

## PHYSIOLOGICAL EFFECTS OF POLYCHLORINATED BIPHENYLS OR A COMBINATION OF DDT, DDD, AND DDE IN PENNED WHITE PELICANS

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The effects of PCBs (polychlorinated biphenyls) or a combination of DDT (1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane), DDD (1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane) and DDE (1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene) on organ weights, liver storage of vitamin A and carotene, selected blood chemistry parameters, and serum protein fractions were determined in penned white pelicans (*Pelecanus erythrorhynchos*) receiving a daily dosage of these compounds. Birds received 100 mg of PCBs or a combination of DDT (20 mg), DDD (15 mg), and DDE (15 mg) injected into the first fish fed each day for ten weeks. A greater percentage of PCB treatment was retained in brain, liver, carcass and feathers than the percentage of DDT + DDD + DDE treatment. Liver weight as percent of body weight decreased ( $p < 0.01$ ) in DDT + DDD + DDE-treated birds and increased ( $p < 0.01$ ) as a total weight in PCB-treated birds. Spleen weight as percent of body weight was greater ( $p < 0.05$ ) in PCB-treated birds. Neither treatment had a significant effect on the weight of the brain, heart, or kidney. Liver vitamin A levels were greater ( $p < 0.01$ ) on a  $\mu\text{g/g}$  of liver basis in the DDT-treated birds than in controls. Significant lowering of serum potassium and protein values was noted in both the PCB- and the combination of DDT, DDD, DDE-treated birds, while serum calcium values were lowered ( $p < 0.01$ ) only in PCB-treated birds. Values of serum inorganic phosphorus, uric acid and magnesium were not significantly changed by either treatment. Globulin fractions were unaltered by either treatment, but albumin fractions were lowered ( $p < 0.01$ ) in the PCB-treated pelicans.

Polychlorinated biphenyl (PCB) and organochlorine insecticide residues have been found in the tissues of piscivorous and raptorial birds (Holmes *et al.* 1967, Walker *et al.* 1967, Woodwell *et al.* 1967, Greichus *et al.* 1973). Double-crested cormorants (*Phalacrocorax auritus*) and white pelicans (*Pelecanus erythrorhynchos*) had the highest organochlorine insecticide and PCB residue levels of several animal species in South Dakota (Greenwood *et al.* 1967, Moore *et al.* 1968, Greichus *et al.* 1968a, Greichus *et al.* 1973). Average body concentrations of insecticides and PCBs, respectively, were 280- and 30-fold greater in pelicans than in fish (Greichus *et al.* 1973).

Anderson and Hickey (1972) stated that brown pelicans (*Pelecanus occidentalis*), double-crested cormorants and black-crowned night herons (*Nycticorax nycticorax*)

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were among species which appeared to have suffered the greatest effects of the eggshell thinning syndrome. DDE (1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene) was the most important pollutant associated with these changes, while effects of PCBs required further investigation. They stated that eggshell thinning had occurred more recently in white pelicans. Residues of chlorinated insecticides and PCBs have been found in bodies and eggs of white pelicans (Greichus *et al.* 1973).

White pelicans were selected as experimental animals because of their close relationship to the endangered brown pelican and their accessibility for study in South Dakota. Biochemical effects of DDT (1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane), DDD (1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane) and DDE on another local fish eating species, the double crested cormorant, have been reported (Greichus and Hannon 1973).

The objectives of this study were to: (1) determine concentrations and distributions of PCB or DDT and its metabolites in tissues of penned white pelicans receiving dietary doses of these compounds; and (2) examine effects of these compounds on organ weights, liver storage of vitamin A and carotene, selected blood chemistry parameters and serum protein fractions.

### Methods

Twenty-seven wild nestling white pelicans were collected from LaCreek National Wildlife Refuge, Bennett County, South Dakota in June and July, 1972. The birds were randomly placed into nine cages 8 ft. x 8 ft. x 6 ft., with three birds in each cage, and started on treatment July 17, 1972. Each bird received one of the following daily treatments injected into the first fish fed each morning:

Groups 1, 2, 3 - Controls - two ml of 85 percent ethanol;

Groups 4, 5, 6 - PCB treated - 100 mg of Aroclor 1254 (Monsanto Chemical Co.) in two ml of 85 percent ethanol;

Groups 7, 8, 9 - DDT, DDD and DDE-treated - 20 mg of *p,p'*-DDT, 15 mg of *p,p'*-DDD and 15 mg of *p,p'*-DDE in two ml of 85 percent ethanol.

Six of the pelicans were females, with control, PCB and DDT groups having one, three and two females, respectively. All fish were weighed and each bird consumed an average of 695 g of fish per day. The average daily dietary intake of PCB and the DDT-complex (DDT + DDD + DDE) were 144 and 72 ppm, respectively. The fish used as feed were taken from Lake Poinsett, South Dakota, and averaged < 0.05 ppm of DDT and metabolites and < 0.5 ppm of PCB. Birds remained on treatment for ten weeks, after which treatments ceased and a two-week food stress was imposed. The stress consisted of a reduction in food intake to 300 g/day. The purpose of this stress was to determine effects of feed reduction on birds previously exposed to dietary PCB or insecticide.

All birds were weighed every two weeks to the nearest 25 g. Weights of brain, heart, liver, spleen, and kidney were taken upon necropsy. Composite feather samples were

combination of DDT (1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane) on red blood chemistry of penned white pelicans. Birds received 100 mg of DDT, 100 mg of DDD, and 100 mg of DDE. A greater percentage of body weight decreased ( $p < 0.01$ ) as the dose of DDT was increased. No significant effect was observed in birds which were greater than controls. Significant differences in both the PCB and DDT serum albumin values of serum inorganic phosphorus were changed by either treatment, but albumin

residues have been found in eggs of double-crested cormorants and in the highest concentrations in South Dakota (Greichus *et al.* 1973). They were

the only species in which the DDT residues were found in the eggs.

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taken from the wings, breast, back and uropygial area of each bird, finely ground, and one-g aliquots taken for analysis. Carcass samples consisted of five-g aliquots of the finely ground entire body less bill, feet and those tissue samples removed for residue analysis. Brain, liver, feather, and carcass samples were stored in a deep freeze ( $-20^{\circ}\text{C}$ ) for later residue analysis. Blood samples were taken from the brachial vein prior to treatment and stress, and by cardiac puncture at necropsy.

Methods for determination of blood chemistries, liver vitamin A and carotene were described in a previous paper (Greichus and Hannon 1973). Serum protein fractions were analyzed by electrophoresis using cellulose acetate strips which were processed on a recording densitometer (Gilford Instruments). Relative amounts of each fraction were determined with a planimeter. Extraction and purification of DDT, DDD and DDE were accomplished by the Florisil column method (Stemp *et al.* 1964) as modified by Greichus *et al.* (1968b). PCB residues were extracted and purified according to the method described by Greichus *et al.* (1973). Quantitation of PCBs by gas-liquid chromatography was accomplished by comparing the areas of seven peaks in the sample to the corresponding peaks in the standard. The retention times of these peaks relative to *p,p'*-DDE at 100 were 71, 84, 100, 107, 129, 154, and 179. Control DDE, DDD and DDT peaks were corrected for interfering PCB peaks. Extract dilutions eliminated any interference between PCB peaks and DDT, DDD or DDE peaks in tissues from treated birds. Procedural efficiencies for insecticides and PCBs were  $90 \pm 6\%$  and  $95 \pm 9\%$ , respectively, and minimum analytical confidence limits were 0.05 ppm and 0.5 ppm, respectively.

Parameters of body and organ weights, liver vitamin A and carotene levels, blood chemistry levels and serum protein fractions in control and treated birds were analyzed statistically by the least-squares analysis of variance in conjunction with Dunnett's test (Steel and Torrie 1960).

### Results and discussion

**Concentrations and distribution of residues.** Average concentrations of DDE, DDD and DDT were highest in carcass, followed by liver, feathers, and brain (Table I). DDT comprised the smallest residue concentration in all tissues. PCB residues were also greatest in the carcass, followed by liver, feather, and brain. The substantial body fat depots of the birds undoubtedly contributed to the high carcass residue levels. PCB residues in cockerels fed 500 ppm Aroclor 1254 for 12 weeks were greatest in adipose tissue, followed in decreasing order by kidney, liver, brain, muscle and blood (Platonow and Funnell 1972).

Although the PCB treatment (100 mg/day) was twice the concentration of the DDT-complex treatment (50 mg/day), the mean PCB residue levels in all tissues were greater than twice the mean level of DDT + metabolites. These data indicated that PCBs were more readily stored in tissues than the DDT-complex and/or were less easily metabolized.

The DDT-complex was comprised of 40% DDT, 30% DDD, and 30% DDE, approximately the same proportions as found in local fish (Hannon *et al.* 1970). Percentage

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Table I. Average Tissue Concentrations in Pelicans Treated Daily with PCBs or a Combination of DDT, DDD and DDE  
(ppm - Wet Weight Basis)

| Treatment<br>9 birds/group |       | Carcass |             | Liver   |               | Brain   |               | Feathers |               |
|----------------------------|-------|---------|-------------|---------|---------------|---------|---------------|----------|---------------|
|                            |       | Average | Range       | Average | Range         | Average | Range         | Average  | Range         |
| Control <sup>a</sup>       |       |         |             |         |               |         |               |          |               |
| Males = 8                  | DDE   | 0.35    | 0.24 - 0.48 | 0.05    | < 0.05 - 0.10 | < 0.05  | < 0.05 - 0.05 | 0.08     | < 0.05 - 0.11 |
| Females = 1                | PCB   | 5.7     | 2.00 - 12.3 | 4.09    | < 0.50 - 7.00 | 2.99    | < 0.50 - 6.62 | 1.74     | 1.20 - 4.14   |
| PCB                        |       |         |             |         |               |         |               |          |               |
| 100 mg/day                 | PCB   | 2130    | 1470 - 3000 | 290     | 110 - 520     | 110     | 82 - 170      | 120      | 28.6 - 350    |
| Males = 6                  |       |         |             |         |               |         |               |          |               |
| Females = 3                |       |         |             |         |               |         |               |          |               |
| DDT Complex                |       |         |             |         |               |         |               |          |               |
| 50 mg/day                  | DDE   | 328     | 262 - 417   | 53.9    | 38.4 - 62.6   | 15.4    | 8.34 - 23.1   | 15.6     | 7.25 - 27.6   |
| Males = 7                  | DDD   | 370     | 273 - 493   | 62.9    | 40.2 - 95.2   | 11.8    | 6.07 - 16.7   | 18.9     | 8.56 - 29.2   |
| Females = 2                | DDT   | 161     | 75.4 - 270  | 18.6    | 11.1 - 30.1   | 7.38    | 2.95 - 12.2   | 13.4     | 5.20 - 20.6   |
|                            | Total | 859     | 610 - 1180  | 135     | 89.7 - 188    | 34.6    | 17.4 - 52.0   | 47.9     | 21.0 - 77.4   |

<sup>a</sup>No samples in controls had concentrations of DDD or DDT above the minimum analytical confidence limit of 0.05 ppm.

compositions of DDT, DDD and DDE residues respectively, were 14, 46 and 40 in liver, 21, 34 and 45 in brain, 19, 43 and 38 in carcass, and 28, 39 and 33 in feathers. Metabolism of DDD from DDT and of DDE from DDT in the pigeon (*Columba livia*) has been reported (Bailey *et al.* 1969a). Bailey *et al.* (1969 a and b) reported that DDT, DDD and DDE had half-lives of 28, 24 and 250 days, respectively, in the pigeon. The percentage increases of DDD and DDE and the percentage decrease of DDT in the pelican tissues from the original treatment percentages indicate a rapid metabolism of DDT and a slower change or elimination of DDD and DDE.

Brain tissue has been reported to be more useful than other tissues in diagnosing poisoning from insecticides (Stickel *et al.* 1966, Greichus and Hannon 1973). In 72% of the present samples, feather concentrations of DDT + DDD + DDE were as close or closer to those of the brain than were the levels in the liver or carcass. In 56% of the samples, concentrations of PCBs in feathers were closer to those in the brain while in 44% of the cases liver concentrations were closer. There were no samples in which carcass concentrations of DDT complex or PCB were as close to those of the brain as concentrations in feather or liver. Whereas liver concentrations of the DDT complex and PCB's were always higher than brain concentrations, feather concentrations varied. These data suggest that feathers, which are relatively easily obtained, may be preferable to other tissues in indicating concentrations of DDT plus metabolites and PCB in the brain.

No gross signs of intoxication were observed in the pelicans. Average brain residue levels and ranges for the DDT complex and PCB's were 35 (17-52) ppm and 110 (82-170) ppm, respectively. Cormorants dying from DDT poisoning had brain residue concentrations of DDT + DDD + DDE ranging from 69 to 302 ppm (Greichus and Hannon 1973), while pheasants (*Phasianus colchicus*) dying from orally administered Aroclor 1254 had ranges of 320 to 770 ppm in the brain (Dahlgren *et al.* 1972). No pelicans had brain residues of either the DDT complex or PCB's as high as the lower limits observed for intoxication of cormorants and pheasants.

**Body and organ weight.** DDT complex and PCB treatments had no significant effects upon pelican body weights (Table II). Females were significantly lighter ( $p < 0.01$ ) than males throughout the study. Control, PCB and DDT complex groups lost 20, 17 and 18%, respectively, of their body weight during the two-week stress. Considerable amounts of body fat remained at necropsy. Fat depletion or weight loss have been reported as signs of poisoning by some insecticides (Stickel *et al.* 1966) and PCBs (Vos *et al.* 1971). Fat reserves and healthy body conditions of the pelicans in this experiment probably enabled the birds to withstand the DDT and PCB treatments without effects upon appetites or body weights.

Birds in the DDT treatment group had decreased ( $p < 0.01$ ) liver weights when analyzed as percent of body weight (Table II). PCB-treated birds had increased ( $p < 0.01$ ) liver weights on a total weight basis. Decreases in liver weight have been reported in the double-crested cormorant fed a combination of DDT + DDD + DDE (Greichus and Hannon 1973). However, large increases in average liver weight were found in pigeons fed DDT and DDE (Bailey *et al.* 1969 a and b). Increases in liver size in birds adminis-

Table II. Organ and Body Weights of Pelicans Treated Daily with PCB's or a Combination of DDT, DDD and DDE

| Organ and treatment | No. | Organ weight (g)   |           | % of body weight  |           |
|---------------------|-----|--------------------|-----------|-------------------|-----------|
|                     |     | Means <sup>a</sup> | Std error | Means             | Std error |
| <b>Brain</b>        |     |                    |           |                   |           |
| Control             | 9   | 17.79              | 0.51      | 0.42              | 0.01      |
| PCB                 | 9   | 17.63              | 0.34      | 0.38              | 0.01      |
| DDT                 | 9   | 18.29              | 0.38      | 0.38              | 0.01      |
| Female              | 6   | 16.86 <sup>b</sup> | 0.43      | 0.42              | 0.01      |
| Male                | 21  | 18.95              | 0.21      | 0.37              | 0.01      |
| <b>Heart</b>        |     |                    |           |                   |           |
| Control             | 9   | 37.45              | 3.76      | 0.87              | 0.06      |
| PCB                 | 9   | 38.48              | 2.51      | 0.81              | 0.04      |
| DDT                 | 9   | 41.50              | 2.84      | 0.86              | 0.05      |
| Female              | 6   | 34.23 <sup>c</sup> | 3.20      | 0.84              | 0.05      |
| Male                | 21  | 44.06              | 1.56      | 0.86              | 0.03      |
| <b>Liver</b>        |     |                    |           |                   |           |
| Control             | 9   | 74.99              | 3.59      | 1.79              | 0.10      |
| PCB                 | 9   | 88.72 <sup>d</sup> | 2.40      | 1.90              | 0.07      |
| DDT                 | 9   | 71.31              | 2.72      | 1.48 <sup>d</sup> | 0.08      |
| Female              | 6   | 72.52 <sup>b</sup> | 3.06      | 1.81              | 0.09      |
| Male                | 21  | 84.16              | 1.49      | 1.64              | 0.04      |
| <b>Spleen</b>       |     |                    |           |                   |           |
| Control             | 9   | 3.04               | 0.53      | 0.07              | 0.01      |
| PCB                 | 9   | 4.33               | 0.35      | 0.09 <sup>e</sup> | 0.01      |
| DDT                 | 9   | 3.33               | 0.40      | 0.07              | 0.01      |
| Female              | 6   | 3.06               | 0.45      | 0.08              | 0.01      |
| Male                | 21  | 4.07               | 0.22      | 0.08              | 0.01      |
| <b>Kidney</b>       |     |                    |           |                   |           |
| Control             | 9   | 35.84              | 4.18      | 0.85              | 0.10      |
| PCB                 | 9   | 37.42              | 2.79      | 0.79              | 0.06      |
| DDT                 | 9   | 35.13              | 3.16      | 0.73              | 0.07      |
| Female              | 6   | 31.41 <sup>c</sup> | 3.56      | 0.79              | 0.08      |
| Male                | 21  | 40.86              | 1.73      | 0.79              | 0.04      |
| <b>Body Weight</b>  |     |                    |           |                   |           |
| Control             | 9   | 4280               | 190       |                   |           |
| PCB                 | 9   | 4720               | 130       |                   |           |
| DDT                 | 9   | 4810               | 150       |                   |           |
| Female              | 6   | 4090               | 160       |                   |           |
| Male                | 21  | 5130               | 80        |                   |           |

<sup>a</sup>Values for means were statistically adjusted for differences in numbers of males and females in each group.

<sup>b</sup>Females significantly different from males at  $p < 0.01$  level.

<sup>c</sup>Females significantly different from males at  $p < 0.05$  level.

<sup>d</sup>Significantly different from control at  $p < 0.01$  level.

<sup>e</sup>Significantly different from control at  $p < 0.05$  level.

tered PCBs have been reported for pigeons (Bailey and Bunyan 1972), Japanese quail (*Coturnix coturnix japonica*) (Vos *et al.* 1971, Bitman *et al.* 1972), pheasants (Dahlgren *et al.* 1972), and chickens (Vos and Koeman 1970).

Spleen weight taken as percent of body weight was significantly greater ( $p < 0.05$ ) in PCB-treated pelicans than in controls. Spleen atrophy induced by feeding PCBs has been reported in chickens (Vos and Koeman 1970, Flick *et al.* 1965), and in pheasants (Dahlgren *et al.* 1972). There was no effect on spleen weight due to DDT treatment in the present experiment.

No significant weight differences due to treatments were detected in brain, heart, or kidney analyzed on the basis of total organ weight, or organ weight taken as percent of body weight. Dahlgren *et al.* (1972) observed decreased heart weights in pheasants administered PCBs. Kidney enlargement with PCB treatment has been reported in pheasants (Dahlgren *et al.* 1972), in Bengalese finches (*Lonchura striata*) (Presst *et al.* 1970) and in chickens (Flick *et al.* 1965). Greichus and Hannon (1973) did not find significant differences in cormorant heart or brain weights due to treatment with a combination of DDT, DDD and DDE.

Female pelicans were lighter in body weight ( $p < 0.01$ ) than male pelicans. Females had smaller brains ( $p < 0.01$ ), hearts ( $p < 0.01$ ), livers ( $p < 0.01$ ), and kidneys ( $p < 0.01$ ) than males. Spleen weight differences between sexes were not significant.

**Liver carotene and vitamin A levels.** No significant differences due to treatments were observed on liver carotene levels (Table III). Liver vitamin A levels were greater ( $p < 0.01$ ) in DDT-treated pelicans than in controls when examined on a  $\mu\text{g/g}$  of liver basis. Cormorant liver stores of carotene and vitamin A did not show any consistent differences due to DDT-complex treatment when compared on  $\mu\text{g/g}$  of liver basis, but total amount of vitamin A in the liver was lower (Greichus and Hannon 1973). Vitamin A content of livers of PCB-treated birds were not significantly different from controls when compared on a  $\mu\text{g/g}$  of liver basis or on total amount present in the liver.

**Serum chemistry evaluations.** Means and standard error values of blood chemistries in control, PCB and DDT-complex treated pelicans are given in Table IV. No significant serum sodium changes were observed due to treatment or sex. However, sodium levels were significantly raised ( $p < 0.05$ ) by stress in this experiment. Sodium values ranged from 145 to 162 millicivalents (meq)/liter and were similar to values presented by Bell and Sturkie (1965) for chickens, turkeys, ducks, and gulls, and with the values of Greichus and Hannon (1973) for double-crested cormorants. These data indicate that serum sodium levels are similar for several avian species.

Serum potassium levels were decreased at the final bleeding in the PCB ( $p < 0.05$ ) and DDT ( $p < 0.01$ ) treatment groups. Stress also produced a significant lowering ( $p < 0.05$ ) of serum potassium. Males had significantly higher levels ( $p < 0.01$ ) than females at the time of pre-stress bleeding. A possible explanation for a lowering of potassium with stress is that the lower intake of food was not compensated for by renal conservation. The

**Table III. Liver Vitamin A and Carotene Values of Pelicans Treated with PCBs or a Combination of DDT, DDD and DDE**

| Treatment | No. | $\mu\text{g/g}$ of liver |           | Total mg/liver |           |
|-----------|-----|--------------------------|-----------|----------------|-----------|
|           |     | Means <sup>a</sup>       | Std error | Means          | Std error |
| Vitamin A |     |                          |           |                |           |
| Control   | 9   | 20.3                     | 4.9       | 1.52           | 0.40      |
| PCB       | 9   | 16.8                     | 3.3       | 1.47           | 0.26      |
| DDT       | 9   | 34.9 <sup>b</sup>        | 3.7       | 2.36           | 0.30      |
| Female    | 6   | 25.8                     | 4.2       | 1.72           | 0.34      |
| Male      | 21  | 22.2                     | 2.0       | 1.85           | 0.16      |
| Carotene  |     |                          |           |                |           |
| Control   | 9   | 74.0                     | 14.3      | 5.55           | 1.22      |
| PCB       | 9   | 64.9                     | 9.5       | 5.95           | 0.81      |
| DDT       | 9   | 64.7                     | 10.8      | 4.54           | 0.92      |
| Female    | 6   | 65.1                     | 12.2      | 4.76           | 1.04      |
| Male      | 21  | 70.6                     | 5.9       | 5.93           | 0.51      |

<sup>a</sup>Values for means were statistically adjusted for differences in numbers of males and females in each group.

<sup>b</sup>Significantly different from control at  $p < 0.01$  level.

kidney is much less able to conserve potassium than sodium during periods of interrupted uptake (Tasker 1971). The further decreases with PCB and DDT treatments may reflect structural changes in the kidney influencing absorption or secretion rates. Histologic and ultrastructural changes in renal tubules of rats have been reported after administering chlorinated hydrocarbon insecticides (Treon *et al.* 1955, Boyd and Chen 1968, Fowler 1972).

Significant decreases in serum protein were observed at the time of the pre-stress bleeding for PCB ( $p < 0.01$ ) and DDT-complex ( $p < 0.05$ ) treatment groups. Slight decreases in serum protein levels have been reported in Japanese quail given DDT (Ernst 1966) or dieldrin (Call and Call 1974), and in dieldrin-treated dogs (Walker *et al.* 1969). The significantly higher ( $p < 0.01$ ) final protein levels in females than in males agrees with findings in chickens (Bell and Sturkie 1965) and Japanese quail (Call and Call 1974). The protein levels for both sexes were quite similar two weeks earlier. However, there was a significantly greater ( $p < 0.05$ ) effect of stress on serum protein levels of males than females.

Serum uric acid levels were not affected by PCB or DDT-complex treatment, but stress significantly lowered ( $p < 0.05$ ) levels from about 20  $\text{mg}/100 \text{ ml}$  to about 8  $\text{mg}/100 \text{ ml}$ . This is in accordance with the findings of Siller (1959) that high protein diets increased plasma urate, while starvation caused a significant depression.

Serum calcium was significantly decreased ( $p < 0.01$ ) in PCB-treated pelicans at the time of final bleeding. This was not due to decreased food as there were no significant

Table IV. Means and Standard Errors of Values of Blood Chemistries in Pelicans Treated Daily with PCB's or a Combination of DDT, DDD and DDE<sup>a</sup>

| Treatment No. <sup>b</sup> | Sodium<br>(meq/liter) |           | Potassium<br>(meq/liter) |           | Protein<br>g/100 ml |           | Uric acid<br>mg/100 ml |           | Calcium<br>mg/100 ml |           | Phosphorus<br>mg/100 ml |           | Magnesium<br>mg/100 ml |           |
|----------------------------|-----------------------|-----------|--------------------------|-----------|---------------------|-----------|------------------------|-----------|----------------------|-----------|-------------------------|-----------|------------------------|-----------|
|                            | Means                 | Std error | Means                    | Std error | Means               | Std error | Means                  | Std error | Means                | Std error | Means                   | Std error | Means                  | Std error |
| Pre-stress Bleeding        |                       |           |                          |           |                     |           |                        |           |                      |           |                         |           |                        |           |
| Control 9                  | 144.7                 | 0.1       | 8.57                     | 0.02      | 3.87                | 0.01      | 19.88                  | 0.08      | 9.69                 | 0.04      | 6.15                    | 0.01      | 0.55                   | 0.05      |
| PCB 9                      | 146.3                 | 0.1       | 8.50                     | 0.01      | 3.23 <sup>c</sup>   | 0.01      | 18.94                  | 0.06      | 9.51                 | 0.03      | 5.50                    | 0.01      | 0.71                   | 0.04      |
| DDT 9                      | 146.4                 | 0.1       | 7.15                     | 0.01      | 3.41 <sup>d</sup>   | 0.01      | 20.02                  | 0.06      | 9.42                 | 0.03      | 5.17                    | 0.01      | 0.71                   | 0.04      |
| Female 6                   | 146.6                 | 0.1       | 7.32 <sup>e</sup>        | 0.01      | 3.40                | 0.01      | 21.60                  | 0.07      | 9.47                 | 0.03      | 5.28                    | 0.01      | 0.52                   | 0.05      |
| Male 21                    | 145.0                 | 0.1       | 8.82                     | 0.01      | 3.60                | 0.01      | 17.63                  | 0.04      | 9.62                 | 0.02      | 5.94                    | 0.01      | 0.79                   | 0.02      |
| Final Bleeding             |                       |           |                          |           |                     |           |                        |           |                      |           |                         |           |                        |           |
| Control 9                  | 160.7                 | 0.2       | 6.28                     | 0.16      | 4.16                | 0.01      | 8.11                   | 0.04      | 10.20                | 0.03      | 5.08                    | 0.02      | 0.42                   | 0.02      |
| PCB 9                      | 156.8                 | 0.1       | 3.92 <sup>d</sup>        | 0.11      | 3.75                | 0.01      | 8.18                   | 0.03      | 8.73 <sup>c</sup>    | 0.02      | 5.39                    | 0.01      | 0.44                   | 0.01      |
| DDT 9                      | 157.9                 | 0.1       | 3.20 <sup>c</sup>        | 0.12      | 3.81                | 0.01      | 8.51                   | 0.03      | 9.58                 | 0.02      | 4.97                    | 0.01      | 0.89                   | 0.01      |
| Female 6                   | 162.4                 | 0.1       | 4.11                     | 0.14      | 4.59 <sup>f</sup>   | 0.01      | 8.93                   | 0.04      | 9.16                 | 0.02      | 5.42                    | 0.02      | 0.65                   | 0.02      |
| Male 21                    | 154.5                 | 0.1       | 4.83                     | 0.07      | 3.22                | 0.01      | 7.60                   | 0.02      | 9.85                 | 0.01      | 4.88                    | 0.01      | 0.65                   | 0.01      |

<sup>a</sup>Values for means were statistically adjusted for differences in numbers of males and females in each group.

<sup>b</sup>All samples were run in duplicate

<sup>c</sup>Significantly different from control at the  $p < 0.01$  level.

<sup>d</sup>Significantly different from control at the  $p < 0.05$  level.

<sup>e</sup>Females significantly different from males at  $p < 0.05$  level.

<sup>f</sup>Females significantly different from males at  $p < 0.01$  level.

<sup>c</sup>Significantly different from control at the  $p < 0.01$  level.  
<sup>d</sup>Significantly different from control at the  $p < 0.05$  level.  
<sup>e</sup>Females significantly different from males at  $p < 0.05$  level.  
<sup>f</sup>Females significantly different from males at  $p < 0.01$  level.

Table V. Serum Protein Fraction Values in Pelicans Treated Daily with PCB's or a Combination of DDT, DDD and DDE<sup>a</sup>

| Treatment              | No. | Albumin          |           | $\alpha$ Globulin |           | $\beta$ Globulin |           | $\gamma$ Globulin |           | Total globulin |           | A/G ratio |           |
|------------------------|-----|------------------|-----------|-------------------|-----------|------------------|-----------|-------------------|-----------|----------------|-----------|-----------|-----------|
|                        |     | Mean             | Std error | Mean              | Std error | Mean             | Std error | Mean              | Std error | Mean           | Std error | Mean      | Std error |
| Pre-treatment Bleeding |     |                  |           |                   |           |                  |           |                   |           |                |           |           |           |
| Control                | 9   | 1.5              | 0.4       | 2.0               | 0.3       | 1.1              | 0.3       | 0.5               | 0.3       | 3.6            | 0.7       | 0.45      | 0.05      |
| PCB                    | 9   | 1.5              | 0.2       | 1.3               | 0.1       | 0.9              | 0.2       | 0.9               | 0.2       | 3.1            | 0.4       | 0.47      | 0.03      |
| DDT                    | 8   | 2.0              | 0.4       | 1.3               | 0.2       | 1.8              | 0.3       | 1.3               | 0.3       | 4.3            | 0.6       | 0.46      | 0.04      |
| Pre-stress Bleeding    |     |                  |           |                   |           |                  |           |                   |           |                |           |           |           |
| Control                | 9   | 1.4              | 0.1       | 1.0               | 0.1       | 1.0              | 0.1       | 0.8               | 0.1       | 2.8            | 0.2       | 0.49      | 0.05      |
| PCB                    | 9   | 1.3              | 0.04      | 0.6               | 0.1       | 0.8              | 0.04      | 0.5               | 0.04      | 2.1            | 0.1       | 0.62      | 0.02      |
| DDT                    | 8   | 1.3              | 0.1       | 0.7               | 0.1       | 1.0              | 0.1       | 0.7               | 0.1       | 2.4            | 0.1       | 0.60      | 0.04      |
| Final Bleeding         |     |                  |           |                   |           |                  |           |                   |           |                |           |           |           |
| Control                | 9   | 1.5              | 0.2       | N.D. <sup>b</sup> |           | N.D.             |           | N.D.              |           | 2.6            | 0.3       | 0.56      | 0.07      |
| PCB                    | 9   | 1.1 <sup>c</sup> | 0.1       | N.D.              |           | N.D.             |           | N.D.              |           | 2.5            | 0.1       | 0.42      | 0.03      |
| DDT                    | 9   | 1.5              | 0.1       | N.D.              |           | N.D.             |           | N.D.              |           | 2.2            | 0.2       | 0.68      | 0.06      |

<sup>a</sup>Data are expressed in grams per 100 ml

<sup>b</sup>No data.

<sup>c</sup>Significantly different from controls at the  $p < 0.01$  level.

339

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effects of this stress on calcium levels. Plasma or serum calcium levels were unchanged in chicks fed 150 ppm of Aroclor 1248 for 2.5 weeks or 100 ppm of Aroclor 1248 for 4.5 weeks (Rehfeld *et al.* 1972), and in Japanese quail fed up to 75 ppm of dieldrin for two weeks (Call and Call 1974).

Serum inorganic phosphorus levels were not affected by treatment, sex, or stress in this experiment. Likewise, Call and Call (1974) found no significant changes in serum inorganic phosphate levels in Japanese quail fed dieldrin.

Serum magnesium levels of the pelicans were not affected by treatment, sex, or stress. Rehfeld *et al.* (1972) reported that chick plasma magnesium levels were unaffected by PCBs in the diet at levels up to 150 ppm.

Serum protein fractions. Albumin was lowered ( $p < 0.01$ ) in PCB-treated pelicans at the time of the final bleeding, but not in DDT-treated birds (Table V). Wassermann *et al.* (1973) reported that Aroclor 1221 lowered rabbit albumin levels while DDT and dieldrin caused slight increases. Lowering of serum albumin was noted in the present experiment after the food stress had been imposed.

No significant changes were noted in globulin fractions at the times of the pre-treatment or pre-stress bleedings, or in total globulin at any time. Individual globulin fractions were not readily separable and identifiable on the spectrophotograms following stress, so a single measure of total globulin was taken. Wassermann *et al.* (1973) reported decreased gamma globulin fractions in rabbits administered organochlorine insecticides and PCBs. There were no significant changes in the albumin/globulin ratio (A/G) due to either treatment.

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