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Organochlorine Contaminants in Common Tern (*Sterna hirundo*) Eggs and Young from the River Rhine Area (France)

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Common terns (*Sterna hirundo*) exhibit a remarkable range of variation in reproductive success. Several factors are known to contribute to reproductive failure either before hatching or between the time of hatching and fledging : predation pressure, food availability, flooding, competition for nesting sites, and toxic chemicals (Morris *et al.*, 1976). Contaminants such as organochlorine pesticides, polychlorinated biphenyls (PCBs), polychlorodibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs), mercury and selenium were proved to significantly impair tern reproduction (Hays and Risebrough, 1972; Gochfeld, 1975; Kubiak *et al.*, 1989; Becker *et al.*, 1993).

During the reproductive period of 1988, an important mortality of common terns was observed in french colonies around the river Rhine. Approximately 50% of the young died a few days after hatching. The objective of the present study was to determine whether the intoxication by chlorinated compounds could have been responsible for the observed reproductive failure.

MATERIALS AND METHODS

Ten eggs and ten dead young of common terns were collected from Rohrschollen colony near Strasbourg (Alsace, France, 48.35 N, 7.45 E) in June 1988. Five eggs contained embryos, one contained a young tern dead just before hatching whereas no embryos were found in the others. The embryo and yolk were analysed separately in embryo-containing eggs whereas albumen and yolk were mixed before analysis for the other eggs.

Young terns and embryos were weighed and ground for 5 min in 40 ml *n*-hexane with 5 g anhydrous sodium sulphate. The homogenate was filtered on a buchner funnel filled with Hyflo-Supercel (Prolabo, France). The sample was re-extracted using 20 ml *n*-hexane. The organic fractions were pooled and concentrated to 1 ml using a rotary evaporator.

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The extract was then transferred to hexane-preeluted column (1 cm diameter, 50 cm long) containing 5 g Florisil (60-100 mesh) deactivated with 3% distilled water and covered with 2-3 g of anhydrous sodium sulphate. The column was first eluted with 20 ml *n*-hexane and with 50 ml *n*-hexane/diethyl ether (95:5, v:v) in a second phase. The eluted fractions were then appropriately diluted before analysis.

The yolk of the embryo containing eggs and the whole content of the other eggs were ground 2 min in 100 ml acetonitrile with 5 g Celite 545 (Prolabo, France). The homogenate was then filtered on a buchner funnel and the extract was transferred to a 1 l separatory funnel for liquid-liquid extraction. 50 ml petroleum spirit, 5 ml sodium chloride saturated water and 300 ml distilled water were then added in the separatory funnel. After 5 min shaking, the organic phase was collected. The extract was then evaporated to 1 ml using a rotary evaporator and purified as described above.

Extracts (5 μl) were injected in an ECD (^{63}Ni source) gas liquid chromatograph (Girdel Série 3000). Two 2.1 m x 2 mm i.d. glass column packed either with OV 210 or OV 225 on Chromosorb WHP (100-120 mesh) were used. Argon/methane (90/10; 40 ml min^{-1}) was the carrier gas. All analyses were performed under isothermal conditions of 210 and 220 °C for OV 210 and OV 225 respectively. Injector and detector temperatures were 240 and 270 °C respectively.

Standards of PCBs and organochlorine pesticides were obtained from Alltech France, Supelco and Aldrich-Chemie. Recoveries of pesticides and PCBs from fortified samples ranged from 89.7-108.1%, using the procedures described above. Residues were not corrected for percent recovery. The lower limit of reportable residues was 0.01 ppm for all pesticides and 0.05 ppm for PCBs.

Mean organochlorine concentrations were compared among samples (whole egg content, yolk, embryos, young) with analysis of variance (ANOVA) when at least half of the samples had detectable levels. Chi-square test was used when less than half of the samples had detectable levels (Henny *et al.*, 1984; Boellstorff *et al.*, 1985). For this analysis, a value of one-half the detection level was assigned to samples in which residues could not be detected (Ohlendorf and Miller, 1984; Custer *et al.*, 1983, 1985; Weseloh *et al.*, 1989).

Residue data in wild populations generally exhibit a skewed distribution (Ohlendorf *et al.*, 1978; Custer *et al.*, 1985). Therefore, the data were log-transformed before statistical analysis. Multiple comparison among groups were made by the Student-Newman-Keuls method when analysis of variance indicated significant differences among samples (Struger and Weseloh, 1985; Weseloh *et al.*, 1990).

RESULTS AND DISCUSSION

DDE and PCBs were detected in every egg and young (Table 1). PCB levels were 100- to 1000-fold higher than those of DDE. γ- HCH was detected in every dead young terns and in more than 65 % of the other samples whereas heptachlor epoxide was detected in 40 % of the eggs and only 20 % of the young terns (Table 1). p,p'-DDT, o,p'-DDD, α-endosulfan and dieldrine were not detected in any samples. DDE and PCB levels were significantly different between the various samples (Table 1). They were significantly higher in the yolks than in the embryos. This could be due to the relatively high lipid content of yolk.

Residue uptake by avian embryos is maximal just before hatching, when the yolk sac is resorbed (Custer *et al.*, 1983). This could account for the higher residue levels observed in young terns. The ingestion of contaminated food could also explains the relatively high PCBs levels in dead young terns (Custer *et al.*, 1985; Scharenberg, 1991). Monitoring studies have demonstrated that fish from the river Rhine were contaminated with PCBs (Thybaud and Ramade, unpublished data).

Short- and long-term toxicity in birds is often observed when the organochlorine pesticides concentrations in eggs are above 0.5 ppm (Fox, 1976; Nisbet and Reynolds, 1984; Hall *et al.*, 1989). However, Switzer *et al.* (1971, 1973) observed that mean DDE levels varying between 4.5 and 7.5 ppm in common tern eggs did not significantly reduce reproductive success. In this study, DDE, γ-HCH and heptachlor epoxide levels were inferior to the threshold value of 0.5 ppm.

PCB levels in eggs, yolks and young terns were always superior to 4 ppm with geometric mean concentrations varying from 6.1 to 14.65 ppm (Table 1). Among sensitive avian species, PCBs disrupt normal patterns of growth, reproduction, metabolism, and behaviour (Peakall *et al.*, 1972; Heinz *et al.* 1984; Eisler, 1986). According to Eisler (1986), reproduction in birds may be impaired when PCB level in eggs exceeds 16 ppm whereas Lorenz & Neumeier (1983 *in* Becker *et al.*, 1993) assume the range critical for affecting breeding success of birds to be between 3 and 5 ppm fresh egg mass. Moreover, pre- and post-hatching mortality is often correlated with PCB content of young birds (Hays and Risebrough, 1972; Hoffman *et al.*, 1987; Becker *et al.*, 1993).

Morris *et al.* (1976), Custer *et al.* (1983) and Nisbet and Reynolds (1984) did not reported any alteration of common tern reproduction with PCB levels in egg ranging from 4.6 to 37.8 ppm, whereas Hays and Risebrough (1972) and Gilberston *et al.* (1976) described morphological abnormalities in embryos contaminated with PCBs at levels ranging from 4.9 to 55.9 ppm. Hoffman *et al.* (1987) similarly reported a decrease in reproductive success of Forster's terns

Table 1 . Geometric mean concentration, range (ppm wet weight) and frequency of occurrence (%) of organochlorine residues in common tern eggs, embryos and young.

Sample	DDE	PCBs	γ-HCH	Heptachlor epoxide
Eggs without embryos (n=5)	0.016 *A*[a] (0.007-0.04) 100	6.1 *A* (4.29-10.11) 100	0.0015 *A* (ND[b]-0.0042) 80	0.0023 *A* (ND-0.0078) 80
Eggs with embryos				
Yolk (n=4)	0.15 *B* (0.07-0.37) 100	8.82 *A* (6.9-12.3) 100	0.001 *A* (ND-0.0045) 75	ND
Embryo (n=3)	0.005 *C* (0.002-0.01) 100	0.33 *B* (0.05-1.05) 100	0.0003 *A* (ND-0.0001) 67	ND
Young dead before hatching (n=1)	- 0.0075 100	- 6.71 100	- 0.0017 100	- ND
Young dead after hatching (n=10)	0.079 *B* (0.023-0.218) 100	14.65 *A* (4.41-49.33) 100	0.0014 *A* (0.001-0.0022) 100	0.0001 *A* (ND-0.0011) 20

[a]means not sharing the same letter are significantly different, $\alpha = 0.05$.
[b]ND - not detected.

(*Sterna forsteri*) following the contamination of eggs by PCBs at a level of 22.5 ppm. Scharenberg (1991) reported PCB levels in dead young terns ranging from 18.2 to 85.7 ppm depending on the age of the animals. He concluded that organochlorine residues alone did not cause the death of the terns but were an important stress factor

In the present study, 50% of the egg samples contained more than 16 ppm PCBs and young terns were heavily contaminated by these compounds. However the latter were less contaminated than the individuals studied by Scharenberg (1991). Therefore, it seems unlikely that organochlorine residues would be the single factor responsible for the observed reproductive failure of common tern in the River Rhine area.

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REFERENCES

Boellstorff DE, Ohlendorf HM, Anderson DW, O'Neill EJ, Keith JO, Prouty RM (1985) Organochlorine chemical residues in white pelicans and western grebes from the Klamath Basin, California. Arch Environ Contam Toxicol 14: 485-493

Becker PH, Schuhmann S, Koepff C (1993) Hatching failure in common terns (*Sterna hirundo*) in relation to environmental chemicals. Environ Pollut 79: 207-213

Custer TW, Erwin RM, Stafford C (1983) Organochlorine residues in common tern eggs from nine Atlantic Coast colonies, 1980. Colonial Waterbirds 6: 197-204

Custer TW, Bunk CM, Stafford CJ (1985) Organochlorine concentrations in prefledging common terns at three Rhode Island colonies. Colonial Waterbirds 8: 150-154

Eisler R (1986) Polychlorinated biphenyl hazards to fish wildlife and invertebrates, a synoptic review. U.S. Fish Widl. Serv. Biol. Rep. 85(1.7)

Fox GA (1976) Eggshell quality: its ecological and physiological significance in a DDE contaminated common tern population. The Wilson Bulletin 88: 459-477

Gilbertson M, Morris RD, Hunter RA (1976) Abnormal chicks and PCB residue levels in eggs of colonial birds on the Lower Great Lakes (1971-73). The Auk 93: 434-442

Gochfeld M (1975) Developmental defects in common terns of Western Long Island, New York. The Auk 92: 58-65

Hall RJ, Haseltine SD, Geissler PH (1989) Monitoring contaminant exposure: relative concentrations of organochlorines in three tissues of american black ducks. Environ Monit Assess 13: 11-19

Hays H, Risebrough W (1972) Pollutant concentrations in abnormal young terns from Long Island Sound. The Auk 89: 19-35

Heinz GH, Swineford DM, Katsma DE (1984) High PCB residues in birds from the Sheboygan River, Wisconsin. Environ Monitor Assess 4: 155-161

Henny C, Blus LJ, Krynitsky AJ, Bunck CM (1984) Current impact of DDE on black-crowned night-herons in the Intermountain West. J Wildl Manage 48: 1-13

Hoffman DJ, Rattner BA, Sileo L, Docherty D, Kubiak TJ (1987) Embryotoxicity, teratogenicity, and arylhydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. Environ Res 42: 176-184

Kubiak TJ, Harris HJ, Smith LM, Schwartz TR, Stalling DL, Trick JA, Sileo L, Docherty DE, Erdman TC (1989) Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan - 1983. Arch Environ Contam Toxicol 18: 706-727

Morris RD, Hunter RA, Mc Elman JF (1976) Factors affecting the reproductive success of common tern (*Sterna hirundo*) colonies on the lower Great Lakes during the summer of 1972. Can J Zool 54: 1850-1862

Nisbet ICT, Reynolds LM (1984) Organochlorine residues in common terns and associated estuarine organisms, Massachusetts, USA, 1971-81. Mar Environ Res 11: 33-66

Ohlendorf HM, Klaas EE, Kaiser TE (1978) Environmental pollutants and eggshell thinning in the black-crowned night heron. In: Sprunt A IV, Ogden JC, Winckler S (eds) Wading birds, Natl Audubon Soc Res Rep n°7, pp. 63-82

Ohlendorf HM, Miller MR (1984) Organochlorine contaminants in California waterfowl. J Wildl Manage 48: 867-877

Peakall DB, Linver JL, Bloom SE (1972) Embryonic mortality and chromosomal alterations caused by Aroclor 1254 in ring doves. Environ Health Perspect 1: 103-104

Scharenberg W (1991) Prefledging terns (*Sterna paradisea, Sterna hirundo*) as bioindicators for organochlorine residues in the German Wadden Sea. Arch Environ Contam Toxicol 21: 102-105

Struger J, Weseloh DV (1985) Great Lakes caspian terns: egg contaminants and biological implications. Colonial Waterbirds 8: 142-149

Switzer B, Lewin V, Wolfe FH (1971) Shell thickness, DDE levels in eggs, and reproductive success in common terns (*Sterna hirundo*), in Alberta. Can J Zool 49: 69-73

Switzer B, Lewin V, Wolfe FH (1973) DDE and reproductive success in some Alberta common terns. Can J Zool 51: 1081-1086

Weseloh DV, Custer TW, Braune BM (1989) Organochlorine contaminants in eggs of common terns from the Canadian Great Lakes, 1981. Environ Pollut 59: 141-160

Weseloh DV, Mineau P, Struger J (1990) Geographical distribution of contaminants and productivity measures of herring gulls in the Great Lakes: Lake erie and connecting channels 1978/79. Sci Total Environ 91: 141-159