Insecticides, Polychlorinated Biphenyls and Mercury in Wild Cormorants, Pelicans, their Eggs, Food and Environment[1]

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The biological concentration of organochlorine insecticides in the food chain of fish and fish-eating birds has been frequently reported (HICKEY *et al.*, 1966; HANNON *et al.*, 1970; GREICHUS *et al.*, 1969; WOODWELL *et al.*, 1967; COLE *et al.*, 1967). Both organochlorine insecticides and polychlorinated biphenyls (PCB's) are stored in the fat of birds and are difficult to eliminate from the body (LAMB *et al.*, 1970; DAHLGREN *et al.*, 1971). Therefore, it could be expected that PCB's would also be concentrated in the food chain of carnivorous birds.

There is little data available relating levels of mercury with its accumulation in animals in the top of the food chain. FIMREITE *et al.*, (1971) have reported a positive correlation between mercury concentration in birds and the proportion of animal food in the diet. Predominately fish-eating birds had mercury levels approximately 10-fold greater than levels in fish.

The purpose of this study was to: (1) examine the levels of organochlorine insecticides, PCB's and mercury in water, bottom sediments, fish and birds in the Lake Poinsett, South Dakota ecosystem, (2) determine whether or not biological concentration was occurring and, (3) report the distribution of these compounds in various tissues.

Methods and Materials

Sample Collection

Samples of water and bottom sediments were collected from six sites on Lake Poinsett and Dry Lake, South Dakota. Twenty fish from each of four species including carp (*Cyprinus carpio*), crappie (*Pomoxis annularis*), black bullhead (*Ictalurus melas*), and bigmouth buffalo (*Ictiobus cyprinellus*) were collected from diverse areas of the lake. Only fish under 30 cm were included since larger fish are not commonly used as food by pelicans and cormorants. These

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fish constitute the major portion of the diet of these birds in this area (TRAUTMAN, 1951; personal observation). The double-crested cormorant (*Phalacrocorax auritus*), white pelican (*Pelecanus erythrorhynchos*) and their eggs were collected from rookeries on Dry Lake and South Waubay Lake, South Dakota. All samples were placed in glass containers which had been thoroughly cleaned and heated in an oven at 520 C.

Insecticide and PCB Analysis

Water samples were filtered and analyzed using the procedure of BREIDENBACH *et al.*, (1964). Bottom sediments were air dried and 100 g samples were extracted with hexane/propanol-2 (Shell Development Company, 1964). After the intestinal tract was removed, ten fish of the same species were finely ground together and a 25 g aliquot was extracted with ethyl-petroleum ether in a Sorvall homogenizer. The ether extract from the fish and one g samples of brain, liver, fat and kidney and five g samples of muscle, body and eggs of birds were extracted and purified by the Florisil column method (STEMP *et al.*, 1964; GREICHUS *et al.*, 1968). The body samples consisted of the finely ground body which remained after the removal of the above samples and included the head, feet, feathers and empty intestinal tract. Eggs were homogenized without the shells.

The PCB's were separated from the insecticides by thin-layer chromatography after the samples were saponified with alcoholic KOH (U.S. Food and Drug Administration, 1971) and oxidized with CrO_3 (MULHERN *et al.*, 1971). The samples were spotted on 0.25 mm silica gel plates and the hexane solvent was advanced 15 cm from the spotting line. This procedure separated PCB's from most organochlorine insecticides but not from aldrin, p,p'-DDE or p,p'-DDT. Aldrin does not commonly occur in animal tissues and in other samples it was determined by using multiple columns on the gas chromatograph. The levels of p,p'-DDE in most bird tissues were such that when the sample was diluted sufficiently to produce a peak similar to that of the DDE standard, the PCB peaks could no longer be seen on the chromatogram. PCB's interfere with p,p'-DDT on most gas chromatography columns, so DDT was converted to p,p'-DDE by saponification and the decrease in the size of the interfering PCB peak was measured. Oxidation converted p,p'-DDE to 4,4'-dichlorobenzophenone which was easily separated from PCB's by thin-layer chromatography.

The instruments used for gas chromatography analysis included two Varian Aerograph HY-FI models 600D equipped with model S-R 1 mv Sargent recorders and an electron capture detector cell with either a 250-millicurie tritium or a 8-millicurie Ni^{63} source. Injector and column temperatures were 210 and 180 C, respectively, and the detector temperatures were 210 C for the tritium and 280 C for the Ni^{63} source. Three 1/8-inch outside diameter by 5 feet borosilicate glass columns were used with packings consisting of: (a) 2% QF-1 Silicone (Fluoro), (b) 5% Dow 11 Silicone and (c) 1:1 mixture of 15% QF-1 and 10% DC-200 Silicone. All packings were on 80/100 mesh

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chromosorb W (H/P A.W. DMCS). The nitrogen carrier gas was operated with a flow rate of 40 ml per minute.

All solvents used in this study were nanograde (Mallinckrodt Chemical Works). Some batches of Florisil (Fisher Scientific Company) and silica gel were found to be contaminated with PCB's so they were washed repeatedly with hexane until no PCB's could be detected in the hexane rinse.

Mass spectrometric analysis of PCB's in cormorant and pelican samples was done by Finnigan Instruments Corporation, Sunnyvale, California employing the coupling of gas chromatography with mass spectrometry (BONELLI, 1971).

Average percent recoveries, standard deviations and minimum confidence levels of organochlorine insecticides and PCB's from fortified samples are given in Table 1.

TABLE 1

Average Recoveries of Insecticides and Polychlorinated Biphenyls from Water, Bottom Sediments, Fish, Birds and Eggs.

Material	Organochlorine Insecticides		Polychlorinated Biphenyls	
	% Recovery ± SD	Minimum Confidence	% Recovery ± SD	Minimum Confidence
		ppb		ppb
Water	85 ± 9	0.05	82 ± 5	0.5
Bottom sediments[1]	71 ± 10	0.1	88 ± 11	5.0
Fish	72 ± 7	1.0	93 ± 7	50
Bird tissues	89 ± 9	50	95 ± 9	100
Eggs	89 ± 5	10	89 ± 10	100

[1]Dry weight basis.

Mercury Analysis

The flameless atomic absorption method for mercury analysis (HATCH *et al.*, 1968) was used on substantially all fish tissues, cormorant muscle tissues and lake-bottom sediments. All the pelican tissues, the remaining cormorant tissues, and pelican and cormorant eggs were analysed by the A.O.A.C. method (1970). These samples were stored frozen until analyzed. Water samples preserved with 10 ml concentrated nitric acid per liter were analysed for mercury using the provisional F.W.Q.A. method (1970) as follows: a 100 ml water sample was acidified with 5 ml concentrated sulfuric acid and 2.5 ml concentrated nitric acid. One ml of a 5% (w/v) potassium permanganate solution was added and allowed to stand for at least 15 min, 2 ml of a 5% (w/v) potassium persulfate solution was added

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and the sample was allowed to stand for at least an additional 30 min. The flameless atomic absorption procedure used to determine mercury content of the oxidized sample was essentially that of HATCH and OTT (1968).

All tissue and bottom sediments analyzed by flameless atomic absorption were subjected to prior exhaustive acid digestion (Personal Communication, FDA, 1970). Body tissue samples weighed from 2 to 7 g (wet basis) and bottom sediment samples weighed 1 g (wet basis). A solution consisting of 25 ml of a 5:4:9 (v/v) mixture of concentrated sulfuric acid, concentrated nitric acid and water and 1 ml of 2% (w/v) sodium molybdate solution was added to the sample in a 24/40 @ 250 ml round bottom flask. Several boiling chips were added and the flask was connected to a 400 mm x 13 mm i.d. water condenser modified to hold 6 mm Raschig rings. The condenser was packed to a height of 100 mm with Raschig rings plus an additional 20 mm with 4 mm glass beads. The digestion mixture was refluxed for 1 hr. The flask was allowed to cool, water was disconnected from the condenser and 10 ml of a 1:1 (v/v) mixture of concentrated nitric and perchloric acids was added through the top of the condenser. The digest was boiled 10 min. beyond the time that white fumes appeared in the flask. After being allowed to cool, the condenser was washed down with approximately 15 ml water. The digest was again boiled for 5 min, allowed to cool, and the condenser was washed down with an additional 10 ml of water. After cooling to room temperature the digest was transferred to a 50 ml volumetric flask and made to volume. Mercury was determined on a 25 ml aliquot.

In a comparison of the flameless atomic absorption and A.O.A.C. methods of analysis for 9 samples of cormorant liver, average mercury values were 4.8 ppm (range, 0.16 to 14.0 ppm) for atomic absorption and 4.5 ppm (range, 0.16 to 15.0 ppm) for the A.O.A.C. method with a correlation coefficient of 0.971 ± 0.018.

Recoveries of 89 to 105% (average, 99.0%) were obtained following the addition of mercuric chloride to breast and liver samples at levels equivalent to 0.4 ppm Hg in the samples. With the addition of mercuric chloride to lake waters at a level equivalent to 10 ppb Hg, recoveries of 98 to 105% (average, 101.7%) were obtained.

Results and Discussion

The concentration of organochlorine insecticide residues in bottom sediments was about 7-fold greater than in the water whereas the concentration of residues in fish over bottom sediments was from 10- to 60-fold (Table 2). PCB's were concentrated in fish an average of 12-fold over bottom sediments. The levels of PCB's in both bottom sediments and fish were higher than those of insecticides. Mercury residues were not detected in water, bottom sediments or fish except carp which also had the highest levels of

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insecticides and PCB residues. Concentrations of total mercury in lake trout were found by BACH *et al.*, (1971) to increase with the age of the fish and ranged from 0.19 to 0.66 ppm.

Bodies of adult cormorants had 250-fold greater levels of insecticides than fish and adult pelicans were 280 times greater (Tables 2 and 3). PCB's were concentrated in bodies of cormorants 60-fold over fish but only 30-fold in pelicans. This could represent a difference in the amount of PCB's in food at their wintering grounds. Levels of insecticides and PCB's in nestling cormorants appeared to reflect the levels in local fish as both the fish and young birds had more PCB residues than insecticides whereas the opposite was true with adult birds (Table 3).

TABLE 2

Average Levels of Insecticides, Polychlorinated Biphelyls and Mercury in Water, Bottom Sediments and Fish

Material Sampled	No. of Samples	Average Total Insecticides	Average PCB's	Average Mercury
		ppm	ppm	ppm
Water	6	0.00023	< 0.0005	< 0.0005
Bottom sediments	6	0.0016[1]	0.0064[1]	< 0.1
Fish[2]				
Carp	2	0.092	0.11	0.11
Crappie	2	0.016	< 0.05	< 0.05
Buffalo	2	0.032	0.06	< 0.05
Bullhead	2	0.031	0.11	< 0.05

[1]Dry weight basis.

[2]Each sample is a composite of 10 fish (wet weight basis).

Insecticide and PCB levels in cormorant eggs appeared to reflect body levels but in pelican eggs they did not. However, because the number of pelican body samples was small, i.e. 3, it is difficult to draw conclusions from the data. RISEBROUGH *et al.*, (1968) reported average total amounts of DDT and its metabolites in California brown pelican eggs to be 53 ug and amount of PCB's to be 10.4 ug. Brandt's cormorant eggs had an average of 326 ug of total DDT and 113 ug of PCB's. The ratio of DDT and its metabolites to PCB was 5:1 for pelican and 3:1 for cormorant eggs, while in the present study the ratio was 2:1 for both cormorant and pelican eggs (Table 4).

Presence of PCB's in bodies of pelicans, cormorants and cormorant eggs was demonstrated by comparing the mass spectra of 10 PCB peaks (Aroclor 1254) with peaks in the samples having the same relative gas chromatographic retention times.

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TABLE 3

Average Levels of Insecticides, Polychlorinated Biphenyls and Mercury in Cormorant and Pelican Tissues.

Material Sampled	No. of Samples	Average Total Insecticides	Average PCB's	Average Mercury
		(ppm - wet weight basis)		
Adult Cormorant				
Body	10	10.7	4.6	0.64
Muscle	7	6.7	2.3	0.78
Liver	10	3.6	2.0	7.98
Kidney	6	---[1]	---	1.51
Eggs[2]	5	10.1	5.7	0.29
Nestling Cormorant[3]				
Body	5	0.24	0.39	0.06
Liver	5	0.39	0.60	0.28
Adult Pelican				
Body	3	12.3	2.3	0.29
Muscle	3	11.3	3.1	0.25
Liver	3	6.8	4.5	0.59
Kidney	2	6.8	3.1	0.37
Eggs[2]	3	3.7	1.7	0.22

[1]Not analysed.
[2]Whole egg minus shell.
[3]Age 2-4 weeks.

Mercury was highest in the liver of cormorants and pelicans. DUSTMAN *et al.*, (1970) found mercury higher in the liver of birds than in the breast muscle, kidney or carcass. Concentration factors of 14- and 6-fold over fish were found in bodies of cormorants and pelicans respectively. This may reflect greater mercury contamination in the food of cormorants in the wintering areas as the concentration in nestling cormorants was only 1.3-times that of fish. Cormorant eggs had higher levels of insecticides, PCB's and mercury than pelican eggs.

Organochlorine insecticides and PCB's were more concentrated in the fat of cormorants and pelicans than in any other tissue with p,p'-DDE constituting the greatest single contaminant (Table 4). Bodies, liver, fat and eggs had greater amounts of total insecticides than PCB's but brain tissue had more PCB's. This was true in 10 of 13 brain samples.

Studies completed in this laboratory on the effects of DDT, DDD and DDE on penned cormorants indicated that 20 ug/g of DDD in the brain was diagnostic of approaching toxicosis (HANNON, 1972). As birds approached death, there occurred a depletion of lipid stores in the body while the amount of brain lipids remained the same. Body insecticide residue levels decreased while brain residues increased. This was also true of cormorants that were stressed by a decrease in food (HANNON, 1972).

Adult cormorants in the present study averaged 2,200 g in weight and had 1.00 ppm of DDD in their bodies (Table 4) or a total of 2,200 ug. Stress conditions such as migration, reproduction or

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disease may cause a decrease in body lipid stores. It appears that the accumulation of 20 ug of DDD per g of brain could possibly occur. Also, there are many types of toxic chemicals in these birds and the effects of the combination of these chemicals is difficult to ascertain.

There was an unknown peak on the chromatograms of cormorant brain which was later identified by mass spectrometry as possibly being a phthalate ester. This peak was also observed in numerous cormorant tissues but less frequently in pelican samples. This type of compound was recently reported as a toxic environmental contaminant found in aquatic ecosystems and may be concentrated in food chains (BOWER *et al.*, 1970; SMEA, 1971; Press Release, U.S. Bureau of Sport Fisheries and Wildlife, 1971).

TABLE 4

Average Levels of Individual Insecticides and Polychlorinated Biphenyls in Cormorant and Pelican Tissues.

Tissue	No.	Hepta. Epoxide	Lindane	Dieldrin	DDE	DDD	DDT	Total	PCB
				(ppm - wet weight basis)					
Cormorant									
Bodies	10	<0.05	<0.05	0.09	9.12	1.00	0.51	10.8	4.6
Brain	10	<0.05	<0.05	0.12	0.56	0.09	0.08	0.9	1.3
Liver	10	<0.05	<0.05	0.13	2.70	0.44	0.31	3.6	2.0
Fat	8	0.25	0.26	1.38	107.84	2.11	2.16	114.0	22.4
Eggs[1]	17	0.05	0.05	0.13	10.38	0.47	0.67	11.7	5.9
Pelican									
Bodies	3	<0.05	<0.05	0.12	11.56	0.53	0.05	12.3	2.3
Brain	3	<0.05	<0.05	0.10	0.87	0.05	0.05	1.1	1.3
Liver	3	<0.05	<0.05	0.27	5.37	0.56	0.60	6.8	4.5
Fat	3	1.33	0.25	1.78	154.50	7.32	2.91	168.1	31.4
Eggs[1]	9	0.05	0.05	0.10	2.07	0.18	0.05	2.5	1.2

[1]Whole egg minus shell.

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