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PRESENCE AND TIME TRENDS OF ORGANOCHLORINE POLLUTANTS AND HEAVY METALS IN EGGS OF PREDATORY BIRDS OF SPAIN

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Organochlorine pesticides, polychlorinated biphenyls, and heavy metals are well known as persistent contaminants that accumulate in the upper trophic levels of food chains. These compounds have many and varied deleterious environmental effects (Edwards 1975, Forstner 1980, Kaiser and Tolg 1980). A close relationship between organochlorine insecticide residues in avian tissues and eggs and reduced reproductive success has been demonstrated for a variety of birds, both in controlled experiments and in field situations (Cooke 1973).

Although DDT was banned in Spain in 1977, DDE and DDT continue at high levels in certain parts of the country (Gonzalez et al. 1984, Hernandez et al. 1985). DDT continues to be used in other countries, including wintering areas of birds that breed in Spain. Due to the persistence of the compounds, as well as their continued introduction into the environment, some predatory birds are probably still ingesting organochlorines at levels that can reduce productivity. A similar situation exists among migratory North American birds (Henny 1977, Wiemeyer et al. 1978).

McEwen and Stephenson (1979) suggest that the following range of problems may be involved in reproductive failure caused by DDT and its derivatives: (1) laying of eggs with thin eggshells; (2) reduced clutch size; (3) high egg loss during incubation; (4) egg breakage; (5) high death rate of embryos; (6) high death rate in unhatched chicks at pipping; (7) high mortality of chicks; (8) late nesting and unusual nesting behavior including aberrant parental behavior and destruction of eggs; and (9) large proportion of adults in the population.

The present paper reports the levels of organochlorine compounds and heavy metals in 84 eggs of 5 species of Falconiformes collected at Doñana National Park, the Toledo Mountains, and the Balearic Islands (Spain). This study was designed to: (1) determine the levels of organochlorine pollutants and heavy metals in the eggs of predatory birds of Spain; (2) evaluate regional patterns and time trends of residues; and (3) evaluate the impact of the contaminants on reproductive potential.

STUDY AREAS AND METHODS

Eighty-four infertile eggs were collected between 1982 and 1985. The Doñana National Park, an area of 50,720 ha, is located in the south-southwest of the Iberian Peninsula between 37°7′ and 36°48′ North latitude and 6°12′ and 7°34′ West longitude. Stabilized sands and marshes constitute the 2 major ecosystems of the Park. The Doñana National Park is a Scientific Reservation in the Biosphere of UNESCO, is included in the International Agreement of Ramsar for the protection of wet areas, is of the utmost importance for sedentary resident birds, and is also important for birds migrating between Europe and Africa. Insecticides are not used in the Park, however, some of the agricultural practices in the surrounding areas require the use of insecticides. The Guadalquivir River flows through a major mining field as well as major urban and industrial centers before it enters the Park.

The Toledo Mountains and the Balearic Islands, located in the centre and the east of the Iberian Peninsula respectively, constitute two important breeding habitats of predatory birds in Spain (Fig. 1).

All eggs were collected after they failed to hatch and identified to species as shown in Table 1. They were kept frozen until preanalytical treatment was performed and analyzed individually for residues of organochlorine pesticides, PCBs, and heavy metals.

Organochlorine compounds.—α-HCH, γ-HCH, aldrin, dieldrin, heptachlor, heptachlor epoxide, dichlorobenzophenone, p,p'-DDE, p,p'-TDE, p,p'DDT, and PCBs were determined. Egg contents were homogenized with anhydrous sodium sulphate, and extracted with hexane in a Soxhlet apparatus for 8 h. The extract was cleaned on a partially deactivated Florisil column. The insecticides and PCBs were separated into 4 fractions (Cromartie et al. 1975, Kaiser et al. 1980), but fraction I was eliminated since it was not analyzed for HCB or mirex. Due to a lack of Silica, silica gel chromatography with the same eluting solvents was substituted in the analysis of the egg contents.

The organochlorine pollutants in each fraction were identified and quantified by injecting 10 μl or less of each eluate into a Perkin-Elmer Model 3920-B gas chromatograph equipped with an electron affinity detector of Ni-63. Instrument parameters and operating conditions were: column of 1.8 m by 4 mm ID, packed with a mixture of 7.5% QF-1 and 5% DC-200 on 80–100 mesh Chromosorb W-HP. The column temperature was 195 C, the injector temperature 250 C, and the detector temperature 275 C. Carrier gas was N_2 with a flow rate of 50 ml/mn. The PCBs were estimated by comparing the total peak area with that of Aroclor 1260.

The lowest limit of reportable residues was 0.01 ppm (mg/kg) for pesticides and 0.08 for PCBs. Recoveries of pesticides and PCBs from fortified chicken eggs ranged from 89% to 106%, but the residue data in the tables were not adjusted on the basis of these recoveries. All residues are expressed as ppm, wet weight. Geometric means were used to express residue levels. For samples in which no residues could be detected, a value of one-half the reportable limit was assigned for statistical analysis.

Heavy metals.—For the mercury analysis, duplicate homogenized samples weighing from 0.3 to 0.6 g (wet weight) were dissolved with 10 ml of 3:1 sulphuric and nitric acid mixture for 2 h at 60 C, followed by 15 ml of 6% MnO_4K_2 and analyzed by the cold vapor spectrometry tech-

FIGURE 1. Study areas and sampling locations.

nique of Hatch and Ott (1968), using a Perkin-Elmer spectrophotometer Model 103.

For the lead, cadmium, copper, and zinc analyses, each 5 g portion of samples was placed in a capsule and, after drying them for 2 h at 110 C, the capsules were placed in a muffle furnace, at 200 C for 2 h. The temperature was then increased to 450 C at a rate of 100 C/h and the sample was left to reduce to ashes overnight. The ashes were cooled, dissolved in approximately 4 ml of nitric and hydrochloric acids over a hot plate, transferred to a propylene tube, and diluted to 10 ml with distilled deionized water. The residues of metals were determined by comparison with aqueous standards on a Perkin-Elmer Model 2280 atomic absorption spectrophotometer (Haseltine et al. 1981).

The limit of detection was 0.01 ppm (mg/kg) for mercury, 0.02 ppm for lead, 0.001 ppm for cadmium, 0.002 ppm for copper, and 0.006 ppm for zinc. Recoveries of mercury, lead, cadmium, copper, and zinc from fortified chicken eggs ranged from 87% to 96%, but the residue data in the tables were not adjusted on the basis of these recoveries. All the residues are expressed as ppm wet weight. Geometric means were used to express the residue levels.

RESULTS

Organochlorine compounds.—All of the 84 egg contents analyzed during the 4 yr of investigation contained residues of DDE and PCBs (Table

TABLE 1. Avian species, location, year, and number of eggs collected.

Species	Location	Year	No. of eggs
Black Kite (*Milvus migrans*)	Doñana	1982	5
Black Kite (*Milvus migrans*)	Doñana	1983	4
Black Kite (*Milvus migrans*)	Doñana	1984	3
Black Kite (*Milvus migrans*)	Doñana	1985	15
Imperial Eagle (*Aquila heliaca*)	Doñana	1982	3
Imperial Eagle (*Aquila heliaca*)	Doñana	1983	10
Imperial Eagle (*Aquila heliaca*)	Doñana	1984	3
Imperial Eagle (*Aquila heliaca*)	Doñana	1985	3
Imperial Eagle (*Aquila heliaca*)	Toledo	1982	2
Imperial Eagle (*Aquila heliaca*)	Toledo	1983	2
Imperial Eagle (*Aquila heliaca*)	Toledo	1984	6
Marsh Harrier (*Circus aeruginosus*)	Toledo	1982	5
Marsh Harrier (*Circus aeruginosus*)	Toledo	1983	3
Marsh Harrier (*Circus aeruginosus*)	Toledo	1984	9
Northern Goshawk (*Accipiter gentilis*)	Toledo	1984	2
European Black Vulture (*Aegypius monachus*)	Balearic	1983	3
European Black Vulture (*Aegypius monachus*)	Balearic	1984	3
European Black Vulture (*Aegypius monachus*)	Balearic	1985	3

2). In addition, 73 samples (86.9%) had detectable amounts of DDT, 61 (72.6%) of γ-HCH, 41 (48.8%) of TDE, 27 (32.1%) of α-HCH, and 8 (9.5%) of dieldrin. Residues detected in individual eggs but not shown in Table 2 were dieldrin at 0.16 and 0.23 ppm in the Northern Goshawk, 0.01, 0.02, 0.02, 0.03, 0.04, and 0.79 in Marsh Harrier, all from the Toledo Mountains.

The maximum concentrations of DDE, PCBs, DDT, γ-HCH, TDE, α-HCH, and dieldrin were 45.89, 20.26, 8.61, 1.19, 0.30, 0.26, and 0.79 ppm, respectively. Levels of the other chlorinated pesticides (aldrin, heptachlor, heptachlor epoxide, and dichlorobenzophenone) included in the analytical survey were below their limits of detection.

Heavy metals.—All of the 84 egg contents analyzed during the 4 yr of investigation contained residues of Hg, Cd, Pb, Cu, and Zn (Table 2). In all cases Cd showed the lowest level with a range from 0.03 (Imperial Eagle, Doñana) to 0.20 ppm (Marsh Harrier, Toledo), followed by Hg with a range from 0.03 (Imperial Eagle, Toledo) to 1.18 ppm (Imperial Eagle, Doñana); Pb, range 0.30 (Imperial Eagle, Doñana) to 1.91 ppm (Imperial Eagle, Toledo); Cu, range 0.44 (Imperial Eagle, Toledo) to 2.78 ppm (Marsh Harrier, Toledo); and Zn, range 6.00 (Black Kite, Doñana) to 29.80 ppm (Marsh Harrier, Toledo).

Correlation of residue levels.—Among 8 chemical residues, there are highly significant positive correlations between each organochlorine pesticide: DDT_t and HCH_t (Table 3), as well as between DDT_t and PCBs. Of the 5 metals, 3 (Cd, Pb, and Zn) demonstrated at least 1 significant positive correlation with an organochlorine compound. In contrast, the

TABLE 2. Geometric mean and range of organochlorine pollutants and heavy metals in eggs of predatory birds from Spain (ppm wet weight).

Species (n)[a]	Location	α-HCH	γ-HCH	DDE	TDE	DDT	PCBs
B.K. (27)	Doñana	0.001 N.D.[b]–0.05	0.033 N.D.–0.32	0.994 0.20–4.43	0.003 N.D.–0.30	0.616 N.D.–6.14	3.522 0.42–20.3
I.E. (19)	Doñana		0.144 N.D.–1.19	1.324 0.08–4.68	0.003 N.D.–0.03	0.093 N.D.–1.96	1.912 0.80–3.36
I.E. (10)	Toledo	0.014 N.D.–0.06	0.023 N.D.–0.14	1.178 0.30–11.6	0.006 N.D.–0.07	0.133 0.04–0.66	0.197 0.08–1.54
M.H. (17)	Toledo	0.019 N.D.–0.26	0.021 N.D.–0.14	1.450 0.20–5.00	0.002 N.D.–0.09	0.449 N.D.–1.68	1.334 0.10–6.35
G. (2)	Toledo	0.012 0.01–0.02	0.053 0.04–0.07	41.449 37.4–45.9	0.049 0.03–0.09	7.232 6.07–8.61	12.958 10.5–16.0
B.V. (9)	Balearic	0.002 N.D.–0.01	0.010 N.D.–0.06	0.071 0.05–0.10	0.004 N.D.–0.01	0.048 N.D.–0.21	0.246 0.12–0.43

Species (n)	Location	Hg	Cd	Pb	Cu	Zn
B.K. (27)	Doñana	0.14 0.04–0.93	0.05 0.04–0.10	0.62 0.33–1.46	1.16 0.74–1.87	10.51 6.00–15.8
I.E. (19)	Doñana	0.32 0.14–1.18	0.05 0.03–0.08	0.52 0.30–0.74	1.29 0.92–1.60	10.55 7.03–20.4
I.E. (10)	Toledo	0.12 0.03–0.46	0.07 0.06–0.14	0.74 0.52–1.91	0.78 0.44–1.97	10.42 6.20–27.6
M.H. (17)	Toledo	0.20 0.05–0.78	0.10 0.06–0.20	0.78 0.48–1.36	0.97 0.37–2.78	9.43 6.48–29.8
G. (2)	Toledo	0.20 0.20–0.21	0.07 0.07–0.08	0.75 0.68–0.83	1.47 1.42–1.53	9.25 8.04–10.6
B.V. (9)	Balearic	0.06 0.04–0.11	0.06 0.04–0.09	0.68 0.53–0.91	1.10 0.93–1.31	11.57 8.28–19.3

[a] B.K. = Black Kite, I.E. = Imperial Eagle, M.H. = Marsh Harrier, G. = Goshawk, B.V. = Black Vulture; n = number of eggs.
[b] Not detected.

TABLE 3. Correlation matrix for residues of three organochlorines and five heavy metals identified in 84 eggs of predatory birds.

	Simple correlation coefficient (r)						
	HCH_t	DDT_t	PCBs	Hg	Cd	Pb	Cu
DDT_t	0.473**						
PCBs	0.229	0.465*					
Hg	0.002	0.063	0.097				
Cd	0.117	0.425*	0.460*	0.059			
Pb	0.007	0.414*	0.222	0.199	0.504**		
Cu	0.018	0.092	0.048	0.032	0.093	0.023	
Zn	0.191	0.351	0.615**	0.053	0.753**	0.457*	0.004

* $P < 0.05$; ** $P < 0.01$.

residues of Hg and Cu are usually not correlated with the residues of organochlorine compounds.

Significant positive correlations were found between Pb and Cd, Pb and Zn, Zn and Cd. Hg and Cu had no definitive tendencies for significant correlations with other heavy metals. The significance of these relationships in eggs of predatory birds is difficult to determine because different metals may follow different pathways in the body of the bird.

DISCUSSION

Organochlorine compounds.—DDE is the predominant insecticide found in our birds, as it was present in all eggs analyzed at levels from 0.05 to 45.89 ppm. As expected, the residue levels varied considerably, even within the same species from the same locality. Difference in age, growth, nutritional status, metabolic capacities and migration pattern between individuals probably contributed to this observed variance. It has been proved that DDE is the main pesticide responsible for decreasing the thickness of the eggshell but DDE's incidence is subjected to interspecific variations. Furthermore, the hazard to predatory birds must be imputed to their high position in the food chain and their sensitivity to DDE induced eggshell thinning. Keith and Grouchy (1972) reported that 12 ppm of DDE was associated with 20% of the thinning for the Prairie Falcon (*Falco mexicanus*), whereas Peakall et al. (1975) calculated comparable figures of 20 ppm for the Peregrine Falcon (*Falco peregrinus*), and Blus et al. (1972) estimated 8 ppm for the Brown Pelican (*Pelecanus occidentalis*). Kiff et al. (1979) confirmed that 5 ppm of DDE was correlated with 20% of the thinning for the California Condor (*Gymnogyps californianus*). Wiemeyer et al. (1984) reported that 5 ppm of DDE was associated with 10% of the shell thinning for the Bald Eagle (*Haliaeetus leucocephalus*) and mean 5-yr production was near normal for breeding areas where eggs contained less than 3 ppm DDE. With regard to the eggs of the White-tailed Eagle (*Haliaeetus albicilla*), 5 ppm of DDE could be associated with about 9% of the shell thinning as well as a reduction in mean production to about 0.6 young per occupied nest, a level below that needed for population stability (Coon 1983). In 8.3% of the instances the levels reported in this study are higher than those reported by Kiff et al. (1979), Coon (1983) and Wiemeyer et al. (1984); about 3.5% exceed those shown by Blus et al. (1972) and 2.4% are higher than those assumed by Keith and Grouchy (1972) and Peakall et al. (1975).

All of the 84 eggs analyzed contained residues of PCBs. Unexpectedly high levels of PCBs were present in 1 egg of the Black Kite (20.3 ppm) collected in Doñana National Park and in 2 eggs of the Northern Goshawk (10.5 and 16.0 ppm) collected in Toledo Mountains.

Several authors have suggested that PCBs have adversely affected reproduction in wild populations of predatory birds (Blus 1982, Newton and Bogan 1978). However, studies with Screech Owls (*Otus asio*) (McLane and Hughes 1980) and American Kestrels (*Falco sparverius*) (Lincer 1972) have shown little or no significant effects of PCBs on shell thickness or reproductive success. It is difficult to separate the effects of PCBs from those of DDE, therefore PCB levels and DDE levels in eggs are commonly evaluated collectively. DDE and PCBs appeared to interact in reducing breeding success of American Kestrels (Lincer 1972).

Residues of other organochlorine pollutants generally occurred much less frequently or they were found at much lower levels; furthermore DDT, TDE and γ-HCH are not significant factors in eggshell thinning (Faber and Hickey 1973).

Wiemeyer et al. (1978) have verified that intermediate levels in Osprey (*Pandion haliaetus*) of 2.4, 0.25, and 2.6 ppm of DDE, dieldrin, and PCBs, respectively, are found in a stable population with reproduction slightly reduced. The residues recorded in the present study are in the same order of magnitude as those supplied by Wiemeyer, with the exception of dieldrin which was detected in only 9.5% of the samples, and was present in minor levels. The present values of DDE and PCBs are considerably lower than the levels found in Peregrine Falcon eggs collected in 1973–79: 23.3 and 5.0 ppm respectively, and a shell thinning of about 16% (Enderson et al. 1982) and in Double-crested Cormorant (*Phalacrocorax auritus*) eggs that showed population declines and severe eggshell thinning at 7–11 ppm DDE and 10–12 ppm PCBs (Postupalsky 1971).

The Imperial Eagle and European Black Vulture were declared endangered species in Spain following major declines in some populations that were associated with low rates of reproductive success. Both populations are small, and concern has recently been expressed about their status. The present levels of organochlorine pollutants in Imperial Eagle and European Black Vulture are low and considerably lower than the levels reported to manifest possible toxicological implications; the levels of DDE, dieldrin, and PCBs found in the eggs in our study are not believed to have caused adverse effects on reproductive success or shell thickness.

The eggs of the Imperial Eagle from Doñana National Park contain lower concentrations of pesticide residues than those of the same species collected in the Toledo Mountains (with the exception of 1982), which may be attributed to the banning of pesticides in the Park. However, PCBs in Doñana reached higher levels than those in Toledo. Migrations do not seem to represent an important contribution to the levels of residues found in migratory species, for example the Marsh Harrier or the Black Kite, given that similar levels occur in sedentary species such as the Imperial Eagle or the Black Vulture.

In Figures 2 and 3, the geometric means of the residue levels of total DDT (DDE + TDE + DDT) and PCBs in predatory bird eggs are plotted vs. time. Egg residues of 1972, 1973–1976, and 1980–1981 have already been published (Baluja and Hernandez 1978, Gonzalez et al. 1984, Hoórn et al. 1973). In Spain, a ban on the general use of DDT was imposed in 1977; as expected a decrease was observed in the amount of total DDT in eggs from 1972 to 1985. The temporary evolution of total DDT in Imperial Eagle and Black Kite eggs from Doñana National Park shows that the total DDT levels fall in successive nestings. However, for both species, this trend is interrupted in 1982, possibly by illegal spraying. The PCBs levels in Imperial Eagle eggs show a slight but steady fall, between 1972–1981 and 1982–1985. For the Black Kite during the same period there is an increase in PCBs, especially in 1982, possibly because this species winters in Africa and breeds in the Doñana National Park. The Imperial Eagle is sedentary in the Park. The residue level of total DDT and PCBs in Imperial Eagle and Marsh Harrier eggs of Toledo Mountains decreased at similar rates.

The ratio of PCBs to DDE increased between 1982 and 1985 in all cases (except for Marsh Harrier in the Toledo Mountains) indicating that DDE concentrations in the foods of those birds decreased more rapidly than PCBs concentrations.

Heavy metals.—Existing data on the levels of heavy metals for falconiforme birds are scarce; for this reason the values found in the present work are compared with those reported by various authors for several predatory bird species. Wiemeyer et al. (1984) reported values of 0.02–1.2 ppm of Hg in Bald Eagle eggs collected in the United States, 1969–1979. Koivusaari et al. (1980) reported that total mercury concentration varied from 0.33 to 2.25 ppm in White-tailed Eagle eggs collected in Finland, 1969–1978. Finally, Henny and Kaiser (1979) have found 0.005–0.012 ppm of Hg in Swainson's Hawk (*Buteo swainsoni*) eggs collected in Oregon and Washington in 1976. Levels of Hg reported in the present study are within the range of these values.

The levels of heavy metals detected in eggs of falconiforme birds are below those reported to detrimentally affect reproduction of captive birds. Fimreite (1971) fed grain treated with a mercurial fungicide to Pheasants (*Phasianus colchicus*), and he found that their eggs containing 0.5–1.5 ppm of the mercury suffered greater mortality than the controls. Mallards (*Anas platyrhynchos*) fed a diet containing mercury laid fewer eggs and produced fewer young than the control group; average residues in eggs from 3 generations ranged from 0.79 to 0.86 ppm (Heinz 1979).

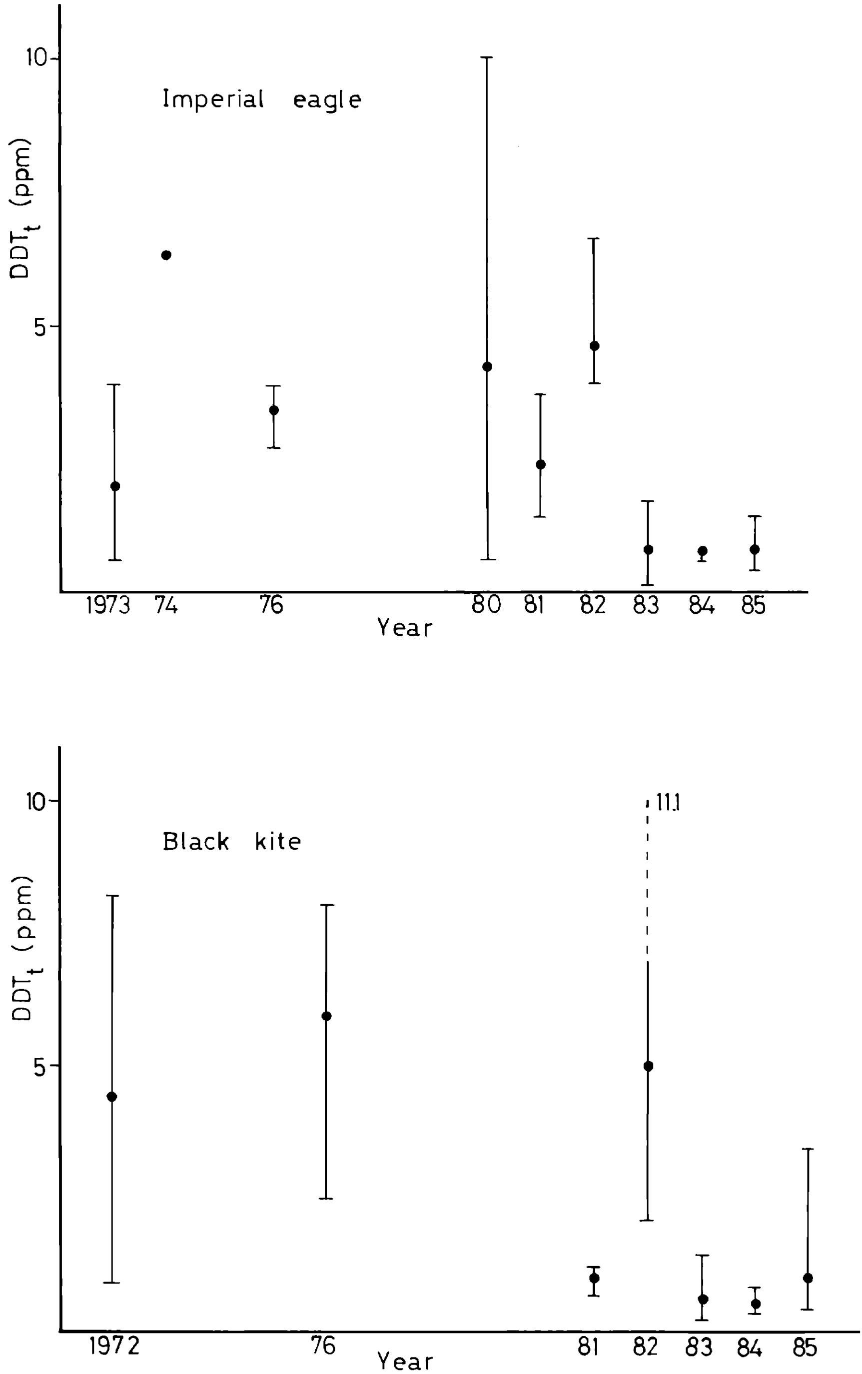

FIGURE 2. Total DDT levels (geometric means and range) in eggs of Imperial Eagle and Black Kite collected at Doñana National Park, 1972–1985.

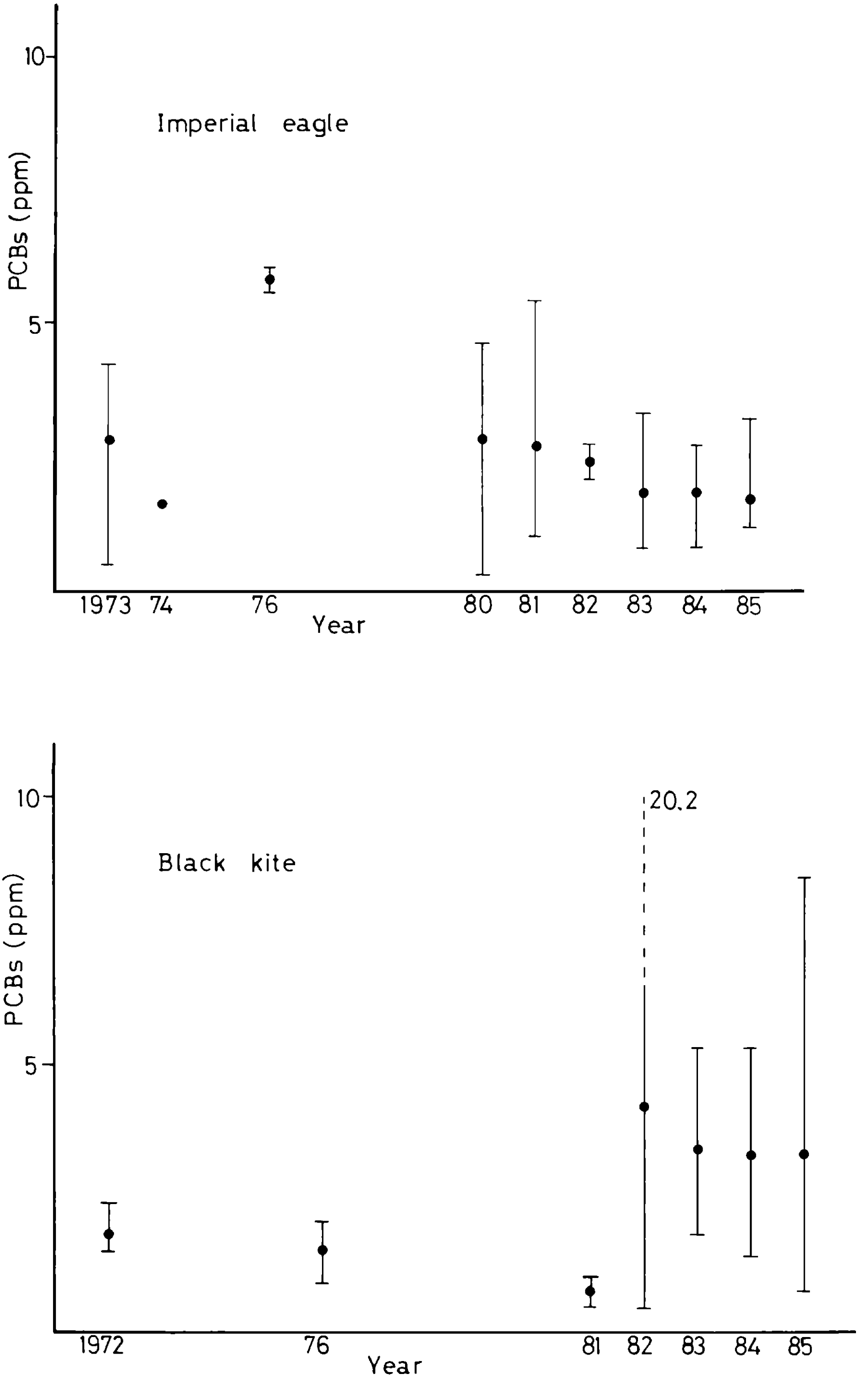

FIGURE 3. PCBs levels (geometric means and range) in eggs of Imperial Eagle and Black Kite collected at Doñana National Park, 1972–1985.

Only 10.7% of predatory bird eggs in our study contained more than 0.50 ppm of mercury and 7.2% contained more than 0.86 ppm of mercury, levels at which adverse effects on reproduction might be expected.

Investigations of Sparrow Hawk (*Accipiter nisus*) eggs indicate that a correlation exists between thin shells and relatively high concentrations of lead (Grandjean 1976). The role of lead as a possible inhibitor of eggshell production needs elucidation. The evolution that levels of heavy metals follow, in function of time, is unclear; more data and time are needed.

SUMMARY

The existence, and time trends of 11 organochlorine compounds and 5 heavy metals in 84 eggs of 5 species of predatory falconiforme birds collected at Doñana National Park, the Toledo Mountains and the Balearic Islands (Spain) during the nesting season between 1982 and 1985 have been investigated. Seven organochlorine compounds and all five heavy metals were present in the samples. The total DDT and PCBs content in eggs decreased with time. The levels of organochlorine pollutants and heavy metals in these eggs are generally below the levels known to cause direct effects on the survival or reproduction of birds.

RESUMEN

Se ha investigado la presencia y evolución temporal de 11 compuestos organoclorados y 5 metales pesados en 84 huevos pertenecientes a 5 especies, pertenecientes al orden de las Falconiformes, recogidos en el Parque Nacional de Doñana, Montes de Toledo e Islas Baleares (España) durante los periodos de incubación comprendidos entre 1982 y 1985. Se ha detectado la presencia en las muestras de siete de los compuestos organoclorados investigados y de los cinco metales pesados. El contenido en DDT total y PCBs de los huevos decrece con el tiempo. Los niveles de contaminantes organoclorados y metales pesados encontrados son inferiores a los que se considera que ejercen efectos deletéreos sobre la supervivencia o reproducción de las aves.

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NOTES AND NEWS

The North American Bluebird Society is proud to announce the presentation of the third annual research grant awards. The 1986 recipients are:

Bluebird Grant.—ANTHONY J. SAVERENO: Movements and habitat use of juvenile Eastern Bluebirds.
R. CRAIG HENSLEY: Predation of Eastern Bluebird nests by black rat snakes based on nestbox location.

General Grant.—JEFFREY R. WATERS: A comparative and experimental study of the nesting habitat of a cavity-nesting bird community.
GREGG ZUBERBIER: Effects of blowfly *Protocalliphora* sp. larvae on nestling passerines in both laboratory and natural environments.
BARRY A. BERMUDEZ: Development of a House Sparrow/Starling-proof nest box design that is acceptable to native cavity nesters.

Student Grant.—KAREN J. WILSON: Validating a Pileated Woodpecker habitat suitability index model for the northern Rocky Mountains.

The North American Bluebird Society annually provides research grants-in-aid for ornithological research directed toward cavity-nesting species of North America with emphasis on the genus *Sialia.* Presently three annual grants of single or multiple awards totalling $5000 are awarded and include:

Bluebird Research Grant.—Available to student, professional, or individual researchers for a suitable research project focused on any of the three species of bluebird from the genus *Sialia.*

General Research Grant.—Available to student, professional, or individual researchers for a suitable research project focused on a North American cavity-nesting species.

Student Research Grant.—Available to full-time college or university students for a suitable research project focused on a North American cavity-nesting species.

Further guidelines and application materials are available upon request from THEODORE W. GUTZKE, *Research Committee Chairman, P.O. Box 121, Kenmare, North Dakota 58746.* Completed applications must be received by **December 1, 1986**; decisions will be announced by January 15, 1987.