REDUCTION OF COURTSHIP BEHAVIOR INDUCED BY DDE IN MALE RINGED TURTLE DOVES

M. A. Haegele and Rick H. Hudson

Some wildlife investigators who have studied declining bird populations have reported what they consider to be aberrant reproductive behavior. For instance, Gress (1970) reported abnormal behavior in the California Brown Pelicans (*Pelecanus occidentalis*) nesting on Anacapa Island, and Snyder et al. (1973) observed abnormal reproductive behavior in Cooper's Hawks (*Accipiter cooperii*). These investigators suggested that the behavior problems were caused by DDE residues. Effects of DDE on behavior have been observed in laboratory studies concerned with reproduction of birds. Lincer (1972) observed possible abnormal parental behavior in American Kestrels (*Falco sparverius*) treated with dietary DDE and polychlorinated biphenyls (PCB's). In our own studies with Ringed Turtle Doves (*Streptopelia risoria*) we found that DDE-treated birds in undisturbed reproductive cycles took an average of 2.5 times longer to renest than did control birds (Haegele and Hudson 1973).

In the laboratory and field studies cited, few quantitative data were available to test if DDE does affect reproductive behavior of birds. The purpose of the present study was to measure the quantitative effects of dietary DDE on the initial courtship behavior of male Ringed Turtle Doves.

METHODS

The 18 pairs of Ringed Turtle Doves used in this study were hatched and raised at the Denver Wildlife Research Center. Each pair had raised at least 1 young to 21 days of age prior to use in this study. After all 18 pairs of birds had successfully completed 1 breeding experience, each bird was isolated from viewing others (sight isolation) for 18 days. At the start of isolation, the outside of each wing of all males was marked with red ink so that we could easily distinguish the male birds when paired for courtship observations. Lights were clock-controlled, turned on at 06:30 and off at 20:00 MST and temperature in all rooms was maintained between 22° and 24°C. Water, food, and mineralized grit were provided *ad libitum*.

After the 18th day of isolation, each male dove was randomly paired with a female dove with which he had never previously mated, and the pairs were placed in observation cages for 12.5 min each day for 5 consecutive days. Courtship behavior displayed during this period was recorded on video tape. This procedure provided a base line for normal courtship behavior. In order to keep assigned pairs separate until the video tape equipment was in operation, each observation cage was divided by an opaque partition. These partitions were removed after our recording equipment was started.

To quantify courtship behavior, the video tapes were later played back and the number of bow-coos performed by each male bird was counted for each observation period. A

stopwatch was used to measure total time each male bird spent in performing sexual behavior. The types of behavior which we timed included driving, bow-coo, wing-flip, hetero-preening, billing, sex-mount, and stick-nest building (Miller and Miller 1958). The first 30 sec of video-taped behavior for all 12.5-min observation periods were not used. This allowed the investigator to leave the room and gave the doves time to adjust to the experimental conditions and their assigned mates. The video tape recorder and TV monitor were kept in an adjacent room so that no one was present in the room with the doves during a taping session. At the end of each observation period, all birds were returned to sight isolation until the next period. During all recording periods, each male dove was always paired with the same female.

After the pretreatment data were gathered, the 18 pairs of doves were randomly assigned to 3 treatment groups each consisting of 6 pairs. One group was given a 10-ppm p,p'-DDE diet and one a 50-ppm diet dry weight basis. The remaining group served as a control and received an uncontaminated pigeon checkers diet. Initiation of treated diets was designated as day 0. Treated diets were made by adding proper amounts of DDE to ground Purina pigeon checkers. (Trade names are provided for identification only. Their mention does not imply endorsement of commercial products by the Federal Government.) All birds were fed *ad libitum* for 63 days at which time the study was terminated.

The 10-ppm dietary treatment was chosen to simulate what was considered a possible field exposure level and the 50-ppm dietary treatment was selected to approximate the exposure level which adversely affected reproduction in our earlier study (Haegele and Hudson 1973). To minimize possible intoxication effects, both treatment levels were selected to be sufficiently below the 20-day LC50 value of 250–300 ppm p,p'-DDE for Ringed Turtle Doves, which we determined before initiating this study.

On days 31–35 and days 59–63, all birds were again paired with the same female and put into observation cages for video taping of courtship behavior. All doves were kept in sight isolation between observation periods. After completion of the observations, on day 63, all birds were sacrificed and the males plucked, eviscerated, and analyzed for residues. All birds were examined internally to confirm that they had been correctly sexed. One pair on the 50-ppm diet consisted of 2 males; therefore, all results for the 50-ppm dietary treatment are based on a sample size of 5 pairs rather than 6.

A 3-factor analysis of variance with repeated measures on the last 2 factors (Winer 1971) was used to determine significant differences in bow-coo frequency and total activity time. Factor 1 was the 3 treatments, factor 2 was the 2 time periods (days 31–35 and 59–63), and factor 3 was the 10 1-day periods. Analysis of variance and Duncan's new multiple range test were used to determine significant differences between percent lipids found in whole body carcasses. Residue values were determined by the method of Peterson et al. (1976).

RESULTS

The mean number of seconds of total courtship activity time displayed by male Ringed Turtle Doves was reduced by the DDE treatment (Fig. 1). Control birds increased their average courtship activity by 25% and 23%, respectively, for the 31–35 and 59–63 day posttreatment observation periods. The 10-ppm-treated birds showed no courtship activity time behavior effects at 31–35 days, but activity time decreased 55% from pretreatment values at 59–63 days. The birds on the 50-ppm DDE-contaminated diet had a 30%

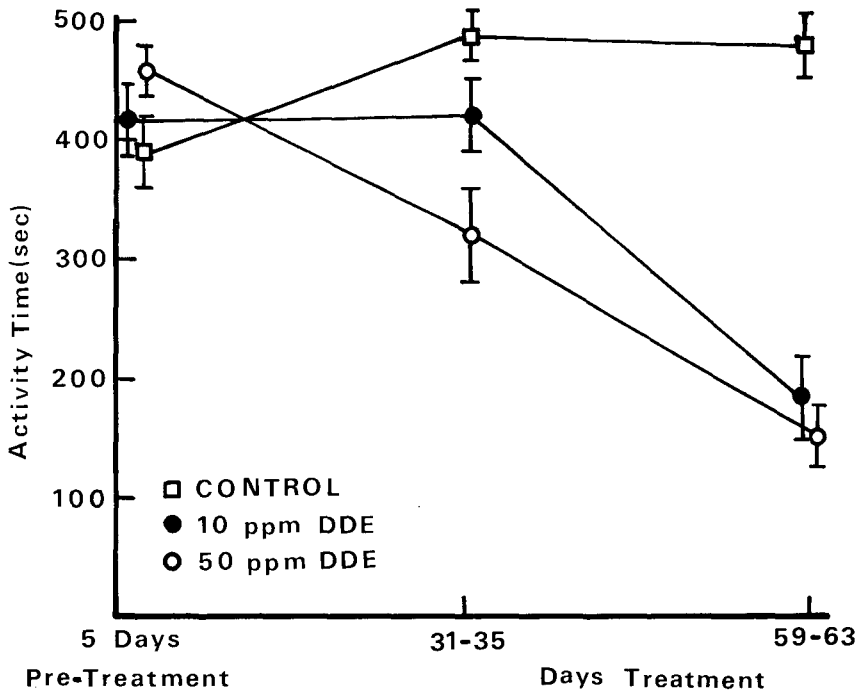

FIG. 1. Mean total courtship activity time displayed by male Ringed Turtle Doves per 12.5-min observation period. The vertical lines indicate ± S.E. of the mean.

reduction in activity time at 31–35 days, and a 67% reduction at days 59–63, when compared to pretreatment activity. The analysis of variance showed: (1) a significant difference among the 3 treatments ($F_{[2,14]} = 6.58$, $p < 0.01$), (2) a significant difference between the 2 time periods ($F_{[1,14]} = 16.51$, $p < 0.005$), (3) a significant difference among the 6 treatment × time period interaction means ($F_{[2,14]} = 4.08$, $p < 0.05$), (4) a significant difference among the five 1-day periods ($F_{[4,56]} = 2.72$, $p < 0.05$), and (5) a significant difference among the 10 time period × 1-day period interaction means ($F_{[4,56]} = 3.81$, $p < 0.01$). In summary, Figure 1 shows that the controls maintained a high level of activity time for both observation periods while the treated birds showed a marked reduction in activity time at the 59–63-day period. These results indicate that the difference in activity time between the 2 observation periods was due to the DDE treatment over time.

The mean bow-coo frequency by male Ringed Turtle Doves treated with the DDE diet was also reduced (Fig. 2). When compared with their pretreatment values, the bow-coo frequency of control males increased 19% at

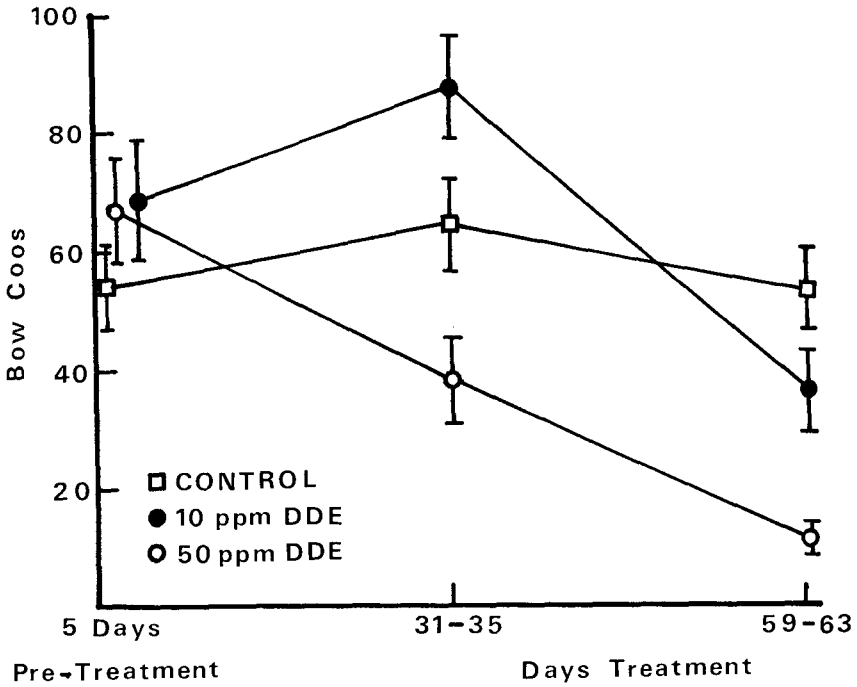

FIG. 2. Mean number of bow-coos performed by male Ringed Turtle Doves per 12.5-min observation period. The vertical lines indicate ± S.E. of the mean.

31–35 days and then decreased 1% at 59–63 days. The 10-ppm group also increased (26%) at 31–35 days; however, after 59–63 days, the 10-ppm treatment had begun to affect bow-coo frequency causing a reduction to 53% fewer bow-coos than were observed during pretreatment observations. The 50-ppm group had a reduced bow-coo frequency of approximately 43% during the first observation period and 84% during the second. The analysis of variance showed a significant difference among the 3 treatments ($F_{[2,14]} = 3.58$, $p < 0.06$) and a significant difference between the 2 time periods ($F_{[1,14]} = 11.05$, $p < 0.01$). The difference between the 2 observation periods indicates that, as DDE residues were increasing in treated birds, bow-coo frequency was decreasing. Figure 2 shows that all groups performed fewer bow-coos at 59–63 days than they did at 31–35 days, but the greatest changes in bow-coo frequency were shown by the DDE-treated groups.

For this study, total activity time, rather than the frequency of bow-coos, was probably a more sensitive index to the effects of the treated diet. Whereas bow-coos are only an early segment of courtship behavior displayed by male

TABLE 1

PERCENT BODY WEIGHT LOSS OF RINGED TURTLE DOVES DURING THE STUDY (0–63 DAYS)

Dietary DDE treatment	N	Males Mean percent body weight loss (S.E.)	Females Mean percent body weight loss (S.E.)
0 ppm	6	6.0 (3.4)	5.3 (3.7)
10 ppm	6	7.7 (2.7)	11.7 (6.2)
50 ppm	5	12.5 (10.6)	14.1 (7.9)

Ringed Turtle Doves, total activity time took into account all types of courtship behavior occurring during the observation period, such as nest site selection, wing-flipping, hetero-preening, billing, driving, bow-cooing, and copulation. The fact that male Ringed Turtle Doves could bow-coo infrequently during the observation period but still score very high in total time spent in courtship activity explains why our control birds showed a 1% decrease in bow-coo frequency at 59–63 days and yet had an increase of 23% in activity time during the same period (Figs. 1 and 2).

Body weight losses for both the male and female Ringed Turtle Doves are given in Table 1. All birds tended to lose weight during the study. A small amount of weight loss during isolation seems to be normal. We have usually observed this effect in our laboratory when we have isolated doves for any extended period of time. Although the doves fed 50 ppm DDE weighed somewhat less than others at the termination of the experiment, group differences were not statistically significant.

Brain and whole-body DDE residues, along with lipid values, are given in Table 2. Small amounts of Aroclor 1254-like residues, which could have accumulated from small amounts of background contamination in the feed, were also found in all birds. Although there may be a possibility of PCB's acting synergistically with DDE, the levels found in the different treatment groups, including controls, were probably too low (0.80 to 0.86 ppm wet weight) to affect the results. The percentage whole-body lipid in the male Ringed Turtle Doves was significantly ($p \leq 0.05$) reduced by the diets contaminated with DDE. The standard errors for whole-body lipids were 0.5, 1.2, and 1.3 for the controls, 10-ppm DDE and 50-ppm treatment groups, respectively. Thus, DDE treatment not only reduced the percentage whole-body lipid found in male Ringed Turtle Doves, but it also increased the within-treatment variance of percentage whole-body lipids. The percentage lipid in brain tissue, not included in the whole-body lipid determination, was constant regardless of treatment (Table 2).

TABLE 2

DDE RESIDUES FOUND IN MALE RINGED TURTLE DOVES AFTER 63 DAYS OF DIETARY TREATMENT

Dietary DDE treatment	N	Brain		
		ppm wet weight (S.E.)	ppm lipid weight (S.E.)	Percent lipid (S.E.)
0 ppm	6	0.09 (0.02)	1.6 (0.3)	6.7 (0.2)
10 ppm	6	2.9 (0.5)	40.9 (7.1)	7.0 (0.0)
50 ppm	5	7.6 (0.9)	116 (14.6)	6.6 (0.2)
		Whole body		
		ppm wet weight (S.E.)	ppm lipid weight (S.E.)	Percent lipid* (S.E.)
0 ppm	6	0.39 (0.16)	1.8 (1.8)	13.4[a] (0.5)
10 ppm	6	37.8 (4.4)	446 (108)	9.9[b] (1.2)
50 ppm	5	153 (25.2)	2477 (1107)	8.4[b] (1.3)

* Those % lipid values with different letters as superscripts are significantly different from each other ($p \leq 0.05$)—Duncan's New Multiple Range Test.

DISCUSSION

Throughout this study, we continued the exposure to the DDE-treated diet and did not assess whether or not or how long it would take for the birds to recover from the treatment. It is not common for a population of wild birds to be continually exposed to levels of DDE of the same magnitude as were used in our experiment. Yet, it is not unusual for wild birds to be exposed to levels of DDE equal to our 10-ppm (dry weight) contaminated diet for fairly long periods of time during some part of the year and build up body residues equal to or greater than those found in our 10-ppm birds (Table 2). Some examples are Brown Pelicans (Keith et al. 1970), Prairie Falcons (*Falco mexicanus*) (Enderson and Berger 1970), and Peregrines (*Falco peregrinus*) (Lincer et al. 1970, Cade et al. 1968, Enderson and Berger 1968).

This study supplements our earlier work (Haegele and Hudson 1973) by showing that dietary DDE at low levels can adversely affect sexual behavior and performance in Ringed Turtle Doves. The 40-ppm DDE-contaminated diet in our earlier study caused a significant delay in renesting and an abundance of single-egg clutches. This delay in renesting could have been caused by a reduction in courtship behavior displayed by the male Ringed Turtle Doves treated with DDE.

The 1-egg clutches observed in our 1973 study could also have been caused

by behavioral effects of DDE. Courtship and nest building behavior are very important in stimulating the female Ringed Turtle Dove to ovulate and lay eggs (Lehrman et al. 1961, Lehrman 1965). The DDE diet could have reduced male and female Ringed Turtle Dove courtship behavior to a level that was insufficient to cause normal egg laying. Embryo development was absent in the eggs from single-egg clutches after 18 days of incubation (14 days is the normal incubation period); this suggests that the pairs of doves with infertile single-egg clutches never copulated before egg laying. The fact that these birds may not have copulated could be related to DDE effects on reproductive behavior.

The reduced intensity of male Ringed Turtle Dove courtship behavior observed in this study supports the suggestion that DDE accumulation in wild birds is causing aberrant reproductive behavior and thereby reducing fecundity. Gress (1970) reported what he felt was aberrant reproductive behavior in Brown Pelicans, and he believed that the presence of large amounts of chlorinated hydrocarbon residues found in pelican tissue should be considered as a potential cause of this erratic behavior. Snyder et al. (1973) suggested that the disturbed behavior they observed in Cooper's Hawks might also have been linked to DDE residues present. Switzer et al. (1971) felt that the cause for the low reproductive success of their study population of Common Terns (*Sterna hirundo*) was attributable to aberrant reproductive behavior caused by DDE residues. Koeman et al. (1972) state that in their study 60% and probably 80% of the reproductive failures were caused by some intrinsic derailing factor in the Sparrow Hawk's (*Accipiter nisus*) breeding process. They felt this might be caused by DDE residues the birds were carrying.

All of the above examples suggest that some wild populations of birds contaminated with DDE are showing altered reproductive behavior and consequent reproductive failure. Judging from the effects dietary DDE had on courtship behavior of male Ringed Turtle Doves in our study, it is likely that DDE is affecting behavior in wild birds such as those mentioned above. The subsequent degree of reproductive inhibition may depend on the importance and complexity of courtship behavior for different species. Whether other pollutants may contribute to these reproductive failures cannot be answered without further study, but DDE may be the most important factor.

A possible mechanism for DDE effects on reproductive behavior has been suggested by Peakall (1970). He found that Ringed Turtle Doves treated daily with DDT had significantly lower estradiol levels in the blood associated with increased hepatic enzyme activities. Since DDE is also a microsomal enzyme inducer (Conney et al. 1967), it could be influencing reproductive behavior in the same manner as DDT. However, no study to date has demon-

strated a sequential occurrence of elevated hepatic microsomal enzyme activity, reduced circulating levels of sex hormones, and altered reproductive behavior in the same birds.

The results of this study demonstrated that environmental levels of dietary DDE can lower the intensity of courtship behavior in male Ringed Turtle Doves. Therefore, we feel that DDE might indeed be a significant factor contributing to reproductive failure in wild birds.

SUMMARY

The effects of p,p'-DDE on the intensity of male Ringed Turtle Doves' courtship behavior were determined for dietary levels of 10 ppm and 50 ppm (dry weight). Pairs of doves were placed in cages for 12.5 min on 5 consecutive days for behavioral observation before dietary treatment and for periods 31–35 and 59–63 days after initiation of the treated diet. Total amount of time spent displaying courtship behavior and bow-coo frequency were analyzed through video tape recording.

The 50-ppm diet caused a reduction in total courtship activity time and in bow-coo frequency for both posttreatment observation periods. The 10-ppm diet did not affect bow-coo frequency and total activity time at 31–35 days but did cause a significant reduction in courtship behavior during the 59–63-day observation period. The DDE residues found in the male Ringed Turtle Doves showing reduced courtship behavior were lower than residues found in many species of birds that have shown reproductive failures in the wild.

ACKNOWLEDGMENTS

This work was conducted at the Denver Wildlife Research Center prior to a program consolidation of 28 March 1976. We thank co-workers at the Center, especially Kristine M. Stahl, for DDE residue analyses.

LITERATURE CITED

CADE, T. J., C. M. WHITE, AND J. R. HAUGH. 1968. Peregrines and pesticides in Alaska. Condor 70:170–178.

CONNEY, A. H., R. M. WELCH, R. KUNTZMAN, AND J. J. BURNS. 1967. Effects of pesticides on drug and steroid metabolism. Clin. Pharmacol. Ther. 8:2–10.

ENDERSON, J. H., AND D. D. BERGER. 1968. Chlorinated hydrocarbon residues in Peregrines and their prey species from northern Canada. Condor 70:149–153.

——— AND ———. 1970. Pesticides: eggshell thinning and lowered production of young in Prairie Falcons. BioScience 20:355–356.

GRESS, F. 1970. Reproductive status of the California Brown Pelican in 1970, with notes on breeding biology and natural history. Wildl. Manage. Branch Admin. Rep. No. 70-6. Calif. Dep. of Fish and Game.

HAEGELE, M. A. AND R. H. HUDSON. 1973. DDE effects on reproduction of Ring Doves. Environ. Pollut. 4:53–57.

KEITH, J. O., L. A. WOODS, AND E. G. HUNT. 1970. Reproductive failure in Brown Pelicans on the Pacific coast. Trans. 35th N. Am. Wildl. Nat. Resour. Conf. 35: 56–64.

KOEMAN, J. H., C. F. VAN BEUSEKOM, AND J. J. M. DE GOEIJ. 1972. Eggshell and population changes in the Sparrow Hawk (*Accipiter nisus*). TNO-News 27:542–550.

LEHRMAN, D. S. 1965. Interaction between internal and external environments in the regulation of the reproductive cycle of the Ring Dove. Pp. 355–380 *in* Sex and Behavior (F. A. Beach, ed.), Wiley, N.Y.

———, P. N. BRODY, AND R. P. WORTIS. 1961. The presence of the mate and of nesting material as stimuli for the development for incubation behavior and for gonadotrophin secretion in the Ring Dove (*Streptopelia risoria*). Endocrinology 68:507–516.

LINCER, J. L. 1972. The effects of organochlorines on the American Kestrel (*Falco sparverius*). Ph.D. thesis, Cornell Univ., Ithaca, N.Y.

———, T. J. CADE, AND J. M. DEVINE. 1970. Organochlorine residues in Alaskan Peregrine Falcons (*Falco peregrinus* Tunstall), Rough-legged Hawks (*Buteo lagopus* Pontoppidon) and their prey. Can. Field-Nat. 84:255–263.

MILLER, W. J., AND L. S. MILLER. 1958. Synopsis of behavior traits of the Ring Neck Dove. Anim. Behav. 6:3–8.

PEAKALL, D. B. 1970. p,p'-DDT: effect on calcium metabolism and concentration of estradiol in the blood. Science 168:592–594.

PETERSON, J. E., K. M. STAHL, AND D. L. MEEKER. 1976. Simplified extraction and cleanup for determining organochlorine pesticides in small biological samples. Bull. Environ. Contam. Toxicol. 15:135–139.

SNYDER, N. F. R., H. A. SNYDER, J. L. LINCER, AND R. T. REYNOLDS. 1973. Organochlorines, heavy metals, and the biology of North American accipiters. BioScience 23:300–305.

SWITZER, B., V. LEWIN, AND F. H. WOLFE. 1971. Shell thickness, DDE levels in eggs, and reproductive success in Common Terns (*Sterna hirundo*), in Alberta. Can. J. Zool. 49:69–73.

WINER, B. J. 1971. Statistical principles in experimental design. McGraw-Hill Book Co., N.Y.

U.S. FISH AND WILDLIFE SERVICE, PATUXENT WILDLIFE RESEARCH CENTER, LAUREL, MD 20811. (PRESENT ADDRESS RHH: DEPT. OF ZOOLOGY, UNIV. OF WASHINGTON, SEATTLE 98195). ACCEPTED 1 AUG. 1976.