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Organochlorine Compounds in Pelicans (*Pelecanus crispus* and *Pelecanus onocrotalus*) Nesting at Lake Mikri Prespa, North Western Greece

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Many studies have provided evidence of the large variety of persistent organic pollutants that are present in marine or continental aquatic ecosystems around the world. Fish-eating birds have been often chosen as bioindicators for such contaminants (Ohlendorf et al. 1978; Walker, 1990; Scharenberg, 1991; Focardi et al. 1992; Furness, 1993; Furness and Camphuysen, 1997). In the Palearctic, two species of large fish-eating birds, the Dalmatian and the Great white Pelicans (*Pelecanus crispus* and *P. onocrotalus*) occurs and are considered as vulnerable species (Crivelli et al. 1998). Both species forage mainly in inland freshwaters, although during migration and wintering they can forage on fish in coastal or marine ecosystems.

In the Palearctic, few studies have assessed the level of concentration of chlorinated hydrocarbons in eggs of pelicans (Fossi et al. 1984; Crivelli et al. 1989; Albanis et al. 1995). The aim of the present paper was to assess the trend of chlorinated hydrocarbons in eggs (1984 versus 1989) of both species of pelicans nesting at Lake Mikri Prespa, north western Greece.

MATERIALS AND METHODS

Eggs were collected in 1989 at the breeding colonies of Lake Mikri Prespa in north western Greece on the border with FYROM (Former Yugoslavian Republic of Macedonia), with a small section stretching into Albania. After collection, the eggs were frozen and brought back to the laboratory. In 1984 five eggs of *P. onocrotalus* from Mikri Prespa were analysed (Crivelli, Focardi, Renzoni, unpublished data) and the 1989 data will be compared with the latter. For *P. crispus* 1989 data will be compared with those of 1984 to 1986 (Crivelli et al. 1989).

For the analysis of chlorinated hydrocarbons, about 1g of the freeze-dried material was extracted for 9h in Soxhlet with n-hexane (pesticide grade) using cellulose thimbles (internal diameter x external length = 33mm x 94mm) preextracted for 7h in Soxhlet. The extract was subjected to sulphuric acid clean-up (Murphy, 1972) followed by Florisil chromatography, and was analysed using a Perkin Elmer gaschromatograph 8700 equipped with Ni63 electron capture detector. A

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SBP-5 bonded phase fused silica capillary column, 0.25 μm film thickness, 30-m long from Supelco was used. The quantification of PCBs was carried out using pure reference mixture of single congeners; the quantification of DDTs was performed using a standard with known quantities of the various components. Pure reference Standard solutions (from Supelco Co.) were used for instrumental calibration, recovery, quantification and confirmation. Recoveries were calculated by adding known quantities of standard to homogeneous replicates of the same samples. For eggs, recovery varies from a minimum of 85% to a maximum of 95%. All values are given in mg/kg dry weight.

Eggshell thickness was measured to the nearest 0.05 mm by calipers at four points on the shell of eggs that have been analysed for chlorinated hydrocarbons, all points located on the equator of these and museum eggshells. The pre-1947 (before DDT use) mean eggshell thickness of eggs of *P. crispus* was calculated using ten eggs collected in Greece in 1880 and in the Volga delta in 1905 and 1908, of *P. onocrotalus* using twelve eggs collected in Greece from 1986 to 1904 and from Danube delta in 1925 (Dr G. Rheinwald, pers. comm.).

RESULTS AND DISCUSSION

The levels of HCB in eggs are significantly higher ($P<0.01$) in *P. crispus* than in *P. onocrotalus* (Table 1 and Table 2). HCB is mainly concentrated in the yolk and is significantly lower in the albumen ($P<0.05$; Table 3). The levels of HCB in Dalmatian Pelican eggs in 1989 are lower than in 1985 and 1986, but similar to 1984 (0.103, 0.123 and 0.067 mg/kg dw, respectively; Crivelli et al. 1989). The levels of HCB in Great white Pelican eggs in 1984 at Lake Mikri Prespa (0.155 ± 0.09 mg/kg dw) and in Danube Delta in 1984 (0.318 mg/kg dw; Fossi et al. 1984) are significantly higher than in 1989 at Lake Mikri Prespa.

Lindane (γ-HCH) concentrations are not significantly different between the two pelican species in 1989 ($P<0.16$), or between yolk and albumen in eggs of Dalmatian Pelicans (Tables 1,2 and 3). The levels of Lindane in Mikri Prespa in eggs of *P. onocrotulus are* much lower than those observed in the Danube delta (1.154 mg/kg; Fossi et al. 1984).

DDT, pp'DDD and pp' DDE levels are more concentrated in yolk than in albumen of *Pelecanus crispus* (Table 3). Differences in levels of DDE between years (1984, 1985, 1986 from one side and 1989 from the other side) are significant ($P<0.001$), with DDE concentrations (Table 1) more than 5 times lower than in previous years in *P. crispus* (Crivelli et al. 1989). Levels of DDE in *P. onocrotalus* in 1984 at Lake Mikri Prespa (13.042 ± 4.06 mg/kg dw) were seven times higher than in 1989 ($P<0.004$; Table 2). Only DDD concentrations are significantly different between the two pelican species in 1989 ($P<0.04$).

The total PCBs are 1.78 mg/kg dw in *P. crispus* and 1.12 mg/kg dw in *P. onocrotalus* . Concentrations of PCBs in *P. crispus* are consistently higher in the yolk than in albumen as expected and differences are significant ($P < 0.01$; Table

6). Important differences (P<0.001) were found between 1984-1986 (Crivelli et al 1989) and 1989: the decrease being 64 % for *P. crispus* and 85% for *P. onocrotalus* (P<0.003; 1984 value: 7.814±2.317mg/kg dw). For the Great white Pelican it represents also a decrease of 80% in comparison of the Danube delta values (5.584 mg/kg dw; Fossi et al. 1984). PCBs composition was also determined (Table 4, 5 and 6): the main components in the total residues are the penta-, hexa- and heptachloro congeners. The most abundant is the isomer 22'44'55' (N° IUPAC = 153) which constitutes about 32% of the total residues in *P. crispus* and 31% in *P. onocrotalus* followed very closely by the isomers 22'344'5' (N° IUPAC= 138), 22'344'55' (N° IUPAC = 180) and 22'33'44'5 (N° IUPAC = 170).

Positive correlations between total PCBs and pp' DDE in eggs has been found for *P. crispus* (r = 0.79, F= 8.33 P<0.03) and also for *P. onocrotalus,* although not significant (r = 0.58, F = 1.8, P>0.21). Such positive correlations have been found previously by Borlakoglu et al. (1990) in adipose tissue of seabirds and cormorants. The ratio PCBs/DDE for 1989 is 0.76 in *P. crispus* against 0.41, 0.22, 0.40 for 1984, 1985, 1986 respectively, and 0.58 in *P. onocrotalus* against 0.56 in 1984 at Lake Mikri Prespa and 0.39 in the Danube delta (Fossi et al. 1984).

Eggshell thickness of *P. crispus* remains similar from 1984 to 1989 (from 0.638 ± 0.09 to 0.631 ± 0.08 mm), and significantly different from the pre-1947 value (0.734 ± 0.066 mm; P<0.009) with a decrease varying from 14 to 18% depending on the year. Probably there is no recovery of the eggshell thickness value in 1989, the decrease in organochlorines being too recent. The eggshell thickness values of *P. onocrotalus* in 1984 (0.757 ± 0.079 mm) and 1989 (0.722 ± 0.103 mm) are not significantly different from the pre-1947 value (0.705 ± 0.074 mm).

Our results suggest that the level of contamination in aquatic birds in Greece has dropped considerably in comparison with the early eighties. Such results are confirmed by the low values of organochlorines observed in *eggs* of *P. crispus* collected in the early nineties in another breeding Greek colony of Dalmatian Pelican (Albanis et al. 1995). Similar drop in organic pollutants has also been observed in eggs of birds in North America (Pearce et al. 1989) and in Europe (Fasola et al. 1998).

Pollutants have been implicated since long time in the decline of many aquatic bird species, among them the Brown Pelican (*Pelecanus occidentalis*) and the American white Pelican (*Pelecanus erythrorhynchos*) in North America (Anderson et al. 1969; Blus et al. 1974; King et al. 1985). Strong reduction in eggshell thickness compared with eggs laid early this century and reduced breeding success have been observed in those species of pelicans (Anderson et al.1969; King et al. 1985). Blus et al. (1974) and Blus (1982, 1984) established a critical level of DDE in eggs of Brown pelican as 3 ppm (fresh wet weight) for impaired reproductive success, accompanied by a 18-20% decrease in eggshell thickness. The levels of DDE observed in both pelican species in 1984 in Greece were similar to that found by Blus (1982) in the southeastern United States from 1969 to 1977 and associated with impaired reproductive success. However, in

Table 1. Organochlorines (mg/kg dw) in whole egg of *Pelecanus crispus*.

YEAR	n		HCB	γ-HCH	pp'DDE	pp'DDD	pp'DDT
1989	8	x	0.072	0.021	2.345	0.759	0.043
		SD	0.031	0.022	0.932	0.536	0.036
		range	0.023-0.108	0.007-0.074	1.197-3.690	0.273-1.716	0.009-0.120

Table 2. Organochlorines (mg/kg dw) in whole egg of *Pelecanus onocrotalus*.

YEAR	n		HCB	γ-HCH	pp'DDE	pp'DDD	pp'DDT
1989		x	0.036	0.009	1.924	0.297	0.025
	9	SD	0.025	0.007	1.020	0.222	0.016
		range	0.013-0.079	0.002-0.025	1.192-4.495	0.028-0.704	0.003-0.048

Table 3. Orgnanochlorines (mg/kg dw) in yolk and albumen of eggs of *Pelecanus crispus*.

YEAR	n			HCB	γ-HCH	pp'DDE	pp'DDD	pp'DDT
	3	yolk	x	0.096	0.022	5.301	1.347	0.0340
1989			SD	0.037	0.014	1.353	1.429	0.0290
		albumen	x	0.022	0.003	0.760	0.188	0.0037
			SD	0.009	0.001	0.193	0.215	0.0031

Table 4. PCB congeners (mg/kg dw) in whole egg of *Pelecanus crispus.*

YEAR	n		153	138	187	183	128	180	170	196	201	195	194	206
1989	8	x	0.564	0.318	0.108	0.058	0.026	0.293	0.169	0.043	0.045	0.034	0.023	0.011
		SD	0.279	0.134	0.051	0.030	0.021	0.157	0.079	0.021	0.021	0.017	0.011	0.006

Table 5. PCB congeners (mg/kg dw) in whole egg of *Pelecanus onocrotalus.*

YEAR	n		153	138	187	183	128	180	170	196	201	195	194	206
1989	7	x	0.310	0.188	0.072	0.040	0.015	0.192	0.119	0.035	0.039	0.030	0.021	0.007
		SD	0.152	0.102	0.044	0.025	0.011	0.121	0.068	0.021	0.023	0.019	0.012	0.003

Table 6. PCB congeners (mg/kg dw) in yolk and albumen of eggs of *Pelecanus crispus.*

YEAR	n			153	138	187	183	128	180	170	196	201	195	194	206
1989		yolk	x	0.981	0.528	0.181	0.093	0.019	0.426	0.272	0.065	0.066	0.044	0.024	0.022
	3		SD	0.437	0.226	0.111	0.058	0.011	0.276	0.168	0.043	0.036	0.034	0.008	0.016
		albumen	x	0.185	0.092	0.019	0.010	0.004	0.229	0.050	0.010	0.012	0.005	0.005	0.002
			SD	0.143	0.078	0.026	0.012	0.001	0.193	0.034	0.005	0.007	0.002	0.003	0.001

1989 in Greece, DDE levels in eggs of pelicans are low by comparison to those toxicologically significant levels described by Blus (1982, 1984), a fact confirmed by the increase of the number of breeding pairs of Dalmatian Pelican at Lake Mikri Prespa and the good breeding success registered for both species of pelicans during the early nineties (Catsadorakis et al.1996). We believe it would be worthwhile to make additional analyses of organochlorines compounds in eggs of pelicans in 1999 (ten years after the drop observed in 1989).

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