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Organochlorine Insecticide Residues in Eggs of the Little Tern *(Sterna albifrons)* in the Axios Delta, Greece

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Organochlorine pesticides such as DDT were long ago banned in America and Europe but due to their persistence their metabolites are still detected in the environment. In Greece, organochlorine insecticides such as DDT were extensively used before 1972 (Albanis et al. 1994). Coastal wetlands, which are widespread in Greece, are areas where pesticides from the agricultural activities of extensive surrounding areas are transported through rivers. In wetlands of the Thermaikos Gulf wetland complex (north-eastern Greece) among 11 pesticides detected in water and sediments three organochlorines, namely α-]BHC, lindane and 4,4'-DDE (a DDT metabolite) were found (Albanis et al. 1994). These and some other organochlorines were found in heron chicks and in their food in the same area (Albanis et al. 1996). Once these compounds were detected, and it was necessary to obtain more information about their presence in the food chains and simultaneously investigate potential effects on bird species that are of interest from a conservation perspective.

The Little Tern *(Sterna albifrons)* is declining in Europe and therefore receives conservation attention (Tucker & Heath 1994). This species and terns in general are suitable biomonitors because organochlorines in their fish prey are reflected in their egg contents (Ohlendorf *et al.* 1985, Hothem & Zador 1995). This study investigated the presence of organochlorine pesticide residues in eggs of Little Terns in the Axios Delta and the detection of potential effects on egg hatchability.

MATERIALS AND METHODS

Eggs in various stages of incubation were collected in June 1989 from clutches that had been destroyed by flooding due to high tides at a colony on a coastal sand bar in the Axios Delta (40° 30' N, 22° 43' E), a coastal wetland of the Thermaikos Gulf complex, in Macedonia, Greece (see Albanis *et al.* , 1994) for map and more information about the area) and only one egg per clutch was analysed. Soon after collection, the eggs were opened in the laboratory and their contents

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were placed in chemically cleaned glass tubes that were then preserved deep frozen (-20°C) until analysis. Eggshells were rinsed with distilled water and were left to dry at room temperature. Their thickness was measured by a micrometer graduated in units of 0.05 mm.

The contents of each egg were analysed for a series of organochlorine contaminants including : 2,4'-DDT, 2,4'-DDD, 2,4'-DDE, 4,4'-DDT, 4,4'-DDD, 4,4'-DDE, α-BHC, β-BHC, Lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin and endrin. Whole eggs were homogenized in a blender and an aliquot of 5 g was homogenized again with 20 g of sodium sulfate. This mixture was Soxhlet-extracted for 12 h with 100 ml n-hexane (pesticide grade). The extract was evapotated in a rotary evaporator to 12 ml and a portion of 10 ml was subjected to sulphuric acid clean-up (Murphy, 1972 ; Veierov and Aharonson 1980) followed by Florisil chromatography. After overnight drying at 200°C, Florisil (50-100 mesh; from Merk) was eluted with 100 ml of n-hexane. The purified sample was evaporated in a rotary evaporator to about 10 ml and in N_2 stream at 35°C to about 0.5 ml for the organochlorine compounds analysis. The purified sample was analysed by a Varian 3300 gas chromatograph equiped with Ni-63 electron capture detector (ECD) and two glass columns (2 m long, 0.3 mm i.d.) packed with 1.5% OV-17 +1.95% OV-210 on chromosorb Q (100-120 mesh) and 4% SE-30 + 6% SP-2401 on Supelcoport (100-120 mesh). Confirmation and the determination of organochlorine pesticides were made by using a Shimadzu gas chromatography equipped with ECD and a capillary column DB-1, 30 m long, i.d. 0.32 mm. Recoveries of pesticides from fortified chicken egg ranged from 93-112 %. The detection limits of selected organochlorine insecticides in fortified chicken eggs were 0.5 µg/Kg for α-BHC, lindane, heptachlor, aldrin, dieldrin and endrin and 1 µg/kg for β-BHC, heptachlor epoxide, 2,4'-DDT, 4,4'-DDT, 2,2'-DDD, 4,4'-DDD and 4,4'-DDE.

Mean concentrations were calculated after transformation of the individual concentration values to common logarithms. The individual concentrations values were adjusted to fresh wet weight and subjected to correlation analysis after the data were log (x+1) transformed for normality.

RESULTS AND DISCUSSION

Of 13 organochlorine compounds which were analysed for in 34 egg samples eight were detected (Table 1). Aldrin, heptachlor, 2,4'-DDT, 2,4'-DDE and 2,4'-DDD were not detected in any sample. There were significantly positive correlations between α-BHC and β-BHC ($r=0.410$, $P<0.02$), and significantly negative between α-BHC and 4,4'-DDT ($r=-0.422$, $P<0.02$). We also detected significantly positive correlations between 4,4'-DDE and endrin ($P<0.05$) but it is probably meaningless as endrin was only found in 3 eggs. Some orgalochlorines detected in other tern species' eggs have also been found to be intercorrelated (Ohlendorf *et al.* 1985, Becker *et al.* 1993).

Table 1. Geometric mean concentrations of organochlorines (μg/Kg wet wt) in Little Tern eggs (N=34) in the Axios Delta.

Contaminant	Mean	Range	% occurrence in the samples
α-BHC	3.94	ND-21.5	76.5
β-BHC	2.02	ND-2.0	35.3
γ-BHC (Lindane)	2.84	ND-44.6	67.6
4,4'-DDT	3.38	ND-34.4	73.5
4,4'-DDD	3.44	ND-41.3	61.8
4,4'-DDE	2.79	ND-22.3	58.8
Dieldrin	1.06	ND-2.6	11.8
Endrin	1.13	ND-4.5	8.8

Eggshell thickness was measured on 32 of the eggs, having an overall mean of 0.23 mm. Of the eggs, 14 were 0.20 mm thick, 13 were 0.25 mm, four were 0.30 mm and one 0.22 mm. Eggshell thickness in the three size-groups was not related to the 4,4' -DDE content of eggs (ANOVA, F=0.665, NS).

Dieldrin was found in lowest mean concentrations of the detected chemicals. Dieldrin concentrations found in the Little Tern and other terns' eggs were low although it occurred in variable proportions of egg samples (Alberto & Cuenca 1979, Ohlendorf *et al.* 1985, Weseloh *et al.* 1989, Hothem & Zador 1995). On some occasions levels appear similar in different geographical areas (Fasola 1987, Weseloh *et al.* 1989). Concentrations about ten times greater than the highest usually recorded in the previously mentioned studies have been found in eggs of the Herring Gull *(Larus argentatus)* in Germany (Becker *et al.* 1989). King et al. (1978) summarizing the effects of this agent on birds, reported a very considerable variation in the resistance of various species, proposing that amounts greater than 1 μg/g must be viewed as hazardous. Consequently, residue levels found in this study pose no danger to the birds of the area.

Endrin has scarcely been found in eggs of the Little Tern (Blus & Prouty 1979, Hothem & Zador 1995) or in other terns' eggs (King *et al.* 1978, Blus *et al.* 1985, Weseloh *et al.* 1989).

In this study, lindane was found in 68% of eggs and, compared to the other compounds detected, it was present in considerable concentrations, although its absolute amounts were very low. The elevated amounts of α-lBHC and β-BHC, which are metabolites of lindane, in our samples seems to reflect the extensive use of lindane in the surrounding agricultural land (Albanis *et al.* 1994). In relation to other organochlorine pesticides, lindane has been given much lower attention at least as far as contamination of tern egg is concerned. For example, among all the above mentioned studies, only in King *et al.* (1978), and Becker *et al.* (1989, 1993) was lindane analysed for. This compound, used as a heptachlor substitute for seed treatment, is safe to wild birds (Blus *et al.*, 1985) and thus, searching for it may not be useful when effects of chemicals on birds are investigated. Contamination by lindane and its metabolites in larid eggs may vary considerably among sampling locations reflecting local contamination (Becker 1989, Becker et al. 1993).

Numerous studies have examined the content of DDT and its metabolites in birds' eggs, their effects (particularly of DDE) on eggshell thickness and reproduction in general. In Little Terns, varying amounts of DDT metabolites have been detected in different geographical areas. Blus & Prouty (1978) found mean DDE residue values to vary from 0.33 µg/g to 0.45 µg/g (fresh weight) in South Carolina. Hothem & Zador (1995) found mean respective values of 1.02 µg/g and 0.936 µg/g in San Francisco and San Diego Bays. In Spain, Alberto & Cuenca (1979) found a mean of 0.623 µg/g. As suggested in these studies, such DDE concentrations had no effect on eggshell thickness or breeding performance. Much higher concentrations have been detected by Boardman (1988, in Hothem & Zador (1995)) in Venice Beach California, ranging from 4.5-23.0 µg/g with a mean of 9.99 µg/g. In an earlier study in Orange County, California, Massey (1971 in Blus & Prouty (1978)) reported concentrations of 6-41 µg/g which according to Blus & Prouty (1978) could have adverse reproductive effects on the reproduction of the Least Tern. Lambertini & Leonzio (1986), summarizing a number of studies in Italy indicate that mean DDE residues ranged from 1.95 to 11.29 µg/g dry weight (Po Delta). Such concentrations could also pose a threat to the Little Terns. In contrast to all studies mentioned, in the Axios Delta DDE concentrations were very low. Because of this, we did not find any relationship between DDE and eggshell thickness. Eggshells were much thicker than in other studies (i.e. Blus & Prouty (1978)). The geometric mean of the total amount of DDT metabolites in our egg samples was 0.011 µg/g, that is very low to have any adverse effects on birds' reproduction. Along with these results it is once more verified that the coastal areas of the eastern Mediterranean are less polluted by organochlorines than those of central-western part (Renzoni & Massa 1993).

The type and relative concentrations of some of the compounds detected in the Little Tern eggs in this study, in part reflect their presence in the wetland ecosystem of the Thermaikos Gulf. Albanis *et al.* (1994) found that α-BHC, Lindane and 4,4'-DDE were the dominant chemicals in the water and sediments in this ecosystem. Additionally, as in their study, heptachlor, aldrin, 2,4'-DDT, 2,4'-DDE and 2,4'-DDD, although analysed for, were not detected in Little Tern eggs. Nevertheless, in this study we detected the DDT metabolites 4,4'-DDT and 4,4'-DDD and also dieldrin and endrin not found by them at all. However, 4,4'-DDD was detected by Albanis *et al.* (1996) in Black-crowned Night-Heron *(Nycticorax nycticorax)* chicks and prey *(Rana* frogs). The occurrence of 4,4'-DDD in both species can be attributed to partly common prey use. Eighteen percent of 62 food items of Little Terns collected in their colony during the study were fresh- and brackish water fish *(Cyprinus, Gambusia, Rutilus*, and four other genera) (whereas the remaining 82% were sea fish species of the genera *Atherina, Sardina, Aphanius, Belone)*. Prey from the former category was frequently found in Black-crowned Night-Heron chicks regurgitates in the Axios Delta (Goutner & Kazantzidis, unpubl.). These imply that 4,4'-DDD may be limited to particular habitats, mainly those associated with fresh water.

The waterbird egg residues frequently reflect local contamination (Fasola 1987, Becker 1989) but other studies on larids have suggested that chemicals found in the eggs may be accumulated by adult females in polluted wintering grounds or during migration (Weseloh *et al.* 1989). The same has been found for herons and egrets (Findholt & Trost 1985, Fitzner *et al.* 1988). At present it is neither known where the populations of Little Terns breeding in the Axios Delta over winter nor their migration routes. If they migrate following the eastern Mediterranean corridor they may reach Somalia (western Africa) for wintering (Cramp 1985). Along this way they probably accumulate pollutants such as 4,4'-DDT, aldrin and endrin, that have been banned in Greece since 1972. In their study (Albanis et al., 1996) attributed to similar reasons dieldrin, found in Squacco Heron eggs in the Axios Delta but in no other sample analysed. At present, eggs of only Squacco Herons and Little Terns have been analysed for pollutants in this area.

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