Levels of Chlorinated Hydrocarbons in Eggs of Double-Crested Cormorants from 1971 to 1975

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Knowledge of long-term trends of chlorinated hydrocarbon levels in biota is important for the understanding of the environmental behaviour of these compounds, for modelling and for predictions of effects of regulatory actions. Reports of temporal trends of chlorinated hydrocarbons in aquatic fauna are relatively rare. Those covering periods from about 1964 to 1971 indicate that the levels of PCB's, DDT and metabolites, and dieldrin passed through a maximum around 1970 and are either decreasing or remaining more or less constant (ZITKO 1974).

This paper presents the results of five years of monitoring chlorinated hydrocarbons in eggs of double-crested cormorants (*Phalacrocorax auritus*) from the Bay of Fundy. A part of the data has been reported previously (ZITKO 1974).

Mirex (1,2,3,4,5,5,6,7,8,9,10,10-dodecachloro-octahydro-1,3,4-metheno-2H-cyclobuta[*c,d*]pentalene) was first detected in 1975 samples and its presence in those collected in 1973 and 1974 was confirmed subsequently. This compound is commonly encountered in wildlife from the southeastern United States, where it is used for fire ant control (see, for example, BAETCKE et al. 1972; BORTHWICK et al. 1973), but on only two other occasions has been reported from areas outside its application. Mirex was detected in seals from The Netherlands (TEN NOEVER DE BRAUW et al. 1973) and in fish from Lake Ontario (KAISER 1974).

EXPERIMENTAL

Samples. From 9 to 10 eggs (1 egg per nest) were collected yearly, in early May, from a colony of double-crested cormorants on Whitehorse Island, Bay of Fundy, Canada. The eggs were boiled and stored frozen (-20°C) until analysis. In 1971 and 1972, combined groups of 2-3 eggs were analyzed. Starting in 1973, the eggs were analyzed individually.

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The 1973 extract of herring gull (*Larus argentatus*) eggs, used in the determination of mirex, was from a sample collected in the same general area (ZITKO 1974).

Extraction and cleanup. Yolks were ground with anhydrous sodium sulfate (weight ratio 1:6) and extracted with hexane. Lipids were removed from aliquots of the extracts by chromatography on alumina. PCB's and DDE were separated from dieldrin, p,p'-DDD, and p,p'-DDT by column chromatography on silica. The details of extraction and cleanup have been described previously (ZITKO et al. 1974). Starting in 1973, the remainder of the extracts was combined, hexane was evaporated in vacuum in a rotatory evaporator, and the lipids stored at -20°C for possible future reference. In 1974 and 1975, only one half of each yolk was extracted. The remaining halves were combined, freeze-dried, and stored at -20°C. After the analysis of the cleaned-up aliquots by gas chromatography, these were combined and examined by gas chromatography-mass spectrometry (1973-1975 extracts).

The performance of column chromatography on silica was checked periodically by chromatographing 100 µℓ of a solution containing p,p'-DDE, p,p'-DDD, and p,p'-DDT in concentrations of 9.48, 4.33, and 3.62 µg/mℓ, respectively. Each year, 2 samples were analyzed in duplicate. PCB's were quantitated on the basis of the 5 major Aroclor 1254 peaks and expressed as Aroclor 1254.

Instrumental techniques. The instrument and conditions of gas chromatography were identical with those described previously (ZITKO et al. 1974). A Finnigan GCMS system (Model 1015D mass spectrometer, Model 9500 gas chromatograph, and Model 6100 data system) was used in mass spectrometric studies. The mass spectrometer parameters were adjusted for optimum resolution and sensitivity. A 4-ft, 1/4-inch glass column, containing 3% OV-1 on HP-Chromosorb W 80/100 mesh was used in the Model 9500 gas chromatograph. The temperature was maintained at 180°C for 7 minutes after injection and then increased at 4°C/min to a final temperature of 250°C. The column effluent was vented the first 2 minutes. Carrier gas was helium at 80 mℓ/min. Mass spectra were scanned from 100 to 600 a.m.u. in 5-second intervals at an integration time of 10 millisec. Mirex was determined by mass fragmentography at 235, 237, 270, and 272 a.m.u. The concentration was calculated from the integrated 272 a.m.u. peak. The identification of all reported chlorinated hydrocarbons was confirmed by mass spectra.

RESULTS AND DISCUSSION

Egg size and lipid content. Egg weight, yolk and lipid content are summarized in Table 1.

TABLE 1. Analyzed eggs.

Year	Number	Weight, g		Yolk, %		Lipid, %	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
1971	11[a]	47.0	3.1	Not Determined			
1972	9[a]	47.2	4.2	16.7	0.6	4.62	0.59
1973	9	46.3	3.9	16.3	1.3	3.89	0.44
1974	9	47.6	3.7	15.8	1.1	4.19	0.35
1975	10	49.3	6.0	16.6	2.3	4.32	0.67

[a] 2-3 eggs analyzed combined.

Concentration of chlorinated hydrocarbons. The data, presented in Table 2, show a decrease of PCB and DDE levels from 1971 to 1973 and no change thereafter. The concentrations of the other chlorinated hydrocarbons are one to two orders of magnitude below those of PCB's and DDE and may indicate the same trend, except that the 1975 concentrations are generally higher than those in 1973 and 1974.

The numbers of eggs analyzed were not large enough to determine whether the statistical distribution of the concentrations of chlorinated hydrocarbons approaches a normal or a lognormal one. Chlorinated hydrocarbons tend to be distributed lognormally in environmental samples (see, for example, ZITKO et al. 1974) and geometric means may better characterize the data than arithmetic means.

The quantitation of PCB's as Aroclor 1254 is not accurate since the patterns of PCB's extracted from the eggs are quite different from those of Aroclor 1254. Some penta-, hexa-, hepta-, and octachlorobiphenyls are much more abundant in the eggs than in Aroclor 1254. Nona- and decachlorobiphenyl were also qualitatively detectable by gas chromatography-mass spectrometry in the 1973-1975 samples. Because of their long retention times, these compounds can be seen only during temperature-programmed gas chromatographic separations. The inaccuracy of the PCB quantitation has no effect on the reported trend of PCB concentration in the eggs if the chlorobiphenyl patterns remain constant. As Figure 1 indicates, this was the case in 1973-1975. The

TABLE 2. Concentration of chlorinated hydrocarbons in cormorant eggs, µg/g wet weight.

Compound	Year				
	1971	1972	1973	1974	1975
Hexachlorobenzene					
arithm. mean			0.016	0.016	0.017
s.d.			0.005	0.005	0.007
geom. mean			0.016	0.015	0.016
s.d.			0.165	0.162	0.186
PCB's (as Aroclor 1254)					
arithm. mean	14.3	9.06	5.57	5.25	5.23
s.d.	0.86	3.42	2.46	1.90	3.67
geom. mean			5.04	4.89	4.42
s.d.			0.218	0.188	0.248
p,p'-DDE					
arithm. mean	9.70	6.72	2.89	1.92	2.01
s.d.	2.80	3.59	1.54	0.45	5.21
geom. mean			2.51	1.86	1.53
s.d.			0.248	0.114	0.323
Dieldrin					
arithm. mean		0.297	0.117	0.041	0.156
s.d.		0.108	0.030	0.030	0.120
geom. mean			0.112	0.035	0.125
s.d.			0.126	0.239	0.293
p,p'-DDD					
arithm. mean		0.113	0.053	0.020	0.068
s.d.		0.071	0.020	0.010	0.039
geom. mean			0.049	0.018	0.060
s.d.			0.198	0.223	0.227
p,p'-DDT					
arithm. mean		0.167	0.073	0.023	0.075
s.d.		0.066	0.020	0.006	0.053
geom. mean			0.071	0.023	0.062
s.d.			0.132	0.111	0.278
Mirex					
single detn. on combined ext.			0.058	0.059	0.113

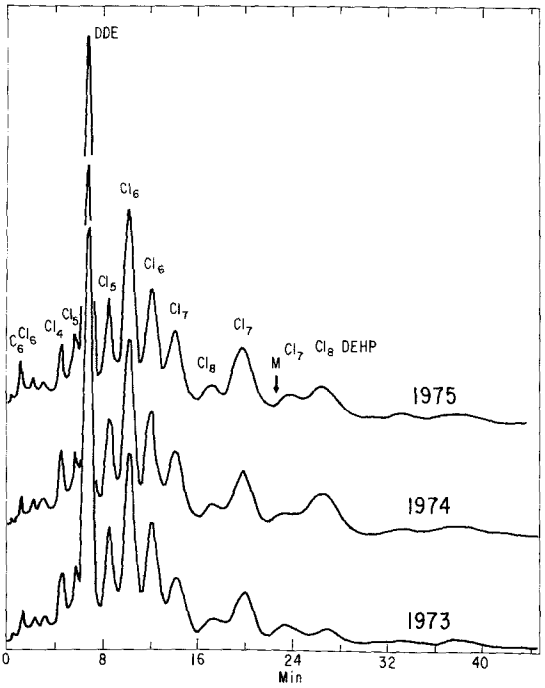

Fig. 1. PCB patterns in cormorant eggs (hexane fraction from the silica column, chromatographed at 200°C on a 4% SE-30 column).

Cl_4-Cl_8 = tetra - octachlorobiphenyl

M = mirex

DEHP = di(2-ethylhexyl)phthalate

Combined 1973-1975 hexane fractions were used.

combined samples were chromatographed under identical conditions in 1975. The 1971 and 1972 samples were recorded under different conditions and cannot be compared directly, but the relative abundances of the individual peaks are identical with those in the 1973-1975 samples.

As can be seen from Figure 1, mirex is eluted from the SE-30 column only partly separated from some heptachlorobiphenyls. Because of its relatively low concentration, mirex cannot be quantitated by the conventional gas chromatography with an electron-capture detector, but it can be confirmed and quantitated by gas chromatography-mass spectrometry. It is impossible to judge whether the increased concentration of mirex in 1975 as compared to 1973 and 1974 is significant.

Cormorants may take up mirex on their wintering grounds in Florida (HANDBOOK OF NORTH AMERICAN BIRDS 1962). On the other hand, the compound is also present in the eggs of herring gulls (*Larus argentatus*), and these birds spend their entire life within the Bay of Fundy area. The concentration of mirex in a combined sample of 12 herring gull eggs, collected in 1973, is 0.028 µg/g egg on a wet weight basis.

According to mass spectra, the p,p'-DDD peak contains, in addition to p,p'-DDD, a compound with a Cl_8 cluster at 405 a.m.u. The retention time and this cluster (M-35) are indicative of a nonachlor (1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane) isomer, a component of technical chlordane. No other chlordane components were detectable in the samples and more data are needed for identification. Due to the presence of this compound the p,p'-DDD concentrations are likely to be somewhat overestimated.

Di(2-ethylhexyl)phthalate, eluted together with an octachlorobiphenyl from the SE-30 column (Fig. 1), is probably a contaminant from the laboratory.

Comparison with other areas and toxicological significance. A summary of levels of PCB's and DDE in cormorant eggs across Canada is available (GILBERTSON and REYNOLDS 1974). In localities west of the Great Lakes the concentrations of PCB's are lower than those of DDE, whereas in the Great Lakes area and to the east the situation is reversed. The 1971 and 1972 data reported in this paper are of the same order of magnitude as those given by Gilbertson and Reynolds for New Brunswick, and 3-4 times lower than those for the Great Lakes. In The Netherlands, the 1971 levels of hexachlorobenzene, PCB's, DDE, and dieldrin were 0.67, 70.0, 5.48, and 1.12 µg/g egg on a wet weight basis and the population of cormorants may be declining (KOEMAN et al. 1973).

It is likely that the present relatively low levels of chlorinated hydrocarbons in eggs of cormorants

from the Bay of Fundy have no detectable effects on the cormorant population. The levels are fairly constant and may remain so for many years. The presence of mirex deserves closer attention.

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