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Abstract

Ring Doves (Streptopelia risoria) received dietary concentrations of a toxic chemical mix containing 2 ppm p,p'-DDE, 8 ppm PCB, 0.3 ppm Mirex and 0.1 ppm Photomirex or 5 ppm p,p'-DDE, 28 ppm PCB, 0.9 ppm Mirex, and 0.3 ppm Photomirex to investigate reproductive behavior and endocrine effects of exposure to these chemicals. Courtship, incubation and care-of-young behavior indices were all reduced in treated groups. Total estrogen, and androgen levels were lower than controls during the courtship phase in treated birds. T_4 levels in treated doves showed a trend to higher levels than controls. The observed poor reproductive performance in treated birds was attributed to toxicant induced endocrine dysfunction and decreased parental attentiveness.

Résumé

Les tourterelles (Streptopelia risoria) ont reçu de la nourriture contaminée avec 2 ppm p,p'-DDE, 8 ppm PCB, 0.3 ppm Mirex, et 0.1 ppm Photomirex ou 5 ppm p,p'-DDE, 28 ppm PCB, 0.9 ppm Mirex et 0.3 ppm Photomirex pour étudier les effets de ces substances sur le comportement reproducteur et les concentrations d'hormones sexuelles. Une réduction des comportements reproducteurs a été notée chez les oiseaux contaminés.

Les concentrations d'estrogène et d'androgène dans le sang des oiseaux traités étaient plus faibles que cells mesurées chez les oiseaux du groupe témoin. Les niveaux de T_4 semblent plus élevés chez les tourterelles traitées que chez celles non traitées.

Nous attribuons les piètres performances reproductrices du groupe expérimental à une diminution des hormones reproductrices causée par l'ingestion de produits toxiques ainsi qu'à une diminution de l'attention parentale. La performance reproductrice des oiseaux contaminés est probablement le résultat d'une déficience endocrine et de comportement parental, déficience causée par les substances toxiques.

Acknowledgements

I would like to thank Dr. D. Peakall for his support and guidance throughout the different phases of my research and for critically reading this thesis. I also extend thanks to my other committee members, Dr. R. Engelhardt and Dr. B. Philogène for their consultations during my course and research program and for their criticism of this manuscript. I would also like to thank G. Fox, Wildlife Toxicology Division of the Canadian Wildlife Service, for his interest and advice throughout this study and especially in the initial planning, literative review, bleeding techniques development and statistical analysis phases of the work.

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Finally, I wish to acknowledge the financial, moral and physical support of my husband Dr. G. McArthur, our sons, Johnathon and Gordon, and my parents, Roy and Annette Brand.

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Introduction

Polychlorinated biphenyls (PCB's) and organochlorines have been implicated in recent reproductive failures of certain species of mammals, fish, and most notably, several species of birds terminating food chains (Stendell, 1975). A most striking example of this was the reproductive failures experienced by colonies of common terns (Sterna hiruudo), herring gulls (Larus argentatus) and double crested cormorants (Phalacrocorax auritus) nesting around Lake Ontario during the early 1970's (Gilbertson, 1975). This author reported that these reproductive failures resulted from early and late embryo deaths, deaths during pipping, high chick mortality, and unusual incubation behavior of the adult birds.

Fox et al. (1978) reported similar findings for a colony of herring gulls in Lake Ontario. Using telemetered eggs they were able to document decreased nest attentiveness. They also observed decreased nest defence. They concluded that parental behavioral abnormalities were the cause of the poor reproductive success of this colony compared to herring gull colonies in the other Great Lakes and the Maritimes.

PCB's, p,p'-DDE(1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene) Mirex (1, 2, 3, 4, 5, 5, 6, 7, 8, 9, 10, 10-dodecachloro-pentacyclo $[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]$ decane) and Photomirex (1, 2, 3, 4, 5, 5, 6, 7, 9, 10, 10-undecachloropentacyclo $[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]$ decane), a breakdown product of Mirex, were present at high levels in the tissues of the adult herring gulls in Lake Ontario in the early 70's (Gilman et al., 1977). Fox et al. (1978) speculated that

the observed behavioral abnormalities probably resulted from pollutant-induced endocrine dysfunction.

The objectives of this study were to determined (1) if a toxic chemical 'soup' consisting of PCB's, p,p'-DDE, Mirex and Photomirex would produce behavioral abnormalities and reduced reproductive success in breeding ring doves (Streptopelia risoria) in a controlled laboratory environment; and (2) if behavioral abnormalities were a result of changes in reproductive hormone levels. Ring Doves were chosen as subjects for study as their consistantly good breeding performance and productivity in captivity and under experimental conditions make them excellent subjects on which to test the effects of specific treatments such as exposure to toxic chemicals (Lehrman, 1965; Lehrman and Wortis, 1967).

The objectives were met by (1) feeding ring doves diets containing an organochlorine coating (2) observing and scoring their breeding behavior displays (3) assaying levels of sex steroids in their plasma during the breeding cycle.

We have demonstrated a definite cause and effect relationship between the toxic chemical 'soup' and abnormal reproductive behavior in ring doves. We also present evidence that these abnormalities were the result of pollutant-induced endocrine dysfunction.

Polychlorinated Biphenyls (PCB's)

PCB's are chemically inert, non-hydrolized by water and resistant to alkali, acids and other corrosive chemicals. They are essentially insoluble in water but soluble in hydrocarbon solvents

(Penning, 1930). PCB's are resistant to environmental breakdown and have longterm stability in the ecosystem when compared to DDT and its metabolites (Greene et al, in Deichmann, 1973).

The uses of PCB's are based on this longterm stability and includes heat transfer fluids, plasticizers for coatings, flame proofing and insecticide extender. PCB's were discovered to be a widespread contaminant by the late 1960's (Riseborough et al., 1968) and by 1972 were found to be the single largest pollutant residue in many world ecosystems (Peakall, 1972).

DDE (1,1-dichloro-2,2 bis (p-chlorophenyl ethelene)

DDE is the most widely distributed in nature of all the DDT isomers and metabolites. DDE is formed in living organisms by the dechlorination of the widely used pesticide DDT. This is an unusual biotransformation in that it does not increase water solubility and the metabolite is more persistent in organisms than the original pesticide (Deichmann, 1973). DDE is chemically stable, persistent, resistant to breakdown by microorganisms, heat, enzymes and ultra-violet light. It undergoes bioaccumulation through the food chains in the environment (Ware, 1975).

Mirex (1, 2, 3, 4, 5, 5, 6, 7, 8, 9, 10, 10-dodecachloropentacyclo pentacyclo $[5.3.0.0.^{2,6}.0^{3,9}.0^{4,8}]$ decane)

Mirex is a stable, white, crystaline and non-volatile solid. It is insoluble in water and moderately soluble in organic solvents. Mirex is biologically active, persistent and dispersed in North America. It enters the environment via losses from the plants of manufacture and by aerial spraying in its use as an

insecticide against fire ants in the south-eastern United States. Mirex has also been used in Canada as a fire retardant (Fisheries and Environment, 1977).

Photomirex (1, 2, 3, 4, 5, 5, 6, 7, 9, 10, 10-undecachloropentacylclo [5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane)

Photomirex is formed by the photodegradation of Mirex. It has only recently been reported as an environmental contaminant and its probable source is the environmental pollutant Mirex (Hallet et al, 1976). Little is known about the toxicity and chronic effects of this compound but it has been shown to accumulate to high levels in the adipose tissues and ovaries of female rats (Villeneuve et al., 1978).

2. Known Effects of PCB's and p,p'-DDE, Mirex and Photomirex on Reproduction in Ring Doves

PCB's and p,p'-DDE have both been shown to affect reproductive behavior and decrease reproductive success in ring doves. Delays in onset of breeding, ovulation, egg laying, decreased parental attentiveness to eggs and squab, and decreased reproductive success have all been reported in ring doves contaminated with PCB's (Peakall, 1970(a); Peakall and Peakall, 1973; (Farve, 1978). Similar reproductive behavior and success effects have also been reported in ring doves contaminated with p,p'-DDE (Keith, 1978) (Richie and Peterle, 1979). In addition, courtship behavior displays were reduced in ring doves contaminated with p,p'-DDE (Haigele and Hudson, 1977).

Richie and Peterle (1978) reported that ring doves fed

40 ppm p,p'-DDE failed to show a strong Luteinizing hormone (L.H.) 'surge' on day 4 post-pairing. Instead a more gradual increase in circulating L.H. to day 6 was observed with an apparent plateau to day 11. This plateau in L.H. level was significantly lower than the peak L.H. level in the control birds in their study.

Farve (1978) concluded that the behavioral abnormalities observed in his PCB-contaminated ring doves were caused by lower levels of circulating reproductive hormones but he did not assay hormone levels.

Two mechanisms have been proposed to account for the delays in initiating nests and ovulation and the decreased parental attentiveness observed in birds contaminated with PCB's and other organochlorines. Jefferies (1967), working with p,p'-DDE contaminated Bengalese Finches (Lonchura striata) proposed a possible hypothalamic-hypophyseal influence of DDE inhibition of gonadotropin secretions. On the basis of subsequent work showing hypo- and hyper-thyroidism induced by varying DDT dosage levels, he also suggested a possible thyroid effect (Jefferies 1969, 1975; Jefferies anf French, 1971). Peakall (1970(b)) showed that both DDE and PCB's cause induction of hepatic enzymes and postulated that these caused the reduction in levels of circulating steroids. He did show lower estradiol levels in DDE contaminated ring doves (Peakall, 1970(a)). He also concluded that decreased incubation of eggs by PCB contaminated female ring doves was the result of PCB-induced endocrine dysfunction- specifically liver hydroxylating enzyme decomposition of circulating steroid hormones (Peakall and Peakall, 1973).

Mirex and Photomirex have not been used to contaminate ring doves to our knowledge and little is known about their effects on reproduction in other species of birds. Mirex has been shown to have a detrimental effect on hatchability and chick survival of domestic chickens (Naber and Ware, 1965) and Mallard ducks (Aras platyryrchos) (Hyde et al., 1973) at doses above 100 ppm. Kendall et al., (1978) report that doses of Mirex of 1, 20 and 40 ppm fed to bobwhite quail (Colinus virinianus) had no deleterious effect on reproductive success of wild quail.

Materials and Methods

Reproductive behavior and maintenance requirements of Ring Doves under experimental conditions have been thoroughly described (Lehrman, 1964; Lehrman 1965; Lehrman and Wortis, 1967). The use of prescribed maintenance techniques can ensure consistantly good performance and productivity against which to test the effect of a specific treatment, such as exposure to toxic chemicals.

Some of the ring doves used in the experiment were progeny of birds originally brought from the Cornell University colony by Dr. D. Peakall. Other ring doves were purchased in July, 1978 from Bill Jansen, Route 3, Woodstock, Ont. and Don Adams, 555 Dupplin Rd., Victoria, B.C.

All birds used in the study were paired and allowed to raise chicks for 21 days in the breeding colony before being used for the experiment.

Reproductive Biology of Ring Doves

Ring doves or ringed turtle doves, as they are also called, are small relatives of the domestic pigeon (Columba livia). This dove has a light grey back, creamy-coloured underparts and a prominant black crescent on the back of its neck. Male and female ring doves appear similar in plumage but can be sexed either internally or by their behavior when mature.

When a male and female ring dove, of previous breeding experience, are placed in a cage containing a nest bowl and nesting material, they begin the reproductive cycle that follows a particular sequence of behavior patterns and a regular time schedule. The first

day post-pairing, the male begins courting-bowcooing and chasing behaviors predominate. After several hours he announces the selection of the nest site with a brief series of nest coos and longer bouts of wing tip flipping. Both sexes are involved in nest construction. During this first week, copulation takes place. Seven to eleven days after the beginning of courtship, the female lays the first egg-usually about 5 p.m. A second egg is usually laid 42 hours later. After 14 days of incubation involving both mates, the young hatch. When the squab are about 10 days old they leave the nest and are self-feeding by two weeks of age. By this time the adults have begun courting again (Lehrman, 1964).

Mei-Fang Cheng (1978) published a summary of present knowledge of the specific reproductive hormones involved in the ring dove reproductive cycle and the particular behavior displays that they are known to affect. The description that follows is summarized from M.F. Cheng's work.

Androgen levels in the male ring dove increase upon exposure to a female. These hormones are considered to be the male courtship inducer and increasing levels lead to bow-coo, chasing and nest coo displays.

Estrogen levels increase in female ring doves exposed to males. Increasing levels of estrogens facilitate in bowl sitting (without eggs) behavior in the female ring dove and lead to displays of wing tip flipping and nest coo behavior in both male and female ring doves.

Luteinizing hormone increases in the female ring dove

with courtship and induces ovulation. Cheng and Follett (1976) report that the LH levels of female ring doves surge to a peak level immediately before ovulation then drop off rapidly after egg-laying and return to baseline pre-courtship levels after the squab are hatched.

Ring dove androgens and estrogens prime and increase the numbers of progestin receptors available. Then the combined actions of these hormones with progesterone lead to the behaviors of nest building and incubation. Once incubation behavior has been initiated, Prolactin is the hormone found to be responsible for maintaining incubation behavior and is responsible for crop enlargement in preparation for feeding squab (Cheng, 1978).

Housing and Maintenance

During the isolation phases of the preliminary trial and the toxic chemical experiment, birds were housed individually in 17" X 9 3/4" X 7" stainless steel 'rat' cages. Isolation was visual but not auditory. The birds had continuous access to food, water and grit. Individual cage feeders were filled twice weekly. Water dishes were washed and disinfected weekly. Papers in trays 2½" beneath the cage floors were used to collect droppings. Papers were changed weekly and the trays holding them were disinfected monthly. All maintenance procedures followed were in keeping with the directives published in 1968 by the Canadian Council of Animal Care.

During the breeding phases of the preliminary trial and the experiment, birds were housed in 3' X 3.5' X 15" 'chick rearing' cages, manufactured by Brower Better-Built and supplied by Dean

Williamson Ltd. London, Ont. These cages were divided by a 3' x 10" plexiglass partition and supplied with external water troughs, feed and grit dishes. Each cage also contained a disposable nest bowl and shredded newsprint for nesting material.

During both isolation and breeding phases of both the preliminary trial and the toxic chemical experiment, the birds were exposed to natural and artificial light on a 14 hour light, 10 hour darkness cycle. Temperatures in the experimental facility were between 18°C and 30°C during the study period but were usually about 22°C. The door between the experimental facility and the NWRC breeding colony was usually open so that the experimental birds could hear the breeding colony at all times.

Preliminary Trial

The purpose of the preliminary trial was to confirm that all ring doves to be used in the experiment would mate and raise chicks successfully under the housing, maintenance, observation and handling conditions to be used in the toxic chemical experiment.

60 experienced ring doves (30 male and 30 female) from the NWRC breeding colony were put into individual isolation cages for 90 days. During this phase and the subsequent breeding phase the birds were fed a diet consisting of: 42% milo; 32% millet; 16% wheat; 4% rice; 6% canary seed and 5% rape seed added after the others were mixed. A commercial multivitamin preparation was added to the doves' water and commercial pigeon grit was available. Pairing for both the preliminary trial and the toxic chemical experiment was done randomly; however, no bird was assigned a mate with which it had

been paired for reproductive experiencing or, in the case of the toxic chemical experiment, with which it had been paired for either reproductive experience or the preliminary trial.

At the end of the isolation phase, the birds were paired, marked with magic marker to identify sex and put into the breeding cages (Figure 1). After the doves were paired, records were made of nesting progress. The number of eggs and young per nest were recorded each week day until all young were fledged. Eggs found out of the nest were put into the nest bowl.

Reproductive behavior was recorded for each pair by the observer, stationed 7' away from the cages, recording observations at one minute intervals for 30 consecutive minutes each week day. Displays recorded were as described by Miller and Miller (1958) and Keith (1978). Behavior scoring was as in Keith (1978) except where noted in the Method of Scoring Reproductive Behavior detailed later in this section. The number of minutes per 30 minute observation period were recorded for individual courtship displays and for periods when birds sat in bowl without eggs, incubated, brooded or fed squab. Behavior scores were calculated daily for each pair based on the observation recorded. Courtship displays timed were: bouts of bowcoo, heteropreening, billing, wing flipping, chasing, and nest material manipulation (acts of picking up nest material that didn't always culminate in nest building).

Blood samples were taken during pre-breeding, on the fourth day post-pairing, mid-courtship, mid-incubation and one week post hatching. The blood sampling technique is described in detail in the

Fig. 1. Time Sequence of Preliminary Trial and Toxic Chemical Experiment.

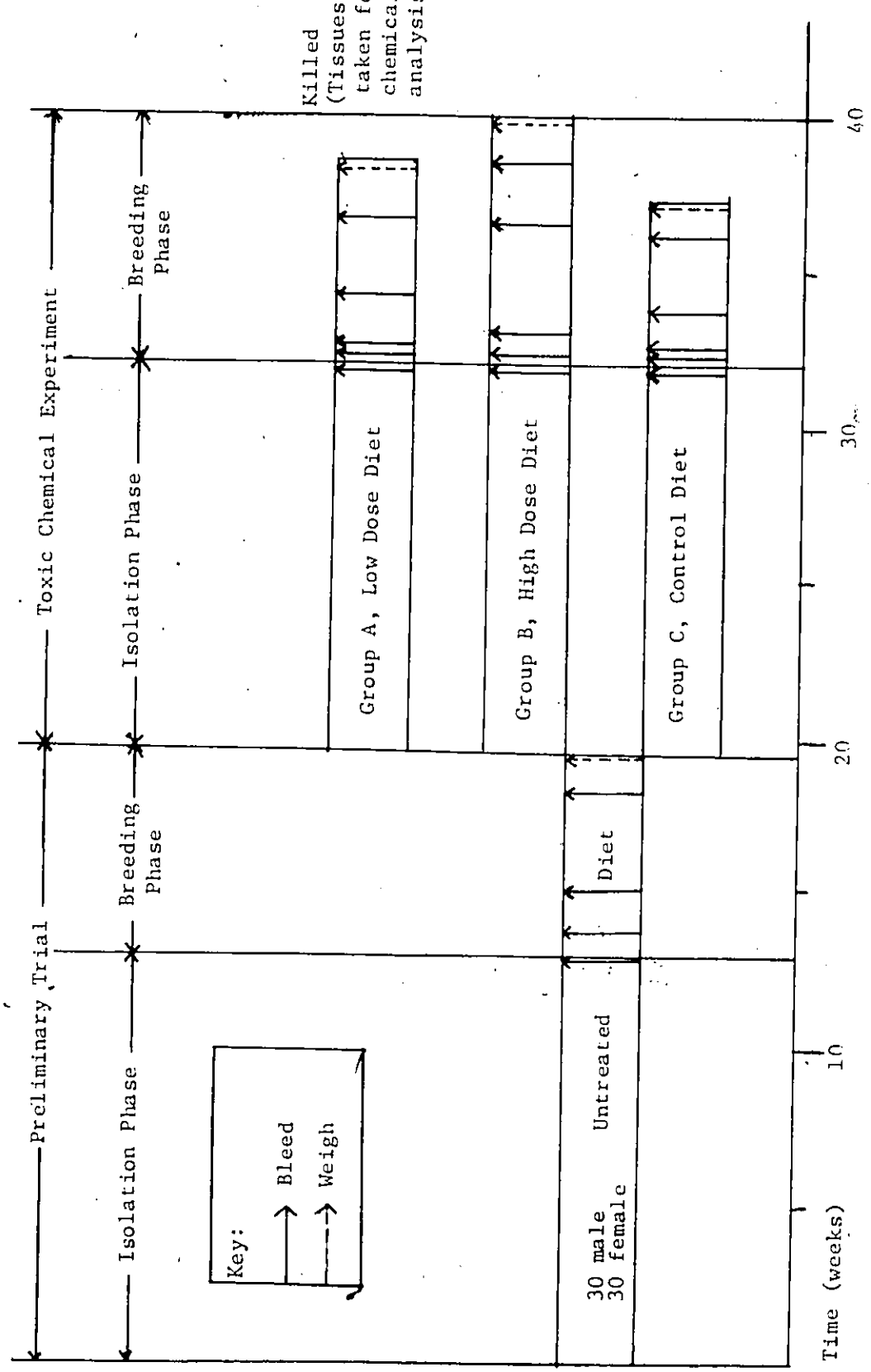

Preliminary Trial
Isolation Phase
Breeding Phase
Toxic Chemical Experiment
Isolation Phase
Breeding Phase
Killed (Tissues taken for chemical analysis)
Group A, Low Dose Diet
Group B, High Dose Diet
Group C, Control Diet
Diet
Untreated
30 male
30 female
Key:
Bleed
Weigh
10
20
30
40
Time (weeks)

Fig. 1

parts headed Method of Blood Sampling and Hormone Assay Techniques.

Toxic Chemical Experiment

The same housing, maintenance, bleeding, scoring and nesting records methods were followed as in the preliminary trial with the following additional methods.

Immediately before being isolated the birds were assigned mates and divided into three groups containing 10 pairs each. These were the low dose group, (group A) the high dose group (group B) and the control group (group C). During the isolation and breeding phases the birds were fed the prepared seed diet containing the following additions: Group A's diet contained 8 ppm dry weight PCB, 1.67 ppm dry weight DDE, 0.297 ppm dry weight Mirex and 0.0954 ppm dry weight Photomirex. Group B's diet contained 28.03 ppm dry weight PCB, 4.61 ppm dry weight DDE, 0.897 ppm dry weight Mirex and 0.324 ppm dry weight Photomirex. Group C's diet contained 22.1 ml of Mazola oil to 1 kg of seed mix.

Toxic chemical doses were calculated in the following manner: 10 ppm dry weight PCB in the food resulted in 50 ppm dry weight in the eggs of ring doves (Peakall et al, 1972). Residue levels in herring gull eggs from Lake Ontario were: 138 ppm wet weight PCB, 17 ppm wet weight DDE, 4.4 ppm wet weight Mirex and 1.6 ppm wet weight (Norstrom et al, 1978). Ppm dry weight levels in the gull eggs were five times these levels (personal communication R. Norstrom).

The toxic chemicals were fed to doves in a pilot project in the amounts calculated for dry weight in the herring gull eggs

divided by the concentration factor of 5 (personal communication D. Peakall). This was based on the assumption that the DDE, Mirex and Photomirex concentrate to the same extent as PCB's. Because doves fed this diet in the pilot project failed to exhibit reproductive behavior, toxic chemicals for Groups A and B were calculated by using 15% and 30% of the levels originally calculated.

In the toxic chemical experiment the amount of food consumed by individual birds was recorded twice weekly throughout the isolation phase.

The adult birds were weighed at the following times in the toxic chemical experiment: before isolation phase, end of isolation phase, end of breeding phase. Weights of chicks fledged were recorded.

A performance index was calculated for each pair. This index was the total number of viable eggs or young that were in each nest during the first 39 days post-pairing. Experimental pairs differed in the time taken to initiate breeding, and some pairs never laid eggs. It was necessary, therefore, to specify a period of time over which comparisons could be made between treatment groups. The 39 day period provided time for a complete reproductive cycle under our experimental conditions as demonstrated in the preliminary trial and by the control doves in the toxic chemical experiment. Ring doves under experimental conditions but on 'clean' food completed their clutches by the eleventh day post-pairing, and incubated for 14 days. Their young were considered fledged at 14 days.

Daily nest records were kept for 54 days post-pairing, at

which time all chicks hatched during the experiment were fledged. At termination of the experiment all toxic chemical treated birds and six control birds were sacrificed. The liver, and mesenteric fat pad were removed and their weights recorded. The liver and a portion of breast muscle were frozen and stored for possible future study. One gram of the fat removed was used for toxic chemical analysis as described in a separate part of this section.

Preparation of Diets

Control Diet: 42 kilograms of seed mix was divided into four 10.5 kg batches. Each batch was mixed in a commercial bread mixer and stirred while 232 ml of 'Mazola' oil was poured slowly into it (Cartwright, 1976). The mixer was left running 15 minutes to ensure even coating. Two, 10 gram samples were taken from each batch and frozen for later analysis.

Experimental diets: For group A -0.1026 gms of DDE, 0.8588 gms of Arochlor 1254, 0.0272 gms of Mirex and 0.0097 gms of Photomirex were individually weighed, dissolved in small amounts of hexane and then dissolved in 930 ml. of Mazola Oil. The Mazola oil and contaminants were stirred for one and a half hours with an automatic stirrer until all hexane was evaporated off and the contaminants were well distributed.

Three 1 ml. samples of the oil mixture was cleaned up and analysed for toxic chemical levels using the methods and equipment described in a separate part of this section.

The group A seed was then mixed with the contaminated oil as described for the control diet. For group B .2097 gm of DDE,

1.714 gms of Arochlor 1254, 0.0548 gms of Mirex and 0.0205 gms of Photomirex were weighed, dissolved in hexane and Mazola oil and the diet was prepared as for group A.

All diets were stored covered in separate galvanized steel garbage cans in the experimental area.

Method of Scoring Reproductive Behavior

Courtship behavior was limited to a score of 20 points per pair per observation period and is defined as behavior that takes place out of the nest bowl and before either bird is in the nest bowl for more than one-half minute during the half hour observation period. The score was calculated by totalling the time spent in courtship by an individual and taking this as a percent of the 30 minute observation period which was then given a score out of 10. For example, a pair where the male courted the female for 10 minutes and the female wing flipped for 10 minutes during the 30 minute observation period would be scored 10/30 x 100/10 plus 10/30 x 100/10 for a total score of 6.6 for the pair for that day.

In-bowl behavior is defined as behavior in the nest bowl before any eggs are laid. At this stage each pair received a base score of ten points for having progressed through mid-courtship and additional activities could increase their score to a maximum of 30 points. For example, a pair in which the male spent 20 minutes courting the female while the female spent 1-30 minutes sitting in the bowl without eggs was scored 20/30 x 100/10 plus 10 for a score of 16.6.

Incubation and brooding behavior were defined as that

behavior in or out of the nest bowl while birds were incubating or brooding young. A base score of 25 points was given to pairs that incubated or brooded young at least 29 minutes during the 30 minute observation period. Scores could be increased by other displays during the period: a pair in which the female incubated for 30 minutes during the observation period while the male added nest material to the nest for a total of ten minutes was scored 25 plus (10/30 x 100/10) for a total of 28.3. Summaries of behavior scores were compiled weekly, for the first 39 days after pairing and for three periods: courtship, incubation and care of the young. The three periods were defined by each pair by its progress through reproduction; the periods do not relate to calendar dates. Scores for the incubation and care-of-young periods were computed even if that period extended beyond 39 days post-pairing. This approach allowed comparison of behavior between treated groups over a fixed period (39 days); but it also permitted assessment of all behavior displayed, including that of pairs that delayed initiation of reproduction.

The amount of time spent per pair in individual behavior displays during the daily observation periods was compiled weekly and for the first 39 days post-pairing. The amount of time spent per pair brooding and feeding was totaled weekly, for the first 39 days post-pairing and for the three reproductive periods.

Method of Evaluating Reproductive Success

Besides the performance index, reproductive success was assessed by determining for each group:

1) the frequency of pairs laying

2) the number of eggs laid

3) the percent hatch

4) the number of young fledged per nesting attempt

Blood sampling

During the preliminary trial and the toxic chemical experiment, blood was collected for hormone analysis at the following times: bleed I, pre-breeding; bleed II, the fourth day post-pairing; bleed III, mid-courtship (in the preliminary trial a daily behavior score of 15-20 was shown to be an accurate indication of the pair's progress to mid-courtship); bleed IV, mid-incubation (this was the seventh or eighth day after the second egg was laid); bleed V, one week post-hatch of the complete clutch.

Approximately 300-500 microlitres of blood were collected each time from leg veins. Veins were punctured with a 23 gauge needle and blood was collected in a 500 microlitre *Microtainer brand capillary blood serum separator. Blood samples were kept on ice for a maximum of 30 minutes after collecting and then were spun in an Eppendorf centrifuge 5412 for 1.5 minutes at 12,000 g's. Plasma was removed and kept frozen, in storage for three to four months until hormone assays were done.

Extraction and Radio Immuno Assay of Hormones from Plasma

Plasma from bleeds I, II and III from female doves was assayed for total estrogen levels. Plasma from bleeds I, II and III

* registered trade mark: Becton; Dickinson and Company, Rutherford, New Jersey.

from male doves was assayed for total androgen levels. Plasma from bleed IV of all doves was assayed for T4 level.

All plasma samples except those from bleed V were analysed by Radio Immuno Assay Technique (Abraham, 1974) using a Beckman L.S. 230 Liquid Scintillation System counter and a Hewlett-Packard 9820 A Calculator for plotting the standard curves. The standard curve program was made by Jim Learning at the NWRC.

Seed, Tissue and Egg Cleanup and Toxic Chemical Analysis

Seed Cleanup and Analysis:

The eight 10 gm samples from each group's diet mix were combined and a 3 gram sub sample was taken for clean up and toxic chemical analysis.

The sub samples were ground in a mortar with a small amount of sodium sulphate then placed on a florisil column where the toxic chemicals, were extracted in hexane. Known concentrations of the four toxic chemicals used were injected into a Hewlett-Packard 5739 dual column gas chromatograph equipped with a SP2100 6 foot column (4 mm I.D.). One micron of each of the cleaned up samples was then injected into the same machine. To calculate the levels of toxic chemicals in the samples the height of the major peaks were divided by the height of the standards' same peaks then divided by the weight of the original sample.

Tissue Cleanup and Analysis

A 1 gram sample of mesenteric fat tissue from each dove was ground with 10 grams of sodium sulphate and 5 grams of flourisil.

Cleanup and extraction procedure was the same as described for the seed sample.

Egg Cleanup and Analysis

The entire contents of each failed clutch, minus egg shells, was homogenized using a Sorvall Omni-Mixer. A 5 gram sub sample was then mixed with 30 grams of sodium sulphate, air dried and ground. Cleanup and extraction procedure was the same as described for seed samples.

Calculations and Statistics

The X^2 test was used to test differences in reproductive parameters (Table II) since these are expressed as frequencies. The significance level accepted is 0.05. Two-tailed and unpaired students T-tests were applied to test group differences in mean adult bird weights, food and pesticide consumption, and hormone levels. Paired T-tests were applied to determine significant differences between sexes within groups. The significance level accepted is 0.05.

Two nonparametric tests were applied to the behavioral data since the scores were ordinal measures. These nonparametric tests determine whether it is likely that two independent samples came from the same population.

The Mann-Whitney U-test and the Kolmogorov-Smirnov both test for differences in central tendency. However, the Mann-Whitney is regarded as the most sensitive for differences in central tendency, whereas the Kolmogorov-Smirnov test is sensitive to any kind of difference in the distributions from which the two samples were drawn-differences in central tendency, in dispersion, or

skewedness (Siegel, 1956). The Kolmogorov-Smirnov Two Sample test was used to illustrate differences in central tendency and dispersion of scores within groups where the Mann-Whitney-U-test showed a statistically significant difference between groups whose median scores appeared similar. Critical values used for both non-parametric tests are those presented in Siegel (1956).

Results

Levels of Toxic Chemicals found in Diets, Adult Dove Tissues and Eggs

There were substantial differences in the levels of toxic chemicals found in the diets, birds and eggs of the three groups of ring doves involved in the toxic chemical experiment. The high dose diet contained approximately three times the amounts of chemicals as the low dose diet (Table 1). The control diet contained an insignificant but detectable level of DDE; DDE has been found in samples of Mazola Oil and are probably the source of the DDE found in our control diet (R. Norstrom, personal communication). The high dose-treated adults were found to have twice the levels of toxic chemicals in their fat tissues as the low-dose-treated doves (Table 2). The unhatched eggs from both treated groups were found to contain similar amounts of toxic chemicals but the samples available (one clutch from the low dose group and three eggs from two clutches in the high dose group) are too small for this result to be considered significant (Table 3).

Female ring doves in the low dose treated group had significantly lower toxic chemical levels in their fat tissues after the breeding phase than the low dose-treated male doves. However, there is no significant difference in the levels of toxic chemicals between the sexes in the high dose group (Table 2a). This indicates that the low dose-treated female doves lost a significant amount of their body burden of toxic chemicals through egg laying but for the high dose group, the eggs did not contain a significant fraction of the adults' toxic chemical load.

Table 1. Amounts of Toxic Chemicals Found in Diets (ppm dry wt.)

Level	DDE	PCB	Mirex	Photomirex	Ratio Mirex/Photomirex
Grp. A	1.67	8.02	0.297	0.0954	3.113
B	4.61	28.03	0.897	0.324	2.767
C	.07	<2	<.01	<.01	

Table 2a. Amount of Toxic Chemicals in Fat Tissue of Adult Doves (ppm liquid wt.)

Level	DDE	PCB	Mirex	Photomirex		Ratio Mirex/Photo Mirex
				Found	Calculated	
Grp. A	110.3	676	31.1	11.02	11.2	3.135
S.D.	23.7	135	8.3			
% R.E.	21.5%	20.0%	23.6%			
Grp. B	226.2	1589	63.7	19.08	21.3	2.990
S.D.	59.9	415	17.2			
% R.E.	26.5%	8.6%	27%			
Grp. C	3.06	<2	<.01	<.01		
S.D.	1.11					
% R.E.	36.2%					

Table 2b. Amount of Toxic Chemicals in Fat Tissues of Treated Male and Female Ring Doves in Toxic Chemical Experiment. (ppm liquid wt.)

Group	DDE		PCB		Mirex	
	♂	♀	♂	♀	♂	♀
A	119.6	101*	739	612*	40.1	30.1*
S.D.	±28.8	±13.2	±152	±81.5	±7.87	±5.36
% R.E.	24%	13.1%	20.6%	13.3%	19.6%	17.8%
B	220.6	231.9	1545	1632	58.6	68.8
S.D.	±54.5	±67.3	±366	±475	±13.1	±19.8
% R.E.	24.7%	29%	23.7%	29.1%	22.5$	28.8%

* indicates statistical significance at 0.05 level

Table 3. Amounts of Toxic Chemicals in Unhatched Eggs. (ppm wet wt.)

Level	DDE	PCB	Mirex	Photomirex		Ratio Mirex/Photomirex
				Found	Cal.	
Grp. A (2E 1 clutch)	15.55	84.87	3.49	.7523	.914	3.819
Grp. B (3E from 2 cluches)	9.45	74.6	2.83			
S.D.	2.45	21.4	1.22	.8955	.912	3.104
% R.E.	25.93%	28.69%	43.11%			

Food and Pesticide Consumption, Body Weights of Adult and Fledged Doves and Weights of Adult Livers and Fat Pads

Treatment caused a dose related, significant decrease in total food consumption by treated female doves i.e. the female doves reacted to the treated diet by consuming less food than control females (Table 4(b)). The high dose male doves also consumed significantly less food than control males (Table 4(a)).

The high dose treated female doves weighed significantly less than the high dose males at the end of the breeding period (Table 8) but were not significantly lighter than the female controls.

The high dose treated female doves had significantly heavier livers than the control females. A trend in this direction was noted in the high dose treated-male doves. This trend is not evident in the low dose treated birds (Table 8a).

Reproductive Success

A dose-related significant increase in courtship period was found in treated birds vs controls. High dose-treated doves took significantly longer to hatch their eggs than either the low dose birds or the controls (Table 10).

The main effects of treatment on reproductive success were 1) that the high dose pairs showed significantly fewer fledged per nesting attempt than controls; and 2) the treatment resulted in a dose-related significant decrease in the performance index of the contaminated doves vs controls (Table 11).

Table 4a. Food Consumption /Sex During Isolation Period of Experiment (Grams food consumed/bird/month)

Group	Month 1		Month 2		Month 3	
	♂	♀	♂	♀	♂	♀
A	392.5±59.2	384.4±47.9*	405.5±60.1	394.2±28.9*	353.9±63.8	336.1±35.5*
B	369.5±35.4*	394.2±47.2	371.8±50.2*	383.8±38.2*	303.5±47.8*	313.1±40.6*
C	399.1±38.3	411.9±31.4	407.2±49.4	415.2±43.9	357.1±34.0	373.2±53.4

* statistically significant at 0.05 level

Table 4b. Total food consumption/sex during isolation period. (grams)

Group	Total Food Consumption ♂	♀
A	1195.3	1157.3
B	1044.8	1091.1*
C	1122.4	1200.3

* statistically significant at 0.05 level

Table 8a. Weights of Adult Ring Doves During Isolation and Breeding Periods

Group	Mean wt./bird (gms)					
	Predose		End of Isolation		End of Breeding	
	♂	♀	♂	♀	♂	♀
A	165.2±15.8	160 ±25.1	149.4±15.2	156.2±15.6	149.4±15	142.0±17.7
B	158.9±10.4	154.4± 8.0	148.5±13.9	144.8± 9.1	145.8±12.6	132.2±113*
C	155.2±16.2	163.3± 9.3	157.5± 9.9	165.4± 9.7	145.4± 9.9	141.2±16.4

* statistically significant at 0.05 level

Table 8b. Weights of fat pads and liver tissues of toxic chemical-analysed birds

Group	wt. fat pad(gm)		wt. liver (gm)		ratio fat:body wt.	
	♂	♀	♂	♀	♂	♀
A	1.16±.48	1.6 ±.5 *	4.3±.77	4.4±.75	.78	1.13
B	1.45±.41	1.43±.45	4.3±.84	4.7±.79*	.99	1.08
C	1.4 ±.36	2.13±.97	3.7±.65	3.9±.45	.96	1.5

* statistically significant at 0.05 level

Table 10. Number days in courtship, incubation and care-of-young phases

Group	Courtship			Incubation		Care of Young	
	Courtship period (days p-p)	Median # days post pairing to 1st Egg	Median # days post pairing to complete clutch	Incubation period (days p-p)	Median # days to hatch clutch	c of y period days p-p	
A	1-12	11	12	12-25	13	26-42	all chicks hatched were considered fledged at 14 days.
B	1-18	17*	16*	18-33	16*	34-55	
C	1-9	10	11	9-24	13	25-39	

* statistically significant at 0.05 level

p-p = post pairing

Table 11. Reproductive Parameters

Group	Frequency of pairs laying	Frequency of Eggs laid/group	Frequency of Eggs hatched/laid	% hatch	Total Fledged per group	Young Fledged per nesting attempt	Performance Index	P.I. successful pairs
A	8/9	16/9	14/16	87.5%	13	13/9	54.29± 3.55	54.29± 3.55*
B	9/10	17/10	13/17	76.4%	7*	7/10*	28.9 ±22.86*	41.29±13.7*
C	10/10	20/10	17/20	85%	17	17/10	53.7 ±19.0	59.67± 2.96

* statistically significant at 0.05 level

Effects of Treatment on Reproductive Behavior

Total Behavior Scores During the Three Phases of the Reproductive Period

i) Courtship Phase

The treated groups showed a dose related increase in the number of days spent in the courtship phase but there are no statistically significant differences in the median daily behavior scores or the total behavior scores per group during each group's courtship phase (Table 12).

ii) Incubation Phase

The treated groups showed a statistically significant dose-related decrease in median daily total behavior scores during the incubation phase (Table 13). There were significantly greater numbers of pairs with low (score less than 25) daily total behavior scores in both the treated groups than the control group (Figure 2). That is to say, the treated groups exhibited significantly less incubation behavior during the incubation phases of their cycles than the controls and the decrease in incubation behavior was greatest in the high dose group.

iii) Care-of-Young Phase

The high dose-treated group had significantly lower median total daily behavior scores during the care-of-young period than controls. The high dose group also had a significantly lower total behavior score for its care-of-young period than control doves for their same period i.e. the high dose birds showed less daily care-of-

Table 12. Total Behavior Scores Courtship Period

Group	Courtship Period (days p-p)	Median # days courtship	Daily Behavior Score/Pair Median	Range	Total
A	1-12	11*	14.3	0-34.6	844.7
B	1-20	17*	15.3	0-33	1365.1
C	1-11	10	15.1	2-28	748.6

* statistically significant at 0.05 level

Table 13. Total Behavior Scores Incubation Period

Group	Incubation Period (days p-p)	Median #days Incubation	Daily Behavior Score/Pair		
			Median	Range	Total (Incubation Period)
A	12-25	13	25.3*	5.3-32	1945.2
B	18-33	16*	25*	0-34	2335.3
C	9-24	13	25.3	25-33	2307.5

* statistically significant at 0.05 level between groups (not medians).

Fig. 2. Frequency Distribution Plot of Accumulated % of Pairs In a Particular Score Interval During Incubation.

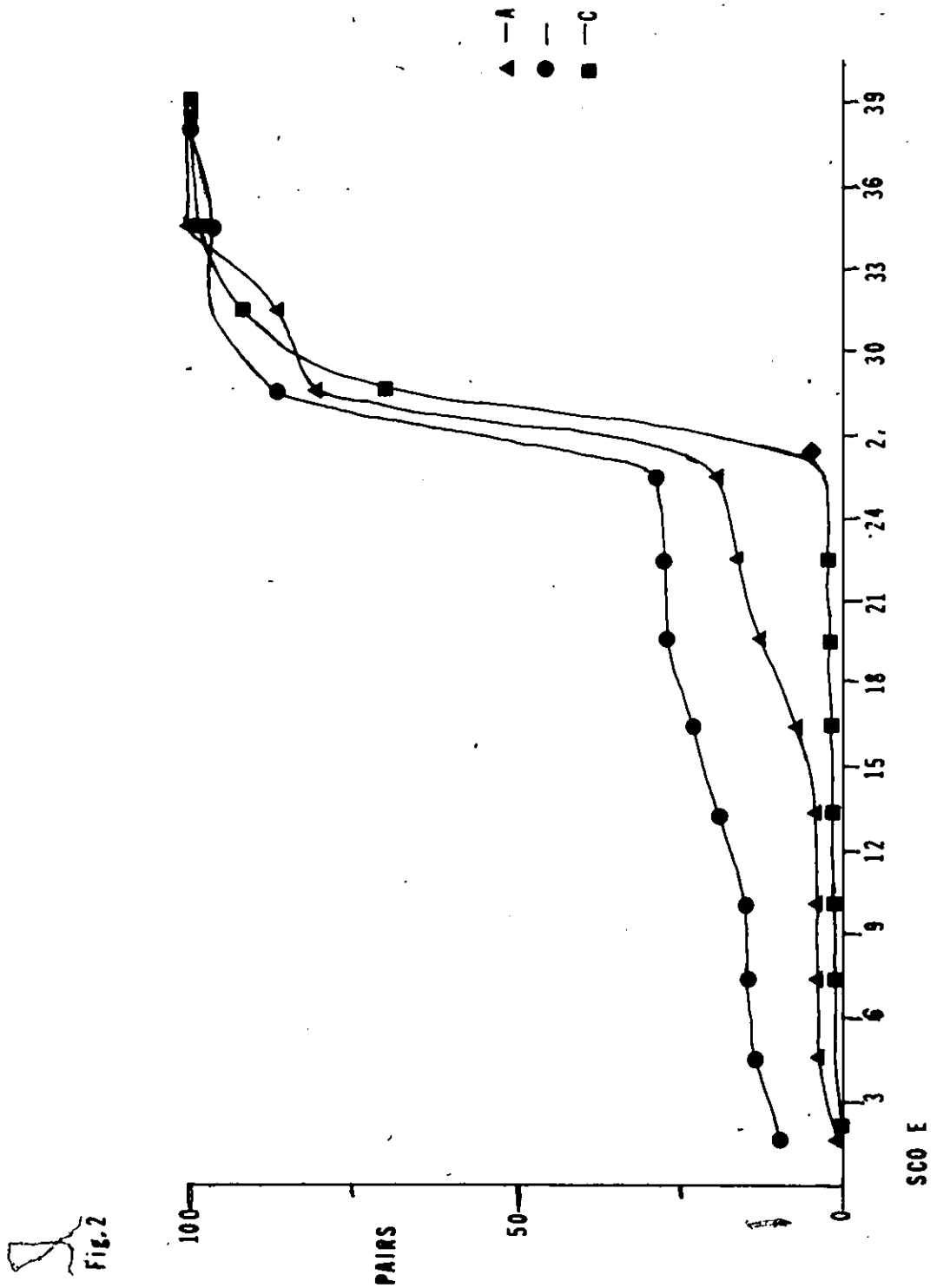


Fig. 2

young behavior during that phase of their cycle and less overall care-of-young behavior than controls (Table 14).

The high dose-treated doves displayed significantly less reproductive behavior, as shown by significantly lower daily total behavior scores during the first 39 days post-pairing, than control doves (Table 15).

Courtship Behavior

Courtship behavior has been previously described in the methods section as including: male wing-flipping displays (♂ W.F.), female wing-flipping displays (♀ W.F.), nest material manipulation by either sex (N.M.) and sitting in the nest bowl before eggs are laid (I.B.).

Amounts of ♀ W.F., N.M. (both sexes), I.B. and total daily behavior score in the treated groups were all significantly less than in the control group during the first week post-pairing (Tables 16, 18, 19, 20; Figures 3-7).

Results indicate that there is a significant dose-related delay in onset of nest building behavior in the treated groups. Nest building was delayed by one week from controls in the low dose-treated group and was two weeks later than controls in the high dose-treated group (Figure 6).

The low dose-treated male doves displayed significantly more W.F. behavior during the first week post-pairing than the control males (Table 17).

Table 14. Total Behavior Scores Care-of-Young Period

Group	C of Y Period (days p-p)	Median # days C of Y	Median Daily Behavior Scores /pair	Range Daily Behavior Scores /pair	Total Behavior Scores /group
A	26-42	14	15	1.3-27.6	1823.3
B	34-55	14	11.3*	0-31.3	1520.3*
C	25-39	14	25	10-29	1967

* statistically significant at 0.05 level

Table 15. Total Behavior Scores First 39 Days

Group	Daily Behavior Scores Median	Range	Total
A	20.6	0-34.6	4482.7
B	18.7*	0-34	4458.3
C	22.9	2-33	5135.3

* statistically significant at 0.05 level

Table 16. Weekly summary of daily behavior scores/pair 1st 39 days post pairing

	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
Group	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total
A	12.3	0-18.6	452.6*	25.2	5.3-34.6	811.2	25.3	5.6-32.6	1098.4	25	3.3-31.6	1072.1	25	1.3-27.6	825.6	20.6	0-34.6	4482.7
B	4.7*	0-19.3	390.9*	15.3*	0-30	583.4*	25*	0.6-33	978.6	25	0-34	1069.6	25*	0.3-31.3	1070.1	18.7*	0-34	4458.3
C	14.3	2-26.3	635.8	26.8	3-33	1049.3	25	4.6-31	1254.0	25	25-29.3	1152.1	25	10-26.6	853.8	22.9	2-33	5135.3

* Statistically Significant at 0.05 level

Table 17. Weekly Summary Number of Minutes Male W.F./Daily Observation Period

	Week 1			Week 2			Week 3			Week 4			Week 5			39-day
Group	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Total
A	6	0-19	313*	0	0-24	101*	0	0-23	83*	0	0-12	51	0	0-12	57*	658*
B	3	0-20	234	.5*	0-24	177*	0.5*	0-17	150*	0	0-15	51	0	0-2	7*	644*
C	3	0-14	232	0	0-4	4	0	0-2	2	0	0-0	0	0	0-1	1	245.5

* statistically significant at 0.05 level

Table 18. Weekly Summary of Number of Minutes Female W.F./Daily Observation Period

	Week 1			Week 2			Week 3			Week 4			Week 5			39-Day
Group	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Total
A	1	0-17	139*	0	0-20	45*	0	0-1	2*	0	0-1	2*	0	0-3	5	199
B	0*	0-16	123*	1*	0-30	129*	0*	0-10	70*	0	0-21	90*	0	0-1	2	415
C	2.5	0-18	183	0	0-15	16	0	0-12	14	0	0-1	1	0	0	0	214

* statistically significant at 0.05 level

Table 19. Weekly Summary of Number of Minutes N.M./Daily Observation Period

	Week 1 p.p			Week 2 p.p			Week 3 p.p			Week 4 p.p			Week 5 p.p			39 Day
Group	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Total
A	1*	0-22	160*	4	0-29	295	0	0-26	167*	0	0-20	45	0	0-7	8*	675
B	0*	0-23	143*	0*	0-26	214*	1	0-24	179*	0*	0-27	229*	0*	0-17	57*	823
C	4.5	0-25	406	6.5	0-28	329	0	0-22	114	0	0-13	37	0	0-12	17	903

* statistically significant at 0.05 level

Table 20. Weekly Summary of Number of Minutes IB/Observation Period

	Week 1 p.p			Week 2 p.p			Week 3 p.p			Week 4 p.p			Week 5 p.p			39-Day
Group	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Median	Range	Total	Total
A	9	0-29	532*	0	0-30	208*	0	0-30	89	0*	0-14	35*	0	0-15	41	918
B	2*	0-30	398*	11.5*	0-30	560*	0*	0-30	304	0	0-30	148*	0	0-1	1	1414
C	12.5	0-30	750	0	0-30	90	0	0-8	0	0	0-0	8	0	0-0	0	848

* statistically significant at 0.05 level

Fig. 3. Group Weekly Behavior Score Expressed as % 39-Day Total Behavior Score.

Fig.3

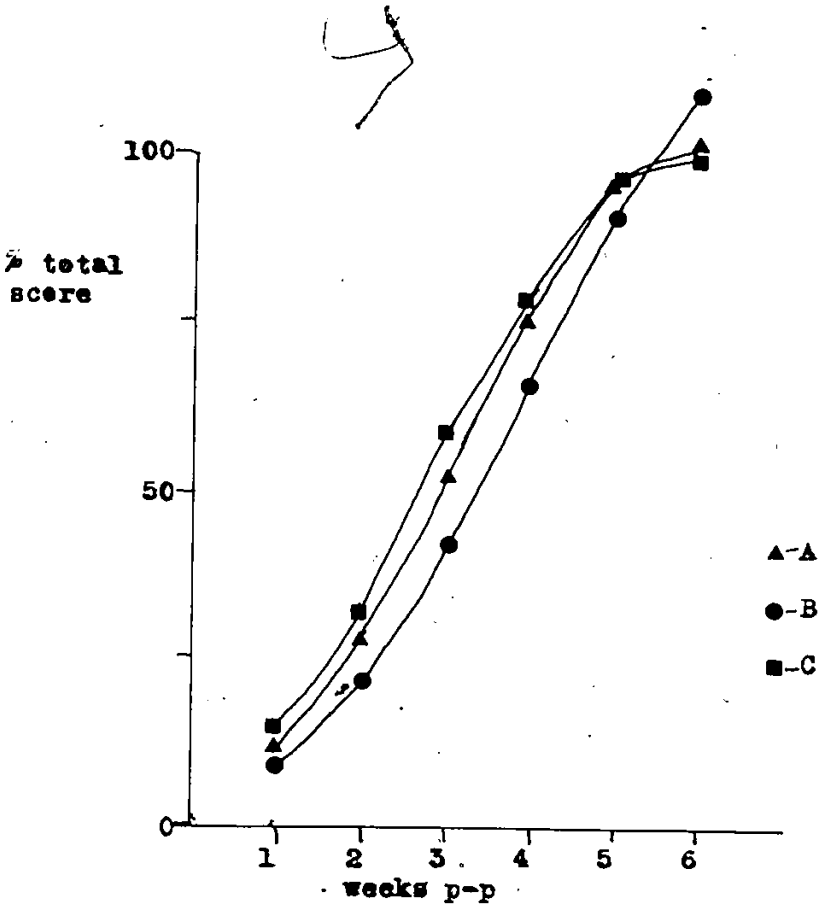


Fig. 4. Accumulated Weekly Total Male W.F. Expressed as % 39-Day Total Male W.F.

Fig. 4

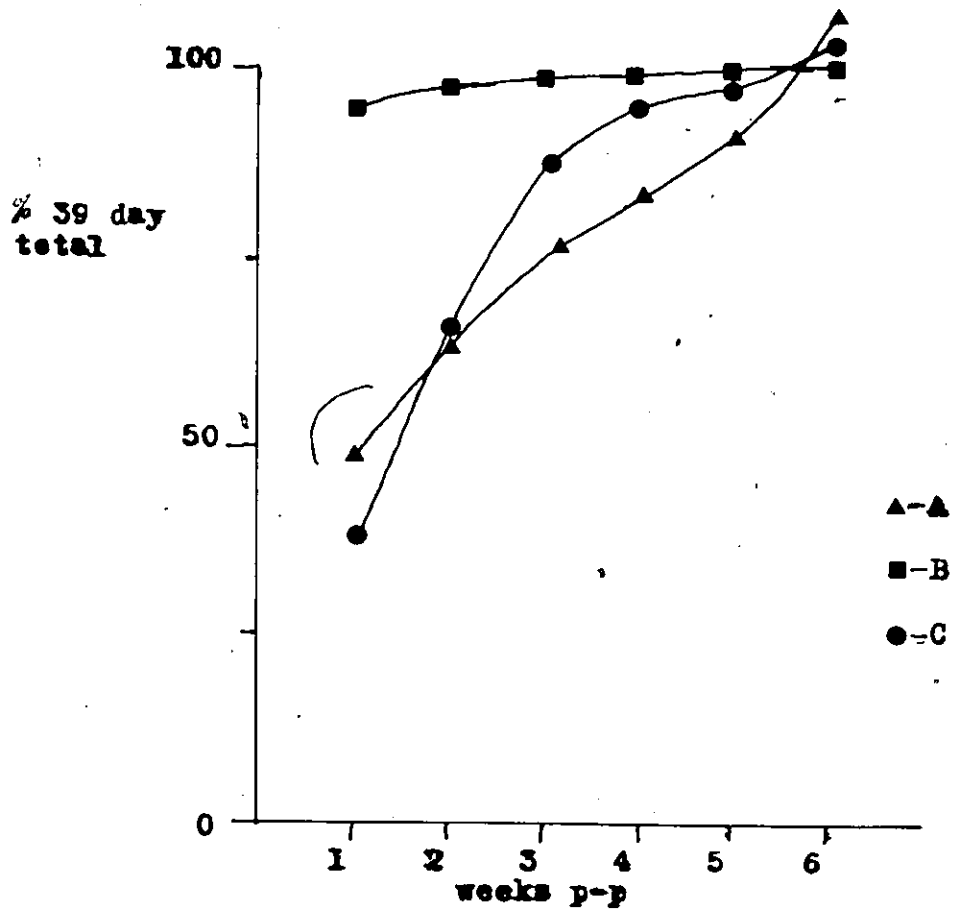


Fig. 5. Accumulated Weekly Total Female W.F. Expressed as %
39-Day Total Female W.F.

Fig. 5

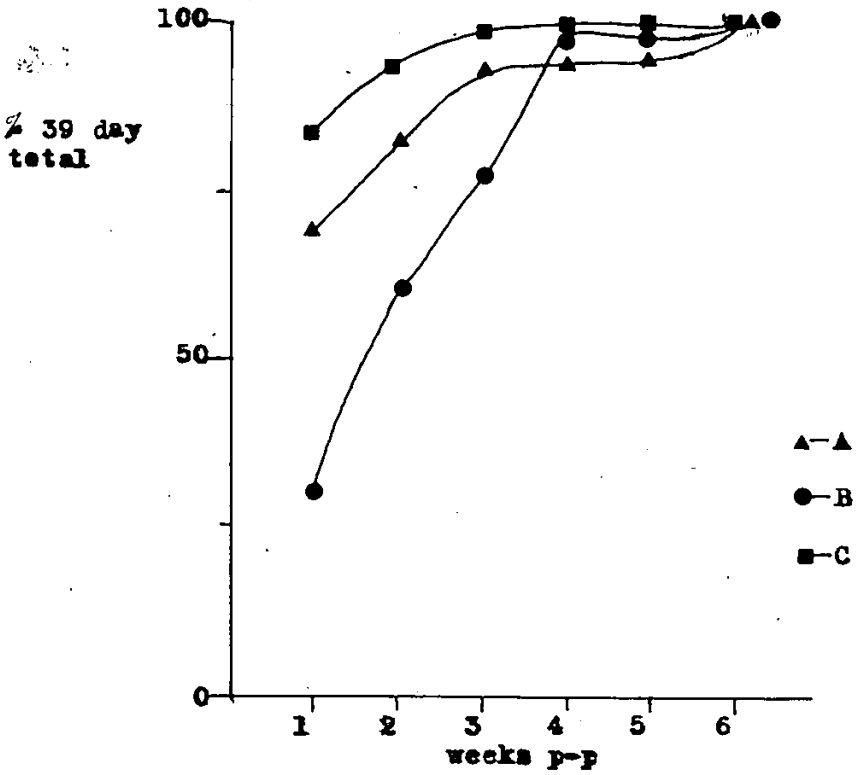


Fig. 6. Accumulated Weekly Total N.M. Expressed as % 39-Day Total N.M.

Fig. 6

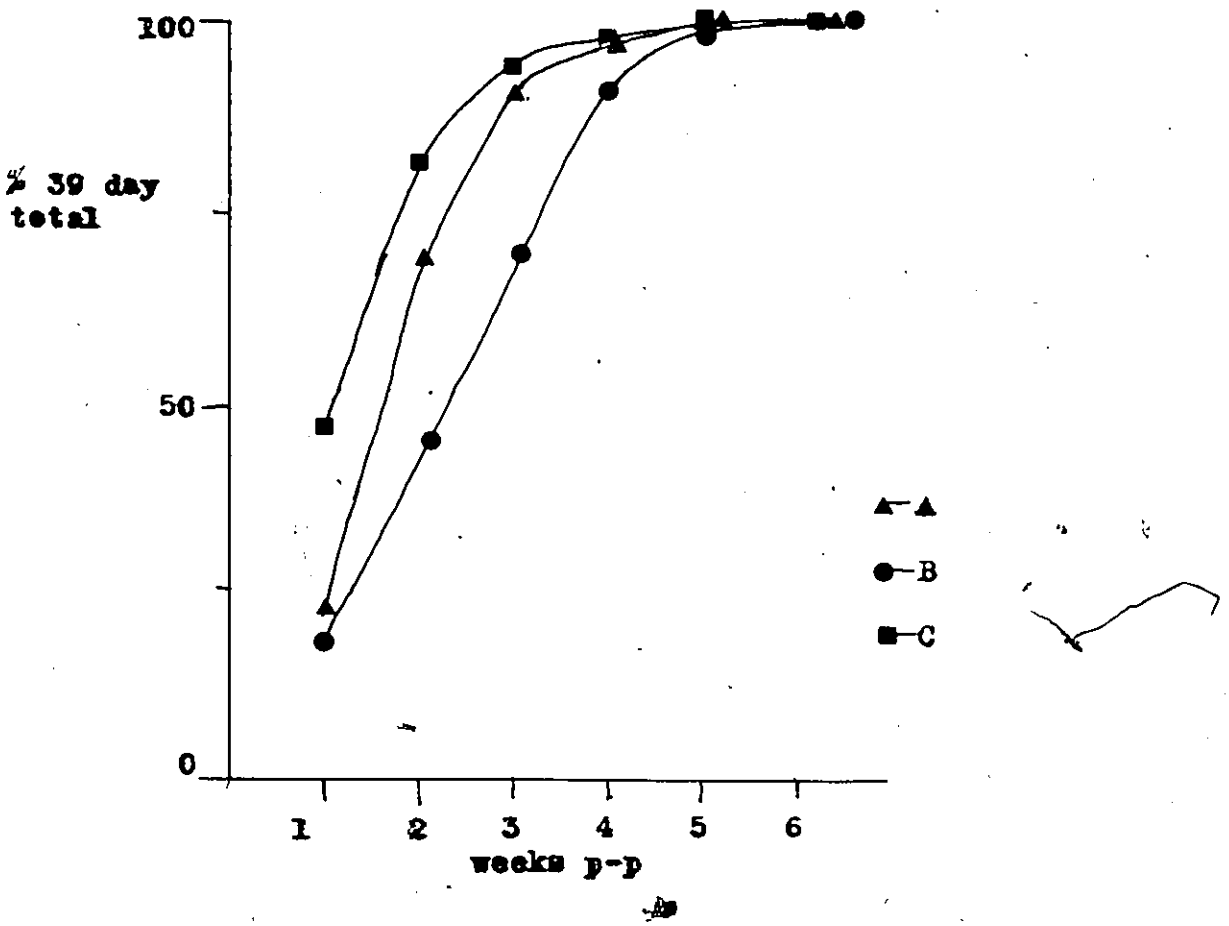
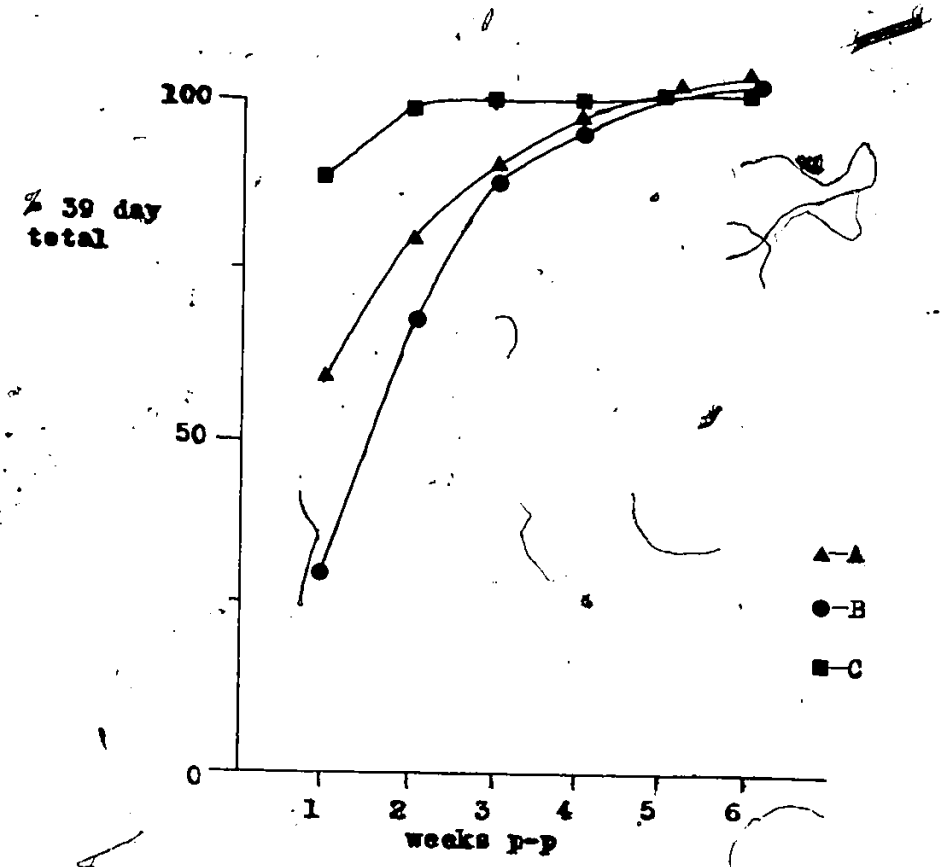


Fig. 7. Accumulated Weekly Total I.B. Expressed as % 39-Day Total I.B.

Fig. 7



Incubation Behavior

The toxic chemical treatment resulted in a dose-related significant decrease in the daily amount of time spent incubating eggs. During their respective incubation periods, control pairs showed an accumulated 98% of their population that incubated eggs for more than 25 minutes during a 30 minute observation period. The high dose-treated group showed an accumulated 29% of its population, and the low dose-treated group showed an accumulated 20% of its population, that displayed less than 25 minutes of incubation behavior during a daily 30 minute observation period (Figure 8). While the Kolmogorov-Smirnov test used to indicate statistically significant differences in Figure 8 showed only a statistically significant difference between groups B and C, the Mann-Whitney U test (Table 21) showed statistically significant dose-related differences between the treated groups and controls. Siegel (1956) indicates that for the large sample sizes we are dealing with, the Mann-Whitney U test is the most accurate non-parametric test. The Kolmogorov-Smirnov test was included to show more clearly the differences between the amounts of daily incubation behavior displayed by the treated and control groups as the median amounts appear similar.

Care-of-Young Behavior

While total behavior scores for this phase include small amounts of courtship behavior during the last week of this phase, the specific behaviors considered as care-of-young were brooding behavior and feeding-chicks.

Both treatment groups did significantly less brooding per

Table 21. Number of Minutes Incubation Behavior/ Daily Observation Period During Incubation Phases.

Group	Incubation Period Days	# Min Incubation Median	Range	Total
A	12-25	30*	5-30	2212
B	18-33	30*	2-30	2361
C	9-24	30	29-30	2698

* statistically significant at 0.05 level between groups (not medians).

Table 22. Number of Minutes Brooding Behavior/Daily Observation Period During Care-of-Young Phases.

Group	Care of Young Period	# Min. Brooding Daily		Total
	Days P.P	Median	Range	
A	26-42	29*	5-30	2264
B	34-55	26*	2-30	1985*
C	25-39	30	3-30	2526

* statistically significant at 0.05 level

Fig. 8. Frequency Distribution Plot of Accumulated % of Pairs with Particular Number of Minutes Incubation Behavior/ Daily Observation Period During Incubation Phases.

Fig. 8

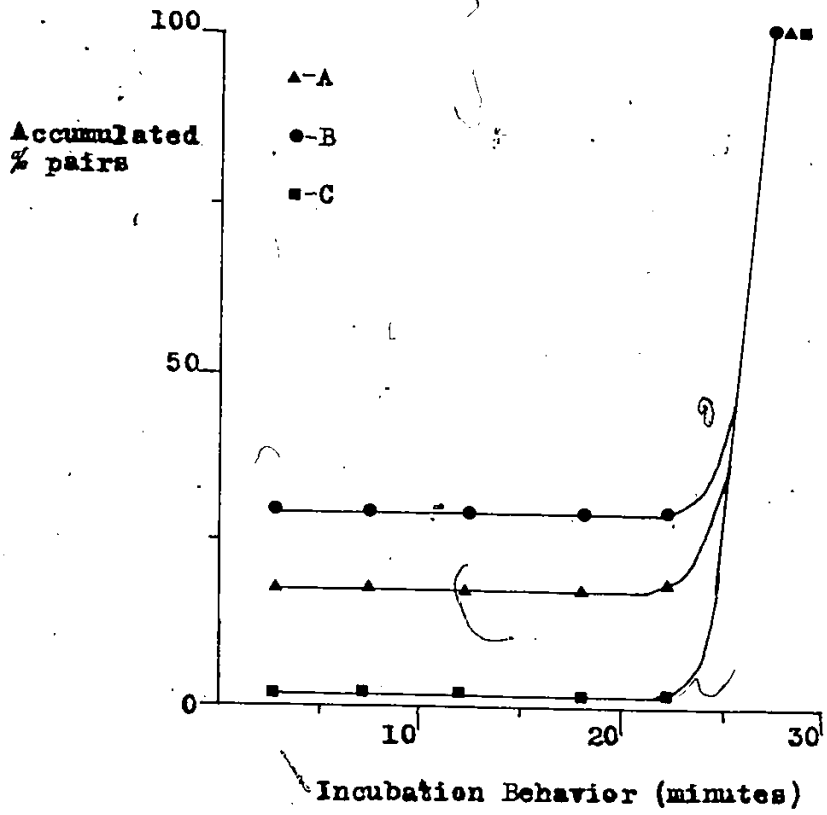
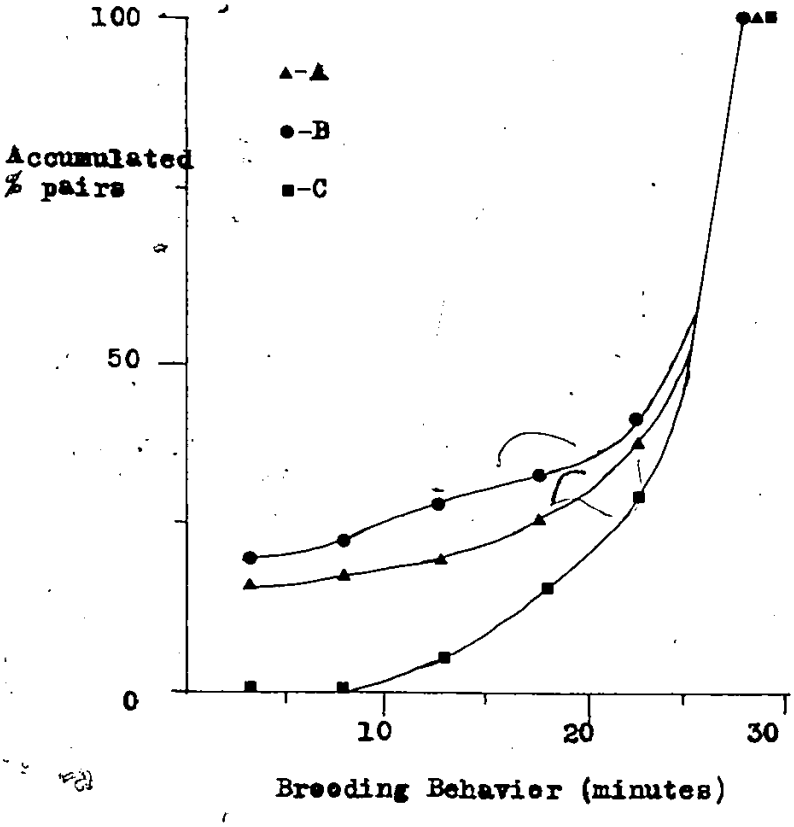

100
50
0
▲-A
●-B
■-C
Accumulated
% pairs
10
20
30
Incubation Behavior (minutes)

Fig. 9. Frequency Distribution Plot of Accumulated % of Pairs with Particular Number, of Minutes Brooding Behavior/ Daily Observation Period During Care-of-Young Phases.

Fig. 9


100
50
0
Accumulated
% pairs
▲-▲
●-B
■-C
10
20
30
Breeding Behavior (minutes)

daily observation period than controls (Table 22). Again the discrepancy in statistical significance between Table 22 and Figure 9, which shows only group B to be significantly different from C, can be attributed to the different statistical tests used. We accept that the differences shown for both groups in Table 22 are accurate, that is that treatment resulted in both treated groups exhibiting less brooding behavior than controls.

The high dose treated adult doves showed significantly less chick feeding behavior during the daily observation period than controls (Table 23).

Hormone Levels Found in Ring Dove Plasma During Toxic Chemical Experiment

Treated males showed significantly lower testosterone levels before pairing and 4 days post-pairing, than control males. Testosterone levels in treated males were not significantly lower than controls by the time they reached their respective mid-courtship periods (Table 26).

Treated female doves had significantly lower levels of total estrogens in their plasma than control females by the fourth day post-pairing but regained similar levels as controls by their respective mid courtship periods (Table 27).

Thyroxin levels in plasma from treated ring doves one week into their respective incubation periods, show a definite trend to higher than control values (Table 28).

Table 23. Number of Minutes Feeding Behavior/Daily Observation Period During Care-of-Young Phases.

Group	Care-of-Young Period (Days p.p)	Number Minutes Feeding Daily		Total
		Median	Range	
A	26-42	0	0-7	87
B	34-55	0*	0-5	37*
C	25-39	0	0-9	76

* Statistically significant at 0.05 level. Application of Mann-Whitney-O-statistical test indicated that the population distribution differs significantly ($p \leq 0.05$) from group C, but not from group A.

Table 26. Testosterone in Plasma From Male Ring Doves During Pre-breeding and Courtship Phases.

Group	Amount of Testosterone in Plasma of ♂ doves (pg/100 µl)					
	Bleed I	n	Bleed II	n	Bleed III	n
A	188±30*	(9)	340±47*	(8)	230±68	(6)
B	174±40*	(9)	244±39*	(9)	244±40	(5)
C	235±42	(7)	396±65	(8)	257±71	(7)

*Statistically significant at 0.05 level

Table 27. Amount of Total Estrogens in Plasma From Female Ring Doves During Pre-breeding, and Courship Phases.

Group	Total Estrogen Amounts in Plasma of o Doves (pg/ml)		
	Bleed I	Bleed II	Bleed III
A	58.1±3.7 (9)	71.3±4.1* (5)	70.7±4.8 (3)
B	56.4±4.8 (10)	65.1±5.1* (8)	73.2±4.8 (7)
C	58.6±3.4 (10)	83.1±3.0 (8)	76.2±3.9 (6)

*statistically significant at 0.05 level

Table 28. Levels of Thyroxine in Plasma of Ring Doves During Mid-Incubation Phases.

Group	Amount of Thyroxine in Plasma (both sexes included) ng/ml	
	Bleed Iv	n
A	8.2±3.7	(20)
B	5.9±2.18	(16)
C	4.1±0.48	(20)

Discussion

The results reported in this thesis demonstrate a cause and effect relationship between sublethal doses of a toxic chemical mix, containing PCB's, p,p'-DDE, Mirex and Photomirex, and abnormal behavior and endocrine dysfunction in breeding ring doves. Treatment caused statistically significant decreases in courtship, incubation and care-of-young behavior in the ring doves used. Treated doves showed dose-related significant reductions in reproductive performance. High-dose treated doves demonstrated a significant decrease in the number of young fledged per nest attempt, compared to controls. Decreased parental attentiveness to eggs and squab was the immediate cause of the poor reproductive performance in both groups of treated ring doves.

Decreased incubation behavior and increased time to hatch eggs have also been observed in PCB and other organochlorine contaminated birds in other control situations (Table 29) (Peakall and Peakall, 1973; Haegle and Hudson, 1973; Farve, 1978; Keith, 1978). Most interestingly these behavioral abnormalities have been described for some colonies of fish-eating birds in Lake Ontario. Gilbertson (1975) studying a number of species of fish-eating birds nesting in Lake Ontario reported reproductive failures followed a general pattern of high egg loss during incubation due to causes unrelated to predation. He reported early and late embryo deaths, deaths during piping, and decreased incubation behavior, by adults in birds contaminated with PCB's and organochloride pesticides from Lake Ontario.

While the present study in a controlled and favourable environment did not produce the embryo deaths Gilbertson observed, one can well

Table 29. Effects of PCB's and p,p'-DDE on Parental Behavior in Ring Doves.

Species	Chemical	Dose	Courtship Behavior	Time to lay	Nest Attentiveness	Reproductive Success	Reference
Ring Doves	p,p'-DDE	100 ppm	decreased	increased	decreased	decreased	Keith, 1978
Ring Doves	p,p'-DDE	10 ppm	decreased	--	--	--	
		50 ppm	decreased	--	--	--	Haegle & Hudson '77
Ring Doves	PCB's	10 ppm	--	increased	decreased	decreased	Peakall and Peakall '73
Ring Doves	PCB's	25 ppm	decreased	increased	decreased	decreased	Farve '78
Ring Doves	PCB's	8 ppm	same	increased	decreased	decreased	This study
	p,p'-DDE	2 ppm					
	Mirex	0.3 ppm					
	Photomirex	0.1 ppm					
	PCB's	28 ppm	decreased	increased	decreased	decreased	
	p,p'-DDE	5 ppm					
	Mirex	0.9 ppm					
	Photomirex	0.3 ppm					

imagine that the decreased parental attentiveness we observed could have resulted in embryo deaths under natural conditions of uncontrolled temperature and humidity fluctuations. The decreased incubation behavior of adults reported by Gilbertson (1975) and in more detail by Fox et al (1978) could therefore have resulted from the presence of PCB, p,p'-DDE, Mirex and Photomirex in the adult birds as the levels reported in their tissues (Gilman et al 1977) were higher than levels in the tissues of the highest dose group in this study.

Decreased parental attentiveness to squab could be the direct cause of the significantly decreased numbers of young fledged per nest attempt observed in the high-dose treated group. Treated doves in both dose groups in this study spent less time brooding young during the daily 30 minute observation period than controls. In addition to this, high dose treated doves also spent significantly less time feeding squab than controls.

The significantly decreased amount of brooding and feeding received by the squab in the high dose group could account for the substantial reduction of young fledged by this group compared to controls.

Fox et al (1978) reported decreased fledging success by the herring gulls in Lake Ontario compared to herring gulls in the other Great Lakes and in the Maritimes. They attributed this to the decreased parental attentiveness that they observed.

Our study indicates that the decreased parental attentiveness to chicks and the decreased fledging success reported by Fox et al

(1978) could be attributed to contamination of adults with the same toxic chemicals.

A performance index- the total number of viable eggs or chicks per nest during the first 39 days post-pairing-was used to summarize overall reproductive performance of treatment groups. Analysis of variance showed that treated ring doves performed poorly compared to control doves and the doves with the highest contaminant load performed more poorly than the less contaminated doves, that is to say, a dose-related effect on the performance index was shown. Keith (1978) used a similar performance index to test reproductive performance of ring doves contaminated with DDE and subjected to food stress. He reported that ring doves fed 100 ppm DDE in their food and not subjected to food stress had, a lower performance index than clean birds. As Keith (1978) used only one dose level of DDE he was unable to demonstrate a dose-related effect on performance index. He reports a performance index of 45.0 ± 22.4 for DDE contaminated doves and 66.5 ± 5.3 for controls. The performance index's in Keith's study are higher than those reported in Table 11 of this report. Keith's indexes however, were calculated using only data from pairs that successfully fledged young. The performance indexes in Table 11 include data from all pairs in our study, including those that failed to lay eggs or fledged chicks.

Our research on ring doves has also shown that treated doves showed significantly less courtship behavior than controls during the first week post-pairing. The time spent in male wing-tip flipping displays however, was the same for high-dose treated birds as for controls and low dose treated males actually showed

significantly more wing-tip-flipping than controls.

Male wing-tip-flipping is one of the earliest courtship displays after pairing and males usually progress from this behavior to nest-building behavior in response to female wing-tip-flipping displays. The fact that treated females did significantly less wing-tip-flipping than controls probably accounts for the low scores of the treated groups during the first week post-pairing and for the observation that nest building was delayed in the treated groups compared to controls.

Peakall and Peakall (1973) in their study of incubating ring doves contaminated with PCB's, concluded that reproductive success was dependant on the contamination of the female while exposure of the male was not important. Our courtship behavior observations would seem to agree with this as female courtship behavior was shown to be affected by treatment but initial male courtship behavior was not.

Decreased female responsiveness to male courtship was probably the result of low levels of circulating estrogens. Estrogen levels usually increase with male courtship and stimulate wing-tip-flipping in female ring doves (Cheng, 1978). Lower than control levels of estrogens in treated doves during the first week post-pairing found in this study could account for the decreased responsiveness of the treated females to male courtship. Testosterone levels in treated males were also significantly lower than in the control doves during the first week post-pairing. This does not seem to have affected their wing-tip-flipping displays. Cheng (1978)

however, has shown that male wing-tip-flipping is an estrogen induced effect.

Delays in initiating nest building and incubation have been observed in many species of birds exposed to DDE or PCB's in experimental set ups (Peakall and Peakall, 1973; Peakall, 1970(a); Keith, 1978; Farve, 1978; Ritchie and Peterle, 1979). These authors have suggested that delays in onset of nest building and incubation can cause serious consequences for contaminated birds in the wild where environmental conditions are suitable for chick rearing for only a specific time period.

Two mechanisms have been proposed in the literature to account for delays in onset of nesting and incubation, and abnormal parental behavior in birds contaminated with PCB's or DDE. Peakall (1970(b)) proposed that pesticide induced increases in hepatic enzymes destroy circulating steroids. This leads to delay in attaining threshold levels necessary to stimulate breeding behavior, ovulation and parental attentiveness. Peakall (1970(a)) showed that ring doves treated daily with 10 ppm DDT have a significantly lower estradiol level in their blood and a much higher level of hepatic enzyme activity. The abnormal delays in breeding and parental behavior can be readily explained in terms of altered hormone concentrations resulting from hepatic enzyme induction. Riseborough et al (1968) have shown that PCB's are even more powerful inducers of hepatic enzymes in birds than DDT. Most recently Richie and Peterle (1979) have shown that DDT-contaminated doves failed to show the strong LH surge on the 4th day post-pairing that was exhibited by the control birds. The LH has been shown to stimulate ovulation in female ring

doves (Cheng 1978). A lack of this surge on the 4th day post-pairing suggests that this is the cause of ovulation delay exhibited by DDT contaminated doves.

Levels of testosterone in plasma from males in both treatment groups were significantly lower than control levels for the pre-breeding and fourth day post-pairing samples. However, treated males showed no significant difference from control males in plasma testosterone levels at the time of mid-courtship bleeds. Thus, we have observed that pesticide-related initial reduction in testosterone level was overcome by the time the treated doves reached mid-courtship. Testosterone levels in male ring doves increase upon exposure to female ring doves (Cheng, 1978). We observed that pesticide-induced reduction of testosterone levels in the treated male doves was eventually overcome by exposure to the females but as female courtship behavior was reduced by the toxic chemical treatment a combination of endocrine and behavior effects resulted in the prolonged courtship periods observed in the treated groups. Haigele and Hudson (1977) reported that ring doves dosed with 10 and 50 ppm DDE displayed reduced total courtship activity compared to controls. Theirs was strictly a behavioral study and no attempt was made to measure endocrine levels during the study. They concluded that both dose levels of the p,p'-DDE diet did cause a significant reduction in courtship behavior during their observation period and speculated that this reflected decreased estrogen and androgen levels.

Estrogen levels in plasma from Bleeds I and II from treated female doves were significantly lower than levels in female

controls. Estrogens facilitate pre-sitting courtship behavior in female ring doves and increase with male courtship (Cheng, 1978).

Peakall (1975) reports that researchers have shown that 10 ppm DDE-dosed ring doves had lower estradiol levels than controls and that PCB-treated pigeons had lower circulating estradiol levels than controls. Our results agree with these findings.

Androgens in male ring doves and estrogens in female ring doves prime and increase the numbers of progestin receptors available. The combined actions of the two hormones with progesterone lead to the behaviors of nest building and incubation (Cheng, 1978). The decreased levels of androgens and estrogens, during the courtship period in the treated doves, combined with the progesterone and could explain the delays in nest building and onset of incubation reported in this study.

Decreased levels of androgens and estrogens measured during the pre-breeding and mid courtship phases in treated doves and the trend towards increased liver weights in the dosed birds, provide evidence that hepatic-enzyme induction and subsequent reduction of circulating steroids may be the mode of action of the toxic chemical mix's effects on the behavior of adult ring doves. Hormone levels reported for control birds during this study are lower than those reported for ring doves in other studies (Silver, Reboulleau and Lehrman, 1974; Silver, 1978; Cheng and Follet, 1976). This is probably the result of collection and separation of the blood samples in microtainers. In another study, using ring dove blood collected and separated in heparanized tubes we obtained higher hormone recovery

levels than for the same blood collected and separated in micro-tainers.

Another mechanism to explain pesticide-induced changes in reproductive hormone levels has been proposed by D.J. Jefferies (1967). He observed delayed ovulation caused by doses of 75 to 1200 ppm p,p'-DDT in Bengalese Finch (Lonchura striata) and proposed that the site of action of DDT was probably the pituitary or hypothalmus. He suggests that DDT has an estrogen-like action on the hypothalmus that results in inhibition of FSH, LH and Prolactin secretion by the pituitary gland.

Jefferies, French and Osborne (1971) fed 3-36 ppm DDT to 3 groups of homing pigeons (Columba livia) and observed that the low dose birds showed signs of hyperthyroidism while the high dosed birds showed signs of hypothyroidism. They also found increased liver weights in the DDT-treated birds. They proposed two possible mechanisms for the observed hyperthyroidism: 1) DDT directly stimulates the pituitary or thyroid glands to produce more thyroid stimulating hormone or thyroxin or 2) a low level of circulating thyroxine, caused by increased liver enzyme activity, stimulates the production of thyroid stimulating hormone by the pituitary.

Jefferies (1975) proposes that PCB's and other organo-chlorines cause hypothyroidism in contaminated birds. He proposes that DDE initially stimulates the pituitary or thyroid gland to produce thyroid stimulating hormone and thyroxin but then DDE competes with thyroxine for thyroxine serum proteins thus leading to symptoms of hypothyroidism. He also proposes that PCB's cause

hypothyroidism via pituitary inhibition of thyroid activity.

We have found increased thyroxin levels in the treated doves in this study and a trend toward increased liver weights in the treated doves. We feel that this evidence supports the idea that pesticides induce increased hepatic enzyme activity. The higher thyroxine levels of the treated birds in our study may have been the result of stimulation of the pituitary secretion of TSH in response to the destruction of circulating thyroxin by hepatic enzymes.

The thyroxine findings along with changes in steroid hormones in the treated groups provide additional support that PCB's and other organochlorines may cause behavioral changes in ring doves via hepatic enzyme induction and the consequent reduction of circulating hormones.

This study indicates that 1) the poor reproductive performance reported in many species of birds contaminated with PCB's, DDE or combinations of PCB's and other organochlorines are the result of toxic chemically induced changes in behavior of breeding adults and 2) reproductive performance of contaminated populations will improve if levels of toxic chemicals in the adults decline.

Norstrom et al. (1978) reports that levels of PCB's and organochlorines in the tissues of herring gulls in Lake Ontario are now lower than levels found during the early 70's (when Gilbertson and Fox made their observations). Reproductive performance of herring gulls in Lake Ontario has been improving since 1975 (Peakall et al., 1978; Weseloh et al., 1979).

The results presented here provide evidence that behavioral

abnormalities observed in the early 70's in herring gulls in Lake Ontario were probably the result of PCB and other organochlorine-induced endocrine dysfunction in the breeding adults.

Eggshell thinning caused by DDT and its metabolites has been associated with reproductive failures in many avian species. Ring doves exposed to 5 ppm or less of DDE in their diets do not show a significant decrease in eggshell thickness (Peakall, 1975). The highest dose group in this study contained 5 ppm in their diet and failed to show any signs of thin shells or egg breakage. PCB's have not been associated with the phenomenon of eggshell thinning in birds (Peakall, 1971).

This study provides evidence that behavioral abnormalities in breeding ring doves are caused by pesticide-induced endocrine dysfunction. It also suggests that further research is needed since PCB's and organochlorines are widespread and persistant pollutants in the environment. The effects of these chemicals should be explored over several generations.

The fact that these chemicals cause significant effects on incubation and care-of-young behaviors in particular suggests that they could cause greatly increased reproductive failures in wild birds due to predation, and hatching failure especially during breeding season with fluctuating or extreme weather conditions.

While it is dangerous to extrapolate to field conditions on the basis of laboratory studies, such studies do illustrate the hazards of environmental contaminants and the need to reduce the exposure of wildlife to such biologically active chemicals.

Summary and Conclusions

Reproductive failure in a colony of Lake Ontario herring gulls are thought to be the result of behavioral abnormalities in the adult gulls. It has been proposed that these behavioral abnormalities are the result of pollutant induced endocrine dysfunction (Fox et al., 1978).

The present study was undertaken to determine if toxic chemicals, currently found in the tissues of adult herring gulls from Lake Ontario would produce behavioral abnormalities, decreased reproductive success and endocrine dysfunction in ring doves in a controlled environment.

Ring doves were fed a toxic chemical mix containing PCB's, p,p'-DDE, Mirex and Photomirex. Treatment resulted in significantly decreased courtship behavior and parental attentiveness. Treated groups had significantly poorer reproductive performance than control doves and significantly lower levels of circulating steroids than controls. Treated birds showed trends towards higher thyroxin levels and higher liver weights during the courtship period than controls.

We have shown that there is a cause and effect relationship between sub-lethal doses of the toxic chemicals used, and behavioral abnormalities that were responsible for the poorer reproductive success of the treated ring doves compared to controls. We conclude that the observed behavioral abnormalities were probably the result of pollutant-induced hepatic enzyme destruction of circulating hormones.

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Appendix

Table 5. Total Food Consumption/Group During Isolation Period

	Total Grams food Consumed/group/month			Total grams food consumed/group during Isolation Period
Group	Month 1	Month 2	Month 3	
A	7,767	7,997	6,210	21,974
B	7,637	7,556	5,163	21,355
C	8,110	8,224	7,293	23,627

Table 6. Rate of Food Consumption During Isolation Period

Group	Mean Rate of Food Consumption in gm/day During Isolation Period		
	♂	♀	Group mean food consumption
A	12.91±1.99	12.27±0.92*	12.59±1.54
B	11.61±1.35	12.12±1.2*	11.87±1.27*
C	12.92±1.28	13.34±1.4	13.12±1.2

* statistically significant at 0.05 level

Table 7. Rate of Pesticide Consumption During Isolation Period

Group	Mean Rate pesticide consumption in mg/kgm body weight/day during isolation period											
	DDE			PCB			Mirex			Photomirex		
	♂	♀	Group	♂	♀	Group	♂	♀	Group	♂	♀	Group
A	.014	.014	.014	.07	.07	.07	.0026	.0026	.0026			.0008
B	.37	.42	.39	2.2	2.6	2.39	.007	.008	.0076			.0025

Table 9. Weights of Fledged Ring Doves

Group	wt. of chicks raised to 14 days(gm)		
	Median	Range	n
A	95	77-104	13
B	86	45-112	7
C	91	78-99	17

Table 24. Total Accumulated Behavior Scores on Bleeding Days Expressed as % of 39 Day Total Behavior Score

Group	Bleeding Days							
	Bleed 2 (4 days p.p)		Bleed 3 (mid c)		Bleed 4 (mid I)		Bleed 5 (mid care of young)	
	Days p.p	% Total Behavior Scores	Days p.p	% Total Behavior Scores	Days p.p	% Total Behavior Scores	Days p.p	% Total Behavior Scores
A	4	3.3%	6	5.4%	19	39.2%	34	87.5%
B	4	2.8%	11	10.5%	26	53.5%*	45	102%*
C	4	3.9%	6	9.2%	17	37.4%	32	87.5%

* statistically significant at 0.05 level

Table 25. Total Accumulated Behavior Score on Bleeding Days Expressed as % of Control Total Accumulated Behavior Score on Same Bleeding Days.

Group	Bleeding Days							
	Bleed 2 (4 days p.p)		Bleed 3 (mid-c)		Bleed 4 (mid I)		Bleed 5 (Mid of Y)	
	days p.p	% Total Behavior Scores	Days p.p	% Total Behavior Scores	Days p.p.	% Total Behavior Scores	Days p.p	% Total Behavior Scores
A	4	77.6%	6	53.8%*	19	94.9%	34	90.7%
B	4	69.9%	11	111.7%	26	138.7%*	45	114.2%*
C	4	100%	6	100%	17	100%	32	100%

* statistically significant at 0.05 level