DDT, *p,p'*-DDD, *o,p'*-DDT and *o,p'*-DDD were found in 6.6%, 17.1%, 18.4%, 28.9% of examined samples, respectively. DDTs composition showed that *p,p'*-DDE was the predominant compound (liver: 85.2%, kidney: 93.6%, lung: 86.4%, muscle tissue: 93.2%) in all tissues and organs, while the proportion of the remaining metabolites differed slightly according to the organs and tissues (Fig. 4). However, the presence of significant proportion of *p,p'*-DDE and of low *p,p'*-DDT concentrations reflected a remote input of this pesticides in the environment. Also the ratio of concentrations of *p,p'*-DDE/DDTs (0.90), indicative of the chronology of DDT input, being above the value of 0.6, which is regarded as a critical threshold (Aguilar, 1984), indicated a lack of new sources of DDT entering the ecosystem. On the other hand, the low in DDT levels in the tissues of marine turtles and the high proportion of metabolised forms is a pattern encountered in worldwide marine turtle populations (Lake et al., 1994; Rybitski et al., 1995; Mckenzie et al., 1999), with the only exception of that reported in sea turtles from the Eastern Pacific, in which 4,4'-DDD was the dominant of the DDT isomers (Gardner et al., 2003).

3. Conclusions

The results from this study indicate that PCBs and DDTs generally accumulate in the following order: liver>kidney>lung>muscle tissues, likely reflecting the relative lipid concentrations of these tissues. Nonetheless, such differences in organochlorine concentrations among tissues are not reflected by congener profiles, congener patterns being similar between liver, kidney, lung and muscle tissue. Our data also show that concentrations of PCBs are significantly higher than DDTs ($P<0.03$), revealing that these organisms might be predominantly exposed to industrial pollution. Further, high of *p,p'*-DDE/DDTs ratio (0.90) indicates the lack of new sources of DDT, as well as high tPCB/tDDT ratio, from 2.88 to 3.43, denotes a proportionally higher contribution of pollutants releases of industrial origin to agricultural origin. Several studies have indicated that Adriatic, in particular the Po Delta, due to the great availability of food and warm shallow waters is an important foraging and over-wintering area for Mediterranean loggerhead turtles (Margaritoulis, 1988; Argano et al., 1992; Lazar et al., 2000). The reduced hydrodynamics, coupled with high riverine inputs and intensive industrial activities make this sea a particularly sensitive area. Several studies have confirmed that the Adriatic Sea is a basin highly exposed to contaminant inputs originating almost exclusively from industrial activities (Viganò et al., 2001; Frignani et al., 2001; 2004; Solis-Weiss et al., 2004). Such an environmental situation can have important toxicological implications for these endangered marine organisms. The toxic equivalents calculated here rank higher when compared to those reported for other loggerhead turtles from the Adriatic Sea, but collected more than ten years ago (Corsolini et al., 2000). Even taking into consideration that TEFs for bird might not be applicable to reptiles, a health risk for these organisms cannot be excluded. The longevity of marine turtles in combination with the fact that the mono-*ortho* congeners are shown to be hazardous congeners with long-term toxic potential and that the total toxic potential in these organisms is largely dominated by the concentrations of non-*ortho* coplanar PCB 77 are all factors increasing the level of health risk.

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