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Chlorobiphenyls, HCB, and Organochlorine Pesticides in Some Tissues of *Caretta caretta* (Linnaeus) Specimens Beached Along the Adriatic Sea, Italy

M. M. Storelli, G. O. Marcotrigiano

Chemistry Institute, Veterinary Medicine Faculty, University of Bari, Italy, strada prov.le per Casamassima Km 3, 70010 Valenzano (BA), Italy

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Marine turtle species present in the Mediterranean Sea are: Loggerhead *(Caretta caretta)*, Green *(Chelonia mydas)* and Leatherback *(Dermochelys coriacea)* (Arnold and Burton 1985). *Caretta caretta* is regarded as threatened, and it is therefore protected by the authorities in all Countries in which this species dwells. In Italy, it has been protected since February 24, 1976 (G.U. 1993). Of the world population of loggerhead adult females estimated about 100.000 specimens (Waldichuk, 1987), about 2000 are present in the Mediteranean sea (Venizelos, 1991). The critical situation of the Mediterranean marine turtle population may be related to different factors: coastal anthropization, that makes places unsuitable for nidification (Jones, 1990), the use of eggs as food in several Countries (Jones, 1990), the casual capture of young and adult specimens during the swordfish and tuna fishing campaign (Venizelos, 1991) and the problem of marine pollution which particularly influences this area. Mediterranean is a semi-enclosed sea in which contamination from anthropogenic sources could generate pollution problems affecting the whole Mediterranean basin. Chlorobiphenyls and chlorinated pesticides are well known as environmental contaminants because of their stability, bioaccumulative capacity and global occurence. The knowledge of PCBs and chlorinated pesticides levels in sea turtles is useful to assess the potential impact of these accumulated compounds on these rare endangered organisms. In order to establish the current levels of chlorobiphenyls and chlorinated pesticides in different organs and tissues of loggerhead turtles we have analysed samples of liver, lung, kidney and muscle from organisms stranded in south Adriatic Sea (Italy).

MATERIALS AND METHODS

Between August 1990 and February 1991 a massive beaching of cetaceans *(S. coeruleoalba)* and sea turtles *(C. caretta* along Apulian coast (South Adriatic Sea, Italy) occurred. Our survey concerns thirteen *Caretta caretta* specimens (sample weight and sex are reported in table 1). Following necropsy, liver, lung, kidney and muscle tissue were removed, wrapped in individual aluminium foils, and kept in deep freeze at - 20 °C until chemical analysis. The organs were analysed to determine chlorobiphenyl (PCBs = sum of 11 congeners), chlorinated pesticide (POCs = p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD) and

Correspondence to: G. O. Marcotrigiano

Table 1. Weight and sex of *Caretta. caretta* specimen.

SAMPLES	LOCATION	WEIGHT (kg)	SEX
C. caretta 1	Lesina	1.8	M
C. caretta 2	Brindisi	2.0	F
C. caretta 3	Lesina	2.2	M
C. caretta 4	Bari	2.5	F
C. caretta 5	Castro marina	2.8	F
C. caretta 6	Bari	54	F
C. caretta 7	Brindisi	50	F
C. caretta 8	Bari	55	F
C. caretta 9	Bari	59.2	F
C. caretta 10	Castro marina	69.4	F
C. caretta 11	Castro marina	100	F
C. caretta 12	Lesina	50	M
C. caretta 13	Lesina	58.7	M

hexachlorobenzene (HCB) concentrations. The following method was used. Aliquots (2-5 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 of the remaining extract was mixed with H_2SO_4 conc. for the clean up, following the procedure described by Murphy (1972). Analytical runs were made on a Carlo Erba HR gas chromatograph 5300 Mega Series with automatic injection system and with an electron capture detector ECD-400, Ni^{63}(temperature: 310 °C). The GC was connected to an IBM PS/2 55SX PC equipped with System Gold version 6.1 software program for integration purposes (Beckman). For all the analysis a fused-silica capillary column SPB-608 Supelco (length = 30 mt, inside diameter 0.25 mm and film thickness 0.25 µm), was used. Helium 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 50 °C. Oven steady for the first 1 min and then an increase from 50 to 180 °C at a rate of 15 °C/min. Oven maintained at steady temperature for 1 min and then an increase from 180 to 220 °C at a rate of 4 °C/min; oven maintained at steady temperature for 20 min and then an increase 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 eleven individual PCB congeners were 8, 20, 28, 35, 52, 101, 118, 138, 153, 180 and 209 IUPAC numbering system (Ballschmiter and Zell, 1980) determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%). The identity of the DDT group compounds was confirmed by an alkali conversion to their respective olefins and re-analysis by GLC. Analytical data, as for DDT group compounds and HCB were obtained by a comparison between sample peak area and external standards peaks area (POCs and HCB mixture, bought from Supelco). Recoveries are determined by adding

known amounts of PCBs, POCs and HCB standards to empty samples and found to be within 80-110%. The limits of quantification were from 0.1 to 0.4 ng/g on a wet wt basis for the pesticides and 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).

RESULTS AND DISCUSSION

Concentration ranges, (mg/kg lipid weight) mean of PCBs, p,p'-DDE, HCB concentrations with standard deviations, lipid percentage and Mann-Whitney test results in different organs of young and adult female specimens are shown in Tables 2. Data for p,p'-DDT, o,p'-DDT, p,p'-DDD and o,p'-DDD are not presented since concentrations of these analytes were below the detection limits in all samples.

The concentrations of PCBs in liver, kidney, lung and heart of young specimens were higher than those detected in adult females; in fact a significant difference was observed between the two groups. The two adult males showed higher values than those of adult females but lower than those found in youngs in liver, kidney, lung and heart except in muscle where the levels found were higher than those detected in adult females but comparable to those of young individuals. However, accumulation order of PCBs was: youngs>adult males>adult females (Fig. 1a).

The same trend was observed for p,p'-DDE with values significantly higher in all organs of youngs with respect to adult females. The two adult males showed in all organs examined values comparable to those of adult females but lower than those found in youngs (Fig. lb). Therefore, the accumulation order of p,p'-DDE was as follows: youngs>adult females=adult males.

Table 3 shows that the dominant congeners have 5-7 chlorine atoms, i.e., numbers 101, 118, 138, 153 and 180 in accordance to as previously observed in marine turtles by Lake et al., (1994) and McKenzie et al., (1999). The other congeners, including some with both less 4 and more 9 chlorine atoms (8, 20, 28, 35, 52 and 209) were below the detection limits in all samples. The chlorobiphenyls are dominated by congeners 153, presents at the highest percentages (37.5-40.0%) and 138 (31.4-35.2%), followed by 180 (16.3-21.4%), 118 (4.4-6.3%) and 101 (4.1-5.6%). There was little difference in the percentage of each congener in the different organs of the same animal. The predominant congeners present in these turtles samples (n°. 101, 118, 138, 153 and 180) have chlorine atoms in both para positions (except for n°. 101 which has only one para chlorine) and lack vicinal hydrogens in the meta and para position. These substitution patterns in PCB congeners confer a resistance to metabolic breakdown (Brown, 1992).

The concentrations of HCB were the lowest of all organochlorine compounds quantified in all tissues. In liver of all specimens the levels varied from 0.02 - 0.08 mg kg. HCB levels in kidney of all specimens were in the range 0.01- 0.04 mg/kg

Table 2. Range, mean and standard deviations of organochlorine compounds concentrations (mg/Kg lipid weight) and % lipid in young (n=5) and adult female (n=6) specimens of *C. caretta*, and Mann-Whitney U test results.

		Young	Adult females	U Test
Liver	LIPID %	0.90-2.42 1.54±0.59	6.58-26.53 15.91±9.64	
	PCBs	3.22-6.54 5.01±1.22	0.21-0.76 0.35±0.21	< 0.06
	DDE	1.15-1.81 1.42±0.25	0.25-0.46 0.41±0.25	< 0.06
	HCB	0.02-0.08 0.04±0.03	0.02-0.08 0.04±0.03	N.S.
Kidney	LIPID %	0.55-0.74 0.64±0.08	7.13-15.05 9.57±2.93	
	PCBs	3.22-5.07 4.20±0.83	0.24-0.42 0.34±0.07	< 0.06
	DDE	0.75-1.59 1.23±0.36	0.09-0.47 0.22±0.19	< 0.06
	HCB	ND-0.03 0.03±0.01	ND-0.03 0.02±0.01	N.S.
Lung	LIPID %	0.22-0.90 0.50±0.33	3.60-7.32 4.98±1.47	
	PCBs	1.59-4.01 2.95±0.97	0.12-0.35 0.25±0.10	< 0.06
	DDE	0.13-0.78 0.48±0.24	0.10-0.30 0.14±0.08	< 0.06
	HCB	ND-0.04 0.03±0.02	0.01-0.04 0.02±0.01	N.S.
Heart	LIPID %	0.20-0.52 0.30±0.13	2.85-5.63 4.19±0.99	
	PCBs	1.05-4.82 3.52±1.60	0.11-0.41 0.25±0.10	< 0.06
	DDE	0.59-1.55 1.13±0.36	0.08-0.20 0.13±0.05	< 0.06
	HCB	0.02-0.06 0.04±0.02	ND-0.06 0.04±0.02	N.S.
Muscle	LIPID %	0.60-1.47 1.01±0.33	1.10-3.92 2.01±1.03	
	PCBs	0.91-3.11 2.03±0.96	0.10-0.23 0.16±0.06	< 0.06
	DDE	0.25-0.77 0.54±0.19	0.10-0.34 0.18±0.10	< 0.06
	HCB	ND-0.05 0.03±0.02	ND-0.05 0.03±0.02	N.S.

with levels below the instrumental detection limits for five specimens. In lung and heart of all specimens the concentrations varied from 0.01 to 0.04 mg/kg and 0.01-0.06 mg/kg respectively. Levels were below the instrumental detection limits in only a specimen for both organs. The level of HCB varied from not detectable

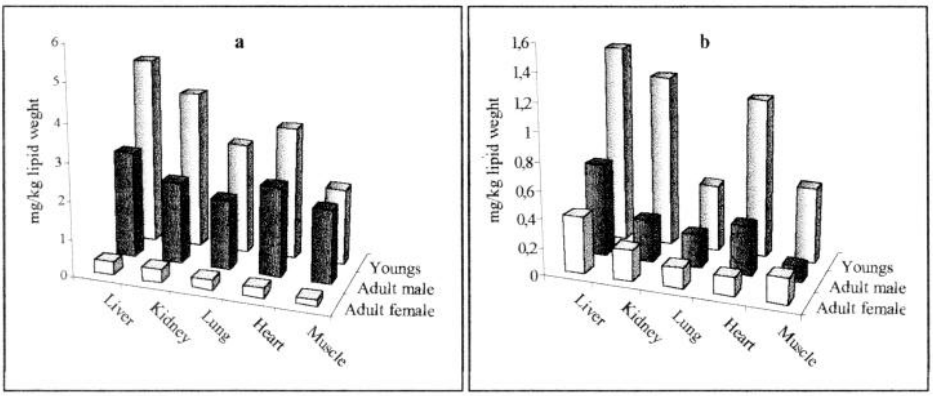

Figure. 1. PCBs (a) and p,p'-DDE (b) mean concentrations in youngs, adult males and adult females.

Table 3. Chlorination level of the PCBs in the organs of *C. caretta* specimens (% of sum-PCBs).

	PCBs Congeners										
	8	20	28	35	52	101	118	138	153	180	209
Liver	ND	ND	ND	ND	ND	5.6	5.0	35.2	37.5	16.7	ND
Kidney	ND	ND	ND	ND	ND	5.2	6.3	31.9	40.0	16.6	ND
Lung	ND	ND	ND	ND	ND	4.2	4.7	33.3	39.6	18.2	ND
Heart	ND	ND	ND	ND	ND	5.6	5.9	34.1	38.1	16.3	ND
Muscle	ND	ND	ND	ND	ND	4.1	4.4	31.4	38.7	21.4	ND

(in 3 specimens) to 0.05 mg/kg in muscle of all turtles. Concentrations observed in youngs were similar to those reported in adult female turtles in all tissues examined. HCB concentrations in various organs of the two adult males were comparable to those in the same organs from females.

The order of concentrations of organochlorine compounds in these specimens was PCBs>p,p'-DDE>HCB. The highest PCBs and p,p'-DDE values were detected in youngs with respect to adult females and males, while HCB concentrations were the same in all the organisms examined. The significant differences between the levels of organochlorine compounds in tissues from juveniles and adult females, can suggests that exist a mobilization of organochlorine compounds through the excretion of these contaminants via reproductive activities in accordance to what reported by McKenzie et al., (1999) in a survey on marine turtles. A datum that further on may confirm the existence in adult females of contaminant excretion via reproductive activities is given by the comparison between males and females of similar weight at about 50 kg (table 4). This comparison indicates that the concentration of PCBs in males are higher than those of the females of similar size. Nevertheless, the limited number of adult males analysed in the present

Table 4. Organochlorine compounds concentrations, mean (mg/Kg lipid weight) and lipid percentages in two male specimens of *C. caretta* compared with two female specimens of similar size

	Sample n.	Sex	Lipids %	PCBs	$\bar{X}$	DDE	$\bar{X}$	HCB
Liver	6	F	6.58	0.76	0.48	0.24	0.28	0.02
	8	F	8.11	0.21		0.32		0.05
	12	M	14.30	2.65	2.83	0.90	0.66	0.02
	13	M	15.01	3.01		0.42		0.03
Kidney	6	F	10.32	0.33	0.37	0.12	0.13	0.02
	8	F	9.26	0.41		0.15		0.05
	12	M	7.21	1.70	2.17	0.32	0.30	0.02
	13	M	10.30	2.65		0.27		0.04
Lung	6	F	7.32	0.33	0.22	0.11	0.11	0.02
	8	F	6.00	0.12		0.10		0.04
	12	M	3.95	2.27	1.86	0.23	0.24	0.01
	13	M	5.12	1.45		0.26		0.01
Heart	6	F	3.62	0.25	0.24	0.17	0.14	0.02
	8	F	4.02	0.24		0.10		0.04
	12	M	4.67	2.50	2.37	0.55	0.35	0.01
	13	M	3.62	2.24		0.15		0.02
Muscle	6	F	1.95	0.23	0.17	0.15	0.13	0.01
	8	F	1.10	0.15		0.10		0.05
	12	M	2.15	2.12	1.93	0.10	0.11	0.01
	13	M	1.95	1.74		0.12		0.01

study, yields uncertain an assessment of organochlorine contamination in marine turtles on the basis of sex and thus a vast scale research would be desirable,

Among DDT metabolites only p,p'-DDE was found in this study. In marine turtles Lake et al., (1994) and McKenzie et al., (1997) found that p,p'-DDE was present in the greatest concentrations 85% and >95% of the total of DDT compounds respectively. DDT, like other lipid soluble xenobiotics, is mostly metabolized by the action of the endoplasmic reticulum enzymes of hepatic cells, commonly known as microsomal enzymes. p,p'- DDT and other organochlorine compounds cause a conspicuous proliferation of the smooth endoplasmic reticulum (Ortega, 1966; Norback and Allen, 1972) and an increase in the liver's amount of the microsomal cytochrome pigments essential for these reactions, such as cytochrome P-450 and NADPH- cytochrome c reductase. (Greim and Remmer, 1966; Parkki *et al.*, 1977). This results in an enhancement of the metabolizing activity of microsomal enzymes which causes an increase in the relative concentrations of metabolic compounds. DDT is transformed into DDE through a dehydrochlorination produced by decomposition reactions. Consequently, DDE becomes progressively more abundant; more, DDE on account of its low polarity is excreted with difficulty and it inclines to accumulate. Therefore the presence of only this metabolite is not surprising; on the other hand studies on the

organochlorine compounds presence in the turtles eggs have pointed out the presence of only p,p'-DDE among DDT metabolites (Clark *et al.*, 1980, 1985).

The harmful effects of these pollutants on reproduction in other marine organisms are well known. Stertility was observed in seals (Reijnders, 1986; Helle et al, 1976a, 1976b), premature parturition in the sea lion (Delong *et al.*, 1973), and decreasing resistance to disease and illness in marine mammals (Morris *et al*, 1989). No direct studies concerning adverse effects of these pollutants on marine turtles are available. On turtles from freshwater environment, in particular *Chelydria serpentina*, Bishop et al. (1991), reported that PCBs levels ranging from 57-72 µg/kg wet weight, caused decreased hatching of eggs, greater incidence of deformities of hatchlings, and unhealthy, disoriented turtles. To establish databases on contaminants in marine turtles will help in understanding the role of contaminants in mortality events and provide a basis for investigating, predicting and mitigating these episodes. Therefore, even if it is not possible to relate these turtles deaths to organochlorine compounds body levels it may be reasonable supposed that these pollutants in synergy with other contaminants like metals, plastic bags, glass, could disturb the normal physiology of the animals.

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