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Organochlorine Pollutants in Peregrines and Merlins Migrating through Wisconsin

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The discovery that the Peregrines (*Falco peregrinus*) breeding in the eastern Arctic are laying thin-shelled eggs and are showing symptoms of reproductive failure (Berger *et al.*, this issue) suggests that this population is beginning to be affected by the same extinction process that has resulted in the extirpation of breeding Peregrines in many areas of North America and Europe (Hickey, 1969; Ratcliffe, 1970). The Peregrines of the eastern United States disappeared before the nature of the extinction process was evident, but subsequent research showed that eggshell thinning had been associated with the population decline (Hickey and Anderson, 1968). In Great Britain there was a considerable amount of evidence that excessive mortality of adult birds had been a major reason for the rapid decline that began around 1955, coinciding with the introduction of highly toxic insecticides such as dieldrin into agricultural use (Ratcliffe, 1963; Jefferies and Prestt, 1966). The surviving birds, however, experienced reproductive failures that were characterized by shell thinning, egg breakage, and eating of the eggs by the adult birds (Ratcliffe, 1967; Ratcliffe, 1970). Although embryonic mortality may be contributing to the decline in reproductive capacity of the Ungava Peregrines (Berger *et al.*, this issue) the pattern of egg breakage associated with shell thinning now observed in this population appears to be identical to the symptoms noted previously in southern Canada (Hall, this issue), the United States (Hickey and Anderson, 1968) and Great Britain (Ratcliffe, 1970).

At the conference convened in Madison, Wisconsin, by J. J. Hickey in 1965 to examine the causes of disappearance of the Peregrine from the eastern United States, all conceivable factors were considered in detail. Among these only environmental pollution was consistent with the pattern of extinction observed in Peregrine populations in both Europe and North America (Hickey, 1969). Since reproductive failure now appears to threaten the survival of the Arctic populations, it has become imperative to determine more precisely the particular pollutants responsible.

In addition to the evidence relating excessive adult mortality to the use of several of the more toxic of the chlorinated hydrocarbon insecticides, Ratcliffe (1970) has accumulated a considerable amount of data relating the shell thinning among British birds of prey to the environmental distribution of organochlorine pollutants, including both insecticides with their derivatives and industrial compounds. The latter consist primarily of polychlorinated biphenyls which have become widespread and locally abundant pollutants (Jensen *et al.*, 1969; Koeman *et al.*, 1969; Risebrough *et al.*, 1968). Other pollutants that might be contributing to the decline of the North American Peregrines include mercury and lead. Mercury

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is more widespread in the environment than was previously suspected (Finnerite *et al.*, this issue). Waterfowl may accumulate lead from ingested shotgun pellets (Bagley and Locke, 1967) and Peregrines preying upon them might acquire dangerous concentrations. There is as yet, however, no evidence that links either metal at environmental concentrations to the shell thinning phenomenon. Proof is rapidly accumulating, however, that one or more of the chlorinated hydrocarbons, particularly the DDT compound DDE, may induce shell thinning under both environmental and experimental conditions (Hickey and Anderson, 1968, Porter and Wiemeyer, 1969; Heath *et al.*, 1969; Anderson *et al.*, 1969). Very small concentrations of DDE are associated with significant changes in shell thickness in eggs of the Brown Pelican (*Pelecanus occidentalis*) (Risebrough, Anderson and Schreiber, unpublished observations). The role of DDE and of other chlorinated hydrocarbons found in the environment such as dieldrin and the polychlorinated biphenyls in producing additional sublethal effects that might interfere with reproduction is as yet unclear. Delayed breeding or abnormalities in behaviour could be associated with the high rate of steroid degradation caused by the liver enzymes induced by these compounds (Jefferies, 1967; Peakall, 1970). The volume that emerged from the 1965 conference in Madison (Hickey, 1969) concludes with the statement by Hickey and Roelle "How much industrial pollutants like the polychlorinated biphenyls have also contributed to some of these phenomena promises to be an absorbing chapter in the research of the immediate future" (Hickey and Roelle, 1969). The present paper reports on the polychlorinated biphenyls and other chlorinated hydrocarbons in fat biopsy samples obtained from ten Peregrines and seven Merlins (Pigeon Hawk, *Falco columbarius*) trapped at the Cedar Grove Ornithological Station on the western shore of Lake Michigan during the fall migrations of 1968 and 1969.

Materials and Methods

All of the Peregrines were immature birds of the year and therefore approximately three months old. Seven were biopsied in 1968 and three in 1969. All of the Merlin samples were obtained in 1969. Three were adult males, one an adult female, and the remaining three were immatures of the year.

The biopsy technique has been described previously (Enderson and Berger, 1968). There have been no indications that this procedure harms the birds in any way. Biopsy samples have been obtained from Harriers (*Circus cyaneus*) that have been observed for many months afterwards (Frances Hamerstrom, personal communication).

The adipose tissue biopsy samples were frozen, either in acetone-washed glass vials capped with aluminum foil, or in sterile pockets lined internally with aluminum foil which are used in medicine for antiseptic gauzes. The samples were weighed in the laboratory to obtain wet weights and then ground in a Waring blendor with anhydrous sodium sulfate. Lipids were obtained by soxhlet extraction for at least six hours with a hot mixture of two parts of hexane and one part of acetone. The organic solvents were removed by evaporation. When small amounts of sodium sulfate were carried over into the extract, the lipid was dissolved in petroleum ether and washed with water.

The biopsy samples were small. The Peregrine samples averaged 0.49 grams, with a range from 0.0496 to 0.983 gm, and the Merlins averaged 0.162 gm, with a range from 0.0497 to 0.5608 gm. Some samples were found to consist mainly of connective tissue rather than lipid. The Peregrine samples consisted of an average of 69% by weight of extractable lipid, with a range from 31% to 91%. One Merlin sample consisted of only 5% lipid; the average percent lipid among the Merlin samples was 35%, and the highest value was 68%.

The lipid extracts were dried at 65° for 8-12 hours, weighed in the beakers, dissolved in hexane and divided into one or more aliquots. The aliquot analysed for the DDT and PCB compounds was passed through a modified Davidow column (Stanley and LeFavoure, 1965). This column, consisting of celite, sulfuric acid and fuming sulfuric acid, permits rapid cleanup with quantitative recov-

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eries of the DDT compounds and PCB. Although not an important factor for the present study, it permits much larger amounts of lipid material to be treated at one time than does the florisil method. This procedure, however destroys dieldrin and to determine this compound another method must be employed. Another aliquot of the lipid extract, in hexane, was partitioned twice with acetonitrile, the combined acetonitrile fractions were evaporated to dryness, redissolved in petroleum ether, and applied to a florisil column. The column was rinsed first with 200 ml of petroleum ether and 200 ml of 6% ethyl ether in petroleum ether. Dieldrin was eluted by passing 200 ml of 15% ethyl ether in petroleum ether through the column. Control had indicated that 90% or more of the dieldrin applied to a florisil column eluted in this fraction.

Blank samples consisting of redistilled petroleum ether analysed through the entire procedure showed no substances that might interfere with the determination of any of the chlorinated hydrocarbons reported here.

The extracts were analysed with a Microtek gas chromatograph equipped with a tritium and a nickel-63 electron capture detector. Glass columns were used containing 3% QF-1, or a mixture of 3% QF-1 and 10% DC-200, on Chromosorb W, 80-100 mesh, acid washed and HMDS treated.

Other laboratories (Reynolds, 1969; Armour and Burke, 1970) have developed methods for separating the polychlorinated biphenyls from some or all of the chlorinated hydrocarbons of insecticide origin. The polychlorinated biphenyl compounds emerge at different times from the gas chromatograph and occasionally a peak may coincide or interfere with the peak produced by one of the insecticides. We have found it possible, however, to quantify PCB, DDE, p,p'-DDD, p,p'-DDT, dieldrin and endrin on the basis of the procedure outlined above, without any attempt at separating the PCB. Dieldrin and DDE emerge at exactly the same time on the DC-200 column, but on the QF-1 column there is no significant interference between dieldrin and DDE, or between dieldrin and any of the polychlorinated biphenyls encountered in environmental samples. DDE frequently constitutes 90% or more of the DDT compounds present in wildlife. The electron capture response of DDE is considerably greater than that of PCB, and although one of the PCB compounds emerges at approximately the same time as DDE, the error introduced in the DDE determination is less than experimental error even when the total PCB concentration is up to five times that of the DDE. Vermeer and Reynold. (1970) have commented upon the difficulty in separating the DDT compounds p,p'-DDD and p,p'-DDT from PCB. On the DC-200 column, both DDT compounds emerge at approximately the same time as a PCB peak (Anderson *et al*., 1969). On the QF-1 column, however, there is no interference between p,p'-DDT and any of the polychlorinated biphenyls usually encountered in environmental samples.

Although many different chlorinated biphenyl compounds are released into the environment, relatively few persist in the higher levels of food chains. Moreover, these are found in approximately the same proportions in environmental samples over wide areas of the world. The chromatograms of the PCB compounds in the Peregrines analysed in the present study are therefore similar to the chromatograms of Peregrine extracts from Mexico, California, and Amchitka Island (Risebrough, unpublished results). Moreover, the chromatograms are similar to those obtained from extracts of Brown Pelican eggs from several areas in both North and South America (Risebrough, unpublished results). It is frequently possible therefore, to treat the PCB mixture as a single compound and to obtain accurate relative concentrations of PCB using any one of the several peaks. The peak emerging at the same time as p,p'-DDD on the QF-1 column is almost always of the same height as two of the other peaks. It is therefore possible to obtain a crude estimate of the DDD present without initial separation of the PCB. This assumption may be tested and the identification of p,p'-DDT and p,p'-DDD confirmed by saponification (Anderson *et al*., 1969, Risebrough *et al*., 1969). An aliquot of the

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extract, in hexane or petroleum ether, is refluxed in 5% KOH in ethyl alcohol. The hexane is then separated from the alcohol and potassium hydroxide by the addition of water and a sample injected into the gas chromatograph. The procedure converts both DDT and DDD to their respective ethylene derivatives, DDE and DDMU, but leaves the PCB profile of the chromatograms unchanged.

Since the profile of the PCB peaks was similar, but not identical to the profile observed in chromatograms of the commercial preparation Aroclor 1254, PCB was quantified by comparing the total area of the PCB peaks emerging after DDE with the areas of the same peaks in chromatograms of standard Aroclor 1254. The procedure is therefore arbitrary, but permits the determination of relative amounts of PCB within the correct order of magnitude of absolute amounts. Moreover, the values obtained can be readjusted as methodology is improved.

Results and Discussion

Enderson and Berger (1968) have previously reported on the chlorinated hydrocarbon residues found in biopsy fat samples from immature Peregrines that were also trapped during fall migration at Cedar Grove, Wisconsin. These samples, analysed by the Wisconsin Alumni Research Foundation in Madison, Wisconsin, contained an average concentration of 19.4 ppm of DDE on a lipid basis. DDE concentrations in the fat of females at the Arctic breeding grounds are much higher, in the order of 300 ppm or higher (Berger *et al*, this issue).

The ten Peregrine samples analysed in the present study contained 17.9 ± 12.3 ppm DDE, 0.23 ± 0.34 ppm p,p'-DDD, 0.71 ± 0.68 ppm p,p'-DDT, 18.8 ± 12.5 ppm total DDT (DDE + p,p'-DDD + p,p'-DDT), and 52.2 ± 32.3 ppm PCB. Seven samples were analysed for dieldrin, which averaged 0.40 ± 0.64 ppm. All residues are expressed on an extractable lipid basis, with the 95% confidence limits of the standard error of the mean. The values for p,p'-DDD and p,p'-DDT are lower than those previously reported by WARF, Inc., presumably a result of interference by PCB in the earlier determinations (Anderson *et al*, 1969). The dieldrin values are equivalent to those previously reported and there is no significant difference in the DDE concentrations. On a lipid basis, the average values reported by WARF, Inc. were 19.3 ppm DDE, 0.8 ppm DDD, 1.2 ppm p,p'-DDT and 0.3 ppm dieldrin (Enderson and Berger, 1968).

First year immature Peregrines migrating from the Arctic have therefore low residues of DDE. Unfortunately little is known about the relationships among dietary levels of DDE and PCB, equilibrium concentrations of these compounds in various tissues, and the times required to achieve an equilibrium concentration. The low concentrations found in these Peregrines presumably reflect therefore both the age of the birds, which possibly has not permitted them to accumulate the high concentrations found in adults, and lower levels of contamination found in some of the prey species. An understanding of the pharmacodynamics of DDE and PCB in birds is one of the conspicuous deficiencies in our knowledge in the field of pollution ecology.

Within several ecosystems so far studied, the concentrations of DDE in birds tends to parallel the concentrations of PCB. The ratio may be approximately one to one, as in San Francisco Bay (Risebrough *et al*., 1968). DDE is several times more abundant than PCB in several Pacific marine ecosystems (Risebrough *et al*., 1968), but in Florida, PCB is approximately three times as abundant as DDE in the Brown Pelicans (Risebrough, Gress, Anderson and Schreiber, unpublished manuscript). The correlation coefficient between the DDE and PCB concentrations is frequently highly significant. These observations have suggested that the ratios observed reflect the local fallout pattern of the DDT and PCB compounds, and that the DDE and PCB show similar patterns of accumulation, concentration and excretion in the birds and possibly other members of the ecosystem.

Among the 10 Peregrine samples analysed, PCB was more abundant than DDE by approximately three times. The correlation co-

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efficient is 0.8897, which is significant at the 0.001 level. Although it is not known from which area of the Arctic these birds have come, the results suggest that over a considerable area of the tundra ecosystems occupied by the Peregrines, this ratio reflects the local fallout pattern of DDE and PCB.

The four adult Merlins averaged 302 ppm DDE (139-704) in the lipid fraction. This value may be compared with an average concentration of 392 ppm in the lipid of 9 breeding Peregrines from the Mackenzie River region (Enderson and Berger, 1968), 725 ppm in the fat of four adult Peregrines from Alaska (Cade *et al.*, 1968) and 334 ppm in the lipid of 9 adult Peregrines from Ungava (Berger *et al.*, this issue). Merlins therefore may possess almost the same level of contamination as the Peregrines and might therefore be expected to show symptoms of shell thinning. The concentrations of total DDT in the adult Merlins averaged 314 ppm. 96% of the DDT residues in these Merlins therefore consisted of DDE. The PCB concentrations averaged 196 ppm (44-467). The samples were not analysed for dieldrin.

In the three immatures, DDE averaged 49.3 ppm (10.8, 18.6 and 119.3), total DDT averaged 50.3 ppm, and PCB averaged 28.6 ppm (5.7, 29.0 and 51.2). With the exception of one immature Merlin that contained 18.6 ppm DDE and 51.2 ppm PCB, a ratio similar to that found in the Peregrines, all other Merlins contained more DDE than PCB. With the one immature excepted, PCB was significantly correlated with DDE ($r = 0.9820$, p less than 0.001, four degrees of freedom). Most of the Merlins, therefore, appear to be coming from a region of the boreal forest where the pollutant fallout pattern is different from that in the tundra ecosystem occupied by the Peregrine.

When PCB was found to be present in Peregrines, occasionally in high concentrations, it seemed likely that these pollutants were contributing to the reproductive failures associated with population decline (Risebrough *et al.*, 1968). The dominant symptom of reproductive failures of the Peregrines in Britain has been shell thinning associated with egg breakage, disappearance of the eggs, and eating of the eggs by the adult birds (Ratcliffe 1970). In the Ungava population of Peregrines in the eastern Arctic, shell thinning and egg breakage are now contributing to reproductive failures (Berger *et al.*, this issue). The evidence available to date suggests, however, that PCB is not a cause of the shell thinning observed in many populations of birds. No correlation between thinning and PCB was found in the eggs of the White Pelican (*Pelecanus erythrorhynchos*) (Anderson *et al.* 1969) or in eggs of the Great Blue Heron (*Ardea herodias*) (Vermeer and Reynolds 1970). An analysis of approximately 200 eggs of the Brown Pelican from both eastern and western North America has shown that DDE rather than PCB is associated with the shell thinning (Risebrough, Gress, Anderson and Schreiber, unpublished ms.). Whenever correlations between PCB and shell thinning have been observed, as in Double-Crested Cormorants (*Phalacrocorax auritus*), PCB is also correlated with DDE (Anderson *et al.*, 1969).

Peakall (1970) has reported that p,p'-DDT delays egg laying in the Ringdove (*Streptopelia risoria*) and that the delay is associated with lower concentrations of estradiol in the blood early in the breeding cycle. Peakall had earlier reported (in Risebrough *et al.* 1968) that technical DDT, p,p'-DDE, and PCB induce the synthesis of liver enzymes that degrade estradiol, and that PCB is a more potent enzyme inducer than is DDE. Delayed breeding may therefore be one of the environmental effects of PCB, an effect that could be critical to Arctic-breeding birds which must complete their reproductive cycle within a restricted period of time.

A recent investigation in Japan of the "Yusho" or Rice Oil Disease has traced the cause to rice oil contaminated by chlorinated biphenyls (Katsuki, 1969). Both the contaminated rice oil and the Japanese commercial PCB produce symptoms when fed to chicks similar to those observed in the "chick edema" syndrome (Goto *et al.*, 1969). This disease of chicks was first observed in the United States in 1957, and among the symptoms were accu-

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mulation of fluid in the pericardial sac and in the abdominal cavity, subcutaneous edema, and liver and kidney damage (Verrett, 1970). Chlorinated dibenzo-p-dioxins have been shown to be one of the groups of chlorinated synthetic compounds that produce this disease (Higginbotham *et. al*, 1968). These compounds have also been shown to be present in the herbicide 2,4,5-T and induce both mortality and embryonic deformities in chicks at very low concentrations (Verrett, 1970). A similar group of compounds, the chlorinated dibenzofurans, are almost as toxic (Schulz, 1970). These resemble the chlorinated biphenyls in structure and might be derived from them. Whether they are now present in the environment and pose a threat to wildlife promises to be "an absorbing chapter in the research of the immediate future" (Hickey and Roelle, 1969).

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