THE PHYSIOLOGICAL EFFECTS OF SELECTED PESTICIDES ON THE JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) AND EMBRYOGENESIS OF THE DOMESTIC FOWL

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THE PHYSIOLOGICAL EFFECTS OF SELECTED PESTICIDES ON THE JAPANESE QUAIL (Coturnix coturnix japonica) AND EMBRYOGENESIS OF THE DOMESTIC FOWL

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ABSTRACT

THE PHYSIOLOGICAL EFFECTS OF SELECTED PESTICIDES ON THE JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) AND EMBRYOGENESIS OF THE DOMESTIC FOWL

by Ralph Ambrose Ernst

The use of pesticides has become commonplace in this country and throughout the world. As the use of pesticides has increased, concern has developed as to how they may affect wildlife. Most present methods of evaluating pesticide toxicity do not measure their sublethal effects.

This study was undertaken to examine various methods of evaluating pesticide toxicity which would be applicable to a wide range of pesticide materials at both lethal and sublethal levels. Selected pesticides were incorporated into the ration of Japanese quail. Feed consumption, mortality, reproductive efficiency, body weight, oxygen consumption, differential leucocyte count, packed cell volume, mean corpuscular volume, total serum protein, electrophoretic pattern of serum proteins and plasma alkaline phosphatase were determined on these quail. Tissues were also examined microscopically. In another series of experiments selected pesticides were injected into the yolk sac of embryonating chicken eggs on the fifth day of incubation and mortality and gross developmental abnormalities were observed.

Zectran and Zytron rations severely reduced feed intake and significantly lowered mean body weight of quail while DDT did not affect mean body weight. Quail females consumed significantly smaller quantities of rations containing Zectran (300 ppm) or Zytron (2000 ppm) than of a control ration when both rations were offered simultaneously at identical feeding sites. In this experiment, consumption of a ration containing DDT (5000 ppm) was not significantly different from a control ration.

None of these pesticides caused a significant change in hen day egg production although Zectran and Zytron appear to have reduced egg production of quail hens. The number of eggs per hen was considerably, but not significantly, reduced by all of the pesticides. Fertility and hatchability did not seem to be affected by any of the pesticides. DDT caused an increase in chick mortality while Zectran and Zytron had no effect on chick mortality.

Oxygen consumption was not significantly altered by any of these pesticides, although DDT may have caused a slight increase in oxygen consumption and Zectran a slight decrease.

Of the pesticides tested only Zytron caused a significant change in the differential leucocyte count of quail. The lymphocyte percentage of males and the basophil percentage of females were significantly lowered following Zytron administration.

Chronic oral administration of DDT, Zectran and Zytron has been shown to significantly reduce the packed cell volume and total erythrocyte count of mature Japanese quail without changing the mean corpuscular volume. These pesticides did not consistently lower the packed cell volume of immature quail although severe anemia appeared in some individual quail preceding their death.

Zectran and Zytron caused a significant drop in total serum protein of quail hens three days after the pesticides were first administered. However, quail hens restricted to the same quantity of basal ration which quail hens receiving the aforementioned Zectran and Zytron rations consumed voluntarily (9 gm/hen/day) also showed a significant drop in total serum protein level three days after the restriction commenced. DDT did not significantly reduce total serum protein; however, after ten days the total serum protein dropped about one-half percent below the control level and remained there.

Zytron did not cause a significant change in any of the components resulting from electrophoretic separation of serum protein of female quail in this study.

Zytron caused a decrease in plasma alkaline phosphatase which approached significance ($P < 0.10$) while DDT and Zectran did not affect this parameter.

Of the various parameters measured in this study, none were definitely shown to be valid measures of pesticide toxicity. Tests of the affect of pesticides on total serum protein indicate that this parameter may be adequately sensitive to pesticides to render it useful as a toxicity test but additional experimentation would be necessary to establish the extent of its applicability for this purpose. The toxicity exhibited by these pesticides when injected into embryonating chicken eggs did not compare well with the toxicity exhibited when they were incorporated into the ration.

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I. INTRODUCTION

The use of pesticides has become commonplace in this country and throughout the world. As more and more of these varied chemicals are utilized, concern has mounted as to how they may affect birds and wildlife. Pesticide residues have been detected in the tissues and eggs of many birds and animals around the world. In numerous instances, dead birds containing pesticides residues have been found following wide scale spray programs. In recent years many new pesticides have been introduced making it more difficult to ascertain the danger of these materials to our many wild avian species. Contributing to this problem is the diversity in the habitat and feeding habits of these many species of birds.

At the present time it is customary to evaluate the toxicity of chemical materials by determining the dose which will cause the death of half of the birds or animals tested. This is usually referred to as the lethal dose fifty (LD_{50}) of the particular chemical for that species. This system of evaluation does not directly measure what effects these materials might have on birds at sublethal levels.

This study was undertaken in an attempt to find some method of evaluating the toxicity of pesticide chemicals which would be applicable to a wide range of pesticide materials and would also measure the relative hazard of these materials at sublethal levels.

II. REVIEW OF LITERATURE

A. Introduction

The origin of pesticides can be traced to the synthesis of DDT* by Zeidler in 1874, or if one prefers, to the discovery of its insecticidal properties by Paul Müller in 1939 (Metcalf, 1955). During the following decade, DDT was widely used on orchards, forests and farms as an insecticide due to its wide spectrum of insecticidal activity, low cost and moderate mammalian toxicity (Metcalf, 1955). As the use of DDT increased, reports of mortality among fish and wildlife began to occur in widely scattered areas across North America (Bossenmaier, 1959). Subsequent studies by Coburn and Treichler (1946), Barker (1958), Wright (1960), Wallace (1962) and many others have implicated DDT as the agent responsible for death. These studies caused many biologists to express concern about the effects of these insect control programs on domestic animals, men and wildlife (Rudd and Genelly, 1956; Barker, 1958 and Bossenmaier, 1959). However, it was not until the publication of "Silent Spring" by Rachel Carson that a large mass of the public became aware of pesticide problems. Her discussion dwelled on one problem, namely, that large volumes of pesticides were being used without extensive investigation into possible hazards to the many organisms which inhabit our lands and waters (Egler, 1964). Certainly if this was the only aspect of the problem, chemical pesticides would never have been cleared for extensive use. Jukes (1963) ably pointed out that chemical insecticides have partially or completely freed the world of some 30 diseases including malaria. Furthermore, without pesticides the world production of food would fall drastically in a world already critically short of food in many areas. Since 1962 the pesticide problem has been carefully reviewed by a special Presidential Committee (Swift, 1964; Weisner, 1964).

*See Appendix for chemical name

The fear of ornithologists that insecticides may place severe pressures on bird populations is based on reports of bird mortality following spray programs (Rudd and Genelly, 1956; Barker, 1958; Bossenmaier, 1959; Wright, 1960; Wilson Ornithological Society, 1961) and on other reports of their toxicity which will subsequently be discussed in more detail. Wallace (1962) has shown that DDT sprayed on elm trees passes into the soil and becomes concentrated in the bodies of earthworms. Robins feeding on these worms succumb from DDT poisoning. The great diversity in the feeding habits and environment of many avian species has made it difficult to determine accurately how wide scale pesticide applications affect their welfare (Rudd and Genelly, 1956). In spite of the problems involved, a great mass of evidence has been accumulated which shows that DDT and other insecticides when used as sprays or dusts can cause mortality among birds (Wilson Ornithological Society, 1961).

The numerous reports of pesticide residues which have been found in birds and animals are another type of evidence which has caused concern. Marsden and Bird (1947) found DDT residues in turkeys four to eight times higher than dietary levels. Gannon et al. (1959) found dieldrin* residues in tissues of steers, lambs, hogs and poultry fed dieldrin in their diets at not more than 2.25 ppm for 12 weeks. Wallace (1962) and Boykins (1965) found residues of DDT in soil, earthworms and robins in areas sprayed for elm bark beetle. Stadelman et al. (1965) have reported residues of lindane*, dieldrin, heptachlor* and DDT in tissues and eggs from chickens receiving 10 - 15 ppm of these chemicals in their diet. These residues were described as extremely persistent.

Dale and Quimby (1963), Quimby et al. (1965) and Zavon et al. (1965) have reported residues of dieldrin, DDT and benzene hexachloride* in humans in the United States. Hunter et al. (1963) have found residues of several chlorinated hydrocarbons in human body fat in England.

*See Appendix for chemical name

The review by Rudd and Genelly (1956) contains many reports of pesticide residues which have been found in bird and animal tissues. However, Durham (1963) and Kraybill (1965) have reported that there was no significant change in the storage of DDT in the general population of the United States between 1950 and 1962.

B. Physiological Effects of Pesticides

Food Consumption and Body Weight

Genelly and Rudd (1956b) reported that pheasants consumed less feed when 300 ppm toxaphene* or 25 or 50 ppm dieldrin was incorporated into the diet. The weight decline in these pheasants on toxaphene and dieldrin was approximately proportional to dietary level (Genelly and Rudd, 1956a), while DDT did not depress feed consumption or body weight. Cross et al. (1962) found that DDT incorporated into the diet of Japanese quail at levels of 500 and 700 ppm reduced feed consumption, while lower levels did not affect consumption.

Shellenberger et al. (1965) have reported that Betasan* fed at levels of 10, 100 or 1000 ppm had no effect on body weight maintenance in Japanese quail, while Imidan* reduced feed consumption and growth at a level of 1000 ppm.

Ross and Sherman (1960) found that Dipterex* (9 ppm), Diazinon* (32 ppm), malathion* (45 ppm) and phenothiazine (1364 ppm) significantly depressed feed consumption of chicken hens but only Diazinon caused a significant drop in weight. However, starting with 9-day-old chicks, Co-Ral* (27 ppm), Diazinon (32 ppm), malathion (45 ppm), phenothiazine (1364 ppm) and Dow ET-15* (27 ppm) significantly reduced weight-gain ratios when compared with control chicks. Lindane (10 ppm) did not depress feed consumption of chicken hens or young chicks when the material was incorporated in the diet for 60 days (Ware and Naber, 1961). Olney et al. (1962) reported that

*See Appendix for chemical name

methoxychlor* did not depress feed consumption or body weight of chicken hens when it was incorporated in the diet at levels as high as 1000 ppm.

Dimethoate*, at a chronic level of 30 ppm in the water for 59 weeks, reduced weight gains and feed consumption of chicken hens (Sherman et al., 1963).

Ball et al. (1953) observed a significant ($P < 0.01$) increase in growth of rats when they received 10 ppm or 20 ppm aldrin* in their diet.

Reproduction

Burlington and Lindeman (1950) found that injecting male chickens with dosages of DDT, which were increased from 15 mg/kg at 8 days of age to 300 mg/kg at 89 days of age, inhibited the normal growth of the comb, wattles and testes. Egg production, fertility and hatchability were relatively unaffected by inclusion of DDT, strobane*, dieldrin or endrin* in diets fed to adult Bobwhite quail, but chicks which hatched showed high mortality rates (DeWitt, 1956). Both hatchability of eggs and survival of chicks were reduced when aldrin, dieldrin or endrin was incorporated into the diet of pheasant breeders (DeWitt, 1956). Genelly and Rudd (1956b) found that 300 ppm toxaphene and either 25 or 50 ppm dieldrin significantly depressed egg production of pheasants. Egg fertility of pheasant breeders receiving 50 ppm dieldrin and hatchability of eggs from the 300 ppm toxaphene group were significantly lower than controls (Genelly and Rudd, 1956b). DDT in the diet of pheasant breeders at a level of 500 ppm did not reduce egg production, fertility or hatchability, but chicks hatched had significantly higher mortality to 46 days of age (Azevedo, 1965).

Sherwood (1959) fed Polybor 3* to chicken hens and found that at 1500 ppm and 3000 ppm it depressed egg production. Ross and

*See Appendix for chemical name

Sherman (1960) reported that Dipterex (60 ppm) and Diazinon (45 ppm) appeared to have had a definite depressing effect on egg production in hens while Co-Ral (27 ppm), malathion (227 ppm), phenothiazine (2373 ppm), ronnel* (45 ppm), and Dow Et-15 (27 ppm) did not. The only consistent abnormalities noted in eggs were dark and mottled egg yolks produced by all hens receiving phenothiazine. Ware and Naber (1961) showed that 10 ppm lindane in the diet of chicken hens did not affect egg production. Feeding up to 1000 ppm methoxychlor to chicken hens for 85 days did not depress egg production (Olney et al., 1962). Sherman et al. (1963) found that dimethoate did not affect egg production when it was mixed in the water of chicken hens at a level of 30 ppm for 59 weeks.

Feeding Japanese quail hens diets containing 500 ppm and 700 ppm DDT resulted in lowered egg production and hatchability but 300 ppm DDT or less did not affect these parameters (Cross et al., 1962). Shellenberger et al. (1965) reported that 10, 100 and 1000 ppm Betasan in the diet of Japanese quail did not affect egg production or fertility of eggs, but 1000 ppm reduced hatchability. Imidan reduced egg production of Japanese quail at a dietary level of 1000 ppm, but had no significant effect at 10 ppm or 100 ppm (Shellenberger et al., 1965).

Ball et al. (1953) have shown that 20 ppm aldrin in the diet of rats caused estrus cycles to be disturbed. Bernard and Gaertner (1964) found that incorporating 300 ppm DDT in the diet of mice reduced fertility but did not affect litter size. Kepone*, however, has reduced both fertility and litter size of mice at levels as low as 10 ppm (Good et al., 1965).

Mosquitofish have been shown to abort their young when sublethal levels of DDT, DDD*, methoxychlor, aldrin, dieldrin, endrin, toxaphene, heptachlor or lindane were present in their water (Boyd, 1964).

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*See Appendix for chemical name

Metabolic Rate

Chronic feeding of DDT to rats resulted in an increase in metabolic rate (Riker et al., 1946). However, this increase was not measured until visual tremors and muscle spasms were present. Rat liver slices excised from rats dispatched during severe tremors, showed a definite increase in oxygen uptake, however, oxygen uptake of liver slices was significantly reduced when the liver exhibited gross abnormalities (Riker et al., 1946). Jandorf et al. (1946) also found an increase in oxygen consumption of rat liver slices when 50 mg DDT per kg weight was administered orally for one to ten days; however, after 30 - 100 days no change in the respiratory rate of liver slices was found. Hukuhara et al. (1962) have shown that adipose tissue, from rats on a diet high in DDT, exhibited the same metabolic activity in vitro as tissue from controls. Skeletal muscle from frogs poisoned with DDT has also shown an increased oxygen consumption (Riker et al., 1946). Neither of these investigators found any increase in the oxygen uptake of brain tissue (Riker et al., 1946; Jandorf et al., 1946). Ozburn and Morrison (1965) found that DDT resistant mice responded to DDT administration with an increase in metabolic rate followed by a quick return to normal while the metabolic rate of control mice increased more quickly and to a greater extent. Many of these control mice died without regaining "normal" rates.

Crevier et al. (1954) found that chronic and acute doses of aldrin decreased the oxygen consumption of rats but failed to accelerate the recovery of the oxygen consumption of thyroxinated rats from augmented levels. They suggested that aldrin may depress metabolic rate by impairing thyroid function.

Blood Parameters

Chronic doses of DDT have been found to cause a drop in hemoglobin in rabbits (Cameron and Burgess, 1945) and in rats (McNamara et al., 1946) while the total number of red blood cells remained unchanged.

In contrast, chronic DDT injection in male chickens reduced red blood cells by an average of 17.8 percent while hemoglobin concentration remained commensurate with control values (Burlington and Lindeman, 1950). Sanchez-Medal et al. (1963) have reported aplastic anemia in human patients severely poisoned with DDT and lindane. Mastromatteo (1964) also observed the development of blood dyscrasias in several humans after exposure to chlorinated hydrocarbons. Anemia and increased sedimentation rate resulted from serious poisoning of human patients by hexachlorocyclohexane (Danopoules et al., 1953). No changes in blood morphology or sedimentation rate were found in rabbits injected with a toxic sublethal dose of trichlorometaphos-3* (Sazonova and Valkova, 1965). Emerson (1965a) found an increase in the packed cell volume and mean corpuscular volume of dogs acutely poisoned by endrin, however, removing the spleen or viscera partially prevented the increase in packed cell volume.

Cameron and Burgess (1945) found a pronounced leucocytosis when DDT approached toxic levels in rabbits, however, Davignon et al. (1965) reported a greater incidence of leukopenia in apple-growers exposed to pesticides than in control populations. Emerson et al. (1964) report leukocytosis in dogs acutely poisoned with endrin. Kunev (1965) found an increased phagocytic action in the blood (leukocytosis) of rats when relatively small amounts of DDT, benzene hexachloride, Dipterex, malathion, Na arsinite* and Na fluosilicate* were injected intragastrically.

Cardiovascular

McNamara et al. (1946) found that prolonged administration of DDT to dogs resulted in an increase in cardiac output while acute doses of endrin given intravenously caused bradycardia and systemic hypertension (Emerson et al., 1964; Reins et al., 1964; Emerson, 1965a). The insecticide DDT injected intravenously into dogs

*See Appendix for chemical name

sensitizes the myocardium so that exogenous epinephrine will cause ventricular fibrillation (Philips et al., 1946). When dogs were infused with DDT, dissolved in peanut oil and emulsified with lecithin in a saline solution, they were observed to die of ventricular fibrillation (Phillips and Gilman, 1946).

Danopoules et al. (1953) have recorded abnormal electrocardiograms in human patients severely poisoned with hexachlorocyclohexane. Emerson (1965b) has measured an increased resistance in both the innervated and denervated isolated limbs of dogs infused with endrin.

Renal Function

Chronic administration of DDT had no effect on glomerular filtration rate or effective renal plasma flow in dogs (Bing et al., 1946; McNamara et al., 1946); however, acute poisoning of dogs with endrin increases renal vascular resistance and decreases glomerular filtration rate and urine flow (Reins et al., 1964). Experiments with adrenalectomized dogs and with drugs indicate this response is hormonal. In the same study, chronic doses of endrin did not appreciably affect kidney function. Kosova (1959) found that chlorindane* given to rabbits in chronic doses caused an alkaline urine which eventually resulted in a depletion in blood alkaline reserve resulting in death.

Hepatic Function

Reports of hepatic lesions which result from DDT poisoning are described in section C. Liver function has been measured in dogs chronically poisoned with DDT by Bing et al. (1946) who found a positive formaldehyde gel test in 7 of 10 dogs tested. This test is indicative of a disturbance in the globulin fraction of plasma and positive gel test values in most dogs occurred 2 to 3 weeks after ingestion of the drug. Some dogs showed elevated serum bilirubin levels indicative of liver damage but only after extreme poisoning.

*See Appendix for chemical name

Cell Cultures

Human cell cultures have been used as a technique for estimating the relative toxicity of the various classes of insecticides (Gabliks and Friedman, 1965). The toxic dose at which half of the cells were affected (TD_{50}) or the dose which inhibited growth and protein synthesis (ID_{50}) were used as end points. Gabliks (1965a) found that when these cell cultures were grown in media, with chronic sublethal levels of insecticides the cultures became 1.7 to 3.0 times more tolerant to subsequent acute doses of the same insecticides. When cells were treated with Cygon*, Dipterex, DI-Syston*, chlordane* and Karathane*, they were more susceptible to poliovirus than untreated cells (Gabliks, 1965b). Malathion did not affect susceptibility of cells to poliovirus. In the same study, Karathane caused a ten-fold increase in the resistance of cells to diphtheria toxin while all of the previously mentioned materials had no effect.

Central Nervous System

Jenkins and Toole (1964) reported observing motor polyneuropathy in two human patients after one was exposed to DDD and aldrin and the other to DDT and endrin. Paralysis has been produced in chickens with Trithion*, malathion, TOCP*, mipafox*, DFP* and EPN but the syndrome with some materials differs in appearance and onset (Barnes and Denz, 1953; Frawley et al., 1956; Witter and Gaines, 1963).

Douglas and Davis (1965) report that when Bobwhite quail, which had been trained to respond to a discrimination program, were fed sublethal levels of DDT for 8 to 16 weeks and then exposed to a new discrimination program control quail committed significantly fewer errors than those receiving 20 ppm DDT in their diet. Durham et al. (1965) tested workers exposed to organophosphorus

*See Appendix for chemical name

insecticides and observed no difference in mental alertness when there was an absence of clinical symptoms of toxicity. Where individuals showed symptoms of organophosphorus poisoning test scores were lowered but progressive improvement occurred as patients showed recovery.

Medved et al. (1964) discuss the use of conditioned reflexes as a measure of safe levels of pesticides. The authors suggest that this method will find wider usage.

Enzymes

Organic phosphate insecticides have been shown to inhibit cholinesterase in cattle (Goulding, 1962), chickens (Witter and Gains, 1963), Japanese quail (Shellenberger et al., 1965) and fish (Weiss, 1961). A more extensive review of this effect in other species by a variety of organic phosphorus materials is described by O'Brien (1960). Crevier et al. (1954) have reported an increase in serum esterase levels in rats given acute or chronic doses of aldrin.

The insecticide DDT has been shown to increase the activity of succinic dehydrogenase, cytochrome oxidase, oxalacetic carboxylase and pyruvic carboxylase in in vitro studies (Torda and Wolff, 1950a, 1950b, 1951). Judah and Williams-Ashman (1949) were unable to show any change in phosphorus utilization, oxygen uptake, or P/O ratios of mammalian kidney extracts when DDT was added to the respiration flasks. Gul'ko et al. (1965) measured a decrease in rat serum carbonic anhydrase following poisoning with hexachlorobutadiene.

Embryogenesis

Marliac (1964) found that organophosphates and carbamate insecticides exhibited marked teratogenic effects on the chick embryo while chlorinated hydrocarbons did not produce this effect. McLaughlin et al. (1963) suggested that injection of insecticides into the yolk sac of the hen's egg prior to incubation provides a

sensitive measure of toxicity and makes it possible to observe how this compound might affect embryogenesis as the yolk sac is much like the placental barrier in mammals. Clegg (1964), on the other hand, found the response of the chick embryo quite variable in relation to its responses to pesticides and suggests that large numbers of eggs would be necessary to overcome this variability.

C. Gross and Microscopic Lesions

Microscopic lesions in the livers of rats poisoned with DDT have been described in detail (Ortega, 1962). Fitzhugh and Nelson (1947) have reported measuring significant increases in mean liver weight when rats were fed a diet containing 400 ppm DDT even though total body weight was significantly reduced at this level. In the same experiment, kidney weights were significantly increased at a dietary level of 600 ppm DDT. Protein dystrophy has been reported in rat livers when hexachlorobutadiene was administered orally (Gul'ko et al., 1965). Reins et al. (1964) reported congestion and swelling of the liver, lungs and spleen following acute or chronic endrin poisoning in dogs. Nelson and Woodard (1949) observed adrenal cortical atrophy and fatty degeneration of the liver of dogs fed 50 to 200 mg of DDD per kg body weight per day.

Durham et al. (1963) were able to detect no clinical signs of illness consistent with DDT poisoning and no liver histopathology in monkeys fed DDT at dietary levels of 200 ppm or less for periods up to 7.5 years. All of six monkeys fed diets containing 5000 ppm of DDT showed classical DDT symptoms.

Coburn and Treichler (1946) have observed necrosis in the liver and kidneys of rabbits fed 2000 ppm DDT in the diet for a period of more than three days. Liver degeneration has also been reported in pheasants poisoned with DDT (Rudd and Genelly, 1955; Genelly and Rudd, 1956a). Burlington and Lindeman (1950)

observed a reduction in the size and development of the testes of White Leghorn cockerels injected with 15 to 30 mg of DDT per kg weight from eight to 89 days of age.

Myelin degeneration has been reported in the spinal cord and peripheral nerves of hens poisoned with TOCP, EPN, and mipafox but hens poisoned with Systox*, parathion*, malathion, isopropyl-parathion* and methyl-parathion* did not exhibit demyelination (Barnes and Denz, 1953; Frawley et al., 1956).

D. Nutritional Interactions

Stickel et al. (1965) found that woodcocks in good condition, as reflected by body weight, were less affected by heptachlor than similar birds in poor body condition. Krishnamurthy et al. (1965) found that when 25 ppm dieldrin was fed to rats, the toxicity, as measured by growth depression, liver degeneration and tissue residue level, was manifested more in rats on a poor rice diet than in rats on a nutritionally adequate synthetic diet.

Sheila et al. (1965) found that, when carotene was used as part of the vitamin A source, liver stores of vitamin A were reduced in adult rats fed diets containing 100 ppm DDT. The vitamin A stores of young rats from these adults were not lowered. Phillips and Hidiroglou (1965) reported that when forage, sprayed with 1.5 pounds of DDT per acre, was fed to yearling steers liver vitamin A stores were significantly reduced and blood serum levels of vitamin A were increased. A similar test with MCPA* did not affect vitamin A levels.

E. Genetic Pesticide Interactions

Ozburn and Morrison (1964) have shown that when a strain of mice was selected under DDT pressure (intraperitoneal injection of DDT in sesame oil) these mice developed a definite DDT tolerance by the tenth generation. This increased tolerance extended to the related chemicals lindane and dieldrin when administered in a like manner.

*See Appendix for chemical name

F. Relation of Pesticide Levels in the Central Nervous System and Toxicity

Dale et al. (1963) have reported that the clinical symptoms of DDT poisoning and the resultant death are closely correlated with the level of DDT in brain lipids but not with levels in the liver, kidney, body fat or plasma. Azevedo (1965) concluded that DDT residue levels in the brain may be used as an indicator of DDT intoxication in pheasants.

G. Carcinogenicity

Oser (1962) reviews the subject of the possible carcinogenicity of pesticides in detail and points out that there are many problems in determining when cancer has been produced in an animal. Furthermore, most pesticides exhibit carcinogenic effects only at very high levels in animals and many compounds which induce cancer in animals have no recognized relation to cancer in man (Oser, 1962; Durham, 1963).

III. OBJECTIVES

A. To determine what effects sublethal dietary levels of pesticides may have on Japanese quail. Special emphasis in these studies is placed on reproduction, liver function, blood parameters, metabolic rate and microscopic examination of tissues.

B. To evaluate physiological tests which may be used to compare the effects of a variety of pesticides on graminivorous birds.

C. To examine the possibility of injecting pesticides into embryonating eggs as a measure of their relative toxicity.

IV. EXPERIMENTAL PROCEDURE

A. Pesticide Materials

A chlorinated hydrocarbon, a carbamate, an organic phosphate and an herbicide were used in these tests. DDT (Dichlorodiphenyltrichloroethane) was selected as a standard chlorinated hydrocarbon because of its extensive commercial use as an insecticide and because its effects in biological organisms have been widely studied.

Zectran* (4-dimethylamino 3,5-xylyl methylcarbamate) was selected as a representative carbamate material. Zectran is a general purpose insecticide for use on ornamental plants. Zytron* (O-(2,4-dichlorophenyl) O-methyl isopropylphosphoramidothioate) was selected as a representative organic phosphate material. Zytron is used to control crab grass and other undesirable plants when applied to lawns. Tordon* (4-amino-3, 5,6-trichloropicolinic acid) was selected as a representative herbicide. Tordon is used to control woody plants.

All of the pesticides used were technical grade materials which contained 98 - 99% active ingredient.

B. Egg Injection Studies

Fertile chicken eggs were obtained from randomly mated Leghorn type hens. These eggs were randomly partitioned into groups for injection with different levels of the pesticide to be studied. The eggs were then incubated as described in section D until the fifth day of incubation, when they were removed for injection. The solutions and equipment used for the injection procedure were sterilized. The eggs were transluminated with a candling light and the air cell of each was outlined with a pencil. A site for injection which was relatively free of blood vessels was selected and marked within a 3/4 inch radius of the air cell. The shell was wiped with alcohol and punctured directly over the air cell

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and at the marked injection site. A Vir Tis precision egg punch was used to puncture the shell. This punch made a small clean hole in the shell without puncturing the outer shell membrane. The outer shell membrane was punctured over the air cell with a sterile dissecting needle to allow air to escape. The pesticide solution was then injected directly into the yolk sac using a 1.0 cc tuberculin syringe and a one inch, 22 gauge needle. After injection, the egg was sealed with melted paraffin and returned to the incubator.

Corn oil was used as a solvent for the pesticide materials to be injected. The dose of pesticide was expressed as mg of pesticide per kg of egg content assuming as average egg content of 50 gms. Eggs which were infertile or abnormal in size, shape, interior quality or shell quality were not used for these studies.

C. Rations

A 25 percent protein basal ration was formulated for use in the quail studies. The formula of this ration is given below.

Ingredient	Percent of ration
Ground yellow corn	41.25
Soybean oil meal, dehulled 50% protein	37.00
Alfalfa meal, 17% protein	5.00
Dried whey	2.50
Meat and bone scraps, 50% protein	2.50
Fish meal, Menhaden 60% protein	2.50
Ground limestone ($CaCO_3$)	5.00
Dicalcium phosphate	1.50
Salt, iodized	0.50
Fat	2.00
Vitamin premix, NOPCO M-4	0.50
	100.25

Pesticide materials were combined with ten grams of powdered sucrose and a sufficient quantity of finely ground

soybean oil meal to bring the total premix to 200 gms. This was accomplished with a mortar and pestle. The premix was then mixed with 3800 gms of basal ration using a twin shell torsion bar mixer. An equal amount of carrier was mixed with the basal ration for a control. Pesticide materials were weighed to the nearest 0.1 mg. The rations were stored in three gallon pasteboard ice cream cartons which were disposable. All rations were fed ad libitum except as noted in special cases. Feed consumption in most studies was measured on a treatment basis, not on individual birds.

Feed Preference Studies

Six female quail were placed in individual cages which had two holes in the front to allow the bird to eat. Next to each hole a five ounce paper Dixie cup was placed with a notch cut in one side so that each quail could easily reach feed placed in either cup. After the basal ration had been fed for several days so that quail were adjusted to the new feeding arrangement, 20 gms of a ration containing pesticide was placed in one cup while the same amount of control ration was placed in the other. The pesticide was placed in the left cup on three cages and in the right cup on the other three. Once each day the feed was weighed to determine consumption and the cups were refilled with feed and reversed in position on each cage.

D. Housing, Equipment and Incubation

Quail were held in individual cages five inches wide and eight inches long. One or two quail were placed in each cage depending upon the objectives of the particular experiment. Cages were constructed from 1 inch x 1/2 inch welded wire with an inclined floor to allow eggs to roll to the front of the cage.

Feeders used were constructed from galvanized steel with a 3/4 inch lip on the edge to prevent feed wastage. The feeder was filled not more than half full and feed was covered with 1/2 inch mesh hardware cloth which virtually eliminated feed wastage.

This made possible a more accurate measure of feed and pesticide intake.

Eggs were incubated in Jamesway 252 forced-draft incubators. Setting units were operated at 99 - 100 degrees Fahrenheit, dry bulb temperature and 85 - 87 degrees Fahrenheit wet bulb temperature. Hatching units were operated at 98 - 99 degrees Fahrenheit dry bulb temperature and 88 - 92 degrees Fahrenheit wet bulb temperature. Chicken eggs were transferred to the hatching unit on the 18th day of incubation. Japanese quail eggs were set in chicken egg flats which were cut into strips to fit into the egg trays. Quail eggs were transferred to a hatching unit on the 14th day of incubation. Any quail eggs which had not hatched by the 18th day of incubation were broken out and examined macroscopically to determine fertility and embryo mortality.

E. Tissue Sections

Tissues were excised as rapidly as possible after quail died or were sacrificed by cervical dislocation. Sections were made from pituitary, adrenals, thyroids, brain, spinal cord, peripheral nerve, lungs, kidneys, liver, spleen, testes, ovary and oviduct. Sections were routinely stained with hematoxylin and eosin stain (H & E) and with a Trichrome stain. Adrenals were also stained for neutral fat with oil red O. In all experiments tissues were taken from appropriate controls for comparison.

F. Oxygen Consumption

Oxygen consumption was determined on a group basis using a closed circuit method as described by MacLagan and Sheahan (1950). A large desiccator jar connected to a mercury manometer was used for determinations. An open petri dish containing 50 ml of Wilson's soda lime was first placed in the bottom of the desiccator. Above this dish, a paper baffle was located such that it would deflect droppings away from the desiccant without obstructing air movement. Six quail were weighed and placed in a specially constructed cage

which fit into the desiccator. The cage floor was oriented in the position which the desiccator plate would normally occupy. The desiccator was then sealed with the cover using stopcock grease and was connected to a mercury manometer with tygon tubing. A three-way stopcock was placed in the line between the desiccator and the manometer and connected to a vacuum line and a rubber expansion bag. With the three-way stopcock closed the expansion bag was filled with oxygen. The circuit was then opened to the vacuum line until the vacuum pressure was equal to -200 mmHg. The stopcock was reversed to charge the chamber with oxygen. When the pressure returned to zero the stopcock was again closed. This entire system was placed inside an incubator which provided complete darkness and maintained a constant air temperature of 72 - 76° F. on the outside of the desiccator.

Thirty minutes were allowed for the system to come to temperature equilibrium and then pressure readings were taken every 15 minutes until three consistent readings were obtained. The temperature was recorded with each reading from thermometers located inside each desiccator. Oxygen consumption was computed directly from the change in pressure, the temperature and the net volume of the unit. Oxygen volumes were adjusted to standard temperature and pressure using appropriate conversion factors. In order to determine the net volume of the desiccator unit the system was first carefully calibrated and the volume of the materials added was subtracted. In this experiment the two units used were calibrated with the cage, paper, petri dish and thermometer in place. The volume of the desiccant and of the quail was deducted therefrom. On the basis of a report by Ernst and Ringer (1966) in which the specific gravity of male and female Japanese quail was found to be 0.977 and 0.995 respectively, a volume of one millimeter was deducted for each gram of body weight.

Quail were fasted for 24 hours before the determinations were made. All determinations were completed in a four to five

hour period to minimize the effect of a possible diurnal rhythm which has been reported to occur in chickens (Barott et al., 1938).

G. Blood Parameters

Blood for smears, total erythrocyte counts, packed erythrocyte volume determinations and serum for total serum protein and electrophoretic separation was obtained by brachial venipuncture. Blood smears were stained with Wright's stain. All differential leucocyte counts were made in duplicate.

Packed erythrocyte volume was determined by collecting two 1.5 mm x 75 mm heparinized capillary tubes of blood from each quail. The tubes were centrifuged at 11,500 rpm for five minutes in an International microhematocrit centrifuge. The mean of the two determinations was used as an observation.

Blood for total red cell counts was diluted 1:200 with Gower's solution in dilution pipettes. Two dilutions were made on blood from each quail. The samples were taken directly from the brachial veni-puncture with aspiration pipettes. One count was made from each pipette in a hemocytometer. The two counts were averaged. Mean corpuscular volume was determined by dividing the packed cell volume by the total erythrocyte count as described by Schalm (1961). The volumes were converted to μ^3 using appropriate conversion factors.

Serum for total serum protein determination was obtained using 1.5 mm x 75 mm plain capillary tubes. A minimum of one hour was allowed for clotting after which the tubes were centrifuged at 11,500 rpm for fifteen minutes in an International microhematocrit centrifuge.

Total serum protein was determined spectrophotometrically as described by Waddell (1956). The procedure involves determination of absorption at 215 mμ and 225 mμ on serum diluted 1:1000 with saline (0.9 gm percent).

A Beckman DU spectrophotometer was used to measure absorption. The difference between the two absorption measurements times 144 gives the protein concentration in micrograms per milliliter.

Serum, to be separated by agar gel electrophoresis, was harvested by the technique previously described for total serum protein determinations. Serum was placed directly on the electrophoresis strip from the capillary tubes.

Agar gel electrophoresis was accomplished as outlined by Brent (1965). Four milliliters of a one percent solution of Oxoid "Ionagar" #2 in buffer was spread on 35 mm film leader strips 16 cm long. After the agar solidified 3 - 10 µl of serum were applied in an even band across the center of the strips. The strips were placed in a modified Beckman-Spinco-Durrum cell with ends submerged in the buffer. A barbital buffer was used in the cell and for the agar solution (pH 8.6, ionic strength 0.037). Electrophoresis was carried out for 45 minutes at 200 volts D.C. and a current of 85 milliamps.

After electrophoresis was completed the strips were removed, placed in a staining rack and dried in an oven at 110° C. After drying the strips were stained with thiazine red and decolorized in two baths of five percent acetic acid. When completely dry they were scanned using a Beckman RB Analytrol equipped with a B-2 cam and 500 mµ interference filters. Strips were driven with a Gelman "Scan-a-tron". Integration with the Analytrol allows quantitation of the fractions.

Plasma alkaline phosphatase was determined by the method of Bessey *et al.* (1946). Blood was collected in a heparinized syringe by direct heart puncture and centrifuged at 0° C. to obtain plasma. The assay is based upon the liberation of the phosphate group from p-nitrophenyl phosphate to yield the yellow salt of p-nitrophenol (absorption maximum, 400 mµ). Plasma samples (0.02 ml) were incubated with the buffered reagent for 30 minutes at 38° C. and

the reaction was stopped by adding 4 ml of 0.02 N NaOH. Optical density was determined with a Spectronic 20 at 400 mμ. Following addition of HCl, which converted the yellow sodium salt of p-nitrophenol to colorless free nitrophenol, a second reading was taken which served as a blank. Appropriate p-nitrophenol standards were prepared and handled in the same manner as serum samples. Values are expressed as mM p-nitrophenol liberated per liter of plasma per hour. One mM of p-nitrophenol liberated per liter of serum per hour is approximately equal to 1.8 Bodansky units (Bessey et al., 1946).

H. Body Weight

In some experiments body weights were measured. When measured quail were weighed to the nearest gram using a Fisher direct reading balance.

V. RESULTS AND DISCUSSION

A. Egg Injection Studies

An investigation was made of the acceptability of solvents in regard to their toxicity to the developing embryo. Ethanol and propylene glycol caused severe mortality of embryos while corn oil and olive oil proved to be non-toxic in doses up to 1/2 cc per egg. One disadvantage of using these oils as solvents for injection tests is their relatively high viscosity which made it necessary to use a large bore needle for injections. Preliminary tests comparing injection sites and procedures indicated that sterile conditions were necessary to obtain consistent results and keep mortality, resulting from the injection technique itself, at a minimum. Four injection approaches were compared. Eggs were injected from the side, small end, large end through the air cell or from the large end close to but not through the air cell. Injecting into the large end near the air cell as described in the procedure gave the best results in our laboratory; injection through the air cell gave acceptable but less consistent results. Injection into the yolk sac was compared with injection into the albumin. The yolk sac proved to be a superior site for injection with less mortality resulting from this technique.

The results of injecting DDT, Zectran, Zytron and Tordon into embryonating eggs are shown in Tables 1 through 4. DDT at levels up to 1000 mg/kg did not cause an increase in embryo mortality; however, chicks which hatched exhibited tremors. Zectran at levels as low as 50 mg/kg killed all of the embryos; however, the length of time the embryos survived after injection with Zectran seemed to be proportional to the dose administered. Thus it appears that by injecting smaller doses of Zectran and using the length of survival after injection as the criteria of evaluation it would be possible to obtain a dose response curve for this material in embryonating eggs. Some embryos which died exhibited extraembryonic hemorrage and deformities of other types.

Zytron was less toxic than Zectran with 100 mg/kg being well tolerated by the embryos. Higher levels caused severe mortality. Some of the embryos which died at the higher levels exhibited various degrees of deformity and extraembryonic hemorrage. Embryo mortality following Zytron injection occurred toward the end of the embryonic period. Tordon was more toxic than Zytron with 50 mg/kg causing severe mortality. Abnormalities were not observed in these dead embryos.

Considerable variation was observed in the response of embryonating eggs to the four classes of pesticides. Furthermore, the apparent toxicity that these materials exhibited when injected into embryonating eggs does not agree with the toxicity exhibited in oral toxicity studies to be described later. These results agree with those of Clegg (1964) who found the response of the chick embryo to pesticides quite variable. Relatively large numbers of eggs were required in this study and the technique required considerable care and experience for consistent results.

B. Oral Toxicity Studies

Feed and Pesticide Intake

An investigation of the quantities of pesticide a bird might obtain from its environment was not within the scope of this project. The pesticide levels administered to quail in this study were based only on the physiological effects and mortality rates observed. In order to search for a physiological test which might be more or less applicable to all pesticide materials, it was deemed most profitable to administer dietary levels which would cause some mortality.

When a pesticide is incorporated into the diet, the dose received by the bird becomes dependent upon the dietary level, daily consumption of feed and the number of days involved. In this

Table 1 -- The effect of injecting DDT into embryonating chicken eggs on the 5th day of incubation

Treatment[1]	Number of Eggs Injected	Dead Embryos 6-10 days	Dead Embryos 11-21 days	Hatched	Pipped
Control[2]	30	1	1	28	--
Control-punctured sealed	30	--	2	28	--
Control-1/2 cc corn oil	30	4	6	20	--
DDT-50 mg/kg in 1/4 cc corn oil	30	7	3	20	--
DDT-100 mg/kg in 1/2 cc corn oil	30	8	6	16	--
DDT-250 mg/kg in 1/4 cc corn oil	30	4	3	23	--
DDT-500 mg/kg in 1/4 cc corn oil	30	1	4	25	--
DDT-1000 mg/kg in 1/2 cc corn oil	30	3	5	20	2

[1] Eggs were randomly partitioned into eight groups for injections

[2] These eggs were held under the same conditions as the treated groups for 21 days

Table 2 -- The effect of injecting Zectran into embryonating chicken eggs on the 5th day of incubation

Treatment[1]	Number of Eggs Injected	Dead Embryos 6-10 days	Dead Embryos 11-21 days	Hatched	Pipped
Control[2]	29	--	4	23	2
Control-punctured sealed	30	2	3	25	--
Control-1/2 cc corn oil	29	7	5	13	4
Zectran-50 mg/kg in 1/4 cc corn oil	29	9	20	--	--
Zectran-100 mg/kg in 1/2 cc corn oil	30	11	19	--	--
Zectran-250 mg/kg in 1/4 cc corn oil	30	14	16	--	--
Zectran-500 mg/kg in 1/4 cc corn oil	30	14	16	--	--
Zectran-1000 mg/kg in 1/2 cc corn oil	30	21	9	--	--

[1] Eggs were randomly partitioned into eight groups for injections

[2] These eggs were held under the same conditions as the treated groups for 21 days

Table 3 -- The effect of injecting Zytron into embryonating chicken eggs on the 5th day of incubation

Treatment[1]	Number of Eggs Injected	Dead Embryos 6-10 days	Dead Embryos 11-21 days	Hatched	Pipped
Control[2]	30	1	1	27	1
Control-punctured sealed	30	--	7	22	1
Control-1/2 cc corn oil	29	4	4	18	3
Zytron-50 mg/kg in 1/4 cc corn oil	30	6	4	19	1
Zytron-100 mg/kg in 1/2 cc corn oil	30	6	4	17	3
Zytron-250 mg/kg in 1/4 cc corn oil	25	2	13	5	5
Zytron-500 mg/kg in 1/4 cc corn oil	30	3	24	--	3
Zytron-1000 mg/kg in 1/2 cc corn oil	30	5	25	--	--

[1] Eggs were randomly partitioned into eight groups for injections

[2] These eggs were held under the same conditions as the treated groups for 21 days

Table 4 -- The effect of injecting Tordon into embryonating chicken eggs on the 5th day of incubation

Treatment[1]	Number of Eggs Injected	Dead Embryos 6-10 days	Dead Embryos 11-21 days	Hatched	Pipped
Control[2]	20	1	4	14	1
Control-punctured sealed	20	2	2	14	2
Control-1/2 cc corn oil	24	7	8	8	1
Tordon-50 mg/kg in 1/4 cc corn oil	30	21	7	2	--
Tordon-100 mg/kg in 1/2 cc corn oil	29	21	7	1	--
Tordon-250 mg/kg in 1/4 cc corn oil	30	23	7	--	--
Tordon-500 mg/kg in 1/2 cc corn oil	23	16	7	--	--

[1] Eggs were randomly divided into seven groups for injections

[2] These eggs were held under the same conditions as the treated groups for 21 days

study the dietary level should not be the only factor considered in comparing these pesticides. In many of these experiments dietary levels were selected on the basis of the level of mortality which they had produced in a previous feeding experiment. This was an attempt to achieve the same degree of toxicity at approximately the same time facilitating easier experimentation, and was not in any way intended to reflect commercial application levels.

Rations containing DDT had little effect on feed consumption (Tables 5 and 6) although 500 ppm for 55 days (Table 5) may have depressed consumption to some extent. The high mortality of females, which normally eat more feed than males, may partially explain this decrease in feed consumption with diets containing 500 ppm DDT. Cross et al. (1962) also found that dietary levels of 500 ppm DDT caused Japanese quail to reduce their voluntary feed intake while lower levels did not affect intake.

Zectran at a level of 300 ppm caused quail to severely reduce their feed intake while a dietary level of 100 ppm Zectran caused only a very small feed restriction (Tables 5 and 6). Zytron also caused quail to severely restrict their feed intake at levels of 2000 ppm and 4000 ppm (Tables 5 and 6). Tordon at levels up to 10,000 ppm had no effect on feed consumption.

Since quail were observed to voluntarily restrict their feed intake when sufficient levels of some pesticides were incorporated into the ration, a series of experiments was conducted to determine whether quail could differentiate between control rations and rations containing pesticides. When examining the results of this test two factors should be kept in mind. These rations were offered to quail in two separate but identical sites which were changed every day so that the location of the control feed could not be learned by quail. Also, only 20 gms of each ration were offered to one quail for a 24 hour period as previous consumption had

averaged less than 20 gms per quail per day. In this experiment, however, feed consumption (including wastage) was greater than 20 gms per quail per day (Table 7). In order to consume feed at this higher level quail had to eat some of the pesticide feed. The higher consumption which was measured in this experiment appeared to be due to increased feed wastage rather than intake.

Quail consumed significantly smaller amounts of rations containing Zectran or Zytron than of a control ration (Table 7). Consumption of rations containing DDT was not significantly different from consumption of the control ration. From these data it appears that birds might reject food contaminated with Zectran or Zytron if clean food was available. This might greatly reduce the hazard of these pesticides to wild birds.

Of course, the conditions in this experiment were somewhat artificial and only one species of birds was tested. In this experiment the pesticides were mixed in a prepared ration while under field conditions the pesticides would be sprayed or dusted on the feed materials. However, it would seem logical that the pesticides would be more likely to repulse birds when sprayed or dusted over the feed materials.

The initial reduction in feed intake which occurred when Zectran or Zytron was incorporated into the ration of quail seemed to have occurred because these rations were lower in palatability. As symptoms of pesticide poisoning began to appear feed consumption dropped even lower and ceased entirely as poisoning became severe. This later type of feed restriction apparently resulted from the toxicity of the pesticide. It was this later type of restriction which seemed to result from certain levels of DDT in the ration. When Zectran and Zytron were incorporated into the ration both types of restriction appeared to occur.

Representative daily pesticide intakes are shown in Tables 5 and 6. These levels of daily pesticide consumption are based on the calculated dietary levels.

Mortality

The dose of a pesticide which will cause the death of half of the subjects treated is widely used to compare the relative toxicity of pesticides to various species of animals. This is usually referred to as the lethal dose fifty (LD_{50}) of the pesticide for that species (Metcalf, 1955). Mortality was used in these experiments as an index of the toxicity of the pesticide which was being administered. As mortality was reasonably consistent throughout these experiments only representative data are presented.

When the insecticide DDT was incorporated into the diet of quail, mortality was higher among females than males probably due to their higher pesticide intake (Tables 5 and 6). Table 6 shows that when Zectran (300 ppm) and Zytron (2000 ppm) were fed for 25 days, mortality of males exceeded that of females. In another experiment, when Zectran (300 ppm) was fed for 23 days, mortality of females exceeded that of males (Table 20). Also, Zytron (300 ppm) when fed for 12 days has caused greater mortality of females than males (Table 21). These differing results may have resulted from the fact that in the later experiments quail were intermittently fasted for a 24 hour period preceding a determination of oxygen consumption.

In all experiments mortality was proportional to the levels of pesticide in the diet and the length of the feeding period. Zectran was the most toxic followed closely by DDT. Zytron was less toxic to quail and levels of 1500 ppm were necessary to cause mortality within 20 days. Quail receiving Tordon in their diets for nearly a year at levels which were increased from

Table 5 -- The effect of feeding rations containing DDT for 55 days, Zectran for 29 days and Zytron for 11 days, on the mean feed consumption, mortality and the mean amount of pesticide ingested by Japanese quail

Ration	Number of Quail at Start of Experiment Male	Female	Percent Mortality	Bird Days	Feed Consumed (gm/bird/day)	Pesticide Consumed (mg/bird/day)
Control	6	6	25.0	423	14.9	----
DDT (300 ppm)	6	6	58.3	310	15.8	4.7
DDT (500 ppm)	6	6	91.6	244	11.4	5.7
Control	6	4	0.0	170	15.5	----
Zectran (100 ppm)	6	6	8.3	204	13.0	1.3
Zectran (300 ppm)	6	5	18.2	176	9.5	2.8
Control	12	10	4.5	235	14.7	----
Zytron (2000 ppm)	12	12	20.8	258	10.2	20.3
Zytron (4000 ppm)	12	12	37.5	248	9.4	37.6

Table 6 -- The effect of feeding rations containing DDT, Zectran and Zytron for 25 days on mortality, feed consumption and the amount of pesticide ingested by Japanese quail

Ration	Sex	Number of Quail at Start of Experiment	Mortality (percent)	Feed Consumed (gm/bird/day)	Pesticide Consumed (mg/bird/day)
Control	Female	12	0.0	18.4	----
Control	Male	12	0.0	13.2	----
DDT (500 ppm)	Female	12	33.3	16.2	8.1
DDT (500 ppm)	Male	12	16.7	13.1	6.6
Zectran (300 ppm)	Female	12	50.0	8.9	2.7
Zectran (300 ppm)	Male	12	66.7	8.0	2.4
Zytron (2000 ppm)	Female	12	83.3	9.6	19.2
Zytron (2000 ppm)	Male	12	91.7	9.5	19.0

Table 7 -- The effect of offering a control ration and rations containing pesticides to Japanese quail females

Ration	Number of Quail	Mean Feed Consumption (gm/quail/day)	F Ratio
Control	6	13.60 ± 0.83[1]	1.30
DDT (500 ppm)	6	12.24 ± 0.32	
Control	6	15.36 ± 0.15	21.71**
Zectran (300 ppm)	6	9.91 ± 0.71	
Control	6	16.96 ± 0.49	273.37**
Zytron (2000 ppm)	6	5.73 ± 0.62	

[1] Mean ± standard error

** Significant difference between control and pesticide ration ($P < 0.01$)

100 ppm to 10,000 ppm had a lower mortality rate than controls. On the basis of these data oral toxicity studies with Tordon were discontinued; however, it is interesting to note that Tordon was very toxic when injected into embryonating eggs causing high embryo mortality at levels as low as 100 mg/kg.

Reproduction

Reproduction is an important function essential to the maintenance of any species of birds. Numerous reports can be found in the literature suggesting or demonstrating that pesticides have adverse effects on avian reproduction.

It is well established that normal reproduction requires the secretion of various hormones by the pituitary and its associated endocrine glands. For this reason observing reproduction is one way of determining whether certain endocrine glands are being adversely affected by pesticide feeding.

The results of feeding rations containing DDT, Zectran and Zytron on reproduction of mature Japanese quail are shown in Tables 8, 9 and 10. The data in each table were collected in two separate experiments. The data shown above the broken line were collected in experiments which were designed to ascertain pesticide toxicity and obtain tissues for histological study. Egg production was not analyzed in this series of experiments due to the small numbers of birds.

The data shown below the broken line in Tables 8, 9 and 10 were collected in an experiment designed to determine the effects of pesticides on oxygen consumption, packed cell volume and differential leucocyte count. The quail in these experiments were subjected to a 24 hour fast at the start of the experimental period. This fast was associated with oxygen consumption measurements.

Fertility, hatchability and livability were determined on a group basis and could not be analyzed statistically in these experiments. When Zytron was fed at levels of 2000 ppm and 4000 ppm, egg numbers were small and eggs were not set to determine fertility or hatchability (Table 10). Egg production of these birds was above 80 percent before the initial fast and the control group produced at a rate of only 46.7 percent during the subsequent 12 day experimental period. This was apparently due to fasting as both control and treated quail were affected.

The intensity and persistency of egg laying in these experiments is indicated by the percent hen day egg production shown in Tables 8, 9 and 10. Diets containing Zectran and Zytron caused a reduction in hen day egg production but diets containing DDT did not. All of the pesticides tested caused a reduction in the number of eggs per hen. This decrease resulted from mortality and/or a reduction in hen day egg production in these experimental groups. Egg production and eggs per hen in the second series of experiments (below the broken lines in Tables 8, 9 and 10) were analyzed and found to be not significantly different.

The incorporation of DDT in the diet of mature quail did not affect fertility, hatchability or percent hatch of fertile eggs but severely reduced the survival of chicks to 13 days of age (Table 8). Dewitt (1956) obtained similar results when feeding DDT to Bobwhite quail and Azevedo (1965) reported that DDT feeding affected pheasant reproduction in a like manner. A similar response has been described following the injection of DDT into embryonating eggs (Section V.A).

Zectran in levels up to 300 ppm had no effect on reproduction in quail (Table 9) other than its previously described effect on egg production. Similarly Zytron did not reduce fertility, hatchability, percent hatch or survival of chicks to 13 days of age at

dietary levels up to 700 ppm (Table 10). Tordon in levels up to 10,000 ppm had no adverse effects on reproduction in this study.

These effects on reproduction indicate that Zectran and Zytron feeding did not cause appreciable residues to be deposited in the eggs while DDT feeding did. This is not surprising considering that Japanese quail hens receiving DDT in their diets continued to lay eggs until they succumbed, often laying an egg on the day preceding death, while Zectran and Zytron diets caused egg production to cease completely after a period of time. Also, DDT is not readily metabolized *in vivo* while carbamate and organophosphate insecticides are (Metcalf, 1955; O'Brien, 1960).

Table 8 -- The effect of feeding rations containing DDT on egg production, fertility, hatchability, percent hatch and the survival of chicks

Ration	Number of Quail Females	Days on Test	Eggs per Hen[1,2]	Percent Egg Production[2,3]	Percent Fertility	Hatch-ability[4]	Percent Hatch[5]	Percent Survival of Chicks to 13 days of age
Control	6	35	30.7	87.6	86.6	71.8	62.2	75.8
DDT (100 ppm)	3	35	34.7	99.0	96.5	72.1	69.6	56.8
DDT (300 ppm)	3	35	25.3	97.4	92.8	61.0	56.5	8.5
Control	6	55	23.0 ± 5.89[6]	59.7 ± 7.86	73.2	32.9	24.1	88.9
DDT (300 ppm)	6	55	16.3 ± 5.07	63.2 ± 9.74	53.7	56.9	30.5	32.1
DDT (500 ppm)	6	55	7.0 ± 1.61	71.2 ± 11.71	74.0	51.4	38.0	26.3

1 Based on the number of hens alive at the start of the experiment

2 No significant differences between means in this group

3 Hen day production

4 Percent of fertile eggs which hatched

5 Percent of total eggs which hatched

6 Mean ± standard error

Table 9 -- The effect of feeding rations containing Zectran on egg production, fertility, hatchability, percent hatch and the survival of chicks

Ration	Number of Quail Females	Days on Test	Eggs per Hen[1,2]	Percent Egg Production[2,3]	Percent Fertility	Hatch-ability[4]	Percent Hatch[5]	Percent Survival of Chicks to 13 days of age
Control	6	39	25.2	89.9	86.6	71.8	62.2	75.8
Zectran (100 ppm)	3	39	18.0	90.0	100	71.7	71.7	71.4
Zectran (300 ppm)	3	39	1.3	13.3	100	75.0	75.0	100
Control	5	29	17.6 ± 4.53[6]	74.6 ± 8.05	43.6	67.6	29.5	87.5
Zectran (100 ppm)	6	29	16.7 ± 2.93	62.9 ± 8.41	93.2	82.9	77.3	93.2
Zectran (300 ppm)	6	29	9.2 ± 2.23	46.2 ± 10.09	67.3	45.5	30.6	85.7

1 Based on the number of hens alive at the start of the experiment

2 No significant differences between means in this group

3 Hen day production

4 Percent of fertile eggs which hatched

5 Percent of total eggs which hatched

6 Mean ± standard error

Table 10 -- The effect of feeding rations containing Zytron on egg production, fertility, hatchability, percent hatch and survival of chicks

Ration	Number of Quail Females	Days on Test	Eggs per Hen[1,2]	Percent Egg Production[2,3]	Percent Fertility	Hatch-ability[4]	Percent Hatch[5]	Percent Survival of Chicks to 13 days of age
Control	3	106	46.3	73.5	53.7	27.6	14.8	75.0
Zytron (100 to 500 ppm)	3	106	26.0	65.5	92.1	68.6	63.2	91.7
Zytron (300 to 700 ppm)	3	106	38.0	75.5	87.9	72.4	63.6	85.7
Control	10	12	6.3 ± 0.80[6]	46.7 ± 5.53				
Zytron (2000 ppm)	12	12	4.6 ± 0.44	40.4 ± 5.83				
Zytron (4000 ppm)	12	12	4.2 ± 0.37	37.0 ± 3.03				

1 Based on the number of hens alive at the start of the experiment

2 No significant differences between means in this group

3 Hen day production

4 Percent of fertile eggs which hatched

5 Percent of total eggs which hatched

6 Mean ± standard error

Body Weight

The body weights of quail were measured in several experiments in which these selected pesticides were administered. In all cases the pattern of weight change was the same as that shown in Table 11 with the weight of quail receiving Zectran and Zytron diets being significantly lower than the weight of quail on control or DDT diets. In the single test in which Tordon was fed it had no effect on body weight of quail at levels up to 10,000 ppm. The decrease in body weight of quail receiving Zectran and Zytron in their diets was undoubtedly due largely to their voluntary reduction in feed consumption described in Section 1.

Histological Lesions

Tissues were examined from quail showing terminal symptoms of DDT, Zectran and Zytron poisoning. Sections were prepared from the lungs, liver, pancreas, spleen, kidneys, thyroids, adrenals, pituitary, testes, ovary, oviduct and the central nervous system of quail receiving control diets or diets containing pesticides. Liver sections from quail on DDT and Zytron diets exhibited definite necrosis of parenchyma cells. None of the other tissues showed definite evidence of lesions.

It appears from these studies that histological examination with the light microscope is of limited value as a tool in evaluating possible effects of pesticides. All of the tissues examined in this study were obtained from quail exhibiting severe symptoms of pesticide poisoning and yet histological damage was observed only in livers of quail on DDT or Zytron rations. Furthermore, liver sections from some individual quail in these groups showed no apparent lesions. However, this evidence does suggest the improbability that permanent damage, of a type which might persist after pesticides were completely removed from the birds' environment, could result from these materials.

Table 11 -- The effect of feeding rations containing DDT, Zectran and Zytron on the body weight of female Japanese quail

Ration	Time of Weight Determination	Number of Quail	Body Weight[1] (gms)
Control	Pretrial[2]	12	116.92
Control	After 10 days	12	126.42
Mean Difference[3]			+ 9.50 ± 1.40[a]
DDT (500 ppm)	Pretrial	11	122.09
DDT (500 ppm)	After 10 days	11	137.18
Mean Difference			+ 15.09 ± 2.41[a]
Zectran (300 ppm)	Pretrial	12	123.58
Zectran (300 ppm)	After 10 days	12	101.08
Mean Difference			- 22.50 ± 4.26[b]
Zytron (2000 ppm)	Pretrial	9	128.00
Zytron (2000 ppm)	After 10 days	9	96.88
Mean Difference			- 31.11 ± 5.83[b]
F Ratio Differences			44.8**

[1] Means having the same superscript are not significantly different ($P > 0.05$); other means are significantly different ($P < 0.01$) by the New Multiple Range Test (Duncan, 1955)

[2] These quail were weighed on the day that the test started

[3] Mean difference ± standard error

** Highly significant ($P < 0.0005$)

Oxygen Consumption

Oxygen consumption measurements of male Japanese quail receiving control rations or rations containing pesticide materials are shown in Table 12. With only one group measurement per mean, no statistical analysis of the data was possible. These pesticide materials were tested individually in separate experiments at different times. From an examination of the data collected in individual experiments, it appears that DDT diets caused a small but consistent increase in oxygen consumption which agrees well with the reported increase in oxygen consumption of rats following chronic poisoning with DDT (Riker et al., 1946). Diets containing Zectran may have depressed oxygen consumption slightly but considering the partial starvation which was gradually occurring in these birds, as exhibited by their reduced feed consumption and body weight (Tables 5 and 12), it is highly possible that this reduction reflects an effect of fasting. Barott et al. (1938) has shown that the oxygen consumption of chickens decreases for as long as 36 hours after feed is removed. Diets containing Zytron do not seem to have caused any consistent change in oxygen consumption.

Considering all of the data in Table 12 it appears that if in fact any of these small differences are true differences then several observations comparable to these would be necessary to demonstrate significance. The differences observed here are not of a magnitude which would suggest a significant change in thyroid function. Oxygen consumption measurements have the advantage of being easy and economical to obtain and can be accomplished without sacrificing the birds. However, they showed little or no value as a measure of pesticide toxicity in this study.

Table 12 -- The effect of feeding rations containing DDT, Zectran and Zytron on the oxygen consumption of male Japanese quail

Ration	Time of Determination	Mean Body Weight[1] (gms)	Oxygen Consumption (ml/100 $gm^{.75}$/min.)
Control	Pretrial[2]	104.0	2.38
Control	After 22 days	105.7	2.36
Mean Difference			-0.02
DDT (300 ppm)	Pretrial	112.7	2.28
DDT (300 ppm)	After 22 days	107.2	2.73
Mean Difference			+0.45
DDT (500 ppm)	Pretrial	106.5	2.40
DDT (500 ppm)	After 22 days	105.2	2.84
Mean Difference			+0.44
Control	Pretrial	110.7	2.46
Control	After 19 days	114.7	2.93
Mean Difference			+0.47
Zectran (100 ppm)	Pretrial	107.5	2.49
Zectran (100 ppm)	After 19 days	102.5	2.30
Mean Difference			-0.19
Zectran (300 ppm)	Pretrial	114.0	2.27
Zectran (300 ppm)	After 19 days	92.0	2.25
Mean Difference			-0.02
Control	Pretrial	100.3	2.12
Control	After 11 days	102.2	2.32
Mean Difference			+0.20
Zytron (2000 ppm)	Pretrial	106.0	2.03
Zytron (2000 ppm)	After 11 days	96.3	2.48
Mean Difference			+0.45
Zytron (4000 ppm)	Pretrial	97.5	2.22
Zytron (4000 ppm)	After 11 days	90.3	2.27
Mean Difference			+0.05

[1] Six male Japanese quail per mean

[2] These determinations were made 24 hours after control rations were removed; after determination, quail were given the test rations

Blood Parameters

a. Differential leucocyte count

It is well known that some pathological conditions cause a characteristic change in the differential leucocyte count of mammals. Differential leucocyte counts were made on the blood of quail to identify characteristic changes which might occur following pesticide administration.

Rations containing DDT and Zectran did not cause any consistent changes in the differential leucocyte counts (Tables 13 through 16). The lymphocyte and heterophil percentages were found to be highly variable. In a group of six quail it was not uncommon to find one bird with 70 percent lymphocytes and another with 30 percent lymphocytes.

All quail receiving rations containing Zytron exhibited a consistent increase in lymphocyte percentage with a nearly parallel drop in heterophil percentage (Tables 17 and 18). This increased lymphocyte percentage was significant in males but not in females due to the greater variability in the counts of females. No attempt was made in this study to determine the exact cause for this shift in the differential count and its significance biologically is not clear. Numerous individual control quail had a lymphocyte percentage as low as 30 percent with no apparent adverse effects; however, lymphocytes are normally the predominant leucocyte in Japanese quail. Since only Zytron caused this shift in differential leucocyte counts it appears that its use as a measure of toxicity would at best be limited to organic phosphate pesticides.

Rations containing Zytron also caused a significant decrease in the basophil percentage of female quail (Table 18). This

decrease is not thought to have any biological significance due to the extremely small numbers of basophils in circulating quail blood.

b. Packed erythrocyte volume, total erythrocyte count and mean corpuscular volume

Some pesticide materials have been reported to affect the blood of animals when they are administered orally or by injection. It is well known that anemia can occur when certain vitamins or minerals are not present in the diet of birds or animals in sufficient amounts. It was thought that pesticides might cause a change in the packed cell volume of quail. When such a change was found total erythrocyte counts were made on the blood of anemic quail and mean corpuscular volume was calculated to determine if it was the size or number of erythrocytes which was altered.

When DDT, Zectran or Zytron was incorporated into the diets of mature male Japanese quail a significant decrease in the packed erythrocyte volume (anemia) occurred (Tables 19, 20 and 21). Mature female quail receiving these rations also showed a decrease in packed erythrocyte volume but this decrease was not significant due to higher mortality. When these same three pesticides were incorporated into the diet of immature quail, which were 40 days of age at the beginning of the feeding period, no decline in packed erythrocyte volume could be measured except in extreme cases of poisoning (Table 22). Individual quail did show a severe anemia but these individuals died within hours of the determination indicating that near lethal levels of pesticide were necessary to cause the effect.

It appears that anemia resulting from pesticide poisoning is less pronounced in young or immature quail probably due to their greater erythropoetic potential. Erythrocytes are formed primarily in the red bone marrow which becomes more fatty as the bird ages. It is possible that the tendency of these pesticides to produce

anemia interacts with the erythropoetic potential of the animal. One would normally expect erythropoesis to be proceeding at about one-tenth its potential rate. Thus it seems probable that an erythropoetic block, regardless of where this block might occur, would have to negate more than nine-tenths of the erythropoetic potential of the bird before anemia would result. In younger quail with more red marrow, higher pesticide levels might be required to produce anemia. At certain ages these pesticide levels might exceed lethal levels and death would precede the appearance of anemia.

In a later experiment in which the feed of female quail was restricted to 9 or 10 grams per quail per day, no significant drop in packed erythrocyte volume had occurred after 24 days of restriction. This is in agreement with data collected by Fox and Harrison (1965) who reported no decrease in hematocrit of Japanese quail as a result of 24 to 48 hours of fasting.

In another experiment, DDT, Zectran and Zytron were again incorporated in the rations of mature male Japanese quail and a significant drop in the packed erythrocyte volume occurred (Table 23). The length of time which expired before a drop in the packed erythrocyte volume was measured varied with the pesticide material. In all experimental groups exhibiting a significantly decreased packed erythrocyte volume, the total erythrocyte count was also significantly decreased. In no instance was there a significant change in the mean corpuscular volume of these groups indicating that a normocytic normochromic anemia was occurring following pesticide administration. This indicates that the anemia observed following chronic administration of these selected pesticides in the diet of quail was due to a decrease in the number of circulating erythrocytes and not to the production of abnormally small red blood cells. In no instance was any hypochromia observed

Table 13 -- The effect of feeding rations containing DDT on the differential leucocyte count of male Japanese quail[1]

Ration	Determination	Number of Quail per Mean	Heterophils (percent)	Lymphocytes (percent)	Monocytes (percent)	Eosinophils (percent)	Basophils (percent)
Control	Pretrial	6	26.0	67.9	1.9	3.6	0.7
Control	After 30 days	6	26.8	69.1	0.2	3.4	0.6
Mean Difference[2]			+ 0.8 ± 3.33	+ 1.2 ± 3.05	-1.7 ± 0.29	-0.2 ± 0.96	-0.1 ± 0.25
DDT (300 ppm)	Pretrial	5	20.5	74.4	1.2	3.6	0.5
DDT (300 ppm)	After 30 days	5	24.5	72.8	0.3	2.1	0.5
Mean Difference			+ 4.0 ± 1.97	- 1.6 ± 2.39	-0.9 ± 0.19	-1.5 ± 0.37	0.0 ± 0.30
DDT (500 ppm)	Pretrial	6	22.9	72.5	0.8	3.3	0.5
DDT (500 ppm)	After 30 days	6	23.1	73.0	0.2	3.1	0.7
Mean Difference			+ 0.2 ± 5.99	+ 0.5 ± 6.00	-0.6 ± 0.28	-0.2 ± 0.30	+0.2 ± 0.17

[1] No significant differences found between means in any of these groups of leucocytes

[2] Mean difference ± standard error

Table 14 -- The effect of feeding rations containing DDT on the differential leucocyte count of female Japanese quail[1]

Ration	Time of Determination	Number of Quail per Mean	Heterophils (percent)	Lymphocytes (percent)	Monocytes (percent)	Eosinophils (percent)	Basophils (percent)
Control	Pretrial	5	41.8	52.5	1.8	3.1	1.1
Control	After 10 days	5	47.2	48.8	0.4	3.1	0.5
Mean Difference[2]			+ 5.4 ± 2.85	- 3.7 ± 2.76	-1.4 ± 0.38	0.0 ± 0.62	-0.6 ± 0.41
DDT (300 ppm)	Pretrial	6	27.2	68.0	1.0	3.5	0.5
DDT (300 ppm)	After 10 days	6	28.8	66.2	0.0	4.7	0.4
Mean Difference			+ 1.6 ± 2.89	- 1.8 ± 3.50	-1.0 ± 0.36	+1.2 ± 0.98	-0.1 ± 0.19
DDT (500 ppm)	Pretrial	4	41.5	52.4	0.7	4.5	1.0
DDT (500 ppm)	After 10 days	4	45.3	51.3	0.0	2.6	0.9
Mean Difference			+ 3.8 ± 3.41	- 1.1 ± 4.22	-0.7 ± 0.24	-1.9 ± 1.01	-0.1 ± 0.22

[1] No significant differences found between means in any of these groups of leucocytes

[2] Mean difference ± standard error

Table 15 -- The effect of feeding rations containing Zectran on the differential leucocyte count of male Japanese quail[1]

Ration	Time of Determination	Number of Quail per Mean	Heterophils (percent)	Lymphocytes (percent)	Monocytes (percent)	Eosinophils (percent)	Basophils (percent)
Control	Pretrial	6	18.4	76.3	1.0	3.5	0.8
Control	After 23 days	6	13.3	82.2	0.6	3.5	0.5
Mean Difference[2]			- 5.1 ± 3.55	+ 5.9 ± 5.38	-0.4 ± 0.83	0.0 ± 1.40	-0.3 ± 0.48
Zectran (100 ppm)	Pretrial	6	42.0	53.8	0.5	3.6	0.3
Zectran (100 ppm)	After 23 days	6	31.6	63.5	0.5	3.9	0.5
Mean Difference			-10.4 ± 9.25	+ 9.7 ± 9.14	0.0 ± 0.31	+0.3 ± 1.34	+0.2 ± 0.33
Zectran (300 ppm)	Pretrial	6	31.7	63.5	0.3	4.0	0.5
Zectran (300 ppm)	After 23 days	6	34.7	59.3	0.6	4.8	0.7
Mean Difference			+ 3.0 ± 7.58	- 4.2 ± 6.33	+0.3 ± 0.21	+0.8 ± 1.68	+0.2 ± 0.17

[1] No significant differences found between means in any of these groups of leucocytes

[2] Mean difference ± standard error

Table 16 -- The effect of feeding rations containing Zectran on the differential leucocyte count of female Japanese quail[1]

Ration	Time of Determination	Number of Quail per Mean	Heterophils (percent)	Lymphocytes (percent)	Monocytes (percent)	Eosinophils (percent)	Basophils (percent)
Control	Pretrial	4	29.5	66.5	0.5	3.3	0.3
Control	After 23 days	4	24.0	70.4	1.1	4.1	0.5
Mean Difference[2]			- 5.5 ± 6.27	+ 3.9 ± 4.92	+0.6 ± 0.83	+0.8 ± 1.24	+0.2 ± 0.14
Zectran (100 ppm)	Pretrial	5	37.6	56.8	0.3	4.2	1.1
Zectran (100 ppm)	After 23 days	5	35.0	58.4	0.3	4.8	1.5
Mean Difference			- 2.6 ± 4.95	+ 1.6 ± 4.72	0.0 ± 0.22	+0.6 ± 0.87	+0.4 ± 0.49
Zectran (300 ppm)	Pretrial	3	47.3	48.7	0.2	3.3	0.5
Zectran (300 ppm)	After 23 days	3	44.0	48.5	0.7	6.0	0.5
Mean Difference			- 3.3 ± 4.57	- 0.2 ± 6.79	+0.5 ± 0.50	+2.7 ± 2.31	0.0 ± 0.50

[1] No significant differences found between means in any of these groups of leucocytes

[2] Mean difference ± standard error

Table 17 -- The effect of feeding rations containing Zytron on the differential leucocyte count of male Japanese quail[1]

Ration	Time of Determination	Number of Quail per Mean	Heterophils (percent)	Lymphocytes (percent)	Monocytes (percent)	Eosinophils (percent)	Basophils (percent)
Control	Pretrial	12	16.1	81.3	0.04	2.1	0.5
Control	After 12 days	12	24.3	71.9	1.17	2.3	0.4
Mean Difference[2]			+ 8.2 ± 4.69	- 9.4 ± 4.31[ab]	+1.13 ± 0.17	+0.2 ± 0.50	-0.1 ± 0.19
Zytron (2000 ppm)	Pretrial	11	16.5	81.5	0.05	1.6	0.4
Zytron (2000 ppm)	After 12 days	11	43.2	54.5	0.59	1.5	0.3
Mean Difference			+26.7 ± 4.90	-27.0 ± 4.50[a]	+0.54 ± 0.18	-0.1 ± 0.52	-0.1 ± 0.20
Zytron (4000 ppm)	Pretrial	11	16.0	82.3	0.05	1.5	0.2
Zytron (4000 ppm)	After 12 days	11	38.7	58.9	1.05	1.0	0.4
Mean Difference			+22.7 ± 4.90	-23.4 ± 4.50[b]	+1.00 ± 0.18	-0.5 ± 0.52	+0.2 ± 0.20
F Ratio			2.37	4.54*	3.02	0.42	0.37

[1] Means having the same superscript are significantly different ($P < 0.05$) by the New Multiple Range Test (Duncan, 1955)

[2] Mean difference ± standard error

* Significant ($P < 0.05$)

Table 18 -- The effect of feeding rations containing Zytron on the differential leucocyte count of female Japanese quail[1]

Ration	Time of Determination	Number of Quail per Mean	Heterophils (percent)	Lymphocytes (percent)	Monocytes (percent)	Eosinophils (percent)	Basophils (percent)
Control	Pretrial	9	37.3	59.8	0.2	2.5	0.2
Control	After 12 days	9	40.0	57.0	1.0	1.6	0.4
Mean Difference[2]			+ 2.7 ± 6.23	- 2.8 ± 6.10	+0.8 ± 3.05	-0.9 ± 2.49	+0.2 ± 0.12^{aB}
Zytron (2000 ppm)	Pretrial	8	38.1	57.5	0.0	3.8	0.6
Zytron (2000 ppm)	After 12 days	8	62.6	35.5	0.4	1.5	0.1
Mean Difference			+24.5 ± 6.61	-22.0 ± 6.48	+0.4 ± 3.24	-2.3 ± 2.64	-0.5 ± 0.13^{B}
Zytron (4000 ppm)	Pretrial	4	37.1	60.3	0.1	2.1	0.4
Zytron (4000 ppm)	After 12 days	4	53.5	44.9	0.4	1.1	0.1
Mean Difference			+16.4 ± 9.35	-15.4 ± 9.16	+0.3 ± 4.58	-1.0 ± 3.73	-0.3 ± 0.19^{a}
F Ratio			2.93	2.38	0.14	1.33	9.44**

[1] Means having the same small case superscript are significantly different ($P < 0.05$); means with large case superscripts are significantly different ($P < 0.01$) by the New Multiple Range Test (Duncan, 1955)

[2] Mean difference ± standard error

** Significant ($P < 0.01$)

Table 19 -- The effect of feeding rations containing DDT on the packed cell volume of mature Japanese quail[1]

Ration	Time of Determination	Males		Females	
		Number of Quail per Mean	Packed Cell Volume (percent)	Number of Quail per Mean	Packed Cell Volume (percent)
Control	Pretrial	6	48.2	6	42.4
Control	After 30 days	6	49.2	4	38.4
Mean Difference[2]			+ 1.0 ± 1.23[a]		
DDT (300 ppm)	Pretrial	5	48.4	6	39.4
DDT (300 ppm)	After 30 days	5	46.1	2	36.5
Mean Difference			- 2.3 ± 0.77		
DDT (500 ppm)	Pretrial	6	51.0	5	45.6
DDT (500 ppm)	After 30 days	6	46.8	-	--
Mean Difference			- 4.2 ± 1.36[a]		
F Ratio			5.08*		

[1] Means having the same superscript are significantly different ($P < 0.05$) by the New Multiple Range Test (Duncan, 1955)

[2] Mean difference ± standard error

* Significant ($P < 0.05$)

Table 20 -- The effect of feeding rations containing Zectran on the packed cell volume of mature Japanese quail[1]

Ration	Time of Determination	Males: Number of Quail per Mean	Males: Packed Cell Volume (percent)	Females: Number of Quail per Mean	Females: Packed Cell Volume (percent)
Control	Pretrial	6	50.5	6	38.7
Control	After 23 days	6	50.8	4	39.2
Mean Difference[2]			+ 0.3 ± 0.83^{A}		
Zectran (100 ppm)	Pretrial	6	50.0	6	39.0
Zectran (100 ppm)	After 23 days	6	49.6	5	39.4
Mean Difference			- 0.4 ± 0.90^{b}		
Zectran (300 ppm)	Pretrial	6	48.1	6	41.3
Zectran (300 ppm)	After 23 days	6	43.3	3	38.5
Mean Difference			- 4.8 ± 1.23^{Ab}		
F Ratio			7.50**		

[1] Means having the same small case superscript are significantly different ($P < 0.05$); means with a large case superscript are significantly different ($P < 0.01$) by the New Multiple Range Test (Duncan, 1955)

[2] Mean difference ± standard error

** Highly significant ($P < 0.01$)

Table 21 -- The effect of feeding rations containing Zytron on the packed cell volume of mature Japanese quail[1]

Ration	Time of Determination	Males: Number of Quail per Mean	Males: Packed Cell Volume (percent)	Females: Number of Quail per Mean	Females: Packed Cell Volume (percent)
Control	Pretrial	12	50.1	9	41.5
Control	After 12 days	12	48.9	9	41.1
Mean Difference[2]			- 1.2 ± 1.77[a]		- 0.4 ± 2.22
Zytron (2000 ppm)	Pretrial	11	48.9	8	40.5
Zytron (2000 ppm)	After 12 days	11	40.2	8	34.1
Mean Difference			- 8.7 ± 1.85[a]		- 6.4 ± 2.29
Zytron (4000 ppm)	Pretrial	11	49.8	4	43.6
Zytron (4000 ppm)	After 12 days	11	43.6	4	40.5
Mean Difference			- 6.2 ± 1.85		- 3.1 ± 3.23
F Ratio Differences			4.49*		1.81

[1] Means having the same superscript are significantly different ($P < 0.05$) by the New Multiple Range Test (Duncan, 1955)

[2] Mean difference ± standard error

* Significant ($P < 0.05$)

Table 22 -- The effect of feeding rations containing DDT, Zectran and Zytron on the packed cell volume of young Japanese quail

Treatment	Time of Determination: Pretrial (percent)	Days after pesticide feeding commenced: 8 (percent)	15 (percent)	18-19 (percent)	22 (percent)	25 (percent)
Control Females	42.5 (12)[1]	43.1 (12)	41.7 (12)	41.0 (12)	40.9 (12)	
Control Males	45.7 (12)	49.0 (12)	49.1 (12)	46.9 (12)	45.3 (12)	46.9 (12)
500 ppm DDT Females	42.2 (11)	43.8 (11)	42.8 (11)	43.0 (10)	41.9 (10)	
500 ppm DDT Males	42.9 (12)	45.4 (12)	45.4 (12)	43.0 (11)	45.4 (10)	46.2 (10)
300 ppm Zectran Females	43.2 (12)	43.3 (7)	42.6 (7)	40.5 (7)	40.6 (6)	
300 ppm Zectran Males	45.0 (12)	46.7 (8)	47.4 (6)	45.3 (4)	43.8 (4)	45.3 (4)
2000 ppm Zytron Females	42.5 (12)	44.0 (12)	40.4 (7)	41.4 (3)	46.1 (2)	
2000 ppm Zytron Males	43.7 (12)	43.6 (12)	42.9 (7)	34.6 (4)	31.9 (4)	39.5 (1)

[1] The number in parentheses indicates the number of Coturnix per mean

Table 23 -- The effect of feeding rations containing DDT, Zectran and Zytron on the packed cell volume, erythrocyte count and mean corpuscular volume of mature male Japanese quail

Ration	Length of Feeding Period (days)	Packed Cell Volume[1] (percent)	Erythrocyte Count[1] (millions)	Mean Corpuscular Volume[1] (μ^3)
Control	31	46.5^{a}	4.00^{A}	116.3
DDT (500 ppm)	31	43.4^{a}	3.83^{A}	113.3
Control	16	45.2^{B}	3.95^{b}	114.4
Zectran (300 ppm)	16	40.7^{B}	3.51^{b}	115.9
Control	13	46.9^{c}	3.99^{C}	117.5
Zytron (2000 ppm)	13	37.6^{c}	3.21^{C}	117.1

[1] Means having the same small case superscript are significantly different ($P < 0.05$); large case superscripts are significantly different ($P < 0.01$) by Student's t Test on differences (Li, 1964)

in erythrocytes which indicates that iron metabolism and hemoglobin formation were not affected by pesticide administration. It is possible that the pesticides inhibit the normal production of erythropoetin by the tissues of these quail and in this way cause a decrease in erythropoesis.

c. Electrophoretic separation of serum protein

An attempt was made to identify the serum fractions of Japanese quail which were separated by agar gel electrophoresis and designated Component 1, 2, 3 or 4 in Table 24. Serum from several other species of fowl, as well as samples of porcine and human serum, were separated with this technique for comparative purposes. Based on the peak height and position Component 4 was identified as the albumin fraction; Components 2 and 3 appear to be α globulins while Component 1 appears to be the β globulin fraction but may include the γ globulins. The γ globulin fraction was either incompletely separated from the β globulin fraction or else was present in very small amounts in Japanese quail sera.

Initial experiments with quail receiving DDT, Zectran and Zytron rations indicated that results were highly variable. In an attempt to obtain data which were less variable, eight female quail were placed on control rations and two separations were effected three days apart to be used as control values. Half of these quail were then placed on a diet containing 2000 ppm Zytron and serum separations were effected after 6, 11 and 15 days. However, variability between measurements on the same quail's serum on different days was still high. It appears that feeding Zytron (2000 ppm) for 15 days caused Component 1 to increase and Component 2 to decrease but with only four observations these were not significant changes. No attempt was made to collect more data to ascertain whether these observed differences were due to the

Zytron treatment because total serum protein proved to be more sensitive to Zytron feeding and less variable.

This technique is tedious and unless improvements in the technique can be made which would provide more consistent measurements it holds little promise as a method of screening pesticides.

d. Total serum protein

Total serum protein measurements were made originally as an adjunct to electrophoretic separations. However, when the data were evaluated, the total serum protein measurements proved to be much less variable than values obtained by quantitation of electrophoretic separations (Table 24). In addition to improved reproducibility these measurements also indicated greater sensitivity to pesticide administration. On the basis of these results, an experiment was designed and carried out to measure total serum protein of quail receiving rations containing DDT, Zectran and Zytron.

The effect of feeding rations containing DDT, Zectran and Zytron on serum protein is shown in Figure 1. Zectran and Zytron rations caused a highly significant decrease in total serum protein levels three days after quail were first fed these rations. Quail receiving a ration containing DDT did not differ significantly from controls but after ten days the total serum protein dropped below control values and remained about one-half percent below for the remainder of the trial period.

Fox and Harrison (1965) have reported that the total serum protein of Japanese quail declined after a 24 or 48 hour fast. Zectran and Zytron rations caused quail to restrict their feed intake to about 9 grams per day (Tables 5 and 6). In order to determine if the decrease in serum protein which occurred with rations containing these pesticides was partially or entirely due to the feed restriction, an additional test was conducted in which quail hens were restricted to 9 grams of feed per quail hen per day.

The results indicate that feed restriction caused an immediate and highly significant drop in the total serum protein of these quail (Figure 2). Serum protein levels seemed to recover almost completely after six days and then declined again after 10 days of feed restriction. The serum protein of the restricted group never declined to levels as low as those resulting from Zectran and Zytron feeding. Also of interest is the fact that all treated groups showed a drop in serum protein three days after the treatment was started and then exhibited a rebound effect the magnitude of which varied with treatment. This phenomenon did not occur in control groups.

It appears from these data that total serum protein is sensitive to certain "stresses" such as toxic dietary ingredients or restricted feed intake. Additional investigations would be necessary to properly evaluate the possible value of this parameter as a measure of pesticide toxicity. However, it has the advantage of being a simple, rapid assay which can be made on small volumes of serum without sacrificing the subjects. Even more important, it has shown greater sensitivity than any other parameter measured in this study.

Table 24 -- The effect of feeding rations containing Zytron on the serum protein of female Japanese quail[1]

Ration	Time of Determination	Total Serum Protein %	Percent Component 1	Component 2	Component 3	Component 4
Control	Pretrial	5.71 ± 0.41	23.6 ± 2.38	31.8 ± 2.56	12.7 ± 0.94	32.1 ± 1.19
Control	After 15 days	5.37 ± 0.24[a]	18.9 ± 1.15	35.7 ± 2.42	11.8 ± 1.38	33.6 ± 1.13
Mean Difference		-0.34	- 4.7	+ 3.9	- 0.9	+ 1.5
Zytron (2000 ppm)	Pretrial	5.63 ± 0.27	22.6 ± 1.48	32.8 ± 1.45	12.2 ± 0.61	32.6 ± 1.13
Zytron (2000 ppm)	After 15 days	2.36 ± 0.69[a]	29.2 ± 4.32	26.2 ± 3.26	11.1 ± 2.05	33.7 ± 4.76
Mean Difference		-3.27	+ 6.6	- 6.6	- 1.1	+ 1.1

[1] Means having the same superscript are significantly different ($P < 0.01$) by the New Multiple Range Test (Duncan, 1955)

Figure 1. The effect of feeding rations containing DDT, Zectran and Zytron on the serum protein of female Japanese quail

● Not analyzed due to small numbers

▲ Not significantly different from the control ($P > 0.05$)

○ Significantly different from control ($P < 0.05$)

△ Significantly different from control ($P < 0.01$)

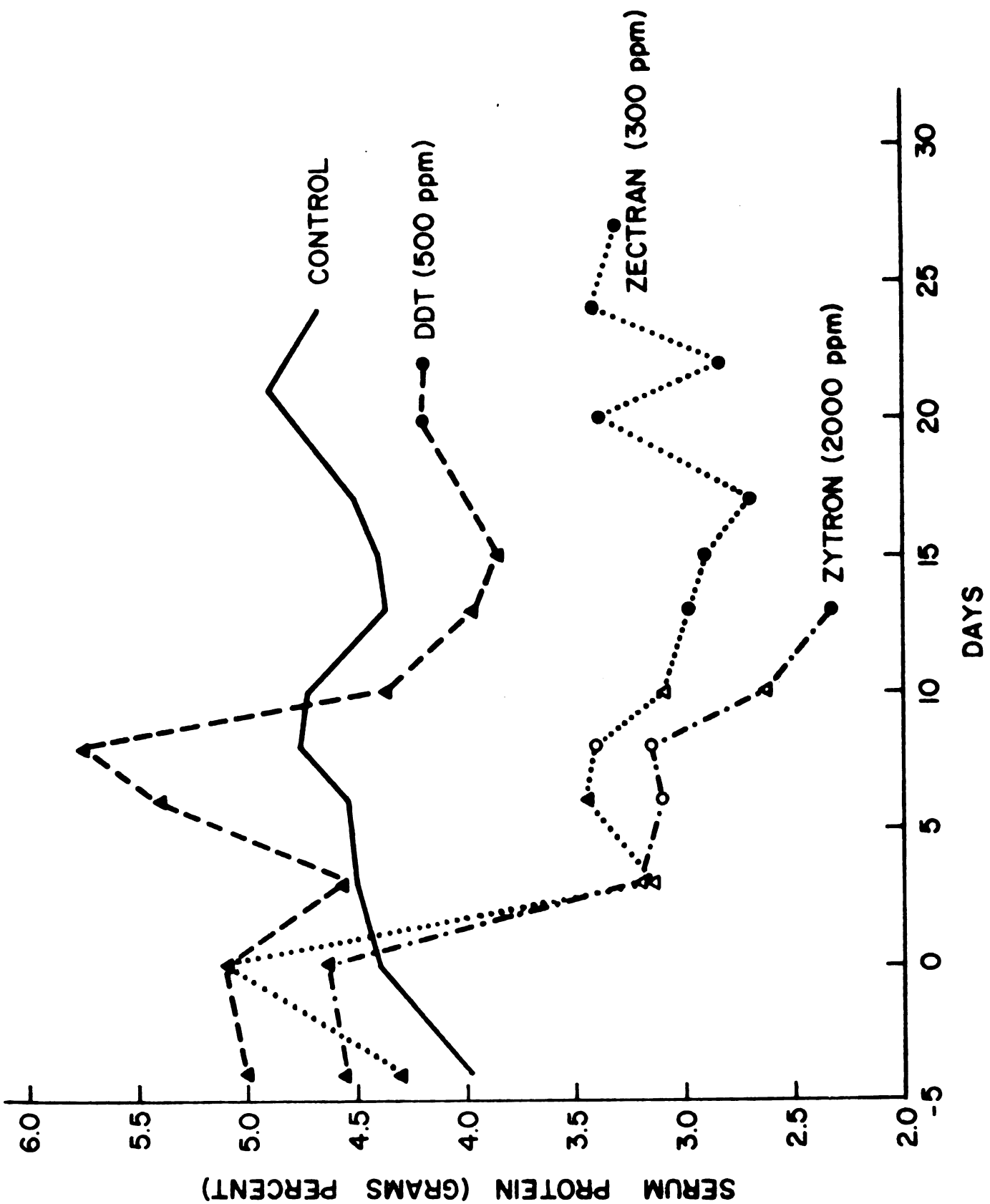

CONTROL
DDT (500 ppm)
ZECTRAN (300 ppm)
ZYTRON (2000 ppm)
SERUM PROTEIN (GRAMS PERCENT)
DAYS
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
-5
0
5
10
15
20
25
30

Figure 2. The effect of feed restriction on the total serum protein of Japanese quail

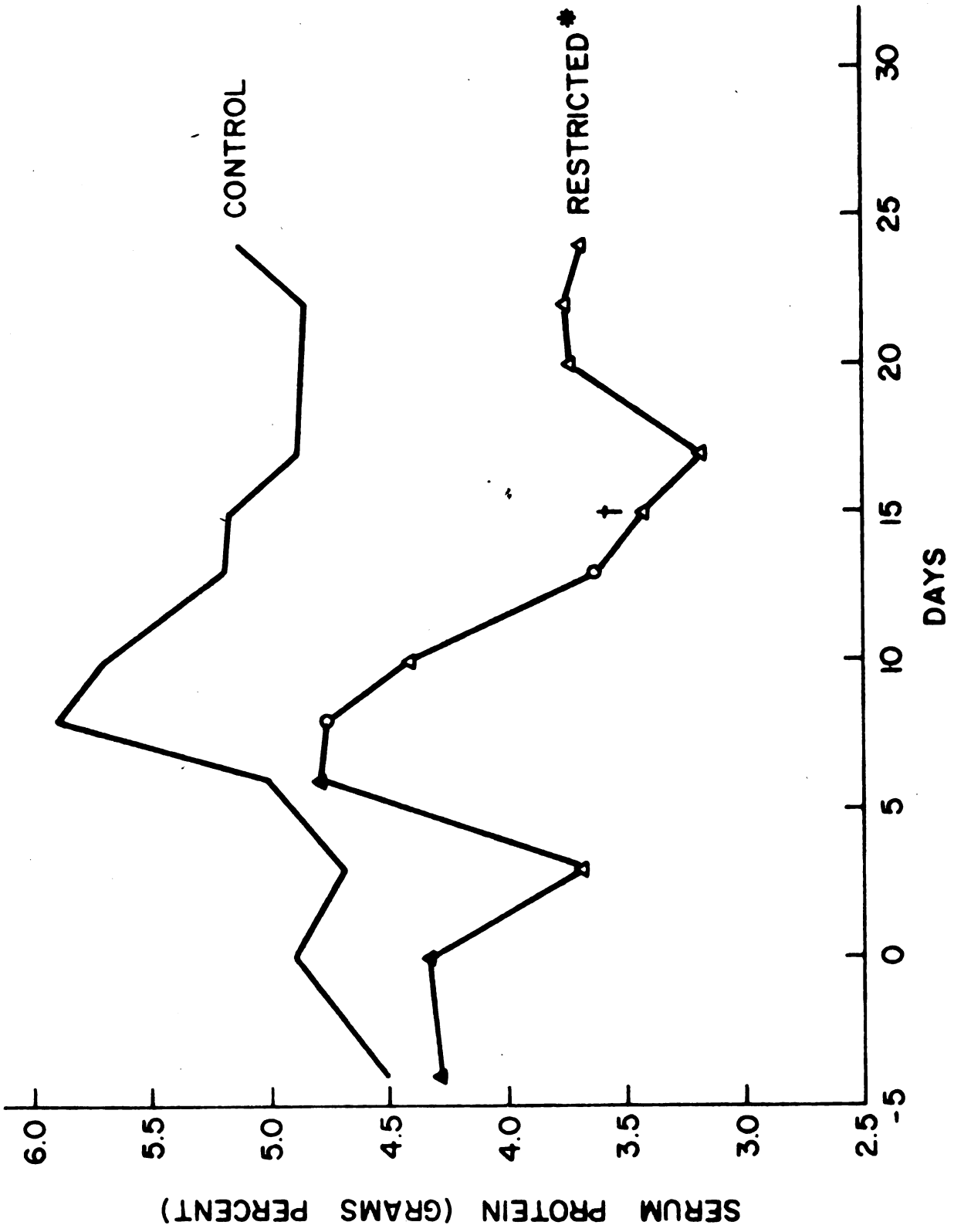
▲ Not significantly different from the control ($P > 0.05$)

● Significantly different from control ($P < 0.05$)

△ Significantly different from control ($P < 0.01$)

* Restricted to 9 grams of feed per quail hen per day

† Daily ration increased to 10 grams of feed per quail hen per day


CONTROL
RESTRICTED*
†
SERUM PROTEIN (GRAMS PERCENT)
DAYS
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
-5
0
5
10
15
20
25
30

Plasma Alkaline Phosphatase

The anticholinesterase activity of organic phosphate insecticides is well known (O'Brien, 1960) and has been used as an assay for organic phosphate insecticides (Gage, 1961). Little has been reported about the effects of pesticides on the levels or activity of various enzymes *in vivo* when these pesticides were chronically administered, with the exception of the enzyme cholinesterase which has been widely investigated. It should be pointed out that extensive investigation has been made into the metabolism of pesticide compounds and the enzymes which catalyze their degradation. This experiment was undertaken to determine if these selected pesticides would affect the circulating level of alkaline phosphatase.

Plasma alkaline phosphatase levels were not affected by rations containing DDT or Zectran but rations containing Zytron caused a decrease in serum alkaline phosphatase which approached significance (Table 25). This decrease is probably due to increased utilization of this enzyme in the metabolism of the organophosphate Zytron rather than to any effect on enzyme synthesis at the cellular level. O'Brien (1960) states that probably all organophosphates can be degradated in the animal body by phosphatases. On the basis of these data this assay would be of no value in measuring the toxicity of other classes of pesticides. Since the anticholinesterase activity of organic phosphate insecticides has proved to be a measure of their toxicity *in vivo* this finding may be only of academic interest.

Table 25 -- The effect of feeding rations containing DDT, Zectran and Zytron on the plasma alkaline phosphatase level of female Japanese quail

Ration	Number of Quail per Mean	Alkaline Phosphatase (mM p-nitrophenol/l. plasma/hr.)
Control	12	5.84 ± 0.93
DDT (500 ppm)	11	5.93 ± 0.71
Zectran (300 ppm)	12	5.30 ± 0.38
Zytron (2000 ppm)	9	3.31 ± 0.80
F Ratio		2.44*

* Significant ($P < 0.10$)

C. General Discussion

In order to make a reasonable interpretation of data such as that which has been presented in this thesis, several factors should be kept in mind. First, many seemingly innocuous materials are toxic to birds or mammals in relatively high concentrations. Two good examples of this are table salt and vitamin A. Both of these materials are common, required dietary constituents but can be very toxic at high levels. Second, any true change in a parameter, no matter how small, can be shown to be a statistically significant change, if enough observations are available. This statistical significance does not necessarily indicate the importance of the change biologically.

In this study, chronic oral administration of DDT, Zectran and Zytron to Japanese quail resulted in differing symptoms of toxicity. Quail receiving DDT exhibited symptoms similar to those widely described in the literature. Tremors appeared first in these quail, followed by tonic and clonic muscle spasms leading to death. The most striking symptom of Zectran and Zytron poisoning was the extreme emaciation which occurred and which undoubtedly resulted from their severely reduced feed consumption which has been previously described. Zytron also affected the epidermis causing it to appear thickened with prolific scaling of keratinized layers. In cases of extreme Zytron poisoning some quail showed lameness and partial paralysis.

When quail were placed on pesticide free rations following severe poisoning with Zectran they rapidly regained body weight and did not show any apparent abnormalities when observed for two months. Quail did not recover as readily following DDT intoxication. If pesticide free feed was offered when only slight tremors were apparent most quail recovered and appeared normal. However, when pronounced tremors were apparent before pesticide free feed

was offered less than half of the quail survived for more than one week. Recovery from Zytron poisoning also varied with the severity of poisoning. However, with the levels at which Zytron was administered most quail died suddenly precluding a large number of observations on their recovery. In one instance when a quail exhibiting lameness and partial paralysis, apparently from the Zytron she was receiving, was placed on clean feed she survived and showed slow recovery from these symptoms.

Quail exhibited considerable variability in their resistance to these pesticide materials. This could have been due to differences in their feed (and therefore pesticide) intake or to their ability to metabolize the materials to less toxic forms. There may also have been differences in the absorption of pesticides from the gut of these quail.

Certainly most methods of evaluating pesticide toxicity, regardless of their limitations, contribute to our knowledge of how pesticides affect the organisms of our environment. However, some methods are more useful and practical than others for evaluating pesticide toxicity.

These studies indicate that egg injection is of little value in screening pesticides for general toxicity. Tordon in these studies had a very low oral toxicity but caused severe mortality of embryos when injected into the yolk sac of embryonating eggs. This result clearly shows that any test used to ascertain the toxicity of pesticides to wildlife must involve the oral administration of the chemical to be valid. Injecting pesticides into embryonating eggs may well be a valuable method of determining what effects these materials have on the developing embryo or fetus; however, the fact that a pesticide produces abnormalities or teratogenic effects when injected into an embryonating egg does not indicate that it will cause this effect under field conditions.

It may be poorly absorbed from the gut, rapidly detoxified in the body, stopped by the placental barrier in mammals or may not be deposited in the eggs of birds. Furthermore, the bird or animal may reject the contaminated food if other food is available for consumption.

Of the various parameters examined, total serum protein measurements seem to hold the most promise as a measure of the relative toxicity of pesticides to birds or mammals. This parameter can be easily and quickly determined without sacrificing or even causing extensive discomfort to the bird or animal. Total serum protein also seems to be extremely sensitive to fasting but this might be overcome by administration of the pesticide orally in a capsule. Hopefully this would prevent the rapid drop in feed intake which occurred when rations containing Zectran or Zytron were fed ad libitum and allow the effect of these two pesticides on total serum protein to be separated from the effects of fasting.

Unfortunately this was the last study undertaken in this project and only limited experimentation was possible. Considerable experimentation would be necessary to properly evaluate just how successful this technique might be as a measure of pesticide toxicity.

VI. SUMMARY AND CONCLUSIONS

A. Chronic oral administration of DDT, Zectran and Zytron has been shown to significantly reduce the packed cell volume and total erythrocyte count of mature Japanese quail without changing the mean corpuscular volume. These pesticides did not consistently lower the packed cell volume of immature quail although severe anemia appeared in some individual quail preceding their death.

B. None of these pesticides caused a significant change in hen day egg production although Zectran and Zytron appear to have reduced egg production of quail hens. The number of eggs per hen was considerably, but not significantly, reduced by all of the pesticides. Fertility and hatchability did not seem to be affected by any of the pesticides. DDT caused an increase in chick mortality while Zectran and Zytron had no effect on chick mortality.

C. Oxygen consumption was not significantly altered by any of these pesticides, although DDT may have caused a slight increase in oxygen consumption and Zectran a slight decrease.

D. Zectran and Zytron rations severely reduced feed intake and significantly lowered mean body weight of quail while DDT did not affect mean body weight. Quail females consumed significantly smaller quantities of rations containing Zectran (300 ppm) or Zytron (2000 ppm) than of a control ration when both rations were offered simultaneously at identical feeding sites. Consumption of a ration containing DDT (500 ppm) in this experiment was not significantly different from a control ration.

E. Zytron caused a decrease in plasma alkaline phosphatase which approached significance ($P<0.10$) while DDT and Zectran did not affect this parameter.

F. Zectran and Zytron caused a significant drop in total serum protein of quail hens three days after the pesticides were first administered. However, quail hens restricted to the same quantity

of basal ration which quail hens receiving the aforementioned Zectran and Zytron rations consumed voluntarily (9 gm/hen/day) also showed a significant drop in total serum protein level three days after the restriction commenced. DDT did not significantly reduce total serum protein; however, after ten days the total serum protein dropped about one-half percent below the control level and remained there.

G. Of the pesticides tested only Zytron caused a significant change in the differential leucocyte count of quail. The lymphocyte percentage of males and the basophil percentage of females were significantly lowered following Zytron administration.

H. Zytron did not cause a significant change in any of the components resulting from electrophoretic separation of the serum protein of female quail in this study.

I. Of the various parameters measured in this study none were definitely shown to be valid measures of pesticide toxicity. Tests of the effect of pesticides on total serum protein indicate that this parameter may be adequately sensitive to pesticides to render it useful as a toxicity test but additional experimentation would be necessary to establish the extent of its applicability for this purpose.

Some of the other parameters exhibited significant changes as a result of pesticide administration but these changes were not observed until poisoning was severe, as evidenced by the mortality which occurred. The egg injection technique proved to be of little value as a method of screening pesticides for general toxicity to wildlife.

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APPENDIX

Aldrin - (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1, 4-endo,exo-5,8-dimethanonaphthalene)

Benzene hexachloride (lindane) - 1,2,3,4,5,6-hexachlorocyclohexane

Betasan - N-(2-mercaptoethyl) benzenesulfonamide S-(O,O-diisopropyl phosphorodithioate)

Chlordane - 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4, 7-methanoindan

Chlorindane-Russian translation; chemical name was not included

Co-Ral - O-3(-chloro-4-methylumbelliferone) O-O-diethyl phosphorothioate

Cygon (dimethoate) - O,O-dimethyl S-(N-methylcarbomoyl)-methyl phosphorodithioate

DDD - 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane

DDT - (dichlorodiphenyltrichloroethane)

DFP - diisopropyl fluorophosphate

Diazinon - O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate

Deldrin - (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7, 8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene)

Dimethoate (Cygon) - O,O-dimethyl S-(N-methyl carbamoyl)-methyl phosphorodithioate

Dipterex - O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate

DI-System - O,O-diethyl S-2-(ethylthio)-ethyl phosphorodithioate

Dow ET-15 - O-methyl O-(2,5,5-trichlorophenyl) phosphoramidothioate

Endrin - (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7, 8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene)

EPN - ethyl p-nitrophenyl thionobenzene phosphonate

Heptachlor - 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4, 7-methanoindene

Imidan - phthalimidomethyl-O,O-dimethyl phosphorodithioate

Isopropyl parathion - O,O-diisopropyl O-p-nitrophenyl- phosphorothioate

Karathane - 25% wettable powder of 4,6-dinitro-2-caprylphenyl crotonate

Kepone - 1,2,3,4,5,6,7,8,9,10,10-decachlorotetracyclo- $(5.2.1.0^{2,6}.0^{3,9}.0^{5,8})$-decane-4-one or decachlorotetracyclodecanone

Lindane (benzene hexachloride) - 1,2,3,4,5,6 hexachlorocyclohexane

Malathion - S - (1,2-bis (ethoxycarbonyl) ethyl) O,O-dimethyl phosphorodithioate

MCPA - 4-chloro-2-methylphenoxyacetic acid

Methoxychlor - 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane

Methyl-parathion - O,O-dimethyl O-p-nitrophenyl phosphorothioate

Mipafox - bis-monoisopropylamino fluorophosphine oxide

Parathion - O,O-diethyl O-p-nitrophenyl phosphorothioate

Polybor 3 - disodium octaborate tetrahydrate

Ronnel - O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate

Na arsenite - ($NaAsO_2$ and Na_2HAsO_3) - sodium arsenite

Na fluosilicate (Na_2SiF_6) - sodium fluosilicate

Strobane - (mixed polychlorinated terpenes)

Systox - O,O-diethyl O-2-ethyl mercaptoethyl thionophosphate

TOCP - tri-orthocresyl phosphate

Tordon - 4-amino-3,5,6-trichloropicolinic acid

Toxaphene - chlorinated camphene containing 67-69% chlorine

Trichlorometaphos-3 - Russian translation; chemical name was not included

Trithion - S-(p-chlorophenylthiomethyl) O,O-diethyl phosphorodithioate

Zectran - 4-dimethylamino 3,5-xylyl methylcarbamate

Zytron - O-(2,4-dichlorophenyl) O-methyl isopropylphosphoroamidothioate