PCBs and organochlorine pesticide residues in eggs of threatened colonial charadriiform species (Aves, Charadriiformes) from wetlands of international impor-

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tance in northeastern Greece

ABSTRACT. The levels of eight PCB congeners (IUPAC 8, 20, 28, 52, 101, 118, 138 and 180) and 13 organochlorine pesticides (α-HCH, β-HCH, Lindane, Heptachlor, Heptachlor epoxide, Aldrin, Dieldrin, Endrin, 2,4'-DDT, 2,4'-DDD, 4,4'-DDT 4,4'-DDD and 4,4'-DDE) were measured in eggs of Mediterranean gulls (Larus melanocephalus) (Lm), avocets (Recurvirostra avosetta) (Ra) and common terns (Sterna hirundo) (Sh) collected at the Evros Delta (Lm, Ra), Porto Lagos (Lm) and Axios Delta (Lm, Sh), north-eastern Greece in 1997. All pollutants were detected in all areas and species, with the exception of Dieldrin in the Mediterranean gull. Percent levels of higher chlorinated PCB congeners (IUPAC 118, 138 and 180) were greater than other compounds in all species and areas, probably due to their bioaccumulative properties. Only the median concentrations of PCB 28 differed significantly among areas in Mediterranean gull eggs. Significant differences between Mediterranean gulls and avocets (Evros) were found with regard to PCB 138 and PCB 180, whereas differences between Mediterranean gulls and common terns (Axios) were found in all PCBs except PCB 8 and PCB 20. These differences were due to the different diets of the species studied. Maximum pesticide concentrations in all samples were below 50 ppb, except for B-HCH and 2,4'-DDD, both of which predominated among all compounds in all areas and species. The same trend has been found in other waterbird species in Greece, suggesting a particular pollution pattern in the region. Significant differences in the median concentrations of most pesticides were found among areas in the Mediterranean gull. Such differences were also found between Mediterranean gulls and avocets and between Mediterranean gulls and common terns, again due to different feeding habits. Our results suggest that, in the wetlands of northeastern Greece, agrochemical sources dominate over industrial pollution. Pollutants still persist in Greek wetlands, but their levels are too low to have any adverse biological effect on the species studied.

KEY WORDS : Polychlorinated biphenyls, organochlorine pesticides, *Larus melanocephalus, Recurvirostra avosetta, Sterna hirundo*, Greek wetlands.

INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (hereafter pesticides) are two groups of substances that have different chemical structure and applications. PCBs were extensively used as plasticizers, as additives in hydraulic and dielectric fluids in industry and as fire retardants. Numerous organochlorine compounds have been used as pesticides. Nevertheless, both groups of chemicals have high toxicity and persistence in the environment. Studies have shown that some compounds of both groups can negatively affect wildlife reproduction and population levels and/or (particularly PCBs) cause various embryonic deformities and mortality (GILBERT-SON et al., 1991 ; YAMASHITA et al., 1993 ; CUSTER et al., 1999). Some of these substances can also pose threats to humans, especially at high levels, but also due to background exposure (LONGNECKER et al., 1997). Due to their lipophilic structure, both groups tend to bioaccumulate along food chains, and thus reach greater concentrations in higher-level consumers (TANABE et al., 1987; HARDING et al., 1997).

In Greece, pesticides and PCBs were banned before the mid-seventies, but studies have indicated that both groups persist in the Greek environment (ALBANIS et al., 1994; KONSTANTINOU et al., 2000; GOUTNER et al., 2001). Monitoring and comparative studies in multiple areas using higher trophic level receptors are scarce. However, Greek wetlands are of particular interest for such studies, as they a) support in their vicinity considerable human populations, who consume local resources such as water and fish and b) they also provide refuge to internationally important populations of wildlife, especially birds.

The levels of organochlorines in seabird eggs reflect the diet of the female and pollutant levels in body reserves, thus constituting a useful indicator of environmental contamination (PEARCE et al., 1989). Waterbirds are top predators in aquatic environments, and, especially fish-eating species, are suitable bioindicators (SCHAREN-BERG, 1991). Nevertheless, due to the complexity of the food chains and particularities in the response of each species to these chemicals, pollution studies must continue.

The aims of this study were : a) to investigate the occurrence of PCBs and pesticides in the eggs of some threatened waterbirds that represent different links in the food chain of Greek wetlands; b) to find out whether these chemicals occur in levels threatening the survival of these species; c) to obtain indirect information about the pollution of these birds' habitats, especially as all wetlands where we collected data are of international importance and protected by the Ramsar Convention.

MATERIAL AND METHODS

This study was carried out in wetlands of northeastern Greece (Fig. 1). The Evros Delta, at the Greek-Turkish border, is the easternmost Greek wetland (40°47'N, 26°05'E), extending over 11,000 ha, and with a great a variety of habitats (BABALONAS, 1980). The river Evros, originating in Bulgaria, is the border between Greece and Turkey for about 200 km and receives considerable amounts of transboundary pollution. Porto Lagos (40°01' N, 25°08' E), is an area in a wide wetland complex including the shallow, polluted, brackish Lake Vistonis and multifarious coastal lagoons. The Axios Delta (40°30'N, 22°53'E) is part of an extensive wetland complex, situated at the west coast of Thermaikos Gulf (KAZANTZIDIS et. al., 1997). This river originates in the former Yugoslavia and suffers considerable pollution, probably being the most highly polluted water body of northeastern Greece (FYTIANOS et al., 1986). All three wetlands are of international importance and protected by the Ramsar convention.

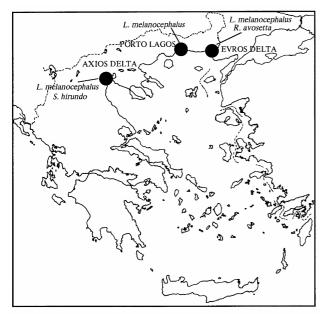


Fig. 1. – Map indicating the areas and species studied within the context of Greece.

Colonies were visited during the egg-laying period in April and May 1997. Under licence, we collected eggs of the Mediterranean gull (*Larus melanocephalus* Temminck 1820) (from the Evros and Axios Deltas and Porto Lagos), avocet (*Recusvirostra avosetta* L. 1758) (Evros Delta) and common tern (*Sterna hirundo* L. 1758) (Axios Delta) (Fig. 1). These species represent different links in the food chains of wetlands, as they have different diets. Eggs collected were either laid outside nests or from nests that were destroyed and abandoned by breeding birds due to flooding. Flooding may happen to these species' nests because they are made at close proximity to water. Eggs were opened in the laboratory on the day of collection and their contents were placed in chemically cleaned jars and deep frozen until analysis.

The following eight PCB congeners were analysed : PCB 8, 20, 28, 52, 101, 118, 138, 180. Of these congeners, five (PCB 28, 52, 101, 138, 180) belong to the group known as "target" or "indicator" PCBs (BACHOUR et al., 1998). The organochlorine pesticides analysed in this study were α -HCH, β -HCH, Lindane, Heptachlor, Heptachlor epoxide, Aldrin, Dieldrin, Endrin, 2,4'-DDT, 2,4'-DDD, 4,4'-DDT 4,4'-DDD 4,4'-DDE.

PCB-standards were obtained from Dr. Ehrensdorfer GmbH laboratory in concentrations of 10 mg/ml. Supelco No. 4-9151 organochlorine pesticides mixture standard in isooctane was used in concentrations of mg/ml for the chromatographic analysis. All solvents used (hexane, acetone, petroleum ether), were pesticide residue analysis grade, purchased from Pestiscan (Labscan Ltd, Dublin, Ireland). Florisil (50-100 mesh) and sodium sulfate (proanalysis) were from Merck (Darmstadt, Germany).

The analytical procedures and chromatographic conditions used have been described in detail in other recent papers of the authors (KONSTANTINOU et al., 2000; GOUT-NER et al., 2001). Here we provide a brief outline of the respective methods. An aliquot of 5-10 g of homogenized egg contents was extracted using sodium sulphate and petroleum ether (1:1) mixture. The centrifuged supernatant was evaporated in a rotary evaporator to 10 ml and lipids were then removed using sulfuric acid. The cleanup was completed by adsorption chromatography, eluting the colorless layer through a chromatography glass column. All solvents used for packing the column were degassed in sonication bath. The purified sample was evaporated in a rotary evaporator to ca. 5 ml and in gentle N₂ stream at 35°C to ca. 0.5 ml. Then samples were stored in silanized vials in a refrigerator (-20°C). Mean recoveries and method detection limits for each congener and compound are given in KONSTANTINOU et al. (2000).

A Shimadzu 14B gas chromatograph equipped with a Ni 63 electron capture detector (ECD) was used for the organochlorine residue analysis. Helium was used as the carrier and nitrogen as the make-up gas. Pure reference standard solutions were used for instrument calibration, recovery, quantification and confirmation. The confirmation of organochlorine residues was performed by using a GC-MSD, QP 5000 Shimadzu equipped with DB-5 capillary column.

Concentrations of pollutants were not normally distributed, thus the median of each pollutant, and the median total concentrations of PCBs (hereafter Σ PCBs) and pesticides (Σ pesticides) were compared. We used the Mann-Whitney U test to compare concentrations between species in the Evros and Axios Deltas and Kruskal-Wallis χ^2 test to compare concentrations in Mediterranean gull eggs from the three areas. We calculated the ratio Σ pesticides/

PCB and organochlorine pesticide concentrations (ppb wet weight) in charadriiform eggs from Greek wetlands (< d. 1.: below detection limits); n.s.: not significant TABLE 1

 Σ PCBs in samples as a measure of agrochemical vs. industrial pollution (Fossi et al., 1984; PASTOR et al., 1995b) and then we compared the median values as previously specified. Separate cluster analyses for PCB and pesticide percentage levels were used to evaluate differences in pollution patterns among the areas studied. As linkage rule we used the "single linkage", where the distance between two clusters is determined by the distance of the two closest objects (nearest neighbors) in the different clusters. This rule will string objects together to form clusters, and the resulting clusters tend to represent long chains. As distance measure we used the "Euclidean distance" that is the geometric distance in the multidimensional space. Euclidean distances are computed from raw data, and this has certain advantages, as the distance between any two objects is not affected by the addition of new objects, which may be outliers, to the analysis (STA-TISTICA, 1999).

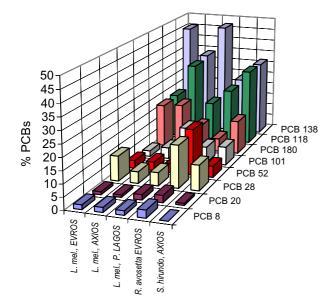


Fig. 2. – Fingerprint of PCB congeners (% of total concentration in each egg sample) detected in the charadriiform species studied in three Greek wetlands.

RESULTS

Polychlorinated biphenyls

With the exception of PCB 8, which was not detected in common tern eggs in the Axios Delta, all other congeners analysed were detected in all species' eggs. In the Evros Delta significant differences were found only in the median concentrations of PCB congeners 138 and 180 between Mediterranean gull and avocet egg samples, due to higher concentrations in the gull eggs (Table 1). In contrast, in the Axios Delta, the median concentrations of all congeners, except 8 and 20, were significantly different between Mediterranean gull and common tern egg samples. In this case, the concentrations of all congeners were higher in the common tern. The only congener with concentrations differing significantly among Mediterranean gull eggs from the three wetlands was PCB 28, showing highest levels in the Evros Delta. Of eight congeners analysed, the maximum levels of PCB 8, 28, 52, 138 and 180

were found in the Evros Delta. The maximum levels of PCB 118 were found in the Axios Delta, whereas of congeners 20 and 101 at Porto Lagos.

The proportions of congeners with a higher substitution pattern (118 and above) predominated in all species and areas, but some of the "lower" congeners occurred in relatively high concentrations, mostly in avocet eggs less so in common tern and Mediterranean gull eggs, resulting in characteristic fingerprints (Fig. 2).

Cluster analysis separated the pollution pattern of avocet eggs from all other samples. Another group included both species sampled in the Axios Delta, while a third joined the pollution patterns of Mediterranean gulls from the Evros Delta and Porto Lagos (Fig. 3).

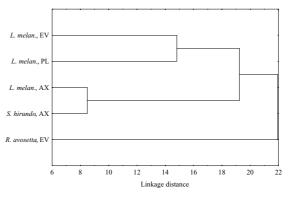
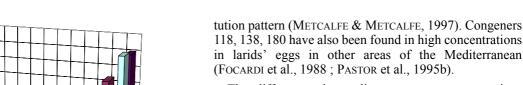


Fig. 3. – Cluster indicating the PCB pollution patterns of the species studied in the three wetlands. Euclidean distance measures and single linkage rule were used.

Organochlorine pesticides

Of the 13 compounds analysed in bird eggs, Dieldrin was below detection limits in Mediterranean gulls in all wetlands, but was detectable in the other two species. Median concentrations of most compounds (except Aldrin, Heptachlor and 2,4'-DDT) differed significantly between Mediterranean gulls and avocets in the Evros Delta (Table 1). Notably, of all samples, the maximum concentrations of Dieldrin, 2,4'-DDT, 4,4'-DDD and 4,4'-DDT were found in avocets. In the Axios Delta, the median concentrations of most compounds (except ß-HCH, Aldrin, Heptachlor 2,4'-DDT and 4,4'-DDD) were significantly different between Mediterranean gulls and common terns. The median concentrations of Aldrin, Endrin, Heptachlor epoxide, 4,4'-DDE, 2,4'-DDD and 2,4'-DDT differed significantly among Mediterranean gull eggs from the three different wetlands (Table 1). The maximum concentrations of six organochlorines, namely β-HCH, Lindane, Aldrin, Heptachlor, 2,4'-DDD and 4,4'-DDT, were found in the Evros Delta, whereas maximum concentrations of α -HCH and Heptachlor epoxide were found in the Axios Delta, and maximum concentrations of Endrin, 4,4'-DDE, 2,4'-DDT and 4,4'-DDD were measured at Porto Lagos.

Of all compounds, the proportions of β -HCH and 2,4'-DDD clearly predominated in all areas and in all three species studied, resulting in a characteristic fingerprint. In addition, levels of Heptachlor epoxide were higher than Heptachlor (Fig. 4).



S. hirundo, AXIOS R. avosetta EVROS

L. mel., P. LAGOS

L. mel., AXIOS

The differences in median congener concentrations detected between Mediterranean gulls and avocets in the Evros Delta, and especially between Mediterranean gulls and common terns in the Axios Delta, were probably due to the different feeding habits of the species involved. Avocets are lower in the food chain than larids feeding mainly on invertebrates (GOUTNER, 1985; FOCARDI et al., 1988; DENKER et al., 1994). The common tern is mainly piscivorous, whereas the Mediterranean gull's diet varies greatly among years and areas. It is mainly composed of insects and fish (GOUTNER, 1986), but may also include considerable amounts of plant material (GOUTNER, 1994). The similarity in PCB pollution of Mediterranean gull eggs from all study areas suggests dietary similarities during the study.

The pattern of pollution found by the cluster analysis, that is the grouping of the Axios Delta samples as a distinct cluster from that of the Mediterranean gull samples from the eastern part of the study area, together, may suggest different historical patterns of management regime of PCBs in the areas studied. The sources of PCB pollution at the Axios Delta have been transboundary and of municipal origin (LARSEN & FYTIANOS, 1989), while the other areas' pollutant sources are unknown at present.

To compare the PCB levels found in this study with results of other studies, we multiplied by two the sum of PCB concentrations in the eggs of the three species sampled (DIRKSEN et al., 1995). In the avocet, the mean (median) concentration multiplied by two was 51 (50) ppb, in the common tern 189 (170) ppb, and in the Mediterranean gull 114 (109) ppb. In the Great Lakes' Herring Gulls (Larus argentatus Pontoppidan 1763), concentrations that have been associated with reduced hatching success are generally higher than 70 ppm (GILMAN et al., 1977; WESELOH et al., 1979). In the Great Lakes' Doublecrested Cormorants, Phalacrocorax auritus (Lesson 1831), total PCB means of c. 4 to 7 ppm were associated with live-deformities (hard tissue malformations, YAMAS-HITA et al., 1993), whereas in Massachusets, USA, total PCB means of 1.4-6.0 ppm were not associated with adverse biological effects in the common tern (NISBET & REYNOLDS, 1984). Consequently, the contaminant levels found in this study seem to pose no threat to the populations of the waterbirds studied.

Although pesticides (with the exception of Lindane) were banned in Greece in 1972 (ALBANIS et al., 1994), most of them were detected in egg samples in our areas, though in low levels. The occurrence of pesticides in bird eggs seems also to be dependent on their feeding habits (FASOLA et al., 1987). Nevertheless, the differences in median concentrations we detected between species in the same area or in the same species among areas, can only in part be attributed to different feeding habits. We suppose that Dieldrin and probably the other drins found in avocet and common tern eggs are accumulated during wintering or migration, as these species follow the eastern flyway to African wintering quarters. In the Axios Delta, drins were also found in eggs of other distant migrants, such as the

Fig. 4. – Fingerprint of organochlorine pesticides (% of total concentration in each egg sample) detected in the charadriiform

species studied in three Greek wetlands.

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Organochlorine 6 8

Cluster analysis distinguished two groups : one joined the pollution patterns of Mediterranean gulls from the Evros Delta and Porto Lagos, while the other connected common terns and avocets, which were separate from the Mediterranean gulls of the Axios Delta (Fig. 5).

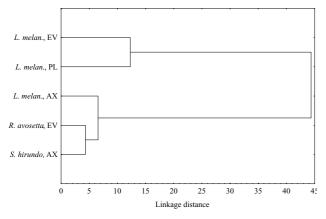


Fig. 5. – Cluster indicating the organochlorine pollution patterns of the species studied in the three wetlands. Eucledian distance measures and single linkage rule were used.

The medians of the ratio Spesticides/SPCBs varied from 2.62 (Mediterranean gull, P. Lagos) to 13.49 (avocet, Evros Delta). The difference of the medians of this ratio was significant between Mediterranean gulls and avocets and also among Mediterranean gulls from all areas (Table 1). Spearman Rank Correlations between Σ pesticides and Σ PCBs for Mediterranean gulls (pairs between areas), for Mediterranean gull and avocet, and Mediterranean gull and common tern were all statistically insignificant.

DISCUSSION

Of PCBs detected in our samples, some mono- and diortho coplanar congeners such as 28, 118, 138, 180 were the most elevated in all species and areas. These congeners exhibit considerable bioaccumulation patterns and are persistent in the environment because of their substilittle tern (*Sterna albifrons* Pallas 1764) and the Squacco heron (*Ardeola ralloides* Scopoli 1769) (ALBANIS et al., 1996; GOUTNER et al., 1997).

The elevated amounts of 2,4'-DDD in comparison to 4,4'-DDE are probably due to its presence in zooplankton and in the water column. Zooplankton possibly acquires DDTs from sediments or from the water column, where the DDD form constitutes the major fraction of DDTs (STRANBERG et al., 1998). Elevated 2,4'-DDD levels may indicate that this compound was a major constituent in a technical mixture used in the region. An important finding is that the characteristic similarity of pesticide finger-prints in all areas and species was also found in previous studies in Greece involving the cormorant, (*Phalacrocorax carbo* (L. 1758) and Audouin's gull (*Larus audouinii* Payraudeau 1826) (KONSTANTINOU et al., 2000; GOUTNER et al., 2001). These suggest a particular pattern of organochlorine pesticide pollution in Greece.

The higher levels of B-HCH in all egg samples were probably due to the relatively high stability of this compound against metabolism (OXYNOS et al., 1993). Lindane is still used as seed and soil insecticide in various cultivations. Lindane levels were lower than those reported in waterbids in other studies, but B-HCH levels (where analysed) were generally higher (Fossi et al., 1984; FASOLA et al., 1998). Lindane is not harmful to birds, in contrast to Heptachlor and especially its metabolite Heptachlor epoxide, which are lethal for birds in concentrations ≤ 9 ppm (BLUS et al., 1985). Concentrations found in our egg samples did not seem to pose any threat to the birds studied. DDE is a compound that can lower the breeding productivity of waterbird populations by reducing eggshell thickness (BLUS, 1984; CUSTER et al., 1999). Levels of DDE that can affect eggshell thickness in the common tern are beyond 4 ppm (WESELOH et al., 1989). For the American avocet (R. americana J. F. Gmelin 1789), the presumptive adverse threshold of DDE in eggs is 3-8 ppm (ROBINSON et al., 1997). Thus, it seems that the DDE levels we found in our study are too low to be harmful for the birds involved.

The median ratio of Σ pesticides/ Σ PCBs was > 1 in the samples from all areas, denoting a dominance of agrochemical over industrial pollution in northeastern Greece. Similar results have been found in other studies in Greece and in the eastern Mediterranean and Black Sea regions (Fossi et al., 1984; PASTOR et al., 1995a; GOUTNER et al., 2001).

CONCLUSIONS

The most heavily polluted wetland area studied was the Evros Delta, where maximum concentrations of most pollutants were found in birds' eggs. Pollution patterns reflected the birds' different positions in the food chain. Levels of both pollutant groups were of the lowest reported, being rather a normal follow up of the ban of these substances in the mid-seventies, and were too low to have adverse effects on birds. The higher agrochemical pollution reflects the underdevelopment of industry in the region. The occurrence of compounds such as drins may reflect differences in bioaccumulation patterns due to different migration routes and/or wintering grounds of some species. Predominance of β -HCH and 2,4-DDD' in all species' eggs and also in others studied in Greece (*P. carbo, L. audouinii*), suggests a particular pattern of agrochemical pollution in this region.

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