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Effects of Polychlorinated Biphenyls on Mourning Dove Reproduction and Circulating Progesterone Levels

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Production of PCBs (polychlorinated biphenyls) was banned by USEPA in July 1979, but because of their stability and persistence they are now ubiquitous environmental contaminants. Contamination results from leaks, accidents, incineration, legal and illegal disposal dumps, and effluent from industries using PCBs in their manufacturing processes.

PCBs acquired in sublethal doses increase activation of the liver's microsomal action and hepatic mixed function oxidase (MFO) enzymes. Physiological effects of increases in the MFO system may delay, if not eliminate, avian reproductive behavior and nesting success (Rattner et al. 1984). No evidence is yet available to clearly link PCBs to lowered levels of circulating steroids in birds. However, data concerning the effects of PCBs on avian reproduction are abundant (Eisler 1986).

Increased degradation of circulating steroids might alter essential hormone dynamics associated with nesting behavior and ovulation. Alternatively, normal feedback regulation by the pituitary-gonadal axis might lead to a full compensatory response to increased MFO such that hormone profiles remain unaltered, suggesting alternative modes of action for PCBs in reproductive disorders. Therefore, we investigated the effect of dietary PCBs on circulating progesterone levels prior to and during egg laying in wild-trapped mourning doves (Zenaida macroura).

Progesterone was selected for study because a preovulatory increase in plasma concentrations of this steroid is known to be a critical component in hormonal control of avian ovulation. In chickens, a slight rise in luteinizing hormone (LH) stimulates progesterone production by the largest ovarian follicle, which, through positive feedback to the pituitary, stimulates an even greater LH surge and ovulation about 6 hours later. Deviations from normal progesterone levels could cause reduced follicular growth and complicate or delay the initiation of the LH surge.

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The mourning dove is widely distributed in North America, is a migratory species, has a high aesthetic value, and is an important game bird. Wild mourning doves have PCBs in fat at 0.4 ppm (Edwards et al. 1983). The dove reproductive behavioral cycle has been well documented, but related endocrine information is limited, to our knowledge, to a report by Mirarchi et al. (1982). The hormonal cycle of a similar species, the ring dove (Streptopelia risoria) has been documented (Silver et al. 1974) and is of comparative value.

MATERIALS AND METHODS

Mourning doves were live-trapped near The Ohio State University, Columbus. All healthy captured birds were transferred to an outside aviary (7.3 x 2.4 x 2.4 m) for an acclimation period of 1-3 weeks. The birds were then moved to inside observation cages (1.8 x 0.7 x 0.5 m) that contained 2 automatic watering bowls, 3 wooden perches, 2 feeder bowls, and 2 nesting bowls. Water and Purina Pigeon Checkers (R) mixed with cracked corn (9:1)(Ralston-Purina, St. Louis, Mo.) were supplied ad libitum . Grit (Kaytee Grit #11, Nhauf & Tesch Co., Chilton, Wis.) was supplied in other containers. A wall constructed with 2" x 4"'s and an opaque plastic sheet with one-way glass separated the cages from observers. Lights were on from 0800 to 2200 hrs; and cage temperature was 20-22°C.

Only mourning dove pairs that laid eggs in the observation cages during the acclimation period were used in the subsequent experiments. Mated pairs were randomly assigned to control or treated groups, and isolated for 28 days by placing a wood divider in the cages. During isolation all birds were provided food containing 10 ppm PCBs (Aroclor 1254) ad libitum , which approximated environmental exposure and was similar to doses used in other studies of PCBs and mourning doves (Tori & Peterle 1983). Treated food was prepared by mixing ground Purina Pigeon Checkers with Aroclor 1254 and re-pelleting the mixture.

At the end of the treatment period, the dividers were removed and behavioral observations and blood sampling were initiated. Observers alternately watched the birds for 2-4 hours everyday beginning 1/2 hour after lights on. Perch-cooing, male nest-cooing, nest site selection, nest site solicitation by the male, mutual grooming, indicating pair-bond formation, copulation (COP), nest building (NB), and occupation of the nest by the female (ON) were recorded. Female ON was followed by oviposition and incubation. When a female dove went ON and proceeded to lay an egg, that ON was considered ON/egg. ON/no egg occurred when a female dove went ON and failed to lay an egg. A dove with ON/no egg might later resume courtship activity and exhibit ON/egg.

Observations were recorded for each bird, and as the pair's behaviors reached COP and NB, blood collection was initiated. Pairs ceased courtship activity if a blood sample was collected before COP (Koval 1986). Blood (1 ml) was collected from a

brachial vein (1000-1200 hr) with a heparinized 3 cc syringe and 25 ga needle; plasma was stored at -10°C until radioimmunoassay (RIA).

Progesterone was extracted twice from 0.5 ml plasma with 4.0 ml diethyl ether. Extract residues were dissolved overnight in 1 ml of assay buffer (0.1 M phosphate-buffered saline containing 0.1% NaN3 and 0.1% Knox gelatin, pH = 7.2). Duplicate standard solutions containing 0.0, 0.025, 0.05, 0.10, 0.25, 0.50, 1.00, 2.50, and 5.00 ng authentic progesterone (Sigma Chemical Co., St. Louis, Mo.) in 0.1 ml assay buffer were included in each RIA. Antibody (1:1200) and 200 pg (2,3-3H)-Progesterone (sp. act. 57.5 Ci/mmol, New England Nuclear, Boston, Mass.), each in 0.1 ml assay buffer, were added to each tube and vortexed prior to incubation at 4°C for 16-24 hours. Free and antibody-bound steroids were separated by mixing cold Dextran-coated charcoal suspension (0.50% charcoal/0.25% Dextran) prior to centrifugation. The supernatant was decanted into 10 ml scintillation fluid (RIA-Solve II, Research Products International, Mt. Prospect, Ill.), and mixed thoroughly prior to measurement of 3H-progesterone in a liquid scintillation spectrometer. Plasma progesterone concentrations were calculated from the standard dose-response curve by logit transformation and weighted linear regression analysis. The antisera, diluted 1:1200, bound 50% of 200 pg 3H-progesterone. The antisera (donated by V.C. Stevens) showed low cross reactivity with other steroids; the highest was 6.5% with 17α-hydroxypregn-4-ene-3,20-dione (Powell & Stevens 1973).

Differences were tested with the F-test, Student's t, T-test, and a nonparametric binomial test. Values are reported as the mean $\pm$ standard error of the mean (SEM). The basal plasma progesterone level in each female was an average of concentrations in plasma samples collected prior to egg-laying or ON behavior. A progesterone concentration was considered peak if the value was at least 2 times greater than the basal level for the individual bird, and coincided with a behavioral event (ON or subsequent events).

Sensitivity of the assay was 0.025-0.05 ng/ml. The mourning dove plasma blank (charcoal treated to remove steroids) averaged 0.065 $\pm$ 0.01 ng per tube (N = 52). Recovery of 10 pg 3H-progesterone added prior to extraction averaged 70.3 $\pm$ 0.5% (N = 275). Coefficient of variation (CV) for 4 plasma samples included in each of the 16 assays averaged 4.0%. Quantitative recovery of authentic progesterone was 68.2 $\pm$ 2.7%. Repeated assays (N = 16) of 2 mourning dove plasma pools (1.00 and 2.50 ng/ml) to measure intra-assay variation yielded averages of 1.02 $\pm$ 0.12 ng/ml, and 2.64 $\pm$ 0.42 ng/ml, respectively. The mean CV for duplicates of the standard curve in 16 assays was 1.7-6.8%.

To assess parallelism between plasma and standard curves, blank plasmas with progesterone added (0.05-5.00 ng/tube) were included in 9 assays. The regression equation for the standards in assay buffer was $y = -0.486 - 2.26x$ ($r = -0.99$). The regression

equation for the standards in dove plasma was y = 0.958 - 2.15x ($r = -0.97$). The curves were parallel ($p < 0.05$; SAS Analysis) in 7 of 9 trials.

RESULTS AND DISCUSSION

Only 50% (5/10) of the birds treated with PCBs laid eggs compared to 77% (10/13) of the control birds. The mean number of days from pairing to oviposition was 32.5 $\pm$ 6.6 for treated birds and 40.0 $\pm$ 4.1 days in the control birds, a non-significant ($p > 0.05$) difference. However, the interval from ON behavior to oviposition (ON/egg) in PCB-treated birds (11.4 $\pm$ 3.0 days) was significantly ($p < 0.05$) longer than for control birds (3.1 $\pm$ 0.44 days).

PCB-treated birds tended to initiate a nesting attempt and then abandon the nest, resulting in a cessation of mating activities for a time (ON/no egg). The mean number of days between ON/no egg and oviposition was greater ($p < 0.05$) for treated doves (39.3 $\pm$ 5.4 days) than for control birds (9.75 $\pm$ 1.9 days). The mean number of days between ON/no egg and ON/egg for control and treated birds with both events was greater ($p < 0.05$) for treated doves (25.6 $\pm$ 6.6 days) than for control birds (6.0 $\pm$ 1.2 days).

Treatment with PCBs caused an increase in the number of days ON per female dove that laid eggs. Treated birds that laid at least 1 egg during the experimental period had a greater ($p < 0.05$) number of days ON/bird (8.4 $\pm$ 2.4) than control birds (2.3 $\pm$ 0.5 days ON/bird). The control birds without eggs spent more ($p < 0.05$) days ON/bird (7.7 $\pm$ 1.7) than treated birds without eggs (4.1 $\pm$ 2.0 days ON/bird). Forty-six percent (6/13) of the control females and 40% (4/10) of the treated females laid a second egg.

Progesterone levels in PCB-treated and control birds were similar at many points in the reproductive cycle. Concentrations of progesterone (ng/ml) in plasma of treated birds were slightly, but not significantly ($p > 0.05$), lower than those in control females at critical points in the reproductive cycle as determined by the female dove's behavior (Fig. 1). However, progesterone levels in control birds were always greater than those for treated birds. The probability of this is low ($p < 0.05$, Binomial Test).

Differences between control and treated birds in circulating progesterone levels were evident when the progesterone values were grouped relative to the day of oviposition of egg 1 (Fig. 2). Significant differences occurred on Days -2 to -1 and Days 3 to 4 ($p < 0.05$). Concentrations of progesterone peaked earlier in treated birds (Days -4 to -3) than in control birds (Days -2 to -1). Progesterone in control birds peaked closer to the day of oviposition, and at a higher level. Peak progesterone levels in individual birds occurred 0-2 days prior to oviposition in control birds whereas this event occurred up to 4 days prior to oviposition for some treated birds.

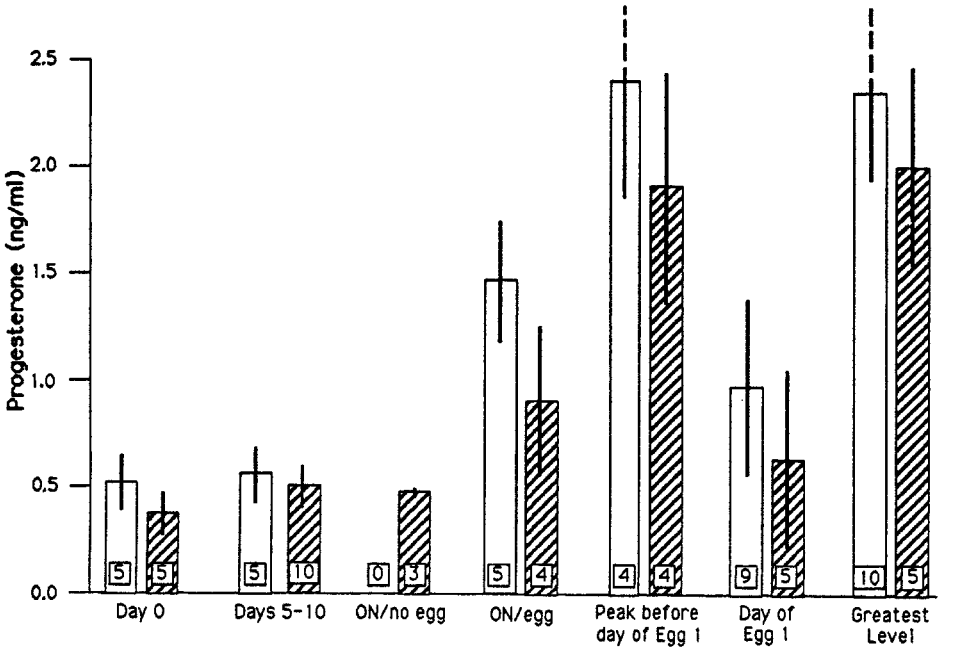

Fig. 1. Plasma progesterone concentrations (mean ± SEM) in female mourning doves treated with PCBs ▨ and control birds □. Birds were paired on Day 0. The sample size is indicated at the base of each bar.

This study provides evidence of delayed reproduction in PCB-treated birds. PCBs, alone and in combination with other contaminants caused delayed egg deposition when ingested by avian species (Tori & Peterle 1983, Farve 1978, McArthur et al. 1983). The general delay of oviposition, seen as a consequence of PCB treatment, might result from alteration in the normal hormonal regime of the nesting cycle. Estrogen and progesterone levels begin to rise at the onset of ON behavior. The levels of plasma progesterone reported here are somewhat lower than those reported (Silver et al. 1974) for the ring dove (0.5 ng/ml basal, 4.0 ng/ml peak), and the rise in progesterone levels occurred closer to the day of oviposition in mourning doves (control birds).

Treatment of Japanese quail (Coturnix coturnix japonica) with PCBs resulted in delayed egg laying, and dose-dependent increased liver weights (Biessmann 1982). Doses of DDE, PCB, mirex and photomirex produced alterations in the behavior of ring doves, and progesterone levels were reduced (McArthur et al. 1983). Ring doves demonstrated a diminished LH peak when 40 ppm DDE was administered (Richie & Peterle 1979).

The relatively small effect of PCBs on plasma progesterone levels observed in this study are consistent with those reported by others (Biessmann 1982, McArthur et al. 1983). Progesterone

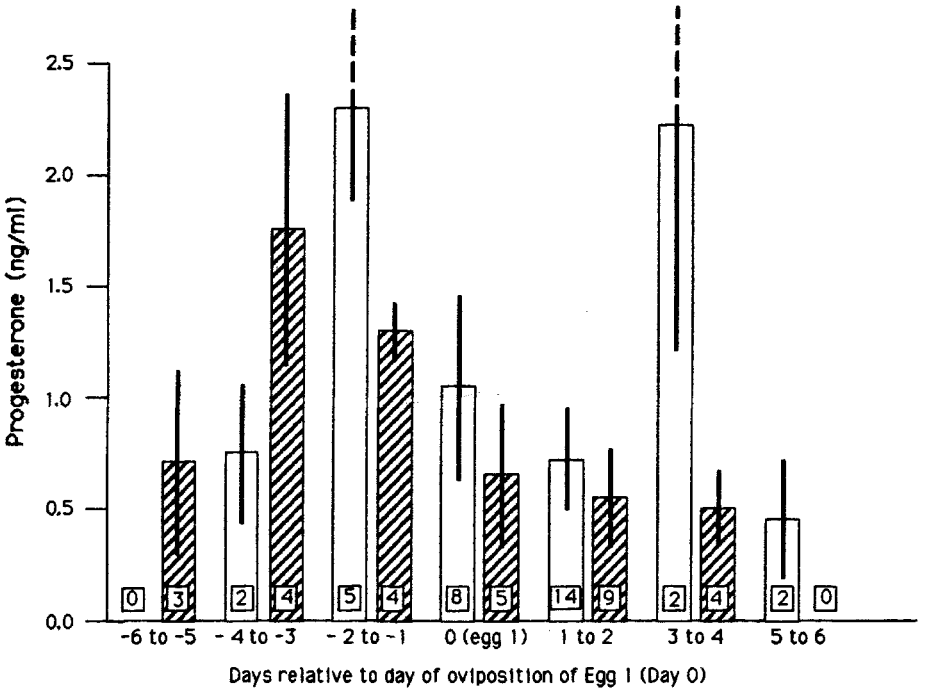

Fig. 2. Plasma progesterone concentrations (mean ± SEM) in female mourning doves treated with PCBs ▨ and in control birds □ relative to day of oviposition of egg 1 (Day 0). Sample size is indicated at the base of each bar. Concentrations are significantly different for control and treated birds on Days -2 to -1 and 3 to 4 ($p < 0.05$).

concentrations in treated birds were lower than in control birds at each stage of the cycle ($p < 0.05$) (Fig. 1), but the physiological level necessary to stimulate the LH surge and ovulation was apparently reached in 50% of the PCB-treated birds. Thus, we agree with Biessmann (1982) that feedback regulation of progesterone secretion appears to be sufficiently robust to compensate for any increased degradation of circulating steroids that might be caused by increased MFO.

Potentially important temporal changes in progesterone dynamics were observed in this study. Progesterone levels for treated mourning doves peaked earlier relative to oviposition than in control birds, an observation also reported by MacArthur et al. (1983). The number of days between the peak in plasma progesterone level and oviposition was greater for PCB-treated birds. Any delay in ovulation from the time of the plasma progesterone peak would increase the number of days between the progesterone peak and oviposition.

The delay in oviposition following the progesterone peak might be related to direct effects of PCBs on the ovary and oviduct.

PCBs accumulate in the ovarian thecal cells and inhibit follicle growth (Biessmann 1982; Rattner et al. 1984). Response of the dominant follicle to LH might also be impaired, resulting in slowed follicular development and increased time to ovulation. Increased time between peak plasma progesterone levels and oviposition might also reflect slowed passage of the egg through the reproductive tract. The effects of PCBs on estrogen levels or the egg shell gland might cause a lengthening in the time required for protein and shell deposition between ovulation and oviposition.

The consequence of sublethal PCB ingestion by mourning doves, and other avian species, is a general decrease in reproductive efficiency. This decrease does not appear to be directly attributable to large quantitative changes in circulating progesterone levels. The delay in oviposition observed in our PCB-treated birds might have resulted from interference with temporal aspects of progesterone feedback regulation or maturation of the preovulatory follicle. More research will be required to understand the mechanisms of PCB action on avian reproduction.

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