Blood Characteristics as Predictors of Reproductive Success in Quail Species Exposed to DDT

by

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(ABSTRACT)

Present sampling techniques are not capable of assessing both contamination with an organochlorine chemical and reproductive success without the need for removing individuals from that population. Experiments were performed to evaluate vitellogenin, vitamin A, and vitamin E as biomarkers of contamination with DDT, an organochlorine pesticide, and reproductive success in Japanese quail (*Coturnix coturnix japonica*) or northern bobwhites (*Colinus virginianus*).

The utility of vitellogenin as a biomarker in northern bobwhites was investigated. Female northern bobwhites were dosed with 0, 1, 10, or 100 μg DDT/g body weight via corn oil intubation. The females were induced to lay eggs by providing the proper daylength, and number of eggs laid was monitored. No differences were found for number of eggs laid, egg morphology, or plasma concentration of vitellogenin among dose groups.

Dietary supplementation and corn oil intubation were evaluated as alternative dosing techniques because handling involved with intubation may have caused decreased egg production in all treatment groups in the first experiment. Nonlaying female bobwhites were dosed with 0 μg DDT, 25 μg DDT/g food, or 25 μg/g body weight (intubation). Bobwhites receiving DDT via intubation accumulated more DDT in brains and livers than did bobwhites receiving DDT via their diet. The two techniques were found not to be equivalent.

Vitamins A and E were investigated as biomarkers of DDT contamination and reproductive success in Japanese quail. Female Japanese quail were given 0, 1, 10, or 25 μg dietary DDT. Significant differences were found among dose groups for reproductive success (number of eggs showing embryonic development), and for plasma concentrations

of vitamin A. Concentrations of vitamin E were not measurable. Correlation and regression analyses failed to show a relationship between reproduction and vitamin A concentrations.

Vitamins A and E also were investigated as biomarkers in northern bobwhites. Female bobwhites were given 0, 1, 10, or 25 ppm dietary DDT. No significant differences were found among dose groups for reproductive success or for concentrations of either vitamin A or vitamin E. Correlation and regression analyses again failed to show any relationship between reproduction and plasma concentrations of vitamins A or E.

During each experiment, concentrations of DDT and its metabolites were measured. DDE/DDT ratios in livers, a means of estimating liver enzyme induction, were calculated. The DDE/DDT ratios in livers of Japanese quail and northern bobwhites were found to be markedly different. More work needs to be done to better evaluate the relationship between differences in enzyme induction and reproductive success among different species.

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CHAPTER 1: INTRODUCTION

DDT AS A TEST CHEMICAL

DDT[1] , the parent compound of DDE, was chosen to investigate blood characteristics that respond to contamination and are important for reproduction and to act as a model of other organochlorine compounds that produce similar reproductive effects. The choice was deliberate for a number of reasons. DDT and its metabolites are still a problem in part due to their persistence in food chains of terrestrial and aquatic species in North America. DDT also presents a problem to many migratory species due to its continued use in other countries. DDE continues to be associated with eggshell thinning in many recent studies of both migratory and nonmigratory species in the USA (Blus et al. 1980, Findholt 1981, 1984, Henny et al. 1984, 1985, White and Krynitsky 1986, Bunck et al. 1987, and Steidl et al. 1991)

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[1] The following abbreviations will appear throughout the text.
p,p′-DDT refers to 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane,
o,p′-DDT refers to 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane,
p,p′-DDE refers to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene,
o,p′-DDE refers to 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethylene,
p,p′-DDD refers to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane,
o,p′-DDD refers to 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane,
and ΣDDT refers to the summation of all the isomers and metabolites of DDT.

despite being banned since the early 1970's. Ohlendorf et al. (1981) reported herons dying from DDT poisoning years after DDT was banned in the United States. DDT's metabolic breakdown and effects also are well known. The large amount of background information known makes it a good choice as a model of other organochlorine pesticides and organochlorine chemicals many of which still present significant environmental problems due to their persistence.

In many areas DDT and DDE residues are decreasing as a result of discontinued use. Longcore and Stendell (1983) report lower concentrations of DDE in black ducks (*Anas rubripes*) from the Atlantic flyway in 1978 than in the 1960's. The U.S. Fish and Wildlife Service's National Contaminant Biomonitoring Program has found decreasing residues of DDE in starlings (*Sturnus vulgaris*) from 1968 (1.64 ppm) to 1982 (0.37 ppm) (Jacknow et al. 1986). In certain other areas, however, it appears that DDE continues to be accumulated in high concentrations (Table 1.1). Peregrine falcons (*Falco peregrinus*) did not show a decreasing trend in DDE contamination as of 1983, but continued to have high concentrations in eggs (up to 130 ppm) (Hunt et al. 1986). An analysis of peregrine falcon eggs that failed to hatch revealed that differential contamination with chlorinated hydrocarbons was not the result of exposure of the falcons on wintering grounds, but rather differential consumption of migratory or residential prey items (Springer et al. 1984). Steidl et al. (1991) reported declining eggshell thickness attributed to organochlorine contamination in peregrine falcon populations reestablished near the Delaware Bay and River. Banning the legal use of DDT has yet to eliminate its detrimental impacts.

As suggested above, the source of contamination may not be local. The measured contamination of black-crowned night herons (*Nycticorax nycticorax*) in the intermountain West was not considered to be of local origin (Henny et al. 1984). Henny and Blus (1986) concluded that wintering grounds were an important source of contamination for black-crowned night herons. Blus et al. (1987) conclude that previous legal use in Washington state is contributing more to present contamination than previously supposed because they were able to discount other sources. In addition to previous use, present sources of

environmental DDT and its metabolites are foreign sources -- both as airborne drift (Jury et al. 1987) and as exposure to migratory species, and illicit use (Blus et al. 1987). Kelthane® (dicofol) is presently used as an acaricide (Hudson et al. 1984), and it was proposed that metabolic breakdown of Kelthane® produced p,p'-DDE (Risebrough et al. 1986), but more recently it has been shown that contamination of Kelthane® with DDT and its metabolites during manufacturing, not metabolic breakdown, was the source of DDE (Clarke 1990).

The above review indicates that although many areas and species show trends of decreasing DDE concentrations, 'hot spots' have persisted from the late 1970's through the late 1980's where considerable contamination remains evident. Additionally, birds migrating to and from highly contaminated areas will be impacted as will their predators. In light of this information, I feel that in addition to being a good model for other organochlorine contaminants, DDT and its metabolite DDE continue to warrant investigation.

RATIONALE

At present, no good method exists to predict the reproductive capacity of birds exposed to environmental contaminants, such as DDT, without the need for destructive sampling of either eggs or adults. In situations where population numbers are decreasing or during the study of endangered species, destructive sampling is undesirable. Reduced reproductive success caused by contamination can be a serious threat to maintaining adequate population numbers. A means by which reproductive capacity of a population can be sampled without exacerbating an existing decline or slowing recovery is most desirable.

To date, attempts at predicting contaminant effects have revolved around thicknesses, strengths, and microscopic malformations of eggshells. To predict contaminant concentrations, Fox (1979) proposed a method using Ratcliffe's shell thickness index. To predict likely reproductive success from contaminant concentrations, an existing data set must

be available to relate shell thickness to contaminant concentrations and reproductive success. Such data sets are available for only a few species. Also, Moriarty et al. (1986) have questioned the ability of Ratcliffe's index to accurately predict the concentration of a contaminant, in their case DDE, in gannets (*Sula bassana*), shags (*Phalacrocorax aristotelis*), and grey herons (*Ardea cinerea*).

Disagreement exists in the literature as to the effect of DDE on eggshell porosity. Cooke (1979) claimed porosity decreased in peregrine falcons exposed to DDE, whereas Burton et al. (1986) claimed porosity increased in grey herons. Carlisle et al. (1986) stated that eggshell breaking strength is a better measure of eggshell quality because they found strength to be significantly reduced in shells laid by mallards (*Anas platyrhynchos*) fed DDE without thickness being significantly reduced. Bennett et al. (1988) have found similar results treating northern bobwhites (*Colinus virginianus*) with sulfanilamide, a chemical that produces DDT-like eggshell thinning.

To standardize the response observed in measurement of eggshell thickness or strength, it is best to use freshly laid eggs. As embryos develop, they utilize calcium from their shells. Use of freshly laid eggs requires sacrificing that embryo, leading to an experimentally induced reduction in reproduction. Also, it can be difficult to collect freshly laid eggs from nests. Alternatively, thickness or strength could be measured on shells producing chicks. This would bias the measurements because only eggs thick or strong enough to have hatched would be measured. In free-living birds, adult birds usually remove shell remnants from the nest shortly after young hatch, making collection difficult.

For the above reasons, more accurate predictors are desirable and blood characteristics offer considerable potential as predictors. Besides, blood samples have the major advantages of being repeatable and are a nondestructive means of sampling. Additionally, blood characteristics have the potential to work with species or compounds that do not exhibit the classical eggshell thinning phenomenon. In this study, it is proposed that blood characteristics of breeding female quail be used to predict reproductive capacity after exposure to an environmental contaminant, DDT.

A sampling technique is proposed using blood characteristics to predict whether a bird exposed to organochlorine pesticides, in this case DDT, will experience reduced reproductive capacity, thus avoiding the possibly less accurate and destructive eggshell strength measuring methods. Data presented in Chapter 3 suggest that DDT does not alter physical characteristics of eggs (e.g. volume, yolk dry weight, albumin dry weight, surface area and Ratcliffe index) of northern bobwhites implying that the chemical characteristics will be very important in predicting the altered reproductive capacity caused by chlorinated hydrocarbons. It is postulated that female blood characteristics will be altered causing changes in egg chemistry. Changes in blood characteristics have resulted from exposure to heavy metals (Cain et al. 1983, Franson et al. 1983a, DiGiulio and Scanlon 1985), organophosphates (Franson et al. 1983b, Grue et al. 1983) and PCB's (Sanders et al. 1974, 1977, Sanders and Kirkpatrick 1975, Montz et al. 1982) and have been shown to have considerable predictive value. Because the value of changes in blood as predictors of well-being and reproductive success has been well established for other groups of environmental contaminants, it is hypothesized that blood chemistry changes will have value as predictors of well-being and reproductive success for organochlorine contaminants as well.

To focus on the effects of sublethal doses of DDT on reproduction, the dose regimen was chosen to include very low doses. It was reasoned that birds exposed to levels approaching lethal burdens would not provide realistic models for reduced reproduction in the wild because these birds would not be likely to survive to reproduce. Considerable variation exists in the literature regarding doses causing mortality from chronic exposure. Bobwhite chicks experienced 53% mortality when exposed to 150 ppm dietary DDT (DeWitt 1956). In the same study, bobwhite in reproduction experienced 25% mortality on diet containing 100 ppm DDT. Lehman et al. (1974) reported no mortality when providing bobwhites with diets containing 150 ppm DDT. Juvenile bobwhite experienced 30% mortality when exposed to either 50 or 100 ppm dietary DDT (Coburn and Treichler 1946). Cottam and Higgins (1946) reported 100% mortality at 500 ppm, 50% mortality at 250 ppm, and 'some losses' at 50 ppm dietary DDT. DeWitt (1955) reported 100% mortality at 250 ppm and 10% mortality at 200 ppm dietary DDT.

A low dose of 5 ppm DDT did not cause mortality, but lowered 6-phosphoglucose dehydrogenase concentrations in Japanese quail (*Coturnix coturnix japonica*) blood (Bunyan et al. 1970). In an attempt to choose a dose range capable of eliciting a toxic response without causing mortality, a dose regimen of 0, 1, 10, and 100 ppm DDT was chosen. To assure precise dosages and to reduce the possibility of cross-contamination, corn oil intubation was chosen as the method of exposure. However, the results presented in Chapter 3 caused a reduction of the high dose to 25 ppm because of high mortality in the 100 ppm dose group bobwhites, and the results presented in Chapter 4 caused a change to dietary exposure because that technique was thought to better reflect exposure in free-living birds.

OBJECTIVES

The overall objective of this project is to find a nondestructive means of predicting potential reproductive output of birds contaminated with organochlorine pesticides. As a means of meeting this objective, egg and blood characteristics of two species of quail were analyzed after being exposed to DDT. One, Japanese quail, is known to exhibit DDT-induced eggshell thinning, whereas the other, northern bobwhite, shows only slight eggshell thinning when exposed to DDT. By studying both a sensitive and a less sensitive species, any change in blood characteristics can be better evaluated for its potential as a predictor of reproductive potential.

A series of four experiments was performed with the overall objective in mind. The first experiment investigated the effects of DDT on plasma vitellogenin concentrations and egg morphology in northern bobwhites. During this first experiment, it appeared as though female bobwhites began laying more regularly after corn oil intubation ceased. This raised the question of whether intubation was comparable to oral exposure in a food source. A second experiment was designed to address this question. The results of the first experiment also

did not support the utility of vitellogenin as a biomarker of exposure to DDT and reproductive potential. The third experiment was designed to evaluate the utility of plasma vitamins A and E as biomarkers in Japanese quail, a species know to be susceptible to DDT's eggshell thinning effects. The fourth experiment was designed to evaluate the utility of both plasma vitamins A and E as biomarkers in northern bobwhites, a species not very susceptible to eggshell thinning.

The specific objectives of each experiment were as follows:

Experiment 1 (Chapter 3): Response of Vitellogenin to DDT in Northern Bobwhites

1) To determine the effects of DDT ingestion on egg morphology.
2) To determine the effects of DDT ingestion on concentrations of vitellogenin in blood plasma.

Experiment 2 (Chapter 4): A Comparison Between Food Addition and Intubation of DDT to Northern Bobwhites

1) To determine whether administering DDT via food addition or corn oil intubation produces similar food intake and tissue concentrations in northern bobwhites.

Experiment 3 (Chapter 5): Response of Vitamins A and E to DDT in Japanese Quail

1) To determine whether changes in egg morphology correspond to dose of ingested DDT.
2) To determine whether decreases in number of eggs laid, number of settable eggs (eggs with uncracked and apparently complete eggshells), number of eggs showing embryonic development, or number of eggs hatching are associated with ingested DDT.
3) To determine whether decreases in plasma concentrations of vitamins A and E are associated with ingested DDT.
4) To determine the ability of plasma vitamin A concentrations to predict reduced reproduction.

Experiment 4 (Chapter 6): Response of Vitamins A and E to DDT in Northern Bobwhites

1) To determine whether decreases in number of eggs laid, number of settable eggs, number of eggs showing embryonic development, or number of eggs hatching are associated with ingested DDT.

2) To determine whether decreases in plasma concentrations of vitamins A and E are associated with ingested DDT.

3) To determine the ability of vitamin concentrations to predict reproduction.

Table 1.1. Extreme concentrations of DDE (wet weight basis) found in bird species in the United States.

Species	DDE (ppm)	Tissue	Year	Location	Reference
Double-crested cormorant *Phalacrocorax auritus*	5.59	egg	1984	Washington	Henny et al. 1989
Olivaceous cormorant *P. olivaceous*	20	carcass	1980-1981	Texas	King and Krynitsky 1986
Olivaceous cormorant	31	egg	1980-1981	Texas	King and Krynitsky 1986
Anhinga *Anhinga anhinga*	16	egg	1984-1985	Mississippi	White et al. 1988
Black-crowned night heron *Nycticorax nycticorax*	17	egg	1980-1981	Tennessee Valley	Fleming et al. 1984a
Black-crowned night heron	26	egg	1982	San Francisco Bay	Ohlendorf et al. 1988
Black-crowned night heron	24	egg	1983	Texas	White and Krynitsky 1986
Great blue heron *Ardea herodias*	26.2	egg	1980	Oregon	Fitzner et al. 1988
Great blue heron	40	egg	1980-1981	Tennessee Valley	Fleming et al. 1984a
Great blue heron	21	carcass	1982	Idaho	Fitzner et al. 1988
Great blue heron	25	brain	1982	Washington	Fitzner et al. 1988
Green-backed heron *Butorides virescens*	12	egg	1980-1981	Tennessee Valley	Fleming et al. 1984a
Green-backed heron	43	egg	1984-1985	Mississippi	White et al. 1988
Little blue heron *Egretta caerulea*	10	egg	1984-1985	Mississippi	White et al. 1988
Wood stork *Mycteria americana*	9.4	egg	1982	Florida	Fleming et al. 1984b
White-faced ibis *Plegadis chihi*	3.1	egg	1985	Texas	Custer and Mitchell 1989
White-faced ibis	29	egg	1986	Nevada	Henny and Herron 1989
Common goldeneye *Bucephala clangula*	6.88	fat	1985	Niagara River	Foley and Batcheller 1988
Bald eagle *Hali&aedip.etus leucocephalus*	9	carcass	1979-1982	California and Oregon	Frenzel and Anthony 1989
Sanderling *Crocethia alba*	32	carcass	1980	California	Schick et al. 1987
Caspian tern *Hydroprogne caspia*	29	egg	1982	San Francisco Bay	Ohlendorf et al. 1988

Table 1.1. (Continued)

Species	DDE (ppm)	Tissue	Year	Location	Reference
Black skimmer *Rhynchops nigra*	30	carcass	1980	Texas	King 1989
Black skimmer	86	egg	1980	Texas	King 1989
Black skimmer	92	carcass	1980-1982	Texas	King and Krynitsky 1986
Black skimmer	86	egg	1980-1982	Texas	King and Krynitsky 1986
Black skimmer	28.4	egg	1984	Texas	Custer and Mitchell 1987
Laughing gull *Larus atricilla*	4.5	egg	1980-1982	Texas	King and Krynitsky 1986
Laughing gull	5.5	carcass	1980-1982	Texas	King and Krynitsky 1986
Western kingbirds *Tyrannus verticalis*	47	carcass	1983	Texas	White and Krynitsky 1986
House sparrow *Passer domesticus*	35	carcass	1983	Texas	White and Krynitsky 1986

CHAPTER 2: LITERATURE REVIEW

GENERAL REPRODUCTIVE EFFECTS IN BIRDS

In addition to the well-recognized effects of DDT and its metabolites on eggshells, DDT decreases reproductive potential through many other means. Recent field studies continue to implicate DDE in cases of impaired reproduction. A negative relationship existed between DDE concentrations and the percent of successful nests, clutch size, and the number of young per nest for black-crowned night herons (*Nycticorax nycticorax*) observed from 1978 to 1980 in the intermountain West (Henny et al. 1984). White-faced ibis (*Plegadis chihi*) eggs collected in 1986 in Nevada continued to show a negative correlation between DDE concentrations and eggshell thickness and with number of young produced per nesting attempt (Henny and Herron 1989). DDE concentrations in black skimmer (*Rynchops niger*) eggs collected in 1984 were correlated with decreased hatching success independent of demonstrable changes in eggshell quality (Custer and Mitchell 1987). Other effects that DDT or DDE elicited in birds relating to reproduction include reduced hatchability in mallards (*Anas platyrhynchos*) (Heath et al. 1969), Japanese quail (*Coturnix coturnix japonica*) (Smith et al. 1969), white-tailed eagles (*Haliæetus albicilla*) (Koivusaari et al. 1980), white-faced ibises (Steele 1984), and American

kestrels (*Falco sparverius*) (Wiemeyer et al. 1986); delayed egglaying in Japanese quail (Cecil et al. 1971); decreased egg production in Japanese quail (Smith et al. 1969) and white-faced ibises (Steele 1984); decreased medullary bone formation in silver king pigeons (*Columba livia*) (Oestreicher et al. 1971); increased embryo mortality in mallards (Heath et al. 1969); decreased fertility in Japanese quail (Smith et al. 1969) and northern bobwhites (*Colinus virginianus*) (DeWitt 1956); decreased blood estradiol in ringed turtle doves (*Streptopelia risoria*) (Peakall 1970); and decreased survivability of young in northern bobwhites (DeWitt 1956), mallards (Heath et al. 1969), and American kestrels (Wiemeyer et al. 1986). Male reproduction was also altered by decreasing sperm production in domestic fowl (*Gallus domesticus*) (Albert 1962), and damaging testicular tissues in bald eagles (*Haliæetus leucocephalus*) (Locke et al. 1966).

DDT also affects other aspects of avian physiology likely to alter reproduction. High doses of DDT (500 ppm) in the diet caused hypertrophy of thyroid glands in northern bobwhites (Hurst et al. 1974). As will be discussed later, hyperthyroidism will likely alter vitamin A physiology. Lehman et al. (1974) failed to find any difference in total adrenal weights in northern bobwhites but did find an increasing cortical/medullary ratio suggesting greater corticosteroid production in response to exposure to DDT. Adrenal glands and livers in pigeons (*Columba livia*) showed increased weights (Jefferies and French 1972). Adrenal glands change weight as a response to many stressors. Stressed birds with greater production of corticosteroids may lose weight and have reduced physical fitness. Therefore, they are less likely to reproduce successfully.

DDT AND REPRODUCTION IN QUAILS AND PHEASANTS

Considerable work has been done investigating the effects of DDT and its metabolites on reproduction in gallinaceous birds such as Japanese quail, northern bobwhite, and

ring-necked pheasants (*Phasianus colchicus*). DeWitt (1955) reported that 200 ppm in the diet reduced the number of eggs laid per hen per day, decreased hatchability, and reduced chick survival in northern bobwhites. DeWitt (1956) reported that 100 ppm DDT in the diet reduced egg fertility and chick survival in northern bobwhites. In the same study, 50 ppm DDT led to fewer eggs per hen, but did not appear to affect other aspects of reproduction in pheasants. Genelly and Rudd (1956) found that 100 ppm or 400 ppm DDT in the diet did not reduce the number of eggs laid, number of fertile eggs, or number of eggs hatching in pheasants, but did reduce chick survival. Japanese quail had a lag in egg laying, produced more broken eggs, and produced eggs with less calcium when fed 100 ppm DDT in the diet (Cecil et al. 1971). Smith et al. (1969) reported reduced fertility, hatchability, and egg production for Japanese quail.

Northern bobwhite do not appear to be very sensitive to DDT-induced eggshell thinning (Tucker and Haegele 1970). Sulfanilamide, a compound known to produce thinner shells in Japanese quail (Chang and Stokstad 1975), failed to thin shells of bobwhites (Bennett et al. 1988). Bennett et al. (1988) did find that eggshell strength was decreased by sulfanilamide, casting doubts on older studies that may have looked only at shell thickness as the indicator of eggshell quality. Because eggshell measurements may not be a reliable indicator of potential reproduction following exposure to a contaminant, another means is needed.

A brief review of only three members of a single family, Phasianidae, indicates considerable variation in response to DDT. This poses a problem for predicting reproductive failure after exposure to a contaminant. Not only were differences reported among species, but different studies reported varying response within a species. If a biochemical response caused by the contaminant and leading directly to reproductive failure can be shown to be less variable, it would allow evaluation of any particular population without needing to rely on extrapolation from other studies.

OTHER EFFECTS OF DDT

Other effects elicited by exposure to DDT include increased liver microsomal enzyme proteins in both Japanese quail and Wistar rats (*Rattus norvegicus*) at doses of 100 ppm in the diet (Bunyan et al. 1971). Gillett et al. (1970), however, failed to show induced microsomal enzyme activity in Japanese quail by measuring UDPGA-o-aminophenyl glucuronyl transferase. They did find reduced activity of a microsomal epoxidase, aldrin epoxidase. Pentobarbital sleeping times, an indirect measure of microsomal enzyme induction, in Wistar rats were reduced by exposure to 100 ppm of the six major isomers and metabolites of DDT, but sleeping times in Japanese quail increased (Bitman et al. 1971). Such anomalous results may at least be partially explained by Vangilder and Drawer's (1983) assertion that granivorous or herbivorous species would likely be less sensitive to organochlorine contamination because their microsomal enzyme systems are pre-adapted to plant defensive secondary compounds reducing the need to increase enzyme activity after exposure to organochlorine contaminants. Also, a less likely explanation for the difference is that Bitman et al. injected the rats with pentobarbital intraperitoneally but injected the quail intramuscularly.

Jefferies (1975) reviewed the effects of organochlorines on thyroid activity. He reported both hyperthyroidism and hypothyroidism for different species. For pigeons, low doses of DDT produced hyperthyroidism as evidenced by an increase in vitamin A storage in livers at doses of 3 and 36 mg DDT/kg body weight/day, whereas higher doses produced hypothyroidism as evidenced by decreased liver storage of vitamin A at a dose of 54 mg DDT/kg body weight/day (Jefferies and French 1972). Such variable responses within and among species suggest that a species' thyroidal response to organochlorine pesticides could be indicative of its sensitivity to organochlorine pollution. A dietary dose of 50 mg DDT/kg in bobwhites failed to affect the thyroid whereas a dose of 500 mg DDT/kg produced thyroid hypertrophy (Hurst et al. 1974). They also found that by the end of the 4-mo dosing period neither dose affected uptake of I^{131}.

Because vitamin A is closely related to thyroid activity and also is important for normal embryonic development (March et al. 1972), it will be monitored in female blood.

VITELLOGENIN

Vitellogenin is an egg yolk protein precursor produced in the avian liver in response to increased concentrations of estrogen in the blood (Gibbins and Robinson 1982). Binding of estradiol to DNA in livers of chickens has been shown to coincide with vitellogenesis (Saluz et al. 1986). Jost et al. (1986) also showed that vitellogenesis could be reactivated *in vitro* in cells that had been stimulated to produce vitellogenin *in vivo*, whereas other genes also induced by estradiol could not be reactivated. They identified calmodulin, a protein important for regulation in many kinds of cells (McGilvery 1983), as one of the necessary components of the reactivation process. Lundholm (1987) identified both DDE and DDT as potent inhibitors of calmodulin. Inhibition of calmodulin, therefore, could cause a decrease in vitellogenesis.

Another possible route of interference of DDT on vitellogenesis would be its effect on estrogen. o,p′-DDT has been shown to have estrogenic effects (Bitman et al. 1968, Robison et al. 1985). Robinson and Gibbins (1984) were unable to induce vitellogenesis in male Japanese quail with DDT but could with estradiol, suggesting that DDT does not exhibit any estrogenic effect involving vitellogenin. Induction of vitellogenesis, therefore, is not likely. DDT also has been shown to decrease concentrations of estradiol in blood (Peakall 1970). This would suggest that DDT could very well inhibit vitellogenesis. Riegal et al. (1986) showed that antiestrogens were able to inhibit vitellogenesis. It is possible, although it has not been shown, that p,p′-DDT could compete with estrogen for its binding sites because it is very similar structurally to o,p′-DDT which will act similarly to estrogen.

Finally, DDT has been shown to bind to vitellogenin and possibly induce its production (Denison et al. 1981, Denison and Yarbrough 1985). This and the evidence presented above

support an interaction between DDT and vitellogenin making vitellogenin a likely candidate as a biomarker reflecting DDT contamination and having an important effect on reproduction in birds.

VITAMIN A

Vitamin A occurs in several forms in the animal body (Ganguly 1989). The four forms of major biological importance are retinaldehyde, retinoic acid, retinol, and retinyl esters. Retinaldehyde is the the active form of vitamin A involved in vision in the vertebrate eye (Bridges 1984). Retinol is an alcoholic form of vitamin A and is the form transported in plasma (Ganguly 1989). Retinol is found in many other tissues as well, but this form does not perform many of vitamin A's numerous functions but can be converted to active forms. Retinyl esters, primarily retinyl palmitate in vertebrates, are the major storage forms of vitamin A (Futterman and Andrews 1964, Frolick 1984, Zachman 1987, Furr and Olson 1989, Ganguly 1989).

Retinoic acid has just recently been identified as the form of vitamin A with biological activity. Roberts and Sporn (1984) said, in their review of cellular biology and biochemistry of retinoids, that the mechanism for cellular or molecular activity for many of vitamin A's effects was not yet known. More recent literature is beginning to provide just such information. Retinoic acid has been shown to induce growth hormone expression (Morita et al. 1990), induce transcription of mRNA from an oncogene (de Verneuil and Metzger 1990), induce homeobox genes which play a role in mammalian development (Cianetti et al. 1990), control expression of the MK gene which is transiently activated during differentiation of embryonal carcinoma cells (Huang et al. 1990), and induce mRNA production for alkaline phosphatase (Rodan et al. 1990).

Retinoic acid cannot perform all of vitamin A's functions. Thompson et al. (1969) showed that retinoic acid supplements will not perform vitamin A's role in vision, presumably because

it cannot be reduced to retinaldehyde. Fertile eggs were produced when both male and female chickens received vitamin A supplements as retinoic acid, but the embryos would not develop past the second day of incubation. It is likely that retinoic acid cannot be deposited in eggs in sufficient amounts for all of vitamin A's activities because Joshi et al. (1973) found that almost 70% of vitamin A content of freshly laid egg yolks was in the form of retinol. Retinoic acid, however, does play a vital role in embryonic development. The exact mechanism remains unclear, but retinoic acid appears important for limb bud development in chicks (Momoi et al. 1990, Tamura et al. 1990, Wanek et al. 1991). Retinoic acid may play a role in the proliferation of chondrocytes and preventing their differentiation (Takishita et al. 1990). Another important role for retinoic acid during embryonic development may be an involvement in the formation of the anterior-posterior axis (Sive et al. 1990). Retinoic acid also can lead to defects of the neural crest if present in high concentrations (Hart et al. 1990).

Additional roles of retinoic acid not related to development include impairment of the biosynthesis of GDP-mannose caused by impaired hexokinase activity when hamsters are deficient in retinoic acid (Shankar et al. 1990), interaction with thyroid hormone to regulate growth factor receptor promoter expression (Hudson et al. 1990), and a reduction in chromosomal instability (Stich et al. 1990). Retinoic acid may play an active role in many of vitamin A's other roles as shown by its ability to inhibit losses from liver and serum pools of retinyl palmitate and retinol, respectively (Shankar and DeLuca 1988).

Not all roles of vitamin A have been attributed to retinoic acid, and it is likely that some functions of vitamin A may be carried out by retinol. Van Beek and Meistrich (1990) showed that diet supplementation with retinol would allow normal testicular development and function in rats, whereas retinoic acid supplementation would not. Thompson et al. (1969) found that dietary supplementation with retinoic acid did allow for normal testicular development and function in domestic fowl. This conflict in results may indicate that retinoic acid may indeed be the active form, but that rat testes are only able to absorb retinol from the plasma and not retinoic acid.

Reproduction in females can be affected by vitamin A status also. Hens fed diets with retinoic acid laid eggs that failed to develop past day 2 of incubation, whereas hens given capsules containing different amounts of retinyl acetate showed varied performance with hens provided 1 mg retinyl acetate laying eggs with high hatchability, but hens given less retinyl acetate exhibited reduced hatchability in their eggs (Thompson et al. 1969). Vitamin A deficiency is known to reduce markedly the hatchability of eggs in poultry (Eddy 1949, p. 30). In addition, excess vitamin A in the diet can reduce egg production and hatchability (March et al. 1972).

Vitamin A also is involved in the immune response. T-lymphocyte proliferative response was decreased at low vitamin A intakes in chicks (Sklan et al. 1989). Friedman and Sklan (1989) found that T-lymphocyte proliferative response was decreased at both high and low dietary concentrations of vitamin A. They also found decreases of antigen specific antibody production at high and low dietary vitamin A concentrations. Bruns and Webb (1990) found that both polyclonal and antigen-specific antibody production was affected by vitamin A status in lambs. Pasatiempo et al. (1990) found that rats deficient in vitamin A produced normal amounts of antibodies to lipopolysaccharide antigens but produced decreased amounts of antibodies to capsular polysaccharides, suggesting that only some immune responses are vitamin A dependent.

Vitamin A is important for growth. Vitamin A deficient lambs (Bruns and Webb 1990), pony fillies (Donoghue et al. 1981), and a fish (*Heteropneustes fossilis*) (Goswami and Basumatari 1988) all grew more slowly and the lambs and fish eventually lost weight. Donoghue et al. (1981) also showed that excess vitamin A decreased growth and caused weight loss in pony fillies. Such an effect of growth and weight gain may be caused by retinoic acid's affect on growth hormone gene expression (Morita et al. 1990).

Vitamin A is important for normal pulmonary activity. Vitamin A deficiency caused atelectasis, bronchopulmonary dysplasia, and pulmonary edema in guinea pigs (Nair et al. 1988). They found microsomal and cytosolic protein content to be diminished by vitamin A deficiency. Enzyme activity was altered. Superoxide dismutase and glutathione peroxidase

showed lower activity, whereas cytochrome P-450, NADPH cytochrome c reductase, analine hydroxylase, and O-demethylase all showed increased activities in lungs. Zachman (1987) exposed newborn rabbits to 70-75% O_2 in their environments and found that lung retinol was decreased as were liver stores of retinyl palmitate, suggesting a role of vitamin A in protection from oxygen.

The above review is not intended to be exhaustive but merely to indicate the varied role vitamin A plays in vision, growth, immunological response, gene control, and reproduction. In his review, Ganguly (1989) describes many more roles such as preventing keratization of epithelia, regulation of cell division, and glycoconjugate biosynthesis. Since the work presented here was initiated, Lewis et al. (1990) proposed a transport scheme that shows the complexity of the many pathways possible for vitamin A. Plasma retinol was shown to be only a small portion of the entire pathway.

A final aspect of the biology of vitamin A that must be discussed is its transport as reviewed by Goodman and Blaner (1984). Vitamin A is absorbed as retinol in the intestine. Intracellular retinol is esterified primarily to retinyl palmitate which is incorporated into chylomicrons and excreted into the lymph. After entering the blood stream, the lymph chylomicron is processed into a chylomicron remnant by removal of triglycerides. Chylomicron remnants are removed from the blood by the liver. During metabolism of the chylomicron remnant, the retinyl esters are hydrolyzed to retinol and may either enter the bloodstream associated with retinol-binding protein (RBP) or are re-esterified for storage in liver parenchymal cells or nonparenchymal fat-storing cells. Hepatic stores generally account for approximately 90% of all body stores.

RBP normally will bind in the plasma with transthyretin (TTR), the carrier molecule for thyroxine. The RBP-TTR complex stabilizes the bond between retinol and RBP (Goodman 1984). Retinol is absorbed into extrahepatic cells through the involvement of cell surface receptors for RBP. Once in the cell, cellular retinol-binding protein will bind the free retinol. Specific binding proteins for retinoic acid and retinaldehyde also exist. The specific form of vitamin A is then transported to its site of activity by these binding proteins (Ganguly 1989).

A number of studies have already reported on effects of organochlorine contamination on vitamin A in birds. Male Japanese quail had decreased liver concentrations of total vitamin A after dietary exposure to either a PCB mixture or p,p′-DDT whereas female quail exhibited decreased total vitamin A concentrations after only dietary PCB (Cecil et al. 1973). Herring gull (*Larus argentatus*) vitamin A (both retinyl palmitate and retinol) stores were reduced in the liver in areas more highly contaminated with dioxins (Spear et al. 1986). In the same study, they reported that only retinol stores were reduced in ring dove livers after being dosed with a dioxin analog (3,4,3′,4′-tetrachlorobiphenyl, TCBP). Spear et al. (1989) exposed ring doves to 40 mg TCBP/kg body weight intraperitoneally causing delayed egglaying and embryo death. The exposed females laying viable eggs had higher concentrations of serum retinol than exposed females laying eggs that failed to hatch. Spear and Moon (1986) found that TCBP also interfered with thyroid activity and serum thyroxin concentrations.

Studies in rats also show that organochlorines will affect vitamin A metabolism. Microsomal enzyme inducers, including phenobarbital (DDT behaves similarly to phenobarbital in microsomal enzyme induction (Bitman et al. 1971)), have been shown to reduce vitamin A stores in livers (Tuchweber et al. 1976). A single oral dose of p,p′-DDT altered the brush border membrane in rat intestines elevating total lipid, phospholipid, and triglyceride content of brush borders (Dudeja and Mahmood 1982). It is unclear how this may affect vitamin A absorption, but exposure to DDT also has been shown to alter liver concentrations of vitamin A (Phillips 1963, Tinsley 1969, Ferrando et al. 1973, de Waziers and Azaïs 1987). In male Wistar rats, injection with 3,3′,4,4′,5,5′-hexabromobiphenyl, a polybrominated biphenyl, reduced vitamin A stores in the liver and increased serum retinol (Spear et al. 1988). A metabolite of TCBP was shown to bind to TTR, thus destabilizing the retinol binding ability of the RBP-TTR complex (Brouwer and van den Berg 1986). TCDD also was shown to interfere with vitamin A metabolism in rats (Håkansson and Ahlborg 1985).

These studies support the contention that halogenated hydrocarbons alter the physiology of vitamin A in both birds and mammals suggesting that the effects are likely to occur across phylogenetic lines. Effects on avian reproduction could arise according to two

scenarios. A contaminant could bind to the TTR-RBP complex thus reducing the amount of circulating vitamin A although adequate stores of vitamin A exist in the liver. Chronic exposure could alternatively deplete liver stores of vitamin A sufficiently that when the stores need to be mobilized during reproduction, hatchability could be reduced because of insufficient vitamin A. The likelihood of DDT affecting vitamin A physiology is high and such an effect would likely alter reproduction. Therefore, vitamin A will be evaluated as a predictor of reduced reproduction caused by exposure to DDT.

VITAMIN E

That vitamin E (α-tocopherol and some of its analogues) acts as an antioxidant has been recognized since the early 1940's (Yang and Desai 1978). Its specific function as free-radical scavenger and its associated protection of membrane-bound lipids from peroxidation was just becoming widely known in the late 1970's and early 1980's (McCay and King 1980, Willson 1983). In addition to its protective function, it appears that vitamin E plays a structural role in membranes (Diplock 1983) and may also function in a regulatory manner in nucleic acid and protein synthesis (Catignani 1980).

The ability to assess vitamin E status from plasma concentrations is still under investigation (Hidiroglou and Charmley 1990). Jensen et al. (1990) found that plasma vitamin E concentrations reflected liver concentrations very well during periods of vitamin E depletion, but during periods of vitamin E supplementation, plasma appeared to become saturated and did not reflect liver stores well. They found that plasma concentrations did not reflect stores in other tissues. Recently it has been shown that after an exposure to contamination, plasma concentrations of vitamin E may not prove appropriate to monitor tissue concentrations because the mobilization and transport of vitamin E is too dynamic a process (Warren and Reed 1991). It is important to be able to assess vitamin status of tissues other than liver

because the liver is only a temporary storage organ whereas skeletal muscle and adipose tissues reflect long-term vitamin E status (Gallo-Torres 1980).

Vitamin E's function as antioxidant has been shown to have a variety of effects. Vitamin E is thought to prevent structural alterations in membranes caused by oxidation (McCay et al. 1978, McCay and King 1980). Membrane protection appears to be the mechanism by which vitamin E prevents inactivation of glucose-6-phosphatase (Kato and Nara 1978, Takeuchi et al. 1978) and prevents erythrocyte lysis (Mino and Sugita 1978, Burton et al. 1983). Barnes et al. (1978) found reduced activities of mitochondrial membrane-bound succinate dehydrogenase and microsomal NADH/cytochrome c reductase to have reduced activities in vitamin E deficient rats. The membrane association of these enzymes would suggest a membrane protective role for vitamin E. Vitamin E was also shown to protect Na^+,K^+-ATPase from inactivation by membrane lipid peroxidation.

Cytosolic antioxidants such as vitamin C and glutathione have been shown to interact with vitamin E to prevent oxidative damage in cells (Sato et al. 1990, Tampo and Yonaha 1990, Schenschonka et al. 1990). Vitamin E, however being membrane bound, would be able to scavenge free radicals produced by membrane-bound enzymes before they could oxidize membrane lipids (McCay and King 1980). Glutathione appears to play an important role in the antioxidation of vitamin E. Leedle and Aust (1990) have shown that reversibly oxidized vitamin E will be reduced by glutathione, thus allowing it to remain active while bound to its membrane. Vitamin E and glutathione are both necessary to prevent oxidative damage from certain toxicants (Maellaro et al. 1990, Tampo and Yonaha 1990). Vitamin E also has been shown to be important to prevent liver damage from carbon tetrachloride (CCl_4) (Miyazawa et al. 1990), reduce the number of liver tumors caused by aflatoxin B_1 (Nyandieka et al. 1990), protect against radical-induced inactivation of Na^+, K^+-ATPase (Thomas and Reed 1990), and protect against lipid peroxidation and loss of protein sulfhydrils in microsomes after exposure to different prooxidants (Scheschonka et al. 1990). The dehalogenation process of CCl_4 and possibly other halogenated hydrocarbons produces halogen free radicals that could be scavenged by vitamin E (Menzel 1980). Phenobarbital, a substance that induces microsomal

enzymes similarly to DDT and PCB's, caused a reduction in liver concentrations of vitamin E (Hendrich et al. 1991), whereas PCB's have been shown to cause an increase in serum vitamin E concentrations (Katayama et al. 1991).

Evidence also exists for a role of vitamin E in regulation of nucleic acid and protein synthesis (Catignani 1980). Vitamin E deficiency leads to an increase in the amount of RNA in tissues probably from reduced degradation rather than increased production. Vitamin E deficiency has been shown to lead to increased protein synthesis and to increase the specific activity of many enzymes. Some of the results of deficiency attributed to regulation could be caused by an antioxidant effect, but the best evidence for a regulatory function is the existence of a binding protein in liver homogenate supernatent (Catignani 1980) and in rat hepatocyte nuclei (Nair et al. 1978).

Structural functions for vitamin E in membranes have also been suggested (Diplock 1983). Vitamin E has been shown to help stabilize membrane fluidity in mitochondria (Corwin 1980). Vitamin E altered the permeability of membranes to glucose and chromate (Lucy 1978). Mino and Sugita (1978) attributed the prevention of hemolysis by vitamin E to a structural role in erythrocyte membranes. Kagen et al. (1990) found that α-tocopherol, the overwhelmingly predominant form of vitamin E was a less efficient antioxidant than its shorter hydrocarbon chain homologues, but it fit into membranes much better adding to their stability.

As with vitamin A, the mechanisms causing many clinical signs of vitamin E deficiency have not been determined. Deficiency can lead to fetal death and resorption, disruption of embryonic development, defective development of yolk sac and allantois, defective fetal blood vessels, testicular atrophy, myopathy, nutritional encephalomalacea, axon and myelin sheath degeneration, exudative diathesis, fatty deposition in livers, spontaneous necrosis possibly with hemorrhage, reduced corticosterone production, and reduced immune response (Kitabchi et al. 1978, Nelson 1980, 1983, Kitabchi 1980, Hardie et al. 1990, Hassan et al. 1990, Moriguchi et al. 1990).

TEST SPECIES

Much recent literature indicates the persistent nature of the problems caused by DDT in not only carnivorous/piscivorous birds but also in such granivores as northern bobwhite. Montz et al. (1983) investigated the problem of contamination of bobwhite in Virginia. More recently, Stinson (1989) found significant concentrations of 24 pesticides and their metabolites in northern bobwhite in Virginia. The most frequently occurring were hexachlorobenzene, dursban, dieldrin, DDE, chlordane, oxychlordane, lindane, and aldrin with concentrations of up to 77 ppm DDE in wing fat.

The northern bobwhite is a species experiencing population declines (Virginia Commission of Game and Inland Fisheries 1986) making the added pressure of destructive sampling undesirable. Reduced reproductive success caused by contamination can be a serious threat to maintaining adequate population numbers. A means by which a population can be sampled and reproductive potential assessed without exacerbating an existing decline is most desirable. Determination, at this time, of the effects of low doses administered over an extended period of time in bobwhites is of interest in light of Stinson's (1989) findings.

Japanese quail is a species commonly used for both toxicological and physiological studies because it has been greatly domesticated and adapts well to the laboratory setting. It will be used in this project because it is a closely related species to the northern bobwhite and exhibits a greater reproductive sensitivity to organochlorine contamination.

CHAPTER 3: RESPONSE OF VITELLOGENIN TO DDT IN NORTHERN BOBWHITES

INTRODUCTION

Organochlorine pesticides are environmentally persistent chemicals most of which have been banned from use in the United States. The organochlorine, DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane), was first used extensively in the 1940's and continued to be used heavily until it was banned from use in the United States in the early 1970's. It continues to be used in many other areas of the world including Latin America. The LC_{50} for DDT in northern bobwhites (*Colinus virginianus*) is 611 ppm in feed (95% C.I. = 514-724 ppm) and is considered moderately toxic (Heath et al. 1972). DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene), the major metabolite of DDT, has an LC_{50} of 825 ppm in feed (95% C.I. = 697-976 ppm) for northern bobwhite.

Locally high amounts can accumulate greatly in species such as the bobwhite which have small home ranges. Ludke (1977) also has shown that DDE has the potential to increase the toxicity of parathion, an organophosphate, to Japanese quail (*Coturnix coturnix japonica*).

For these reasons, the hazard of DDT and related organochlorines continues to be a cause for concern and continued study of nondestructive sampling techniques is warranted.

This study investigated the effects on egg morphology for bobwhite orally dosed with DDT. Gallinaceous birds tend not to experience severe eggshell thinning in response to DDT exposure (Stickel 1973). Other characteristics such as yolk weight, albumen weight, and percent yolk fat were investigated to determine whether these characteristics were altered. Concentrations of plasma vitellogenin also were investigated in an effort to develop a nondestructive sampling technique to evaluate a hen's reproductive potential. As discussed in Chapter 2, it is highly probable that DDT will cause a decrease in plasma concentrations of vitellogenin. Egg morphology and plasma vitellogenin were compared to DDT concentrations in both egg yolks and the liver, the site of vitellogenin production.

METHODS

Animal Care

Female northern bobwhites were purchased in mid-April, 1987 from a commercial breeder (Charles C. Brown, Fayetteville, NC). They were used in a behavioral study of food choices before being used in the present study. During the behavioral study and through the present research, all birds were caged individually and held in an open-air polebarn on the campus of Virginia Polytechnic Institute and State University.

Bobwhites were acclimated to laying cages (G.Q.F. Manufacturing Co., Savannah, GA) for at least two weeks. The cages measured 20.3 x 30.5 cm at the back of the cage; 25.4 x 30.5 cm in the front of the cage and were 50.8 cm long. The floors of the cages were sloped to facilitate egg collection. From mid-June through the end of the study, bobwhites were

provided with commercial laying mash (15% crude protein) and water *ad libitum*. To induce laying, natural light was supplemented by two incandescent bulbs on a 15L:9D light cycle.

From 30 July through 20 August, 1987, 36 bobwhites were randomly assigned to 3 dose groups and weighed and dosed every third day. Bobwhites were dosed a total of eight times during this period. Technical grade DDT (courtesy of J.L. Eaton, Department of Entomology, Virginia Polytechnic Institute and State University) was intubated in corn oil to provide doses of approximately 0, 1, 10 μg DDT/g body weight. Twelve female bobwhites also were assigned to a group receiving 100 μg DDT/g body weight, but this dose was abandoned after 6 of the 12 bobwhites died after only two dose periods. As verified on a gas chromatograph, doses were 0, 0.48, and 10.12 μg DDT/g bw, respectively. Bobwhites were allowed a 14-day recovery period without exposure to DDT before being bled and sacrificed.

Eggs

Eggs were collected daily during the treatment period (30 June - 20 August) and the recovery period (21 August - 3 September) and refrigerated at 1.5 C until processed. Length and width measurements were taken on each egg and volumes calculated. Because many eggs were cracked or dented, volume could not be measured by water displacement so the volume of a prolate spheroid according to Moriarty et al. (1986) was calculated as

$$V = \frac{4}{3}\pi ab^2$$

where a is half the maximum length and b is half the maximum width. Eggs were then boiled for 6 min to facilitate separation into yolk, albumen, and shell plus membranes components. All components were placed into separate 6 oz whirl-pak plastic bags and frozen at -4 C. Components were freeze-dried and dry weights were taken. Percent yolk fat also was measured. Yolks from the first, middle, and last laid eggs from each bobwhite were analyzed

for DDT. First laid eggs were collected from 30 July - 1 September for 0 ppm group, 30 July - 2 August for 1 ppm group, and 30 July - 8 August for 10 ppm group. Middle laid eggs were collected from 15 August - 3 September for 0 ppm group, 4 August - 21 August for 1 ppm group, and 13 August - 21 August for 10 ppm group. Last laid eggs were collected from 1 September - 3 September for 0 ppm group, 23 August - 3 September for 1 ppm group, and 27 August - 3 September for 10 ppm group.

Removal of eggshells and membranes from hard-boiled eggs precluded measurement of eggshell thickness directly because the shell too often broke into small fragments and no standardized measurements were possible. To analyze shell thickness, Ratcliffe's (1967) thickness index was used and defined as

$$Index = \frac{W}{L \times B}$$

where W is the freeze-dried weight of the shell plus associated membranes, L is the maximum egg length measured in mm, and B is the maximum egg width measured in mm.

Bleeding and Sacrifice

On 3 September, 1987, bobwhite were bled via heart puncture and immediately sacrificed by cervical dislocation. Carcasses were refrigerated overnight at 1.5 C. Livers were extracted the following day and frozen at -4 C in 6 oz whirl-pak plastic bags. Livers were freeze-dried and dry weights were taken. Livers were later analyzed for DDT content.

Blood

Immediately after blood was drawn, packed cell volumes were measured and the remaining blood was centrifuged to separate cells from plasma. Vitellogenin was measured in the plasma. To measure vitellogenin, phosphoprotein was precipitated from the plasma with trichloroacetic acid, and phospholipids were removed by acetone and ethanol/ether/ chloroform washes. The initial isolation of acid insoluble phosphorus was performed as suggested by Bergink et al. (1973). Quantitative determination of plasma protein-bound phosphorus was performed as suggested by K.K. Stewart (Dept. Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, pers. comm.). Flow injection analysis with colorimetry was used. Carrier solution was 1 N NaCl; 7.5% ammonium molybdate ($(NH_4)_6 Mo_4O_{24} \bullet 4H_2O$) in 3 N H_2SO_4 was reduced with 0.3% hydrazine sulfate/0.03% stannous chloride in 1 N H_2SO_4. The blue color produced was compared to a phosphate (KH_2PO_4) standard curve to quantify phosphorus present in the plasma sample.

Pesticide Analysis

The fat containing the pesticides was extracted from the liver and yolk samples with petroleum ether, using a Soxhlet apparatus (Pyrex®, Fisher Scientific Co., Norcross, GA). Sample clean-up, using activated florisil columns (U.S. Silica Co., Berkeley Springs, WV), was performed with 6% ethyl ether/petroleum ether eluting the pesticides of interest from the clean-up column.

The analysis of pesticides was performed on a Tracor 540® (Tracor Corp., Austin, TX) gas chromatograph, equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas, a 1.83 m x 4 mm i.d. glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport® (Supelco, Inc., Bellefonte, PA). All samples were verified on either a Hewlett

Packard 5820A® (Hewlett Packard, Greensboro, NC) gas chromatograph, equipped with a Ni_{63} electron capture detector, using a carrier gas of 95% argon-5% methane, using a 2.44 m x 6.4 mm i.d. glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport®, or on a Tracor 550® gas chromatograph, equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas, a 1.83 m x 4 mm i.d. glass column, packed with 3% OV-1 on 80/100 mesh Supelcoport®.

Statistics

Statistical analyses of data were conducted using the SAS® statistical package (SAS Institute, Inc. 1985a, 1985b). Analysis of variance was used to test for differences in body weight among groups and through time. Analysis of variance was used to test whether differences existed among dose groups or changed over time during the dose period for all egg components measured. Analysis of variance also was used to test whether concentrations of DDT isomers and metabolites differed among dose groups or changed over time in the first, middle, and last laid eggs. Analysis of variance was used to determine whether dose groups laid different numbers of eggs. Pearson correlation coefficients were calculated to determine the relationships between each of the egg components and each of the DDT isomers and metabolites as measured in either the egg yolks or the livers. When DDT from livers was correlated with egg morphological components, only the last laid eggs were included. Pearson correlation coefficients also were used to investigate the relationships between both liver dry weights and percent liver fat and DDT isomers and metabolites as measured in the liver. Plasma vitellogenin and packed cell volume (PCV) were compared to DDT isomers and metabolites measured in both livers and egg yolks using Pearson correlation coefficients. Analysis of variance was used to test for differences among dose groups for initial body weights, body weights after last dose period, final body weights, liver dry weights, percent liver fat, plasma vitellogenin, and PCVs.

RESULTS AND DISCUSSION

Body Weights

Figure 3.1 shows the mean body weights for the three dose groups both during the dose period (Days 1-22) and the recovery period. No significant differences were found for starting bobwhite weights ($F_{2,33} = 0.12$, $P = 0.891$), weights after last dose period ($F_{2,33} = 0.13$, $P = 0.880$), and final body weights ($F_{2,33} = 0.32$, P 0.730). No significant change was observed among dose groups ($F_{2,33} = 0.15$, $P = 0.877$) throughout the entire experiment. No time effect was observed either ($F_{7,231} = 1.46$, $P = 0.182$). The dose x time interaction was not significant ($F_{14,231} = 1.46$, $P = 0.1269$).

Eggs

The isomer o,p′-DDD was not found in detectable amounts (Table 3.1). The concentrations found in yolks and livers from control bobwhites was surprisingly high. No measurable amounts of any DDT isomer or metabolite were found in the corn oil used for intubation or in any batch of food. ΣDDT, o,p′-DDE, o,p′-DDT, p,p′-DDD, p,p′-DDE, and p,p′-DDT were significantly different among dose groups for yolks (Table 3.2) with there being no difference between the 0 ppm and 1 ppm groups and the 10 ppm group being significantly greater than both the 0 ppm and 1 ppm groups for ΣDDT, and each isomer and metabolite. Concentrations of o,p′-DDE, o,p′-DDT, p,p′-DDE, and ΣDDT changed significantly through time, increasing through the middle egg and decreasing after dosing ceased. o,p′-DDE, o,p′-DDT, p,p′-DDE, and ΣDDT also showed significant dose x time interactions.

It is of interest to note that whereas the concentrations of p,p′-DDT were significantly different among dose groups, concentrations did not significantly change through time even though it was the largest component of the dose the bobwhites received. The less marked change with laying sequence for p,p′-DDT indicates that p,p′-DDT does not accumulate in eggs to the extent that other metabolites do. The decrease shown for all isomers and metabolites in the last laid egg in all dose groups indicates that deposition in eggs decreases fairly quickly after exposure ceased even after repeated exposures. This differs from the findings of Cummings et al. (1966) that there was greater deposition of DDT than DDE in domestic fowl when DDT was administered in the food. They also found that whereas DDT deposition in eggs decreased after dosing stopped, DDE deposition continued to increase. DDE/DDT ratios increased, indicating a more rapid decrease in DDT, but DDE also was definitely decreasing. Differences between the results reported here and those reported by Cummings et al. can be attributed to dosing in food or intubating in oil, dosing for 98 instead of 23 days as in this study, the lower doses (0.05, 0.15, 0.45 ppm) that they used, or the difference in species. The DDE/DDT ratios in livers of Japanese quail and bobwhites reported in Chapters 5 and 6 (Tables 5.1 and 6.1) are also considerably different. The best explanation is a species difference.

Ten of the 12 bobwhites in the 0 ppm dose group laid a total of 155 eggs ($\bar{x}$ = 13.0, SE = 3.7); 11 of the 12 bobwhites in the 1 ppm dose group laid a total of 217 eggs ($\bar{x}$ = 17.8, SE = 2.9); 10 of the 12 bobwhites in the 10 ppm dose group laid 176 eggs ($\bar{x}$ = 14.8, SE = 2.4). No difference existed for the number of eggs laid among groups ($F_{2,33}$ = 0.58, P = 0.566). Bobwhite dosed twice with 100 ppm DDT, in preliminary work, produced 21.8 eggs per surviving female over a 36 day period. Egg production of individual bobwhites appears in Appendix Table A.1.

No significant differences were noted for any morphological characteristic among dose groups (Figs. 3.2 and 3.3). No significant changes were observed for any egg morphological characteristics from the beginning of the treatment period to the end of the experiment (Table

3.3). No significant dose x time interactions were observed for any morphological measurement.

Moriarty et al. (1986) strongly questioned the ability of the Ratcliffe Index to accurately reflect eggshell thinning because of its dependence on length and width measurements rather than a direct measure of thickness. In this experiment, however, the method of shell removal from the hard-boiled eggs precluded the standardized method of measuring the thickness at the equator. Another difficulty for the analysis in the present study is that cracked or dented eggs could not be included in the analysis because such deformities prevented accurate length and width measurements thus causing a bias towards thicker shells. The 1 ppm group was more greatly affected with 3 hens laying a total of 17 cracked or dented eggs. The 0 ppm and 10 ppm groups both laid 4 dented or cracked eggs from 4 and 3 hens, respectively.

Albumen weights correlated negatively with ΣDDT, p,p′-DDD, and p,p′-DDE (Table 3.4). p,p′-DDT did not correlate significantly with any of the egg morphological components. The only other DDT metabolite that did not differ significantly among dose groups was p,p′-DDD. While highly significant, the negative correlations of albumen weights with the DDT metabolites account for very little of the variation.

Other studies have found results similar to those presented here. Custer and Mitchell (1987) found no difference in lipid content of black skimmer (*Rynchops niger*) eggs differing in DDE concentrations. Vangilder and Peterle (1981) hard-boiled mallard eggs and separated them into components. They found no differences for yolk weight, albumen weight, shell weight, egg length, egg width, or yolk lipid content between controls and a group fed 10 ppm DDE. Crude oil did effect these characteristics in their study.

Pearson correlation coefficients were also calculated for all egg morphological characteristics and concentrations of DDT isomers and metabolites in the liver (Table 3.5). No egg characteristic correlated significantly with liver concentrations of any DDT isomer or metabolite.

Livers

Liver weights differed slightly ($F_{2,32} = 2.69$, $P = 0.083$) with mean dry weights of 1.63 g, 2.05 g, and 1.57 g for 0 ppm, 1 ppm, and 10 ppm dose groups, respectively. Percent fat of livers also differed slightly ($F_{2,33} = 2.58$, $P = 0.092$) with means of 18.4%, 27.9%, and 19.2% for the same three dose groups, respectively.

Table 3.1 contains mean concentrations of DDT isomers and metabolites measured in livers. Those that were significantly different among dose groups were o,p′-DDE, p,p′-DDE, ppDDE, and ΣDDT (Table 3.6). The metabolite o,p′-DDD was not found in livers in a detectable amount. DDE/DDT ratios were significantly different among dose groups with the 10 ppm group being the greatest. This suggests considerable enzymatic induction in bobwhites receiving the higher doses of DDT. Pearson correlation coefficients for liver weight and liver percent fat with DDT isomers and metabolites found in the liver were calculated (Table 3.6). No significant correlations were found.

Blood

Comparisons of vitellogenin values among dose groups also were not significant ($F_{2,29} = 0.37$, $P = 0.691$). Hematocrit values were also not significantly different among dose groups ($F_{2,27} = 0.14$, $P = 0.871$). No significant correlations were found in comparisons of plasma vitellogenin with DDT isomers and metabolites for both egg yolks and livers (Table 3.7). The Pearson correlation coefficient comparing vitellogenin with yolk weight was 0.2760 ($P = 0.2389$) and was not significant. Pearson correlation coefficients comparing PCVs with DDT isomers and metabolites from both egg yolks and livers were calculated and found to be not significant (Table 3.7).

The lack of significance in both ANOVA and correlation analyses is strong evidence that DDT does not affect vitellogenin production in bobwhites. Robinson and Gibbins (1984) found that the estrogenic effect of o,p′-DDT was not strong enough to induce vitellogenin production in male Japanese quail as does estradiol-17β. Vitellogenin does not appear to be a good biomarker of contamination with DDT-like chemicals. Nor does it hold promise as a biomarker of reproductive potential as affected by DDT-like chemicals. DDT also does not affect or correlate with PCVs.

CONCLUSIONS

The only egg morphological characteristic to correlate with egg DDT concentrations was albumen weight. None of the correlations found was strong enough to allow confident prediction of albumen weight from DDT concentrations. Also, without fertility or hatchability information for the eggs in this study, it is not possible to ascribe any biological significance to the correlations. No significant difference in Ratcliffe Index of eggshell thinning among bobwhites receiving DDT and controls has led to the conclusion that northern bobwhites are not very sensitive to the eggshell thinning action of DDE.

Finally, it does not appear that either plasma vitellogenin or blood PCVs respond to DDT contamination. Therefore, they would not be very useful biomarkers for determining reproductive success in response to exposure to DDT-like chemicals.

Table 3.1. Mean values for DDT (μg/g dry weight), its isomers and metabolites, and mean DDE/DDT ratios as found in the first, middle, and last eggs laid and in bobwhite livers.

Dose	Egg	ΣDDT	o,p′-DDE	o,p′-DDT	p,p′-DDD	p,p′-DDE	p,p′-DDT	DDE/DDT
Yolks								
0	First	0.993 (0.051)[2]	N.D.[1] -	N.D. -	N.D. -	0.083 (0.034)	0.074 (0.025)	1.470 (0.561)
	Middle	1.203 (0.093)	N.D. -	N.D. -	N.D. -	0.241 (0.071)	0.128 (0.041)	2.992 (1.313)
	Last	1.026 (0.065)	N.D. -	N.D. -	0.002 (0.000)	0.088 (0.022)	0.089 (0.034)	1.331 (0.287)
1	First	1.135 (0.130)	N.D. -	N.D. -	0.002 (0.000)	0.182 (0.088)	0.117 (0.047)	2.135 (0.756)
	Middle	4.655 (0.315)	0.065 (0.017)	0.145 (0.033)	0.122 (0.057)	2.809 (0.292)	0.853 (0.275)	20.666 (12.067)
	Last	2.327 (0.547)	0.033 (0.003)	0.076 (0.008)	0.048 (0.040)	1.096 (0.299)	0.282 (0.173)	10.586 (2.314)
10	First	22.941 (16.350)	0.117 (0.088)	0.603 (0.354)	0.922 (0.732)	12.697 (8.697)	4.279 (3.335)	2.247 (0.728)
	Middle	96.142 (20.474)	0.660 (0.135)	2.302 (0.622)	3.628 (1.949)	63.268 (9.665)	25.549 (11.523)	6.947 (2.290)
	Last	34.914 (5.997)	0.309 (0.043)	0.336 (0.095)	0.951 (0.460)	28.251 (4.630)	5.760 (1.770)	11.623 (4.985)
Livers								
0		0.748 (0.115)	0.026 (0.002)	N.D. -	0.040 (0.019)	0.386 (0.088)	0.117 (0.026)	4.090 (1.176)
1		1.256 (0.158)	0.029 (0.007)	N.D. -	0.054 (0.022)	0.822 (0.134)	0.170 (0.055)	8.251 (1.982)
10		7.911 (1.686)	0.079 (0.025)	0.243 (0.047)	0.642 (0.173)	6.448 (1.384)	0.499 (0.241)	46.305 (15.011)

[1] N.D. = not detectable.
[2] Values in parentheses are the standard error of the mean.

Table 3.2. Analyses of variance for the effect of dose or laying sequence on concentrations of DDT isomers and metabolites in first, middle and last eggs laid during the dosing period.

	Dose		Sequence	
	F	P	F	P
ΣDDT	5.52	0.0107	5.84	0.0058
o,p′-DDE	4.84	0.0168	9.13	0.0005
o,p′-DDT	3.43	0.0490	6.75	0.0028
p,p′-DDD	1.35	0.2775	1.59	0.2161
p,p′-DDE	8.49	0.0016	13.26	0.0001
p,p′-DDT	6.13	0.0074	2.69	0.0797
DDE/DDT	0.61	0.2034	1.79	0.1794

Table 3.3. Analyses of variance for the effect of dose or laying sequence on morphological measurements taken on all eggs laid during the dosing period.

	Dose		Sequence	
	F	P	F	P
Yolk wts.	<0.01	0.9990	1.30	0.1799
Albumin wts.	0.10	0.9052	1.42	0.1152
Shell wts.	0.88	0.4280	1.55	0.0652
Width	0.02	0.9802	0.51	0.9636
Length	0.04	0.9608	0.79	0.7387
Volume	<0.01	0.9985	0.64	0.8882
R.I.[1]	0.16	0.8530	1.58	0.0586
Yolk %Fat[2]	0.04	0.9606	0.67	0.8085

[1] Ratcliffe Index = Shell weight / (Length x Width)
[2] Yolk %Fat was measured only on the first, middle, and last laid eggs (i.e. those eggs analyzed for pesticide residues).

Table 3.4. Pearson correlation coefficients for egg morphological measurements with DDT isomers and metabolites for first, middle and last laid eggs.

	ΣDDT	o,p′-DDE	o,p′-DDT	p,p′-DDD	p,p′-DDE	p,p′-DDT
Yolk wts.	0.7992 (0.4925)[1]	0.1432 (0.2170)	0.1570 (0.1785)	0.0075 (0.9487)	0.0644 (0.5806)	0.0958 (0.4168)
Albumen wts.	-0.2911 (0.0107)	-0.1730 (0.1352)	-0.2084 (0.0728)	-0.3004 (0.0084)	-0.3187 (0.0050)	-0.1805 (0.1239)
Shell wts.	-0.0318 (0.7851)	-0.1243 (0.2845)	0.0150 (0.8984)	-0.0553 (0.6354)	-0.0503 (0.6659)	-0.0138 (0.9070)
Width	0.0373 (0.7488)	-0.0057 (0.9608)	0.1196 (0.3066)	-0.0209 (0.8575)	0.0285 (0.8072)	0.0493 (0.6768)
Length	0.1265 (0.2764)	0.1131 (0.3305)	0.1184 (0.3116)	0.1373 (0.2369)	0.0791 (0.4965)	0.1740 (0.1382)
Volume	0.1041 (0.3708)	0.0598 (0.6075)	0.1660 (0.1547)	0.0616 (0.5972)	0.0720 (0.5366)	0.0377 (0.2415)
R.I.[2]	-0.0936 (0.4179)	-0.0053 (0.9639)	0.0126 (0.9146)	-0.0017 (0.9884)	-0.0396 (0.7322)	-0.0210 (0.8578)
Yolk %Fat[3]	-0.0893 (0.4338)	0.2114 (0.0668)	0.1260 (0.2813)	-0.0236 (0.8388)	0.1119 (0.3324)	0.0325 (0.7819)

[1] Values in parentheses are P-values for correlation coefficients.
[2] Ratcliffe Index = Shell weight / (Length x Width)
[3] Yolk %Fat was measured only in those eggs also analyzed for DDT content.

Table 3.5. Pearson correlation coefficients for egg morphological measurements from the last laid eggs with DDT isomers and metabolites from livers.

	ΣDDT	o,p′-DDE	o,p′-DDT	p,p′-DDD	p,p′-DDE	p,p′-DDT
Yolk wts.	-0.0790	-0.0974	-0.1970	-0.1651	-0.0690	-0.0178
	(0.7076)[1]	(0.6433)	(0.3452)	(0.4302)	(0.7433)	(0.9326)
Albumin wts.	-0.2495	0.1444	-0.3849	-0.2889	-0.2473	-0.0938
	(0.2290)	(0.4911)	(0.0574)	(0.1613)	(0.2334)	(0.6556)
Shell wts.	-0.2127	-0.2764	-0.1139	-0.2198	-0.2186	-0.0218
	(0.3075)	(0.1811)	(0.5879)	(0.2912)	(0.2939)	(0.9177)
Width	-0.2159	-0.1239	-0.1555	-0.2978	-0.2135	-0.0033
	(0.3000)	(0.5551)	(0.4578)	(0.1483)	(0.3055)	(0.9875)
Length	0.0512	0.1100	-0.2575	-0.0188	0.0619	0.0016
	(0.8081)	(0.6005)	(0.2139)	(0.9288)	(0.7688)	(0.9940)
Volume	-0.1424	-0.0501	-0.2475	-0.2369	-0.1352	-0.0063
	(0.4971)	(0.8119)	(0.2330)	(0.2542)	(0.5192)	(0.9760)
R.I.[2]	-0.1967	-0.3225	0.0158	-0.1531	-0.2089	0.0344
	(0.3460)	(0.1159)	(0.9401)	(0.4649)	(0.3162)	(0.8701)

[1] Values in parentheses are P-values for correlation coefficients.
[2] Ratcliffe Index = Shell weight / (Length x Width)

Table 3.6. Pearson correlation coefficients for liver weight and percent fat in the liver with DDT isomers and metabolites from the liver with the analysis of variance for DDT isomers and metabolites and DDE/DDT ratios.

	Weight	Percent fat	
	Coefficient	Coefficient	F
o,p′-DDE	-0.0243 (0.8934)[1]	0.0455 (0.8013)	3.61 (0.0391)
o,p′-DDT	-0.2478 (0.1643)	-0.0110 (0.9516)	1.60 (0.2180)
p,p′-DDD	-0.2345 (0.1890)	0.0143 (0.9371)	10.51 (0.0003)
p,p′-DDE	-0.1076 (0.5510)	0.1164 (0.5190)	16.14 (<0.0001)
p,p′-DDT	-0.0604 (0.7386)	0.2303 (0.1973)	1.93 (0.1621)
ΣDDT	-0.1118 (0.5292)	0.1354 (0.4453)	15.19 (<0.0001)
DDE/DDT			6.44 (0.0046)

[1] P-values for correlation coefficients.

Table 3.7. Pearson correlation coefficients for plasma vitellogenin and PCVs with DDT isomers and metabolites from the liver and first, middle, and last laid eggs.

	Liver		Yolk	
	Coefficient	P	Coefficient	P
Vitellogenin				
o,p′-DDE	0.0335	0.8604	-0.0145	0.9503
o,p′-DDT	-0.1244	0.5125	-0.0367	0.8851
p,p′-DDD	-0.1923	0.3086	-0.0648	0.7920
p,p′-DDE	-0.1863	0.3243	0.0705	0.7744
p,p′-DDT	-0.1969	0.2970	-0.0155	0.9531
ΣDDT	-0.1673	0.2961	0.0342	0.8832
Hematocrits				
o,p′-DDE	-0.1186	0.5402	-0.2683	0.2397
o,p′-DDT	0.0188	0.9229	0.0148	0.9536
p,p′-DDD	0.0195	0.9202	0.0117	0.9621
p,p′-DDE	0.1084	0.5756	0.2250	0.3544
p,p′-DDT	-0.0893	0.6451	0.1645	0.5282
ΣDDT	0.0979	0.6135	0.3275	0.1473

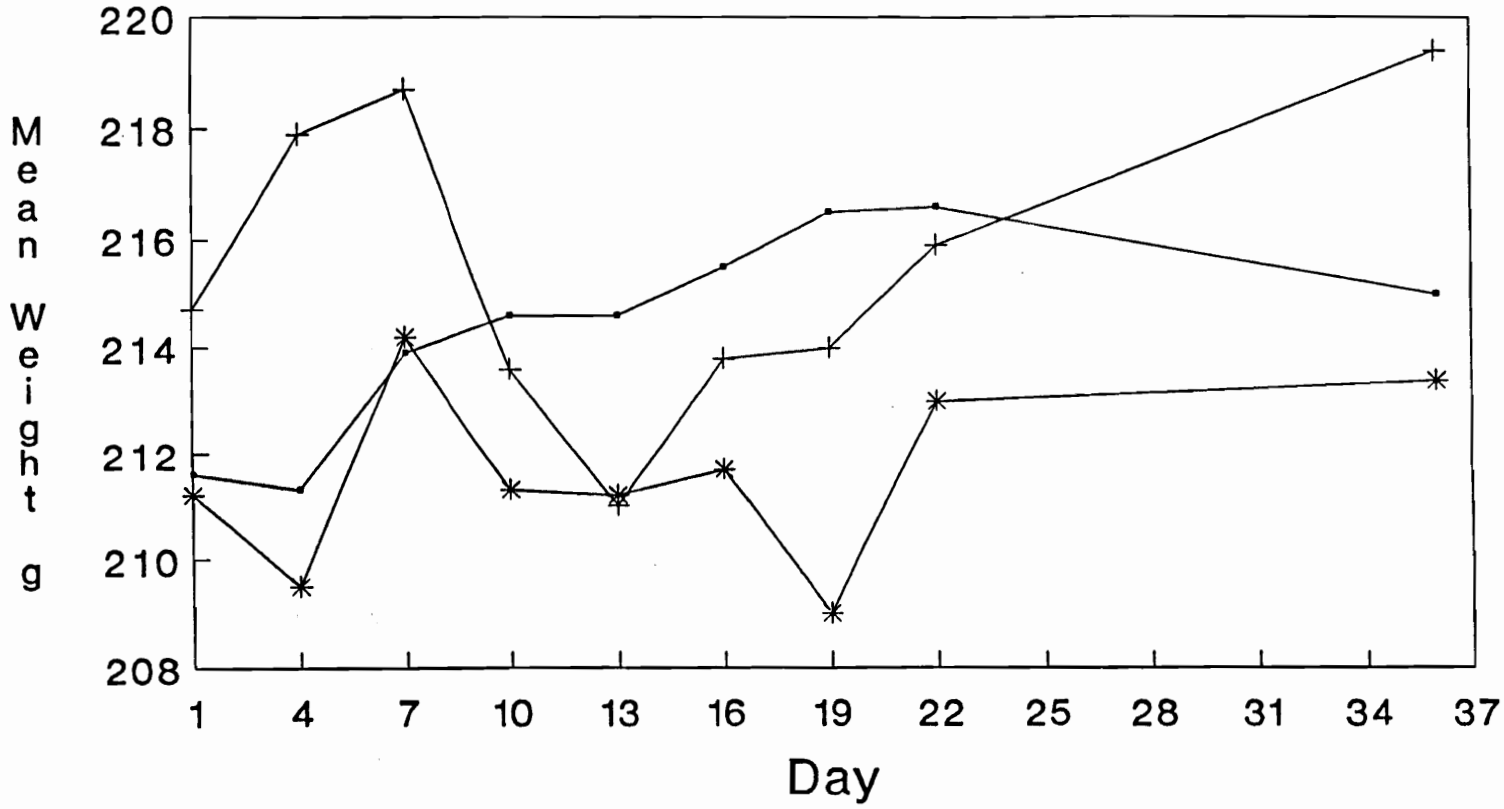

Figure 3.1. Northern bobwhite mean body weights (grams) for all treatment groups (■ for 0 ppm, + for 1 ppm, and * for 10 ppm) throughout both the dosing period (days 1-22) and post-dosing period (days 23-36).

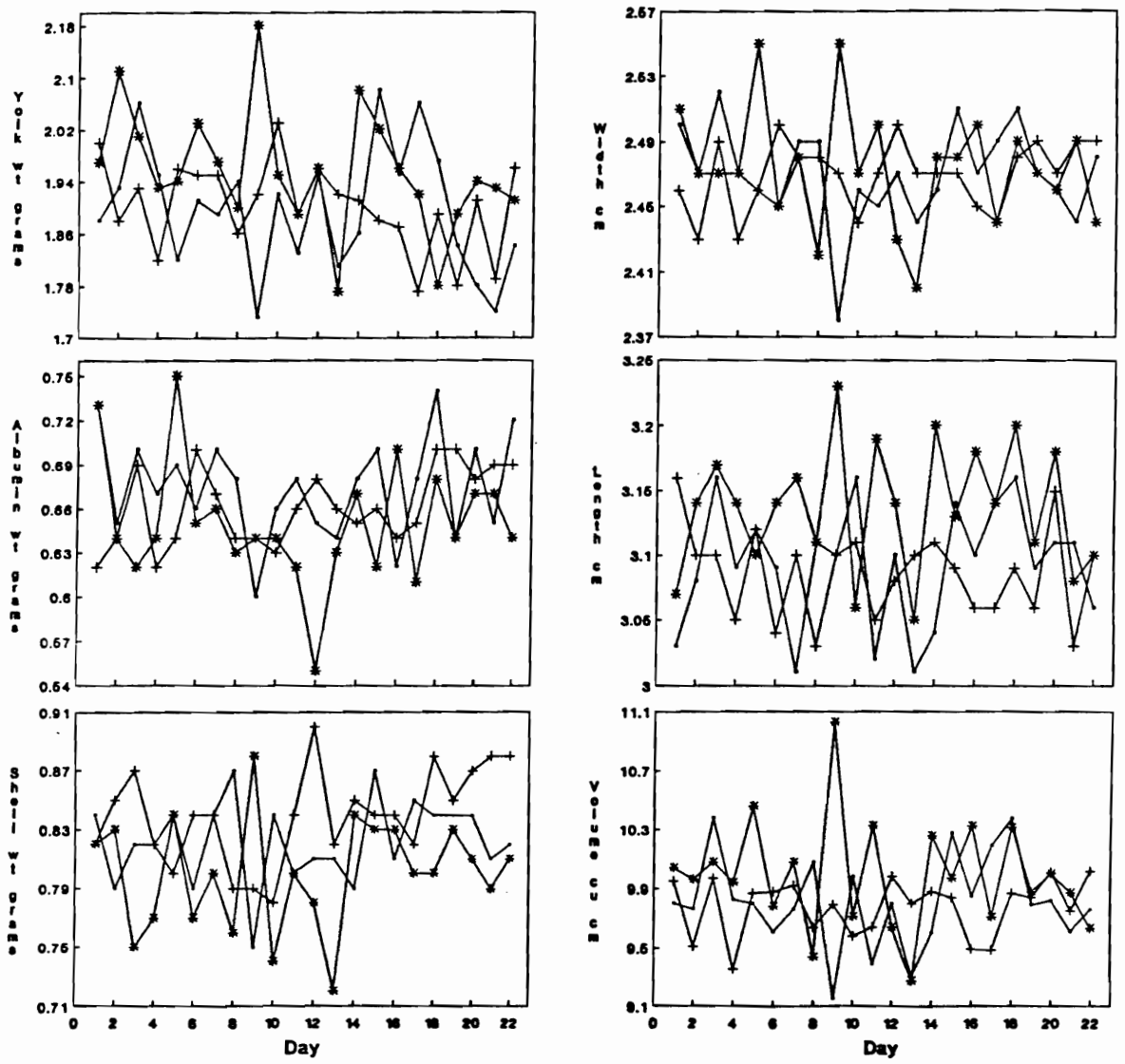

Figure 3.2. Mean values for egg morphological characteristics measured during the dose period for all treatment groups (■ for 0 ppm, + for 1ppm, and * for 10 ppm).

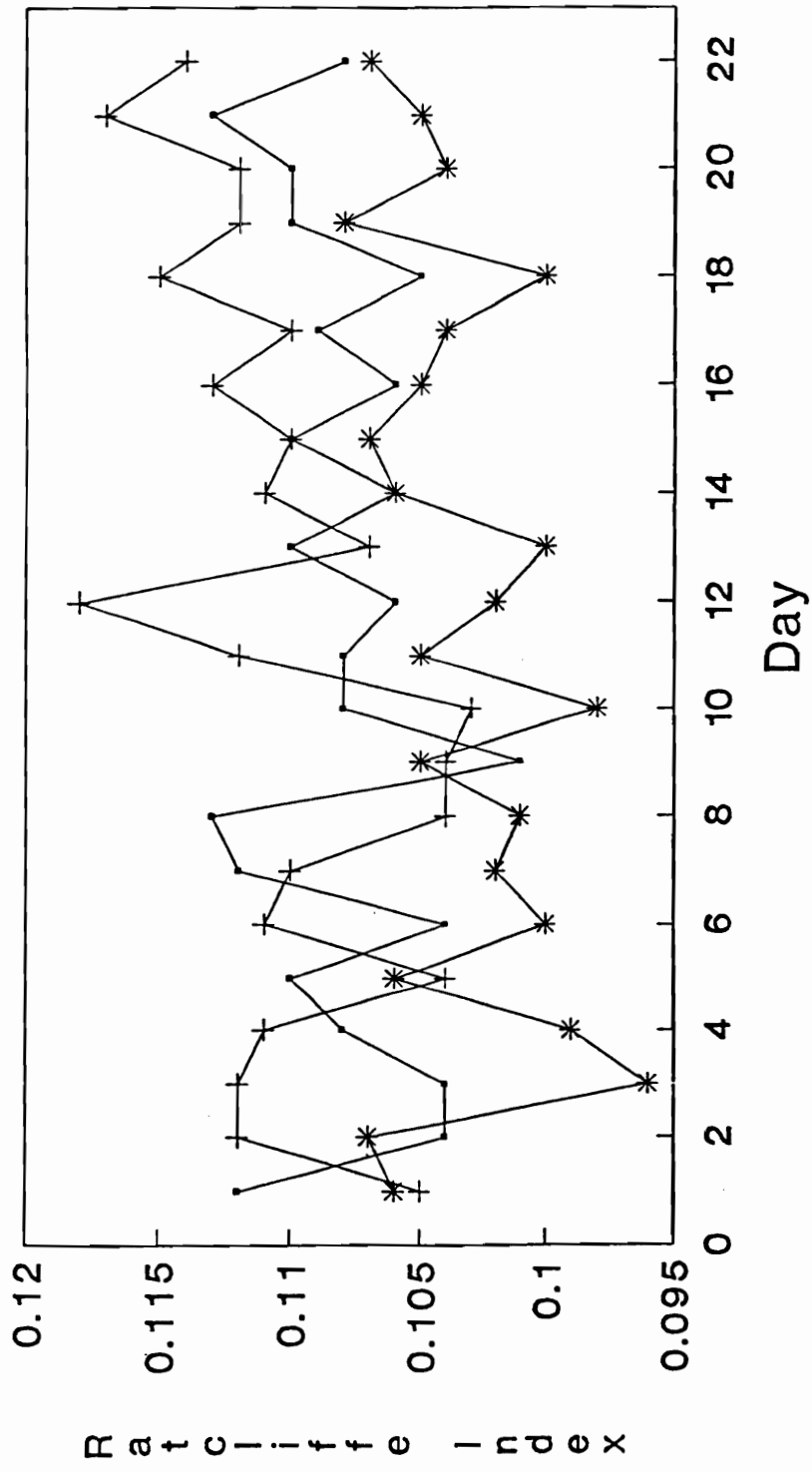

Figure 3.3. Ratcliffe shell thickness index for dose period for all treatment groups (■ for 0 ppm, + for 1 ppm, and * for 10 ppm).

CHAPTER 4: A COMPARISON BETWEEN FOOD ADDITION AND INTUBATION OF DDT TO NORTHERN BOBWHITES

INTRODUCTION

Two commonly used techniques to administer a toxicant orally are food addition and intubation. Each method has certain advantages in experimental situations. Food addition eliminates confounding effects of a carrier, better simulates contamination of an animal's food source, is more continuous because the animal will receive a comparable dose every time it eats, and causes less stress associated with handling. In comparison, intubation allows greater precision in dosing, allows oral administration of less palatable chemicals, and minimizes worker exposure to chemicals during mixing, daily feeding, and clean-up of unused food. Also, peak concentrations in blood or at target organs resulting from bolus doses are more likely to exceed an animal's ability to detoxify a substance than the sustained lower levels resulting from dosage in the diet (Nutrition Foundation 1983).

Little work has been done directly comparing techniques for administering contaminants to test animals. In a study comparing the effects of theophylline dosed via food addition or corn oil intubation, Collins et al. (1988) reported that the target organs (liver in mice and arteries in rats) were similar regardless of dosing technique for both laboratory mice and rats, but the techniques caused differing degrees of toxicity as measured by mortality and body mass changes with intubation being more toxic. Tests comparing carriers are more common. The carrier (corn oil or water) was found to alter the toxicity of trichloroethylene in laboratory mice (Merrick et al. 1989). Two other studies (Chieco et al. 1981, Withey et al. 1983) found that the carrier altered the rate of gastro-intestinal uptake for a variety of organochlorine chemicals.

This study uses northern bobwhite (*Colinus virginianus*) females to compare effects on tissue retention of p,p′-DDT (Sigma Chemical Co., St. Louis, MO) given by food addition and corn oil intubation as oral administration techniques. Only females were used because other work was focusing on effects of contamination on female reproduction.

METHODS

Female northern bobwhites were purchased in mid-April 1987 from a commercial breeder (Charles C. Brown, Fayetteville, NC). They were used in a behavioral study of food choices and kept in an open-air polebarn on the campus of Virginia Polytechnic Institute and State University before use in the present study. They were brought inside in early October 1987 and acclimated to their surroundings for 3 wk before the start of the experiment. Bobwhites were housed individually in galvanized steel cages measuring 51 x 56 x 35 cm and maintained at approximately 21 C with a 9L:15D light cycle.

Thirty nonlaying bobwhites were randomly assigned to three groups of 10 each. A control group received no DDT. A second group (DDT-F) received food treated with DDT

dissolved in acetone at a concentration of 24.81 mg DDT/kg food. The third group (DDT-I) received intubated corn oil containing 6622 mg DDT/ml corn oil. Food for control and DDT-I bobwhites was treated with comparable amounts of acetone. Acetone treatment was assumed not to affect food intake. Food for control and DDT-I groups was found not to contain measurable amounts of DDT. All bobwhites were given corn oil and weighed at 7-day intervals. Individual bobwhites in all groups received corn oil via intubation in amounts adjusted for body mass. Corn oil doses were adjusted so that DDT-I group members each received a dose of 25 mg DDT/kg body mass. The control and DDT-F bobwhites received equivalent amounts of corn oil on a body mass basis. Corn oil for control and DDT-F groups was tested and found not to contain measurable amounts of DDT. Food and water were provided *ad libitum*. Amount of food provided and orts were weighed daily. Spillage was similar among bobwhites and was ignored. The study lasted 56 days with eight dosing periods.

All bobwhites were killed on day 56 by cervical dislocation. Carcasses were refrigerated overnight at 4 C, and livers and brains were removed the next day. The tissues were freeze-dried and weighed. Fat, with associated DDT and metabolites, was extracted from the liver and brain samples with petroleum ether, using a Soxhlet apparatus (Pyrex®, Fisher Scientific Co., Norcross, GA). Samples were extracted for 8 h. Mean recoveries for DDT and its metabolites, based on internal spikes using Aldrin, were 96.7% for brain and 101.8% for liver tissues. Sample clean-up, using activated florisil columns (U.S. Silica Company, Berkeley Springs, WV), was performed with 150 ml 6% ethyl ether-petroleum ether.

Concentrations of o,p′-DDD, o,p′-DDE, o,p′-DDT, p,p′-DDD, p,p′-DDE, and p,p′-DDT were analyzed on a Tracor 540® gas chromatograph (Tracor Corporation, Austin, TX), equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas, and a 1.83 m x 4 mm i.d. glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport® (Supelco, Inc., Bellefonte, PA). All samples were verified on either a Hewlett Packard 5820A® gas chromatograph (Hewlett Packard, Greensboro, NC), equipped with a Ni_{63} electron capture detector, using a carrier gas of 95% argon-5% methane, and a 2.44 m x 6.4 mm i.d. glass

column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport®, or on a Tracor 550® gas chromatograph, equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas, and a 1.83 m x 4 mm i.d. glass column packed with 3% OV-1 on 80/100 mesh Supelcoport®. Limits of detection for each isomer in either brain or liver tissue were determined as the smallest concentration of that isomer measured in that tissue. Liver tissue had smaller limits of detection for each isomer because more tissue was available for analysis. In determining total DDT (ΣDDT), all isomers and metabolites were added; isomer values below the limit of detection were assigned midpoint values between zero and the limit of detection. For comparisons between tissues or between treatment groups, the program UNCENSOR V2.0m (Newman et al. 1989) was used to generate means and standard errors when some samples contained values for some metabolites or isomers below the limit of detection.

Statistical analyses were performed using the SAS® statistical package (SAS Institute, Inc. 1985a, 1985b). Analyses of variance were used to analyze the amount of food eaten, and for differences in body masses among dose groups and over time. A series of t-tests were performed to determine which days were significantly different from others for both body mass and food consumption. Data were tested for normality using the Shapiro-Wilk statistic (SAS Institute, Inc. 1985a). Comparisons for each isomer in livers and brains within each treatment group were made using paired t-tests. Comparisons between DDT-F and DDT-I groups for adjusted residue concentrations were performed using Student's t-tests.

During a previous behavioral study, food intake for these bobwhites was approximately 19 g/day under natural light conditions in May and June (Stinson 1989). Food consumption of $<$ 16 g/day, possibly influenced by the 9 h daylength, and decreasing food intake during the first part of the experiment led to lower DDT exposure in the DDT-F group than anticipated. To account for this, adjusted residue concentrations were calculated by dividing concentrations found in brain or liver (μg DDT/g tissue) by the total amount of DDT (μg) which the individual received. DDE/DDT ratios were calculated by dividing the concentrations of p,p′-DDE by the sum of p,p′-DDD plus p,p′-DDT (used because p,p′-DDT is known to convert

to p,p'-DDD under anoxic conditions as cold as -20 C (Walker and Jefferies 1978)). ΣDDT is the summation of all DDT isomers and metabolites measured in the samples.

RESULTS

Two DDT-F bobwhites died from unknown causes during the experiment. They had no overt signs of DDT poisoning, such as tremors, and their food contained similar amounts of DDT to that found in the entire batch. Because they did not complete the experiment, data relevant to them were dropped from all statistical analyses.

Body masses differed significantly among groups throughout the entire experiment ($F_{2,25} = 3.51$, $P = 0.0452$). However, initial masses (Day 0 of Figure 4.1) of bobwhites randomly assigned to the three dose groups were slightly different ($F_{2,25} = 2.92$, $P = 0.0725$). When initial body masses were used as covariants, body masses no longer were found to differ among dose groups ($F_{2,24} = 0.84$, $P = 0.4440$). Thus it appears that differences in body mass resulted from chance, rather than from a treatment.

When body masses were analyzed through time, a significant effect for daily body mass was noted ($F_{2,200} = 5.01$, $P < 0.0001$). A series of t-tests for paired comparisons of body masses among days showed that masses from Days 7, 14, and 21 differed significantly from masses on Days 28, 35, 42, 49, and 56. No other combination of days showed significance. There was no time x dose interaction ($F_{16,200} = 0.90$, $P = 0.5691$).

The amount of food consumed by bobwhites (Figure 4.2) did not differ significantly among dose groups ($P = 0.1267$). The amount of food consumed did change through time ($P < 0.0001$). A series of t-tests performed on food consumption among days found food consumption differed. Days 28, 35, and 42 were significantly different from the greatest number of other days (≥ 50 days). Bobwhites ate less on these days presumably because they received corn oil on these days.

Brain concentrations of DDT isomers and metabolites did not differ significantly from liver concentrations in the control bobwhites (Table 4.1). In DDT-F bobwhites, ΣDDT ($P < 0.0001$), p,p′-DDE ($P = 0.0002$), and p,p′-DDT ($P = 0.0679$) concentrations all differed significantly between brains and livers. Concentrations in brains and livers of the DDT-I bobwhites differed significantly for ΣDDT ($P < 0.0001$), p,p′-DDD ($P = 0.0008$), p,p′-DDE ($P = 0.0002$), and p,p′-DDT ($P = 0.0388$). When measurable amounts were found, the liver contained greater concentrations of DDT metabolites and isomers, except for p,p′-DDT, than the brain from the same individual.

Significant differences occurred between DDT-treatment methods for adjusted brain concentrations (i.e. concentration measured in the brain / total amount of p,p′-DDT received during the experiment) (Table 4.2) for ΣDDT ($P = 0.0022$), p,p′-DDD ($P = 0.0125$), and p,p-DDE ($P = 0.0016$). Significant differences of adjusted liver concentrations (Table 4.2) were found for ΣDDT ($P = 0.0191$), p,p′-DDE ($P = 0.0931$), and p,p′-DDT ($P = 0.0588$).

DDE/DDT ratios from brains were significantly different ($P = 0.0055$) between DDT-F and DDT-I bobwhites (Table 4.3), but ratios from livers showed no difference. Comparisons of DDE/DDT ratios between brain and liver tissues showed that tissues from DDT-F bobwhites ($P = 0.0017$) and DDT-I bobwhites ($P = 0.0188$) were significantly different. No significant differences were observed for dry liver masses, relative liver masses, or percent liver fat (Table 4.4).

DISCUSSION

The amount of food consumed was not altered by addition of DDT to the food indicating little or no taste aversion. However, handling the bobwhites and/or giving them corn oil via intubation temporarily affected the amount of food consumed for all groups on some days when corn oil was given. Significantly less food was consumed on Days 28, 35, and 42 when

oil was given, subsequently followed by increased food consumption (Figure 4.2). Day 28, the first day that showed a severe reduction in food consumption, was also the beginning of the period of significantly lower body masses. Food consumption after Day 28 was more erratic than early in the study. The change in feeding behavior is the likely cause of the reduction in body masses.

To simulate continual exposure to a contaminant, the chemical must be administered more often than once weekly. In this study, all bobwhites were handled similarly each week when given corn oil. Even with such an intermittent dosing regimen, handling and/or administration of corn oil disrupted eating patterns and led to body mass loss in all treatment groups including controls, possibly confounding potential effects of interest caused by the contaminant. Reducing the amount of corn oil or using a different carrier (i.e. non-nutritional carrier) may alleviate this potential problem. If the problem is related to handling, fewer doses would be indicated, but fewer doses would constrain experimental design and testing protocols by making them more artificial.

As p,p′-DDE is the primary metabolite, it reflects "background" DDT exposure not related to treatment. Only p,p′-DDE was found in detectable amounts in brains of control bobwhites. In DDT-treated groups (DDT-F and DDT-I), the liver had higher concentrations of DDT isomers and metabolites after adjustments for amount of exposure. p,p′-DDT was an exception. From this, it appears that when exposure is relatively high, brains may have a greater tendency to store p,p′-DDT than the liver which has a greater ability to metabolize p,p′-DDT. Also, because livers of some control bobwhites had detectable concentrations of p,p′-DDD, p,p′-DDE, and p,p′-DDT, it appears that under normal conditions and low exposure, the liver is capable of metabolizing most DDT before it reaches the brain.

Methods of dosing did not change the distribution between tissues (i.e. the tissue with a greater concentration for the DDT-F bobwhites also had a higher concentration in DDT-I bobwhites), but did affect the concentrations found. Because adjusted ΣDDT values were greater for DDT-I bobwhite in both brains and livers, but adjusted p,p′-DDT concentrations did not differ between treatment groups in brain tissue, it appears that the technique used to

expose the bobwhite to DDT affected the amount absorbed through the gut wall but did not affect the amount passing through the liver, into systemic circulation, and being absorbed by the brain.

DDE/DDT ratios, a method to compare rates of metabolism, suggest that no difference existed in the rate of metabolism in livers between the techniques but a difference did exist in brains. This, at least partially, may be an artifact of the experimental design. The DDT-I bobwhites had not received any DDT for 7 days when killed whereas the DDT-F bobwhites were continually exposed to treated food until killed. Smaller differences in the DDE/DDT ratio between tissues for DDT-I bobwhites suggests that concentrations in the body had equilibrated, but because the DDT-F bobwhite were continually dosed until the end of the experiment, they had not yet been able to equilibrate between the brains and livers. This could be avoided in future studies by withdrawing treated food from the DDT-F group at the time of the last intubation for the DDT-I group.

CONCLUSIONS

When designing experiments that require oral dosing of a toxicant, addition to the food and oil intubation should not be considered equivalent. Greater quantities of DDT administered via corn oil intubation were measured in brains and livers. Intubation of oil and/or associated handling disrupted feeding patterns and may have been the cause of body mass losses. DDE/DDT ratios indicated that a period of contaminant withdrawal will allow equilibration among tissues within a bobwhite's body.

Table 4.1. Concentrations (μg DDT/g tissue) of DDT isomers and metabolites found in brains and livers of northern bobwhite receiving no DDT (C) or 25 ppm DDT as a food additive (DDT-F) or in corn oil (DDT-I). Results of paired t-tests comparing concentrations of each metabolite measured in both the brain and liver of the same individual are reported.

		Brain			Liver				
Compound	Treatment	n[a]	Mean	S.E.	n	Mean	S.E.	t	P
ΣDDT	C		ND[b]	0	10	0.8384	0.0753		
	DDT-F	8	2.6498	0.1744	8	10.6011	0.9094	9.45	<0.0001
	DDT-I	9	14.7329	1.8998	8	33.4725	2.9662	7.05	<0.0001
o,p′-DDE	C		ND	0		ND	0		
	DDT-F		ND	0	7	0.3481	0.0162		
	DDT-I	1	0.3822	0	9	0.8836	0.2094		
o,p′-DDT	C		ND	0		ND	0		
	DDT-F		ND	0	1	0.3623	0		
	DDT-I		ND	0		ND	0		
p,p′-DDD	C		ND	0	1	0.0053	0		
	DDT-F	2	0.2045	0.0058	8	1.8418	0.1104	4.13	0.1513
	DDT-I	9	0.8339	0.1878	10	6.2249	1.0201	5.25	0.0008
p,p′-DDE	C		ND	0	10	0.5164	0.0350		
	DDT-F	8	1.5430	0.1329	8	7.5087	0.9638	6.82	0.0002
	DDT-I	9	10.9421	1.4007	10	23.3799	1.8544	6.65	0.0002
p,p′-DDT	C		ND	0	1	0.51382	0		
	DDT-F	7	1.2949	0.0887	6	0.9628	0.1505	-2.48	0.0679
	DDT-I	9	4.7825	0.9974	9	3.2101	0.6666	-2.47	0.0388

[a] n = number of samples containing measurable amounts of the compound [b] ND = None Detected

Table 4.2. Adjusted (concentration in organ/total amount fed or dosed) DDT isomers and metabolites found in brains and livers of northern bobwhite receiving 25 ppm DDT as a food additive (DDT-F) or in corn oil (DDT-I). The results Student's t-tests comparing DDT-F and DDT-I groups within either brains or livers are reported.

		Brain				Liver			
Compound	Treatment	Mean	S.E.	t	P	Mean	S.E.	t	P
ΣDDT	DDT-F	0.0001610	0.0000110	4.306	0.0022	0.0006314	0.0000580	2.605	0.0191
	DDT-I	0.0003991	0.0000542			0.0008805	0.0000716		
o,p'-DDE	DDT-F	ND*	0			0.0000228	0.0000001	0.640	0.7309
	DDT-I	0.0000101	0			0.0000190	0.0000053		
o,p'-DDT	DDT-F	ND	0			0.0000288	0		
	DDT-I	ND	0			ND	0		
p,p'-DDD	DDT-F	0.0000011	0.0000003	2.686	0.0125	0.0001203	0.0001151	1.228	0.1253
	DDT-I	0.0000046	0.0000012			0.0001552	0.0000236		
p,p'-DDE	DDT-F	0.0000213	0.0000019	3.962	0.0016	0.0004877	0.0000692	1.431	0.0931
	DDT-I	0.0006206	0.0000095			0.0006039	0.0000472		
p,p'-DDT	DDT-F	0.0000121	0.0000009	1.118	0.1463	0.0000403	0.0000153	1.730	0.0588
	DDT-I	0.0000158	0.0000030			0.0000810	0.0000182		

* ND = None Detected

Table 4.3. DDE/DDT ratios found in both brains and livers of bobwhite quail receiving 25 ppm DDT in corn oil (DDT-I) or as a food additive (DDT-F). Results of Student's t-tests between treatment groups and paired t-tests between tissues are reported.

	Brain				Liver			
Treatment	Mean	S.E.	t	P	Mean	S.E.	t	P
DDT-F	0.6904[a]	0.1333	3.6004	0.0055	2.4256	0.4418	1.2407	0.2326
DDT-I	2.4448[a]	0.4696			3.5610	0.8014		

[a] Indicates a significant difference ($P < 0.05$) between mean ratios of livers and brains for DDE/DDT ratios with that treatment group using Paired t-tests.

Table 4.4. Results of Analyses of Variance for liver dry weights, relative liver weights (liver dry weights / final body weights), and percent fat in liver on a dry weights basis of bobwhite quail receiving no DDT (C) or 25 ppm DDT in corn oil (DDT-l) or as a food additive (DDT-F).

	Mean	S.E.	F	P
Liver weight				
C	1.240	0.150		
DDT-F	1.085	0.035	0.61	0.5515
DDT-l	1.129	0.062		
Relative liver weight				
C	0.0065	0.0011		
DDT-F	0.0053	0.0002	0.61	0.5497
DDT-l	0.0058	0.0004		
Fat as a percentage of dry liver weight				
C	11.80	0.76		
DDT-F	12.86	1.07	1.77	0.1934
DDT-l	10.37	0.97		

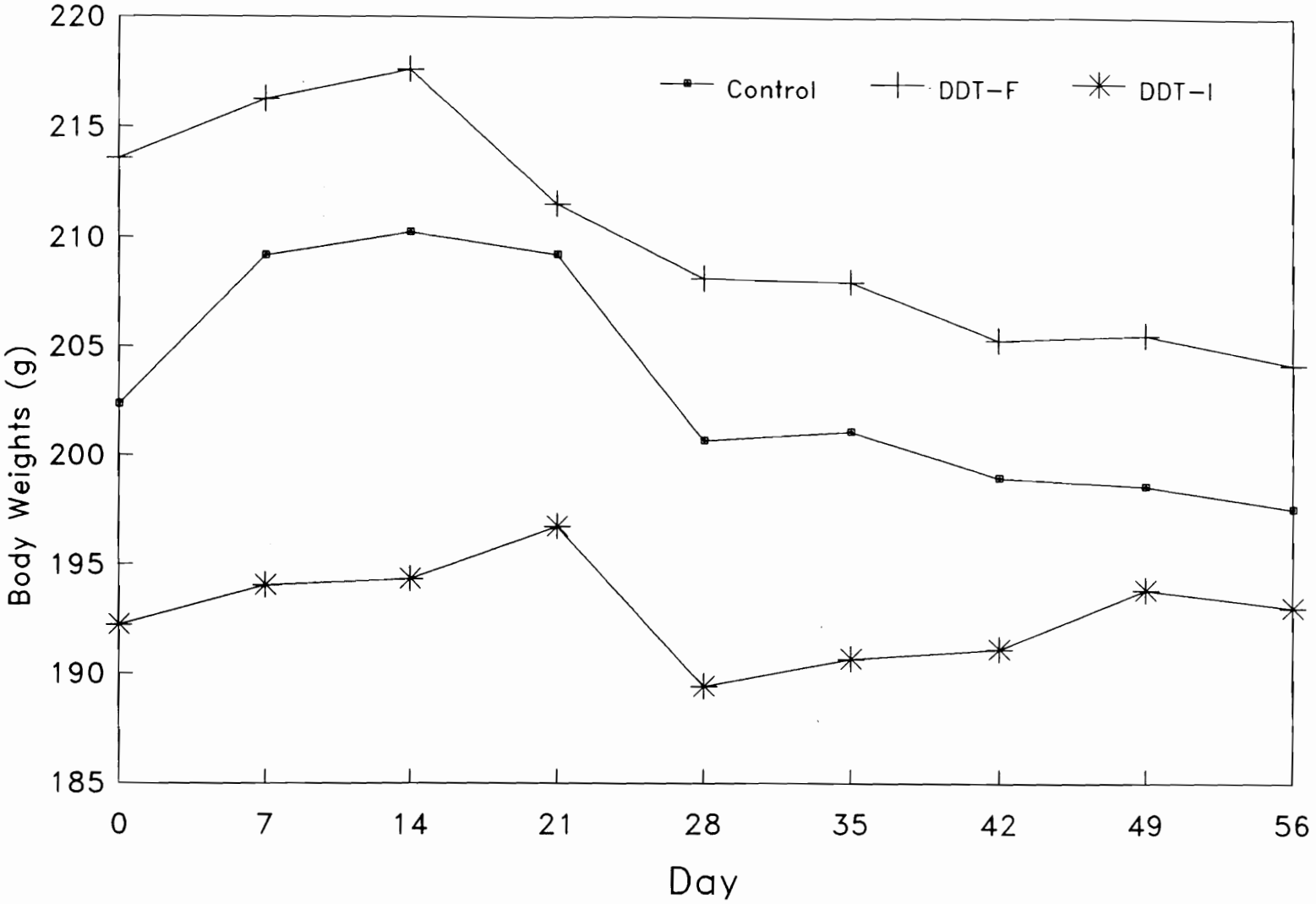

Figure 4.1. Mean body masses of bobwhites receiving no DDT (Controls), or 25 ppm DDT in food (DDT-F) or intubated in corn oil (DDT-I) measured every 7 days. Vertical lines represent 1 S.E.

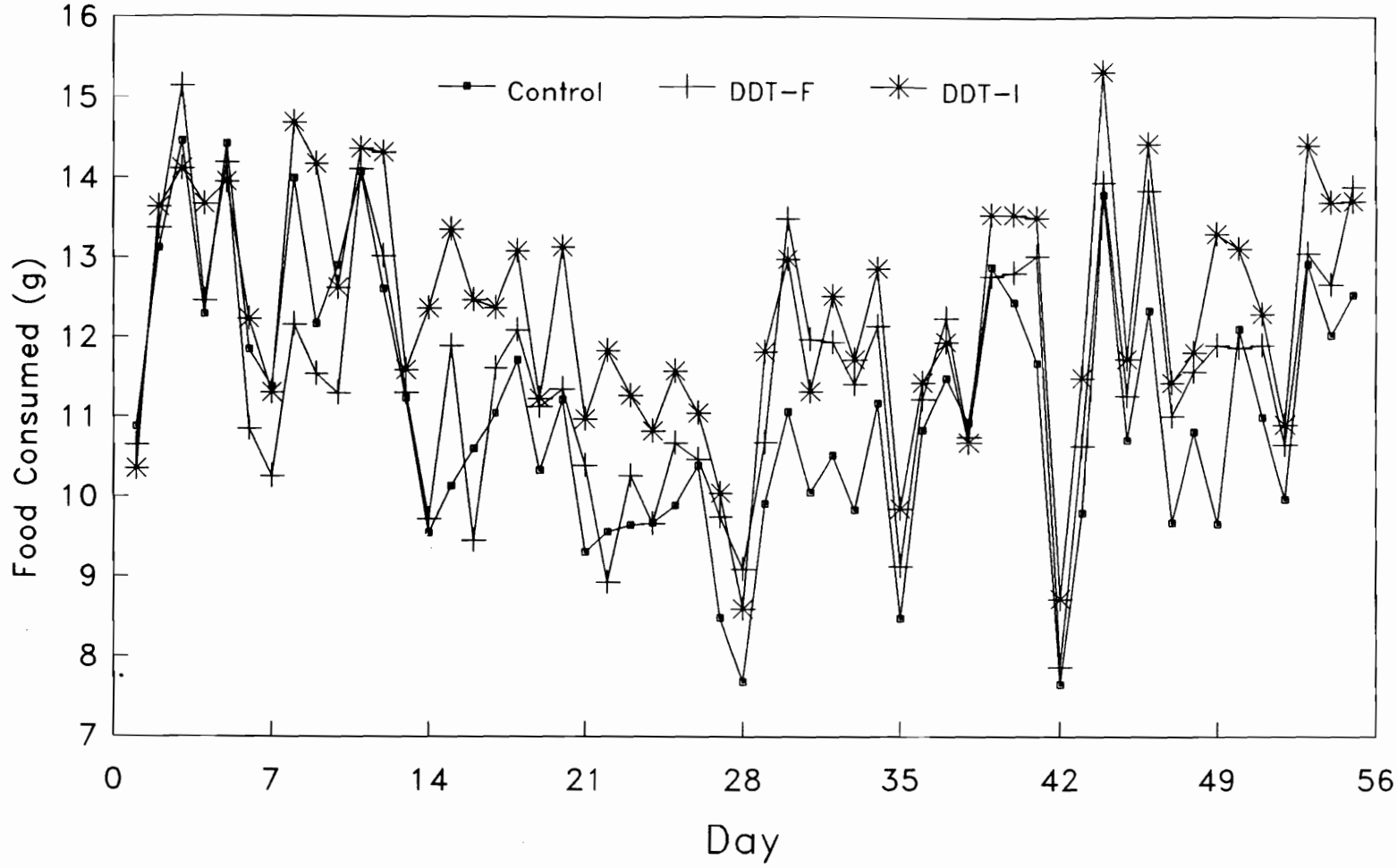

Figure 4.2. Mean daily food consumption of bobwhite receiving no DDT (Control), or 25 ppm DDT in food (DDT-F) or intubated in corn oil (DDT-I).

CHAPTER 5: RESPONSE OF VITAMINS A AND E TO DDT IN JAPANESE QUAIL

INTRODUCTION

Relationships between reproductive success and contamination in birds usually are determined by measuring a reproductive characteristic such as number of eggs laid, number of eggs hatching, or number of young fledging and comparing that to the amount of contamination found in adult birds or eggs. Drawbacks exist to this approach. Determination of contamination that requires removal of eggs from nests or breeding age adults potentially could affect populations adversely and would be infeasible for highly impacted populations. Secondly, field collected samples present a combination of contaminants, and it is often difficult to determine a single causative agent or quantify the possible interactions among the contaminants. Thirdly, different species react differently to contaminants making it necessary to repeat this process each time a new species is of concern.

A better approach would be to measure the concentrations of a biomarker in the blood that is both vital for reproduction and affected by contamination. Many advantages exist to

this approach. Destructive sampling of eggs or adult birds can be avoided. If the biomarker is the causal agent for the contaminants' impact on reproduction, the concentrations of the biomarker would be indicative of the impact on reproduction of a single contaminant or a mixture. Plasma concentrations of many biologically important compounds are maintained at constant values. Measurements outside the normal range would indicate potential problems with reproduction and also may be more constant across species than are responses to contaminants.

Vitamin A is a nutrient of vital importance to overall health and reproduction. Vitamin A deficiencies have been shown to cause reduced chick survivability (Nestler 1946, 1949), reduced hatchability (Nestler 1946), reduced egg production (Nestler 1946), and may have caused delayed egglaying (Lehmann 1953) in bobwhites and reduced development of sex organs in Gambel's quail (*Lophortyx gambelii*) (Hungerford 1964). Because vitamin A deficiencies exhibit similar symptoms to DDT contamination, it is proposed here that vitamin A may be a link between DDT exposure and reproductive impairment and that measures of plasma retinol may be useful as biomarkers to indicate reproductive potential.

This chapter reports on an experiment in which Japanese quail (*Coturnix coturnix japonica*) were dosed chronically with p,p'-DDT in their food. Plasma concentrations of vitamin A were evaluated as a possible biomarker for nondestructive sampling of birds for testing reproductive potential after exposure to DDT. DDT was used because of its continuing effects on avian reproduction in wild populations (e.g. Steidl et al. 1991) and as a model for other organochlorine contaminants. Regression models of reproductive measurements that include plasma and liver vitamin A, DDT contamination, and morphological measurements are presented and their predictive potential is discussed.

METHODS

Animal Care

Forty-eight laying female and 18 reproductively mature male Japanese quail were acquired from an existing colony in the Poultry Science Department at Virginia Polytechnic Institute and State University. The quail were kept in the animal room in the Department of Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University at approximately 21 C. Quail were held individually in standard laying cages (G.Q.F. Manufacturing Co., Savannah, GA) with sloping floors measuring 20.3 x 30.5 cm in the back, 25.4 x 30.5 cm in the front, and 50.8 cm in length. The light cycle was maintained at 15L:9D. Commercial laying mash (15% crude protein) and water were provided *ad libitum*.

Females were randomly assigned to one of four treatment groups. Groups received food treated with 0, 1, 10, or 25 mg DDT/kg food (ppm). Females were paired every third day with a different male. A 2-d interval was used to reduce the amount of handling to which the females were exposed during their laying cycles. Females were paired with different males during each mating session to reduce the effect of possible pair incompatibility or male dysfunction. The females were transferred to males' cages at approximately 0900 and returned to their cages after 90 min. Pairing began on Day 16 after dosing with treated food began. Two mating opportunities with 2-d intervals between each (for a total of 6 d) were allowed before egg collection commenced. Egg collection for reproductive evaluation began on Day 22 of the experiment and continued for 45 d.

Eggs were collected daily. Eggs with apparently complete and uncracked eggshells were placed in a poultry incubator (Humidaire Incubator Company, New Madison, OH) set at 37-38 C and approximately 55% relative humidity and automatically turned every 2 h for

16 d. On day 16, eggs were transferred to a stationary hatching incubator at 38 C and approximately 70% relative humidity. Hatching occurred on days 17 - 20. Eggs from Days 30, 44, and 58 were held for DDT analyses.

Vitamin A and Vitamin E Analysis

Blood samples for plasma were collected 1 d before treated food was given, 31 d after treatment began, and 62 d after treatment began. Plasma samples were analyzed for retinol and α-tocopherol content according to the method proposed by Bieri et al. (1979). For this method, equal volumes (100 μl) of plasma and an internal ethanol standard solution containing 1 μg/ml of retinyl acetate (Sigma Chemical Company, St. Louis, MO) and 10.2 μg/ml of α-tocopheryl acetate (Sigma Chemical Company) were vortexed for 30 sec along with 200 μl of ethanol in a 10 x 75 mm test tube. The additional ethanol was added to precipitate plasma proteins. The retinol and α-tocopherol were extracted from this mixture with 200 μl of hexane by vortexing for 1 min. The phases were separated by centrifugation for 5 min. As much of the hexane layer as possible was transferred to a 6 x 50 mm test tube and evaporated to dryness under a stream of N_2 at room temperature. The residue was dissolved in 80 μl of cold ethanol and vortexed for 30 sec. Fifty μl of this sample was injected into an HPLC (detector model 440, Waters Associates, Inc., Milford, MA) equipped with a 5 x 30 mm guard column and a 5 x 300 mm analytical column both packed with Bondeclone C-18 reverse phase packing (Phenomenex Inc., Torrance, CA).

Liver samples were analyzed for retinol and retinyl palmitate. After freeze-drying, liver portions weighing approximately 0.15 g were crushed into powder using mortar and pestle. The liver samples were transferred to 15 ml centrifuge tubes, and 10 ml hexane was added. After 10 min of gentle mixing by hand to extract the retinol and retinyl palmitate, the mixture was centrifuged for 5 min. A 600 μl aliquot of hexane was transferred to a 10 x 75 mm test tube and evaporated to dryness at room temperature under a stream of N_2. A 250 μl volume of

ethanol containing retinyl acetate internal standard was added to the dry residue. Fifty μl of this sample was injected into an HPLC as was done for the plasma analysis.

Retinol concentrations in both plasma and liver tissue were calculated using the ratio technique described in Bieri et al. (1979). A standard curve was developed for retinyl palmitate (Sigma Chemical Company) and retinyl palmitate concentrations were calculated by direct comparison to the standard curve.

DDT Analysis

The tissues were freeze-dried and weighed. Fat, with associated DDT and metabolites, was extracted from the liver and egg samples with petroleum ether, using a Soxhlet apparatus (Pyrex®, Fisher Scientific Co., Norcross, GA). Samples were extracted for 8 h. Sample clean-up, using activated florisil columns (U.S. Silica Company, Berkeley Springs, WV), was performed with 150 ml 6% ethyl ether-petroleum ether.

The analysis of o,p′-DDD, o,p′-DDE, o,p′-DDT, p,p′-DDD, p,p′-DDE, and p,p-DDT was performed on a Tracor 540® gas chromatograph (Tracor Corporation, Austin, TX), equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas and a 1.83 m x 6.35 mm i.d. glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport® (Supelco, Inc., Bellefonte, PA). All samples were verified on a Tracor 540® gas chromatograph equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas and a 1.98 m x 6.35 mm i.d. glass column packed with 1.5%/1.95% OV-17/OV-210 on 100/120 mesh Chromosorb WHP® (Supelco, Inc.) p,p′-DDD was not detectable in some liver and yolk samples. Because p,p′-DDD is derived from p,p′-DDT during storage (Walker and Jefferies 1978) and its importance to the results presented is for calculation of a DDE/DDT ratio, those samples with below detectable amounts of p,p′-DDD were excluded from statistical comparisons for p,p′-DDD. Most samples did not contain detectable amounts of o,p′-DDD,

o,p'-DDE, or o,p'-DDT. Those samples that did, contained only very small quantities and were not reported.

Statistical Analyses

All data were tested for normality using the Shapiro-Wilk statistic (SAS Institute, Inc. 1985a). Nonparametric procedures were used whenever the data deviated from normal. Statistical analyses were performed using the SAS® statistical package (SAS Institute, Inc., 1985a, 1985b) and a nonparametric statistical package, NPSP (NPSP, 1986). Kruskal-Wallis tests were used to test for differences among dose groups for concentrations of DDT isomers and metabolites and for differences in DDE/(DDD + DDT) ratios. Protected LSD tests on ranks were used for pairwise comparisons. Wilcoxon ranked sum tests were used to test for paired differences between DDT concentrations in livers and yolks. Analysis of variance tests were used to determine whether plasma retinol concentrations were different on any sampling day, and were used to test for differences over time for plasma retinol concentrations. Kruskal-Wallis tests were used to test for differences among treatment groups for liver retinol and retinyl palmitate because concentrations of liver retinol and retinyl palmitate were not normally distributed. Kruskal-Wallis tests were used to test for differences among treatment groups for body weight, percent liver fat, dry liver weight, wet liver weight, and relative liver weight. Analyses of variance were used to test for differences among groups for reproductive measurements with Fisher's LSD test used to test for pairwise differences.

Spearman's rank correlations were used to compare reproductive measurements with liver and yolk concentrations of DDT, plasma and liver concentrations of vitamin A, liver morphology measurements, and body weights. Spearman's rank correlations were also used to compare relationships among DDT measurements, vitamin A measurements, liver morphological measurements, and body weights. Regression analyses were made using a

nonparametric multiple regression technique, general linear models by rank dispersion (Hettmansperger and McKean 1978, NPSP, 1986).

RESULTS

During the experiment, 4 female Japanese quail died. Two, one control bird and one 10 ppm group bird, died from injuries sustained in their cages during the predosing period. Another control bird died during the second bleeding session, and a 1 ppm group bird died from unknown causes. The 1 ppm group female had been laying eggs until only eight days before dying.

DDT Contamination

All DDT residue measurements increased significantly with dose as expected (Table 5.1). However, the amount accumulated in yolks was generally greater than that in livers. Liver p,p′-DDE / (p,p′-DDD + p,p′-DDT) ratios (DDE/DDT ratios) were found to differ significantly among dose groups but yolk DDE/DDT ratios did not differ (Table 5.1). The 25 ppm group had a higher mean liver DDE/DDT ratio than the other treatment groups indicating that at a higher dose, comparatively more p,p′-DDT was metabolized to p,p′-DDE.

In paired comparisons between concentrations found in livers with those found in yolks, control birds showed significant differences only for p,p′-DDE concentrations. Low dose (1 ppm) group birds exhibited higher concentrations of ΣDDT and p,p′-DDE in the yolks, whereas p,p′-DDD concentrations were higher in livers, and p,p′-DDT concentrations did not differ. In the two higher dose groups (10 ppm and 25 ppm), higher concentrations of ΣDDT, p,p′-DDE, and p,p′-DDT were measured in yolks, whereas livers contained higher

concentrations of p,p'-DDD. Yolk ratios were higher than liver ratios except for the 25 ppm group.

Reproduction

The results of reproductive measurements for individual females are in Appendix Table B.1. Production ranged from 13 to 42 eggs laid, from 1 to 42 settable eggs, from 0 to 35 eggs showing embryonic development, and 0 to 18 eggs hatching. Number of eggs showing embryonic development differed significantly among groups ($P = 0.0259$) (Table 5.2). This can more easily be seen in Table 5.3 where simple percentages based on the preceding reproductive stage are shown. The control and 10 ppm dose groups performed similarly for all reproductive characteristics. The 1 ppm and 25 ppm groups also performed similarly for all reproductive characteristics. The 1 ppm group produced significantly fewer eggs showing embryonic development than the control and 10 ppm groups. The 25 ppm group produced significantly fewer eggs showing development than the 10 ppm group.

Vitamins A and E

Plasma concentrations of retinol differed slightly among dosage groups after 1 month of dosing (Day 31) ($P = 0.0848$) and showed a significant difference at the end of the experiment (Day 64) ($P = 0.0002$) (Table 5.4). The high dose group (25 ppm) showed the greatest decline. Analysis of variance showed a significant change among groups when all sampling days were combined ($F_{3,44} = 4.14$, $P = 0.0114$). There was a significant dose x time interaction ($F_{6,44} = 2.93$, $P = 0.0124$) and a significant time effect for all groups combined

($F_{2,44}$ = 12.90, P < 0.0001). Liver concentrations of retinol and retinyl palmitate did not differ among dose groups (Table 5.4). Measurements for total liver retinol and total liver retinyl palmitate (Table 5.5) also did not differ.

No measurable amounts of vitamin E (α-tocopherol) were found in the quail plasma or livers. Whether this was caused by lengthy storage of samples or the contamination of the quail with DDT cannot be determined. However, loss during storage seems unlikely as storage at -20 c was shown not to cause a decrease in either plasma retinol or α-tocopherol concentrations for a period of up to 15 mo (Craft et al. 1988).

Morphological Measurements

Body weight measurements and four liver morphology measurements, percent fat, dry weight, wet weight, and relative liver weight were made at the end of the experiment, and yolk percent fat measurements were made 3 times, once each at the beginning (Day 30), middle (Day 44), and end (Day 58) of the experiment (Table 5.6). Kruskal-Wallis analyses failed to find statistical differences for any of these measurements among treatment groups.

Correlation Between Reproduction and Vitamin A, DDT Contamination, or Morphological Measurements

Correlation analyses were performed to investigate the relationships among all variables measured and each of the measurements of reproductive performance. Correlation analyses were deemed necessary to determine whether the changes seen in reproductive performance were related to the changes observed in plasma retinol concentrations or whether other characteristics were more closely related.

Liver DDE/DDT ratios correlated significantly and negatively with number of eggs laid and number of settable eggs (Table 5.7). Wet liver weights correlated significantly and negatively with number of eggs showing embryonic development. Correlation coefficients for each dose group are in Appendix Tables B.2-B.5.

Correlation Analyses Among Vitamin A, DDT Contamination, and Morphological Measurements

Correlation analyses were performed comparing vitamin A concentrations, DDT concentrations, and morphological measurements to better understand their interrelationships and to investigate how they may affect reproductive potential (Table 5.8). (See Appendix Tables B.6-B.9 for correlation analyses of individual dose groups.) Of greatest interest is the relationships of vitamin A in plasma to vitamin A in the liver, DDT contamination, and morphological measurements. Plasma retinol showed significant negative correlations with liver DDE, ΣDDT, and DDE/DDT ratios and yolk DDE and ΣDDT. Plasma retinol showed significant positive correlations with liver dry weights and wet weights. Plasma retinol failed to correlate significantly with either liver retinol or liver retinyl palmitate.

Regression Analyses

A single variable, yolk DDE/DDT ratios appeared to be of greatest importance for all reproductive measurements as seen in a nonparametric multiple regression procedure, general linear models by rank dispersion (Table 5.9). Yolk DDE/DDT ratios appeared in all but one model, a model for number of eggs showing embryonic development. Plasma retinol concentrations appeared in the only significant model for number of eggs laid, in 15 of 25

significant models for number of settable eggs, in 9 of 16 significant models for number of eggs showing embryonic development, and in 4 of 8 significant models for numbers of eggs hatching. Except for the number of eggs laid, the best models for the other reproductive measurements were those with yolk DDE/DDT ratios as the only variable included in the model. Other variables included in significant models were female body weight, wet or dry liver weight, liver retinol concentrations, and liver or yolk DDT contamination. Because none of the models containing these variables were better than the model for that reproductive measurement with only yolk DDE/DDT ratios, it would not appear that these other variables make important contributions.

DISCUSSION

DDT Contamination

The larger concentrations of ΣDDT, p,p′-DDT, and p,p′-DDE in yolks versus livers is likely a result of the greater amounts of fat in the yolk tissues. DDT also has been shown to bind to vitellogenin (Denison and Yarbrough 1985) and as long as bound DDT does not prevent incorporation of vitellogenin into the developing yolk, this would be an additional means for DDT to accumulate in the yolk. Livers contained larger concentrations of p,p′-DDD. This was probably caused by conversion from p,p′-DDT to p,p′-DDD during storage. Walker and Jefferies (1978) have shown that such conversion occurs at temperatures as low as -20 C in livers but not in eggs without embryos. They did not specify, however, whether the eggs may have been fertilized or how they determined the existence of embryos.

Vitamin A

Plasma retinol differed among groups and through time as a result of exposure to DDT in the diet. The dose x time interaction resulted from variation in concentrations of vitamin A among the dose groups through time. The 25 ppm group steadily decreased, but the other dose groups did not produce any consistent trend. Lower plasma retinol concentrations were associated with higher liver DDE, liver ΣDDT, yolk DDE, and yolk ΣDDT as well as higher liver DDE/DDT ratios as seen in correlation analyses. The negative association with liver DDE/DDT ratios suggests that those Japanese quail with greater liver enzyme activity were not releasing as much retinol into the bloodstream as those with lower enzyme activity. It is not likely that the liver was metabolizing and excreting retinol because both retinol and retinyl palmitate stores in the liver were not depleted in higher DDT dose groups and were not negatively associated with liver DDE, liver ΣDDT or liver ratios. Brouwer and van den Berg (1986) found that a metabolite of TCBP binds to transthyretin in rats and prevents the association of transthyretin with retinol-binding protein (RBP). They suggested that this could lead to greater kidney clearance of RBP with its bound retinol because RBP not associated with transthyretin is small enough to be cleared by the kidney. If DDT or a metabolite also binds to transthyretin, this could be the mechanism for reduced plasma concentrations found in this experiment. Spear et al. (1989), however, failed to find a difference between serum retinol concentrations from female ringed turtle doves (*Streptopelia risoria*) after a single intraperitoneal injection of TCBP. The reason Spear et al. failed to find reduced retinol concentrations may not have been because no effect occurred, but rather because the individual dosed birds responded in such varying degrees that statistical analyses failed to discern a biological effect.

Reproduction

The reason for the unexpectedly low reproductive success of the 1 ppm group is unknown, however, Kendall (1976) found that northern bobwhite exposed to Mirex (dodecachloro-octahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene) at 0 ppm, 1 ppm, 20 ppm, and 40 ppm in feed had more eggs showing embryonic development and more eggs hatching in the 20 ppm and 40 ppm groups than in groups receiving no or 1 ppm dietary Mirex. He also was unable to explain why females receiving higher doses should show better reproductive success than those receiving no or very small quantities of an organochlorine insecticide.

The number of eggs hatching was unexpectedly low for all groups, including the control group, but as seen in Table 5.2, the group experiencing the greatest reduction in hatchability was the 25 ppm group. The results presented in Appendix D suggests that the poor hatchability observed in all treatment groups was not a result of the short, infrequent pairing periods. Better care taken in the handling of the eggs during the transfer between incubators and more rigid control of humidity in the hatching incubator appeared to greatly improve the hatching success. Even with the poor hatchability in mind, it again would appear that alterations of the liver, such as changes in liver weights, are more closely related to hatchability than is plasma retinol or amount of liver or yolk DDT contamination.

Two stages of reproductive response exist. DDE/DDT ratios showed stronger associations with number of eggs laid and number of settable eggs laid, whereas liver weights showed stronger correlations with number of eggs showing embryonic development and number of eggs hatching.

The correlation analyses indicated that wet liver weights were more closely correlated with number of eggs showing embryonic development, the only reproductive stage to show a significant difference among dose groups, than were measurements of liver contamination. In the regression analyses, the only models to be significant included yolk DDE/DDT ratios.

That plasma concentrations of retinol did not correlate with number of eggs showing embryonic development and could not produce a significant regression model without other variables that required destructive sampling of eggs, diminishes the utility of plasma concentration as a biomarker of number of eggs showing embryonic development.

The preceding discussion supports an important observation. DDT contamination of yolks is of minor importance in determining reproductive success of Japanese quail, whereas liver contamination provides useful additional information. Japanese quail are continuous layers. Determinant layers may experience greater effects related to egg contamination because any contaminants mobilized from fat stores would be deposited in a few eggs, whereas with Japanese quail, the first few eggs may be more heavily contaminated than subsequent eggs. Such a hypothesis cannot be tested here because the females were already in production at the beginning of the dosing period.

CONCLUSIONS

The only reproductive stage to be significantly different among dose groups was the number of eggs showing embryonic development. The subsequent stage, number of eggs hatching, failed to be significantly different, possibly because of the overall poor hatching success.

At the end of the experiment, the 25 ppm group had significantly reduced concentrations of plasma retinol. Both correlation and regression analyses failed to show that plasma concentrations of retinol associated closely with any stage of reproduction, whereas plasma concentrations of retinol did correlate strongly with DDT concentrations in livers. The failure of plasma concentrations of retinol to associate closely with reproductive measurements renders questionable its utility as a biomarker of both DDT contamination and reproductive

success in Japanese quail. The failure of retinol to work in Japanese quail creates doubt that it also will not be a useful biomarker in other species as well.

Table 5.1. Concentrations (μg p,p′-DDT/g tissue) of DDT isomers and metabolites and DDE/DDT ratios found in livers and yolks of Japanese quail receiving food containing 0, 1, 10, 25 ppm DDT for 64 days. Kruskal-Wallis test results are reported for among group comparisons.

		Liver						Yolk					
Compound	Treatment	n[a]	Mean	S.E.	C.V.	χ^2	P	n	Mean	S.E.	C.V.	χ^2	P
ΣDDT	Control	10	1.419[c]	0.163	36.34	38.67	<0.0001	26	1.790[c]	0.198	57.40	84.46	<0.0001
	1 ppm	11	5.378[d,x]	0.284	17.49			19	7.032[d]	0.363	21.52		
	10 ppm	11	33.503[e,x]	5.099	50.48			29	72.227[e]	6.444	48.19		
	25 ppm	12	82.293[f,x]	12.290	51.73			22	141.926[f]	7.747	26.04		
p,p′-DDT	Control	10	0.855[c]	0.098	36.26	36.59	<0.0001	26	0.760[c]	0.095	63.54	81.09	<0.0001
	1 ppm	11	2.981[d]	0.295	32.83			19	3.645[d]	0.251	29.97		
	10 ppm	11	19.399[e,x]	4.251	72.69			29	39.038[e]	3.758	51.83		
	25 ppm	12	38.903[f]	9.202	81.94			22	83.419[f]	7.443	41.85		
p,p′-DDE	Control	10	0.218[c,x]	0.030	44.15	38.43	<0.0001	26	0.631[c]	0.098	78.80	82.51	<0.0001
	1 ppm	11	0.916[d,x]	0.117	42.36			19	2.973[d]	0.226	33.20		
	10 ppm	11	4.141[e,x]	0.639	51.21			29	31.019[e]	3.068	53.83		
	25 ppm	12	13.780[f,x]	3.320	83.45			22	53.963[f]	3.270	28.42		
p,p′-DDD	Control	7	0.227[c]	0.041	48.10	37.28	<0.0001	2	0.208[c]	0.070	47.57	38.25	<0.0001
	1 ppm	11	1.284[d,x]	0.216	55.78			12	0.254[c]	0.016	21.36		
	10 ppm	11	9.783[e,x]	0.942	31.92			29	1.901[d]	0.234	66.17		
	25 ppm	12	28.188[f,x]	2.689	33.04			22	3.934[e]	0.452	53.91		
Ratio[b]	Control	10	0.098[c,x]	0.023	74.31	8.11	0.0438	26	0.745	0.067	49.34	3.08	0.3791
	1 ppm	11	0.160[c,x]	0.043	89.01			19	0.827	0.077	40.33		
	10 ppm	11	0.152[c,x]	0.152	30.08			29	0.820	0.070	45.96		
	25 ppm	12	0.305[d]	0.067	76.25			22	2.074	1.419	320.80		

[a] n = the number of samples with measurable amounts of that DDT isomer

[b] Ratio = p,p′-DDE / (p,p′-DDT + p,p′-DDD)

[c,d,e,f] Columnar means, within each compound, followed by different superscripts are significantly different (α = 0.05) according to Fisher's protected LSD on ranks.

[x] Means are significantly different (α = 0.05) from the corresponding values for yolks.

Table 5.2. Reproductive success of Japanese quail after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 64 days. Analyses of variance results are reported for among group comparisons.

Reproduction	Treatment	n	Mean	S.E.	C.V.	F	P
Number laid	Control	10	36.70	1.770	15.25	2.37	0.0850
	1 ppm	12	28.75	2.355	25.57		
	10 ppm	11	34.64	1.825	17.48		
	25 ppm	12	32.00	2.602	28.17		
Number settable	Control	10	34.70	2.261	20.61	2.82	0.0508
	1 ppm	12	22.67	3.600	44.44		
	10 ppm	11	30.82	2.766	29.77		
	25 ppm	12	24.67	3.779	53.07		
Number developing	Control	10	$20.80^{a,b}$	2.590	39.37	3.42	0.0259
	1 ppm	12	12.17^{c}	2.510	63.80		
	10 ppm	11	21.81^{a}	2.795	42.92		
	25 ppm	12	$13.17^{b,c}$	2.899	76.92		
Number hatching	Control	10	7.30	2.087	90.42	2.65	0.0612
	1 ppm	12	3.08	1.145	119.86		
	10 ppm	11	7.64	1.435	61.41		
	25 ppm	12	3.58	1.258	123.34		

^{a,b,c} Means followed by different superscripts are significantly different ($P < 0.05$).

Table 5.3. Total numbers of eggs laid, settable, showing embryonic development, and hatching and the percentages based on the immediately previous reproductive stage from Japanese quail after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 64 days.

	Control	1 ppm	10 ppm	25 ppm
Number laid	367	345	381	384
Number settable	347	272	339	296
Settable/Laid (%)	94.6	78.8	89.0	77.1
Number developing	208	144	238	156
Developing/Settable (%)	59.9	52.9	70.2	52.7
Number hatching	73	45	83	42
Hatching/Developing (%)	35.1	31.3	34.9	26.9

Table 5.4. Concentrations of retinol (μg/ml) in plasma of Japanese quail after they received food containing 0, 1, 10 or 25 ppm p,p′-DDT for 64 days. Analyses of variance test results are reported for among group comparisons.

Day	Treatment	n	Mean	S.E.	C.V.	F	P
0	Control	12	0.622	0.040	22.52	0.28	0.8362
	1 ppm	12	0.567	0.059	36.28		
	10 ppm	12	0.600	0.038	22.11		
	25 ppm	12	0.581	0.034	20.20		
31	Control	10	0.570	0.050	27.75	2.38	0.0848
	1 ppm	11	0.481	0.089	61.43		
	10 ppm	10	0.623	0.082	41.74		
	25 ppm	12	0.370	0.062	58.36		
62	Control	10	0.473[a]	0.049	33.04	8.30	0.0002
	1 ppm	11	0.536[a]	0.048	29.75		
	10 ppm	11	0.453[a]	0.046	33.46		
	25 ppm	12	0.225[b]	0.050	76.78		

[a,b] Means followed by different superscripts are significantly different ($P < 0.05$).

Table 5.5. Concentrations (μg/g) and total amounts (μg) of vitamin A forms in livers of Japanese quail after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 64 days. Kruskal-Wallis results are reported for among group among group comparisons.

Vitamin A	Treatment	n	Mean	S.E.	C.V.	χ^2	P
Retinol	Control	9	2.511	1.161	138.71	0.62	0.8907
	1 ppm	11	1.353	0.567	138.97		
	10 ppm	11	1.652	0.689	138.43		
	25 ppm	12	1.862	1.401	260.54		
Retinyl palmitate	Control	9	29.204	12.861	132.12	3.96	0.2663
	1 ppm	11	69.223	24.504	117.40		
	10 ppm	11	50.488	26.383	173.31		
	25 ppm	12	71.508	50.362	243.97		
Total retinol	Control	9	4.523	1.831	121.61	0.13	0.9877
	1 ppm	11	3.112	1.487	158.51		
	10 ppm	11	3.624	1.521	139.18		
	25 ppm	12	3.007	2.126	244.98		
Total retinyl palmitate	Control	9	49.739	22.389	135.04	4.59	0.2040
	1 ppm	11	142.182	48.241	112.53		
	10 ppm	11	86.730	39.237	150.04		
	25 ppm	12	117.516	76.640	225.92		

Table 5.6. Body weights, liver weights and percent fat content of livers and yolks from Japanese quail after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 64 days. Kruskal-Wallis results are reported for among group comparisons.

	Treatment	n	Mean	S.E.	C.V.	χ^2	P
Body weight	Control	10	170.801	5.958	11.03	2.00	0.5724
	1 ppm	11	179.270	4.452	8.24		
	10 ppm	11	174.196	5.463	10.40		
	25 ppm	12	174.705	5.635	11.17		
Dry liver weight	Control	10	1.783	0.120	21.20	4.38	0.2229
	1 ppm	11	2.291	0.212	30.73		
	10 ppm	11	2.022	0.110	18.07		
	25 ppm	12	2.108	0.149	24.54		
Wet liver weight	Control	10	5.856	0.406	21.92	3.70	0.2956
	1 ppm	11	7.151	0.554	25.68		
	10 ppm	11	6.318	0.289	15.18		
	25 ppm	12	6.454	0.375	20.12		
Dry weight / Wet weight	Control	10	30.64	0.981	10.12	1.96	0.5808
	1 ppm	11	31.76	0.890	9.29		
	10 ppm	11	31.89	0.562	5.84		
	25 ppm	12	32.48	0.930	9.92		
Relative liver weight	Control	10	0.034	0.0022	19.82	0.50	0.9181
	1 ppm	11	0.040	0.0031	26.06		
	10 ppm	11	0.036	0.0013	11.93		
	25 ppm	12	0.037	0.0016	15.11		
Liver percent fat	Control	10	24.850	2.901	36.92	2.50	0.4754
	1 ppm	11	29.202	3.593	40.81		
	10 ppm	10	29.671	2.900	30.90		
	25 ppm	12	31.975	2.764	29.95		
Yolk percent fat, Day 30	Control	10	65.810	0.690	3.31	1.20	0.7541
	1 ppm	8	66.011	0.535	2.29		
	10 ppm	11	66.520	0.796	3.97		
	25 ppm	9	65.487	0.792	3.63		
Yolk percent fat,Day 44	Control	7	65.347	0.741	3.00	4.58	0.2053
	1 ppm	5	66.820	1.089	3.64		
	10 ppm	7	65.407	0.405	1.64		
	25 ppm	6	66.894	0.582	2.13		
Yolk percent fat, Day 58	Control	6	65.489	0.706	2.64	1.93	0.5876
	1 ppm	5	65.022	0.615	2.11		
	10 ppm	9	65.964	0.635	2.89		
	25 ppm	5	64.280	0.859	2.95		

Table 5.7. Spearman rank correlation coefficients relating the number of Japanese quail eggs laid, number of settable eggs, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, liver weights and body weights for all treatment groups combined (n = 44).

Treat	LΣDDT[a]	LPPDDE	LRATIO	YΣDDT	YPPDDE	YRATIO	PLASRET	LIVRRET	LIVRPALM	DRYWT	WETWT	PCTFAT	BIRDWT
Number Laid	-0.368 (0.0781)[b]	-0.275 (0.0712)	-0.498 (0.0006)	-0.157 (0.3266)	-0.153 (0.3401)	-0.029 (0.8564)	0.238 (0.1159)	-0.133 (0.3905)	-0.046 (0.7650)	-0.052 (0.7394)	-0.117 (0.4507)	0.035 (0.8221)	-0.243 (0.1126)
Number Settable	-0.211 (0.1684)	-0.215 (0.1604)	-0.502 (0.0005)	-0.089 (0.5809)	-0.074 (0.6438)	0.048 (0.7650)	-0.230 (0.1291)	-0.172 (0.2641)	0.059 (0.7015)	0.015 (0.9229)	-0.062 (0.6875)	0.082 (0.6000)	-0.074 (0.6438)
Number Develop.	-0.203 (0.1856)	-0.198 (0.1967)	-0.203 (0.1858)	-0.067 (0.6783)	-0.070 (0.6616)	0.065 (0.6886)	0.142 (0.3519)	-0.022 (0.8891)	0.144 (0.3525)	-0.296 (0.0511)	-0.382 (0.0105)	-0.101 (0.5181)	-0.228 (0.1361)
Number Hatching	-0.141 (0.3604)	-0.135 (0.3827)	-0.192 (0.2127)	-0.034 (0.8333)	-0.038 (0.8154)	-0.038 (0.6775)	0.067 (0.3596)	0.140 (0.7969)	-0.040 (0.4515)	0.116 (0.0917)	-0.352 (0.0193)	0.006 (0.9693)	-0.083 (0.5911)

[a] LΣDDT = liver ΣDDT, LPPDDE = liver p,p′-DDE concentrations, LRATIO = liver DDE/DDT ratios, YΣDDT = yolk ΣDDT, YPPDDE = yolk p,p′-DDE concentrations, YRATIO = yolk DDE/DDT ratios, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, DRYWT = liver dry weights, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

[b] Values in parentheses are p-values corresponding to the correlation coefficients.

Table 5.8. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for all treatment groups combined.

	YPPDDE	LΣDDT	YΣDDT	PLASRET	LIVRRET	LIVRPALM	YRATIO	LRATIO	PERCFAT	DRYWT	WETWT	BIRDWT
LPPDDE	0.927 (0.0001)[a]	0.984 (0.0001)	0.915 (0.0001)	-0.464 (0.0015)	-0.137 (0.3754)	-0.123 (0.4282)	0.468 (0.0021)	0.492 (0.0007)	0.387 (0.0103)	0.281 (0.0645)	0.151 (0.3267)	0.003 (0.9855)
YPPDDE		0.922 (0.0001)	0.966 (0.0001)	-0.419 (0.0064)	-0.164 (0.3069)	-0.189 (0.2360)	0.511 (0.0006)	0.432 (0.0048)	0.229 (0.1554)	0.188 (0.2388)	0.085 (0.5979)	-0.062 (0.7001)
LΣDDT			0.927 (0.0001)	-0.441 (0.0028)	-0.072 (0.6443)	-0.133 (0.3904)	0.400 (0.0096)	0.435 (0.0032)	0.432 (0.0038)	0.328 (0.0298)	0.194 (0.2058)	0.053 (0.7319)
YΣDDT				-0.366 (0.0188)	-0.086 (0.5946)	-0.161 (0.3158)	0.371 (0.0169)	0.385 (0.0128)	0.224 (0.1643)	0.216 (0.1749)	0.134 (0.4049)	-0.002 (0.9914)
PLASRET					0.063 (0.6848)	0.060 (0.6998)	-0.135 (0.4011)	-0.474 (0.0011)	0.120 (0.4445)	0.318 (0.0359)	0.310 (0.0404)	0.207 (0.1784)
LIVRRET						0.058 (0.7077)	-0.249 (0.1168)	-0.195 (0.2051)	-0.049 (0.7557)	-0.033 (0.8329)	-0.037 (0.8109)	0.172 (0.2038)
LIVRPALM							0.084 (0.6028)	0.052 (0.7351)	-0.108 (0.4921)	-0.294 (0.0525)	-0.400 (0.0072)	-0.059 (0.7054)
YRATIO								0.216 (0.1753)	0.015 (0.9286)	0.090 (0.5747)	0.035 (0.8296)	-0.046 (0.7744)
LRATIO									-0.069 (0.6587)	-0.149 (0.3335)	-0.190 (0.2178)	-0.040 (0.7971)
PERCFAT										0.645 (0.0001)	0.388 (0.0102)	0.211 (0.1754)
DRYWT											0.924 (0.0001)	0.444 (0.0025)
WETWT												0.487 (0.0008)

[a] Values in parentheses are p-values corresponding to the correlation coefficients.

Table 5.9. General linear models by rank dispersion, multiple regression models relating reprodution measurement to DDT contamination, vitamin A concentrations, and morphological measurements for Japanese quail that received 0, 1, 10, or 25 ppm p,p'-DDT in their food for 64 days.

Model	F	P
Number of Eggs Laid		
Y = 26.71 + 7.26 PLASRET* + 7.95 YRATIO	2.67	0.0817
Number of Settable Eggs		
Y = 20.07 + 19.37 YRATIO - 0.01 YΣDDT	4.88	0.0124
Y = 18.29 + 21.63 YRATIO	9.90	0.0030
Y = 16.88 + 0.01 BIRDWT + 22.13 YRATIO	4.90	0.0122
Y = 17.12 + 0.01 BIRDWT + 20.25 YRATIO - 0.01 YΣDDT	3.22	0.0324
Y = 17.59 + 0.50 DRYWT + 21.05 YRATIO	4.81	0.0132
Y = 19.25 - 0.14 WETWT + 21.65 YRATIO	4.85	0.0127
Y = 17.44 - 0.03 BIRDWT + 3.87 DRYWT + 17.44 YRATIO	2.78	0.0531
Y = 20.31 - 0.50 LIVRRET + 19.57 YRATIO	5.51	0.0075
Y = 15.02 + 9.27 PLASRET + 20.39 YRATIO	5.51	0.0075
Y = 16.21 + 8.13 PLASRET + 19.25 YRATIO - 0.003 YΣDDT	3.58	0.0193
Y = 14.74 + 8.65 PLASRET + 19.89 YRATIO + 0.03 YPPDDE	3.69	0.0193
Y = 16.78 - 0.01 BIRDWT + 8.53 PLASRET + 20.91 YRATIO	3.65	0.0202
Y = 20.01 + 0.31 DRYWT - 0.48 LIVRRET + 19.58 YRATIO	3.65	0.0202
Y = 15.55 - 0.29 DRYWT + 9.20 PLASRET + 20.51 YRATIO	3.65	0.0202
Y = 15.99 + 0.34 WETWT - 0.24 LIVRRET + 21.84 YRATIO + 0.03 YPPDDE	2.40	0.0659
Y = 16.67 - 0.34 WETWT + 9.78 PLASRET + 21.02 YRATIO	3.65	0.0202
Y = 16.26 - 0.01 BIRDWT + 1.72 DRYWT + 6.39 PLASRET + 15.69 YRATIO	2.47	0.0600
Y = 15.66 + 10.68 PLASRET - 0.51 LIVRRET + 19.66 YRATIO	4.02	0.0135
Y = 10.23 + 13.25 PLASRET - 0.21 LIVRRET + 23.95 YRATIO + 0.06 YPPDDE	2.76	0.0407
Y = 17.66 - 0.87 DRYWT + 10.69 PLASRET - 0.55 LIVRRET + 19.37 YRATIO	3.02	0.0288
Y = 20.39 - 0.63 WETWT + 10.29 PLASRET - 0.56 LIVRRET + 18.96 YRATIO	3.10	0.0259
Y = 16.96 - 0.01 BIRDWT + 8.02 PLASRET + 19.86 YRATIO - 0.003 YΣDDT	2.62	0.0491
Y = 16.26 + 0.01 BIRDWT + 7.14 PLASRET + 15.59 YRATIO + 0.03 YPPDDE	2.63	0.0484
Y = 19.73 - 0.10 WETWT + 5.98 PLASRET + 13.14 YRATIO + 0.04 YPPDDE	2.32	0.0734
Y = 16.71 - 0.004 BIRDWT + 0.12 DRYWT + 7.93 PLASRET + 17.57 YRATIO + 0.04 YPPDDE	2.21	0.0727

Table 5.9. Continued

Model	F	P
Number of Eggs Showing Embryonic Development		
Y = 9.56 + 15.88 YRATIO	7.53	0.0088
Y = 10.09 + 0.004 BIRDWT + 12.52 YRATIO + 0.01 YPPDDE	2.38	0.0835
Y = 11.10 + 0.08 BIRDWT - 5.63 DRYWT + 6.65 YRATIO	2.58	0.0665
Y = 20.72 - 1.79 WETWT + 16.26 YRATIO	5.73	0.0063
Y = 10.54 + 0.13 BIRDWT - 2.16 WETWT - 0.06 LΣDDT	2.27	0.0947
Y = 8.65 + 0.30 LIVRRET + 16.47 YRATIO	3.88	0.0284
Y = 6.34 + 6.15 PLASRET + 16.69 YRATIO	4.08	0.0240
Y = 6.88 + 4.96 PLASRET + 15.75 YRATIO + 0.02 YPPDDE	2.71	0.0574
Y = 14.33 - 4.64 DRYWT + 7.14 PLASRET + 0.15 LIVRRET + 18.23 YRATIO	2.95	0.0316
Y = 8.72 + 0.04 BIRDWT - 3.24 DRYWT + 4.78 PLASRET + 11.05 YRATIO + 0.03 YPPDDE	2.12	0.0834
Y = 14.43 - 2.51 DRYWT + 4.20 PLASRET + 10.08 YRATIO + 0.03 YPPDDE	2.33	0.0725
Y = 17.19 - 1.94 WETWT + 8.27 PLASRET + 16.34 YRATIO	4.54	0.0077
Y = 9.27 + 0.04 BIRDWT - 0.99 WETWT + 3.57 PLASRET + 11.96 YRATIO	2.73	0.0423
Y = 11.73 + 0.09 BIRDWT - 2.16 WETWT + 4.86 YRATIO	2.95	0.0438
Y = 5.65 + 6.23 PLASRET + 0.26 LIVRRET + 17.18 YRATIO	2.75	0.0549
Y = 17.27 - 1.85 WETWT + 5.86 PLASRET + 0.19 LIVRRET + 17.87 YRATIO	3.15	0.0242
Number of Eggs Hatching		
Y = 1.81 + 6.99 YRATIO	4.41	0.0416
Y = 4.65 - 1.47 DRYWT + 7.28 YRATIO	3.22	0.0500
Y = 6.05 - 0.69 WETWT + 7.38 YRATIO	3.75	0.0317
Y = 6.82 - 0.71 WETWT - 0.02 LIVRRET + 5.98 YRATIO	2.32	0.0895
Y = 4.71 - 0.83 WETWT + 5.13 PLASRET + 7.08 YRATIO	3.30	0.0297
Y = 3.28 - 2.05 DRYWT + 6.02 PLASRET + 6.75 YRATIO	2.87	0.0479
Y = 4.29 - 2.15 DRYWT + 4.59 PLASRET - 0.05 LIVRRET + 6.75 YRATIO	2.15	0.0924
Y = 6.46 - 0.95 WETWT + 3.57 PLASRET - 0.05 LIVRRET + 6.86 YRATIO	2.40	0.0659

• LΣDDT = liver ΣDDT, LPPDDE = liver p,p′-DDE concentrations, LRATIO = liver DDE/DDT ratios, YΣDDT = yolk ΣDDT, YPPDDE = yolk p,p′-DDE concentrations, YRATIO = yolk DDE/DDT ratios, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, DRYWT = liver dry weights, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

CHAPTER 6: RESPONSE OF VITAMINS A AND E TO DDT IN NORTHERN BOBWHITES

INTRODUCTION

The experiment described here is very similar to that described in Chapter 5. Rather than using Japanese quail (*Coturnix coturnix japonica*), northern bobwhites (*Colinus virginianus*) were used. Japanese quail are known to be sensitive to the eggshell thinning effects of DDT, whereas northern bobwhites are thought not to be very sensitive (Bennett et al. 1988). Also, because vitamin E concentrations could not be measured in plasma of Japanese quail, the effect of DDT on vitamin E was investigated in this experiment.

This chapter reports on an experiment in which northern bobwhite were dosed chronically with p,p′-DDT in their food. In the previous chapter, it was shown that plasma concentrations of vitamin A were reduced in Japanese quail after exposure to 25 ppm dietary DDT. Vitamin A, however was not strongly associated with reproductive success. Plasma concentrations of both vitamins A and E were evaluated as possible biomarkers for nondestructive sampling of bobwhites for testing reproductive potential after exposure to DDT.

The status of both these vitamins influences the other (Frigg and Broz 1984), making it possible that the status of vitamin E prevented reduced plasma vitamin A from affecting reproduction in the previous experiment. It is also of interest to compare the effects of DDT on vitamin status between a species known to be susceptible to DDT-influenced eggshell thinning, Japanese quail, and a species that is not, northern bobwhites. DDT is being used because of its continuing effects on avian reproduction in wild populations (Steidl et al. 1991) and as a model for other organochlorine contaminants. Models including plasma and liver vitamins A and E, DDT contamination, and morphological measurements are presented and their predictive potential is discussed.

METHODS

Animal Care

Forty-eight female and 48 male northern bobwhite were acquired from a commercial gamebird breeder (Shady Knoll Bird Corp., Ashboro, NC). The bobwhites were kept in the animal room in the Department of Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University at approximately 21 C. Bobwhites were held individually in standard laying cages (G.Q.F. Manufacturing Co., Savannah, GA) with a sloping floor measuring 20.3 x 30.5 cm in the back, 25.4 x 30.5 cm in the front, and 50.8 cm in length. The light cycle was maintained at 15L:9D. Water and turkey starter mash (22% protein) supplemented with oyster shell were provided *ad libitum*.

Females were randomly assigned to one of four treatment groups. Groups received food treated with 0, 1, 10, or 25 mg p,p′-DDT/kg food (ppm). Females received treated food for

24 d before pairing with males began and 26 d before egg collection began. Females were paired daily with a different male for the duration of the experiment. Females were exposed to different males during each mating session to reduce the effect of possible pair incompatibility or male dysfunction. The females were transferred to males' cages at approximately 0900 and returned to their cages after 90 min. Two mating opportunities were allowed before egg collection. Egg collection for reproductive evaluation began on Day 26 of the experiment and continued for 22 d. Female bobwhites were maintained on treated food through Day 69.

Eggs were collected daily and placed in a poultry incubator (Humidaire Incubator Company, New Madison, OH) set at 37-38 C and approximately 55% relative humidity and automatically turned every 2 h for 18 d. On day 18, eggs were transferred to a stationary hatching incubator at 38 C and approximately 70% relative humidity. Hatching occurred on days 19 - 22.

Vitamin A and Vitamin E Analysis

Plasma samples were collected 1 d before bobwhites were given treated food, 39 d after treatment began, and 69 d after treatment began. Plasma samples were analyzed for retinol and α-tocopherol content according the method proposed by Bieri et al. (1979). For this method, equal volumes (100 μl) of plasma and an internal ethanol standard solution containing 1 μg/ml of retinyl acetate (Sigma Chemical Company, St. Louis, MO) and 10.2 μg/ml of α-tocopheryl acetate (Sigma Chemical Company) were vortexed for 30 sec along with 200 μl of ethanol in a 10 x 75 mm test tube. The additional ethanol was added to precipitate plasma proteins. The retinol and α-tocopherol were extracted from this mixture with 200 μl of hexane by vortexing for 1 min. The phases were separated by centrifugation for 5 min. As much of the hexane layer as was possible was transferred to a 6 x 50 mm test tube and evaporated to dryness under a stream of N_2 at room temperature. The residue was dissolved in 80 μl of cold

ethanol and vortexed for 30 sec. Fifty μl of this sample was injected into an HPLC (detector model 440, Waters Associates, Inc., Milford, MA) equipped with a 5 x 30 mm guard column and a 5 x 300 mm analytical column both packed with Bondeclone C-18 reverse phase packing (Phenomenex Inc., Torrance, CA).

Liver samples were analyzed for retinol and retinyl palmitate. After freeze-drying, liver portions weighing approximately 0.15 g were crushed into powder using mortar and pestle. The liver samples were transferred to 15 ml centrifuge tubes, and 10 ml hexane was added. After 10 min of gentle mixing by hand to extract the retinol and retinyl palmitate, the mixture was centrifuged for 5 min. A 600 μl aliquot of hexane was transferred to a 10 x 75 mm test tube and evaporated to dryness at room temperature under a stream of N_2. A 250 μl volume of ethanol containing retinyl acetate internal standard was added to the dry residue. Fifty μl of this sample was injected into an HPLC as was done for the plasma analysis.

Retinol concentrations in both plasma and liver tissue were calculated using the ratio technique described in Bieri et al. (1979). A standard curve was developed for retinyl palmitate (Sigma Chemical Company) and retinyl palmitate concentrations were calculated by direct comparison to the standard curve.

DDT Analysis

The tissues were freeze-dried and weighed. Fat, with associated DDT and metabolites, was extracted from the liver samples with petroleum ether, using a Soxhlet apparatus (Pyrex®, Fisher Scientific Co., Norcross, GA). Samples were extracted for 8 h. Sample clean-up, using activated florisil columns (U.S. Silica Company, Berkeley Springs, WV), was performed with 150 ml 6% ethyl ether-petroleum ether.

The analysis of o,p'-DDD, o,p'-DDE, o,p'-DDT, p,p'-DDD, p,p'-DDE, and p,p-DDT was performed on a Tracor 540® gas chromatograph (Tracor Corporation, Austin, TX), equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas and a 1.83 m x 6.35 mm i.d.

glass column packed with 1.5%/1.95% SP-2250/SP-2401 on 100/120 mesh Supelcoport® (Supelco, Inc., Bellefonte, PA). All samples were verified on a Tracor 540® gas chromatograph equipped with a Ni_{63} electron capture detector, using N_2 as the carrier gas and a 1.98 m x 6.35 mm i.d. glass column packed with 1.5%/1.95% OV-17/OV-210 on 100/120 mesh Chromosorb WHP® (Supelco, Inc.). Limits of detection for each isomer were determined as the smallest concentration of that isomer measured.

Statistical Analyses

All data were tested for normality using the Shapiro-Wilk statistic (SAS Institute, Inc. 1985a). Nonparametric procedures were used whenever the data deviated from normal. Statistical analyses were performed using the SAS® statistical package (SAS Institute, Inc. 1985a, 1985b) and NPSP (NPSP 1986). Kruskal-Wallis tests were used to test for differences among dose groups for concentrations of DDT isomers and metabolites and for differences in DDE/(DDD + DDT) ratios. Protected LSD tests on ranks were used for pairwise comparisons. Wilcoxon ranked sum tests were used to test for paired differences between DDT concentrations in livers. Analyses of variance were used to determine whether plasma retinol concentrations were different on any sampling day and used to test for differences over time for plasma retinol concentrations. Kruskal-Wallis tests were used to test for differences among treatment groups for liver retinol and retinyl palmitate because liver retinol and retinyl palmitate concentration distributions were significantly different from normal. Kruskal-Wallis tests were used to test for differences among treatment groups for body weight, percent liver fat, wet liver weight, and relative liver weight. Analyses of variances were used to test for differences among groups for reproductive measurements. Spearman's rank correlations were used to compare reproductive measurements with liver concentrations of DDT, plasma and liver concentrations of vitamin A, liver morphology measurements, and body weights. Rank correlations were also used to compare relationships among DDT measurements,

vitamin A measurements, liver morphological measurements, and body weights. Regression analyses were made using a nonparametric regression technique, general linear models by rank dispersion (Hettmansperger and McKean 1978, NPSP 1986).

RESULTS

Two female bobwhites died during the initial bleeding period. One control group female and one 25 ppm group female died during handling while acquiring blood samples.

DDT Contamination

Liver concentrations of p,p′-DDT ($P = 0.0152$) and its metabolite p,p′-DDE ($P < 0.0001$) differed significantly among the treatment groups (Table 6.1). For p,p′-DDT, the control and 1 ppm groups were not different from each other but did differ ($P < 0.05$) from both the 10 ppm and 25 ppm groups. The 10 ppm and 25 ppm groups also failed to differ for p,p′-DDT concentrations. All four groups were significantly different from each other for liver p,p′-DDE concentrations ($P < 0.05$). Measurable amounts of liver p,p′-DDD were found only in 10 ppm and 25 ppm groups, and no difference was found between the p,p′-DDD concentrations for these groups. ΣDDT (the summation of measurable concentrations of p,p′-DDD, liver p,p′-DDE, and p,p′-DDT within a sample) values also differed significantly ($P < 0.0001$) among all dose groups with each dose group being significantly different from the others. Values for DDE/DDT ratios (p,p′-DDE / (p,p′-DDT + p,p′-DDD)) also were found to differ ($P < 0.0001$) among all dose groups with each dose group being significantly different from the others with their order of increasing magnitude following the order of increasing concentrations in food.

Reproduction

The results of reproductive measurements (Table 6.2) showed no difference among treatment groups. Total numbers of eggs laid, settable eggs, eggs showing embryonic development, and eggs hatching and their percentages based on the previous reproductive stage are in Table 6.3. Very few eggs were cracked or otherwise apparently malformed producing great similarity between mean values of both number of eggs laid and number of settable eggs for all treatment groups (see Appendix Table C.1). Because of the similarity between these measurements, number of settable eggs will not be discussed subsequently.

The failure to find significant differences was caused by the high degree of variation in the reproductive success among females within each group (see Appendix Table C.1). The 25 ppm group showed the greatest variability for number of eggs laid and number of settable eggs, whereas the 10 ppm group was the most variable for number of eggs showing embryonic development and number of eggs hatching. In each instance, the most variable group was also the group with the lowest mean value. The 10 ppm group, the group showing the greatest variability for number of eggs showing embryonic development and number of eggs hatching, also showed the greatest variability for DDE/DDT ratios. Inherent genetic variability may be responsible for the variable response observed and makes it difficult to discern statistical differences among groups.

Vitamin A and Vitamin E

Concentrations of plasma retinol were not found to differ among treatment groups for any sampling day (Table 6.4). Plasma concentrations of α-tocopherol also did not differ (Table 6.4) for any sampling day. Analyses of variance showed that neither plasma concentrations of retinol ($F_{3,42} = 0.65$, $P = 0.5843$) nor plasma concentrations of α-tocopherol ($F_{3,42} = 0.40$,

P = 0.7542) differed when all dates were combined. Both retinol ($F_{2,42}$ = 11.69, P < 0.0001) and α-tocopherol ($F_{2,42}$ = 13.95, P < 0.0001) changed significantly through time with the first sampling period having lower concentrations than the second and third sampling periods. Neither plasma retinol ($F_{6,42}$ = 1.68, P = 0.1358) nor α-tocopherol ($F_{6,42}$ = 0.35, P = 0.9087) showed significant dose x time interactions. Liver concentrations of retinol and retinyl palmitate failed to show any significant differences among treatment groups (Table 6.5). No detectable amounts of α-tocopherol were found in livers.

Morphological Measurements

The morphological measurements, wet liver weight, final body weight, percent fat content of livers, and relative liver weight (wet liver weights / body weights) are reported in Table 6.6. No significant differences were found among treatment groups for any of these measurements.

Correlation Between Reproduction and Vitamins A and E, DDT Contamination, or Morphological Measurements

Correlation coefficients were calculated for nine variables measuring vitamin stores, DDT and metabolites, and morphological measures with number of eggs laid, number of eggs showing embryonic development, and number of eggs hatching (Table 6.7). Plasma concentrations of vitamin A and vitamin E both failed to correlate significantly with any of these measurements of reproduction. However, liver concentrations of retinol and retinyl palmitate and liver concentrations of p,p′-DDE and ΣDDT showed significant negative correlations with number of eggs laid; female body weight showed significant positive

correlation with number of eggs laid; and wet liver weight showed significant positive correlation with number of eggs laid, number of eggs showing embryonic development, and number of eggs hatching. Correlation coefficients for each dose group are found in Appendix Tables C.2-C.5.

Correlation Analyses Among Vitamins A and E, DDT Contamination, and Morphological Measurements

In Spearman's rank correlation analyses relating the same nine variables as presented in Table 6.7 with each other (Tables 6.8), plasma concentrations of neither vitamin A nor vitamin E showed significant correlations with DDT contamination. However, liver concentrations of retinyl palmitate showed a significant positive correlation with liver concentrations of p,p′-DDE, ΣDDT, and DDE/DDT ratios. Plasma concentrations of retinol failed to correlate significantly with either liver concentrations of retinol or retinyl palmitate, except in the control group where liver retinyl palmitate showed a significant negative correlation with plasma retinol. Correlation coefficients for each dose group appear in Appendix Tables C.6-C.9.

Regression Analyses

Regression models to predict the number of eggs laid containing either liver concentrations of p,p′-DDE or ΣDDT were always significant except when they also contained liver retinyl palmitate concentrations in combination with other variables. Those models with only either liver concentrations of p,p′-DDE or ΣDDT were best (Table 6.9). The best model for number of eggs showing embryonic development was the model containing only liver

concentrations of p,p′-DDE. The effect of DDT contamination on number of eggs showing embryonic development was not great enough to cause all models containing DDT contamination measures to be significant as for models for number of eggs laid. For number of eggs hatching, the best model was again the one containing only liver concentrations of p,p′-DDE. Very few other models showed significance. Only one model predicting one of the reproductive measurements that did not contain variables that would require destructive sampling showed significance. It was a model for number of eggs laid that contained only female body weights.

DISCUSSION

DDT Contamination

ΣDDT, p,p′-DDE, p,p′-DDT and DDE/DDT ratios were all found to differ significantly among treatment groups. Total contamination, ΣDDT, varied more in higher dose groups than in low dose or control groups. This indicates greater inherent variability in bobwhites' ability to eliminate DDT when exposed to higher chronic dose concentrations of p,p′-DDT in food. Variation in bobwhites' ability to eliminate residues of p,p′-DDT and its metabolites could likely produce considerable within group variation for responses to contamination such as vitamin concentrations or reproductive success. The change in mean value of DDE/DDT ratios from < 1 to > 1 between the 1 ppm group and 10 ppm group indicates that the 1 ppm group experienced little if any enzyme induction, whereas the 10 ppm and 25 ppm groups experienced enough enzyme induction to transform most of p,p′-DDT to p,p′-DDE.

Vitamins A and E

Concentrations of plasma retinol reported here are slightly higher than serum concentrations of retinol (0.49 μg/ml) reported for ringed turtle doves (*Streptopelia risoria*) (Spear et al. 1989). Concentrations of liver retinol were much lower than those reported for ringed turtle doves (257 μg/g, Spear et al. 1989; 258 μg/g, Spear et al. 1986) or herring gulls (*Larus argentatus*) collected from the Great Lakes region (131-864 μg/g, Spear et al. 1986). The retinyl palmitate values reported here were lower than values reported for ringed turtle doves (3784 μg/g, Spear et al. 1986). The cause of the lower liver values in this study may be a depletion of liver stores from prolonged egg laying. Glover et al. (1980) found that Japanese quail that were kept under natural lighting conditions had lowest plasma concentrations of retinol-binding holoprotein from September to October, after the breeding season. The secretion of retinol-binding holoprotein is strongly controlled by the availability of liver retinol (Goodman 1984). Plasma concentrations could have been maintained through dietary intake (P.A. Spear, pers. comm.).

Reproduction

DDT is known to produce reductions in number of eggs laid (Smith et al. 1969, Henny et al. 1984, Steele 1984), number of eggs showing embryonic development (Dewitt 1956, Heath et al. 1969, Smith et al. 1969), and number of eggs hatching (Heath et al. 1969, Smith et al. 1969, Koivusaari 1980, Custer and Mitchell 1987) in a variety of species. The relatively small amounts of p,p′-DDT and its metabolites stored in the liver probably contributed to the small differences among groups for reproductive measurements observed for bobwhites in this chapter, as compared to the Japanese quail in Chapter 5. Because males were not given contaminated food, any reduction in fertility normally resulting from poor male performance

caused by contamination was not present in this study and therefore increased the potential success of dosed females.

The two plasma characteristics, concentrations of retinol and α-tocopherol, that were measured both failed to correlate significantly with any of the reproductive measurements. Also, the regression models that attempted to predict reproduction could not do so while solely incorporating nondestructive measurements. From this, it seems doubtful that either vitamin A or vitamin E are useful biomarkers of both DDT contamination and reproduction in northern bobwhites.

CONCLUSIONS

The preceding discussion indicates that DDT contamination as measured by either liver p,p′-DDE or ΣDDT in livers did not strongly affect reproduction in northern bobwhites. Plasma concentrations of retinol, failed to have an affect on any measurement of reproduction. As a blood measurement, its potential for directly affecting reproduction is greater than that of either liver concentrations of retinol or liver concentrations of retinyl palmitate. However, liver concentrations of retinyl palmitate exhibited stronger relationships with measurements of reproduction than did plasma concentrations of retinol.

The relationship among liver morphological measurements and reproductive measurements suggests that altered liver metabolism plays a role in accounting for some of the variation in reproduction. These changes in liver metabolism or morphology cannot directly affect reproduction. Some factor reaching the egg in an abnormal amount is more likely the cause. The liver appears to be a good starting point to look for biomarkers in plasma that affect reproduction. More investigations of liver products that are passed to eggs should be pursued.

Table 6.1. Concentrations (μg DDT/g tissue) of DDT isomers and metabolites and DDE/DDT ratios found in livers northern bobwhite receiving food containing 0, 1, 10, 25 ppm DDT. Kruskal-Wallis test results are reported for among group comparisons.

Compound	Treatment	n[a]	Mean	S.E.	C.V.	χ^2	P
ΣDDT	Control	11	0.418[d]	0.041	32.2	29.40	<0.0001
	1 ppm	12	0.638[e]	0.141	76.8		
	10 ppm	11	2.660[f]	0.693	86.5		
	25 ppm	11	4.330[g]	1.310	100.4		
p,p′-DDT	Control	11	0.298[d]	0.029	32.7	10.43	0.0152
	1 ppm	12	0.364[d]	0.103	98.0		
	10 ppm	11	0.673[e]	0.155	76.1		
	25 ppm	11	1.098[e]	0.361	108.9		
p,p′-DDE	Control	11	0.120[d]	0.033	90.6	35.03	<0.0001
	1 ppm	12	0.254[e]	0.050	68.0		
	10 ppm	11	1.613[f]	0.544	112.0		
	25 ppm	11	3.097[g]	0.935	100.2		
p,p′-DDD[c]	Control	11	0	0			
	1 ppm	12	0	0			
	10 ppm	11	0.339[e]	0.269	263.5		
	25 ppm	11	0.126[e]	0.052	136.5		
Ratio[b]	Control	11	0.450[d]	0.140	103.4	25.39	<0.0001
	1 ppm	11	0.795[e]	0.162	67.0		
	10 ppm	11	2.358[f]	0.607	85.4		
	25 ppm	11	3.269[g]	0.464	47.1		

[a] n = the number of samples with measurable amounts of that DDT isomer
[b] Ratio = p,p′-DDE / (p,p′-DDT + p,p′-DDD)
[c] A Wilcoxon rank sum test indicated no difference (α = 0.1) between the 10 ppm and 25 ppm groups, the only groups with samples containing measurable amounts of p,p′-DDD.
[d,e,f,g] Values followed by different superscripts are significantly different (α = 0.05) according to Fisher's protected LSD on ranks.

Table 6.2. Reproductive success of northern bobwhite after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT. Analyses of variance results are reported for among group comparisons.

Reproduction	Treatment	n	Mean	S.E.	C.V.	F	P
Number laid	Control	11	15.64	1.43	30.3	1.07	0.3705
	1 ppm	12	16.42	1.23	25.9		
	10 ppm	12	15.83	1.85	40.4		
	25 ppm	11	12.64	1.88	49.5		
Number settable	Control	11	15.64	1.43	30.3	1.07	0.3722
	1 ppm	12	16.33	1.28	27.0		
	10 ppm	12	15.83	1.85	40.4		
	25 ppm	11	12.54	1.94	51.3		
Number developing	Control	12	12.46	1.84	48.9	0.69	0.5657
	1 ppm	12	10.92	2.19	69.6		
	10 ppm	12	8.50	2.26	92.0		
	25 ppm	11	9.46	1.93	67.8		
Number hatching	Control	11	11.00	1.65	49.6	0.84	0.4789
	1 ppm	12	9.33	1.92	71.4		
	10 ppm	12	7.33	2.06	97.2		
	25 ppm	11	7.54	1.69	74.4		

Table 6.3. Total numbers of eggs laid, settable, showing embryonic development, and hatching and the percentages based on the immediately previous reproductive stage from Northern bobwhite after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 69 days.

	Control	1 ppm	10 ppm	25 ppm
Number laid	172	197	190	139
Number settable	172	196	190	138
Settable/Laid (%)	100.0	99.5	100.0	99.3
Number developing	137	131	102	104
Developing/Settable (%)	79.7	66.8	53.7	75.4
Number hatching	121	112	88	83
Hatching/Developing (%)	88.3	85.5	86.3	79.8

Table 6.4. Concentrations of retinol (μg/ml) and α-tocopherol in northern bobwhite plasma after receiving food containing 0, 1, 10, or 25 ppm p,p'-DDT. Kruskal-Wallis test results are reported for among group comparisons.

Day	Treatment	n	Mean	C.V.	S.E.	F	P
Retinol							
0	Control	11	0.755	0.095	41.9	1.94	0.5842
	1 ppm	12	0.574	0.132	79.6		
	10 ppm	12	0.770	0.120	53.9		
	25 ppm	11	0.760	0.121	52.7		
39	Control	11	1.105[a]	0.073	21.8	6.40	0.0935
	1 ppm	12	1.043[a]	0.131	43.6		
	10 ppm	12	1.025[a]	0.080	27.2		
	25 ppm	11	0.830[b]	0.068	27.3		
69	Control	11	1.055	0.054	17.0	4.63	0.2007
	1 ppm	12	1.034	0.077	25.8		
	10 ppm	12	0.800	0.090	39.0		
	25 ppm	11	0.934	0.119	42.3		
α-Tocopherol							
0	Control	11	13.617	4.345	21.8	0.68	0.8783
	1 ppm	12	9.902	3.069	107.4		
	10 ppm	12	10.947	3.119	98.7		
	25 ppm	11	8.104	3.451	141.2		
39	Control	11	21.579	4.949	76.1	0.07	0.9954
	1 ppm	12	16.980	2.581	52.7		
	10 ppm	12	18.990	1.402	25.6		
	25 ppm	11	16.254	2.691	54.9		
69	Control	11	20.860	1.458	23.2	0.47	0.9260
	1 ppm	12	20.651	3.944	66.2		
	10 ppm	12	20.583	2.644	44.5		
	25 ppm	11	22.641	3.382	49.5		

[a,b] Values followed by different superscripts are significantly different (α = 0.1) according to Fisher's protected LSD on ranks.

Table 6.5. Concentrations (μg/g) and total amounts (μg) of vitamin A forms in livers of northern bobwhite after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT. Kruskal-Wallis results are reported for among group comparisons.

Vitamin A	Treatment	n	Mean	S.E.	C.V.	χ^2	P
LIVRRET	Control	11	15.75	3.66	77.0	1.84	0.6065
	1 ppm	12	13.51	3.97	101.7		
	10 ppm	12	16.83	3.08	63.5		
	25 ppm	11	15.86	3.65	76.2		
LIVRPALM	Control	11	1445.32	339.43	77.9	2.34	0.5054
	1 ppm	12	1194.66	219.44	63.6		
	10 ppm	12	1795.11	320.28	61.8		
	25 ppm	11	1658.68	273.31	54.6		

Table 6.6. Body weights, liver weights and percent fat content of livers from northern bobwhite after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT. Kruskal-Wallis results are reported for among group comparisons.

	Treatment	n	Mean	S.E.	C.V.	χ^2	P
WETWT	Control	11	5.482	0.510	30.9	2.04	0.5632
	1 ppm	12	5.191	0.336	22.4		
	10 ppm	12	4.613	0.301	22.6		
	25 ppm	11	5.512	0.574	34.5		
BIRDWT	Control	11	230.491	5.586	8.0	2.77	0.4281
	1 ppm	12	237.817	5.905	8.6		
	10 ppm	12	222.667	5.367	8.3		
	25 ppm	11	231.064	6.592	9.5		
PCTFAT	Control	11	34.706	4.205	40.2	1.975	0.5777
	1 ppm	12	32.226	3.160	34.0		
	10 ppm	12	31.528	1.686	18.5		
	25 ppm	11	37.186	3.871	34.5		
RELLIVR	Control	11	0.024	0.002	25.1	1.67	0.6445
	1 ppm	12	0.022	0.001	19.1		
	10 ppm	12	0.021	0.001	17.7		
	25 ppm	11	0.024	0.003	36.6		

Table 6.7. Spearman rank correlation coefficients relating the number of northern bobwhite eggs laid, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, vitamin E concentrations, liver weights, and body weights for all treatment groups combined.

Treatment	PPDDE	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
Number Laid	-0.296 (0.0457)*	-0.376 (0.0100)	-0.060 (0.6949)	-0.301 (0.0422)	-0.321 (0.0294)	-0.051 (0.7476)	-0.100 (0.5196)	0.064 (0.6723)	0.436 (0.0025)	0.325 (0.0273)
Number Developing	-0.207 (0.1672)	-0.240 (0.1085)	0.027 (0.8582)	-0.204 (0.1734)	-0.273 (0.0669)	0.000 (1.0000)	-0.185 (0.2286)	-0.012 (0.9349)	0.333 (0.0238)	0.218 (0.1457)
Number Hatching	-0.203 (0.1751)	-0.213 (0.1552)	0.008 (0.9563)	-0.211 (0.1584)	-0.284 (0.0559)	0.022 (0.8925)	-0.252 (0.0983)	-0.003 (0.9834)	0.331 (0.0248)	0.240 (0.1076)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table 6.8. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the all treatment groups combined.

	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
PPDDE	0.934 (0.0001)*	-0.195 (0.1996)	0.221 (0.1403)	0.377 (0.0099)	0.224 (0.1531)	0.794 (0.0001)	0.225 (0.1333)	-0.249 (0.0952)	-0.160 (0.2890)
ΣDDT		-0.196 (0.1980)	0.172 (0.2527)	0.381 (0.0090)	0.175 (0.2669)	0.602 (0.0001)	0.314 (0.0337)	-0.309 (0.0366)	-0.163 (0.2806)
PLASRET			-0.242 (0.1100)	-0.170 (0.2647)	-0.215 (0.1712)	-0.180 (0.2473)	0.183 (0.2282)	0.146 (0.3377)	0.210 (0.1659)
LIVRRET				0.720 (0.0001)	-0.257 (0.1005)	0.328 (0.0296)	-0.619 (0.0001)	-0.553 (0.0001)	-0.447 (0.0019)
LIVRPALM					-0.108 (0.4940)	0.328 (0.0298)	-0.176 (0.2425)	-0.744 (0.0001)	-0.418 (0.0039)
PLASTOC						0.171 (0.2927)	0.013 (0.9371)	0.235 (0.1339)	-0.103 (0.5170)
RATIO							-0.030 (0.8452)	-0.123 (0.4278)	-0.173 (0.2620)
PCTFAT								0.192 (0.2010)	0.274 (0.0652)
WETWT									0.488 (0.0006)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table 6.9. General linear models by rank dispersion, multiple regression models relating reprodution measurement to DDT contamination, vitamin A concentrations, and morphological measurements for northern bobwhite that received 0, 1, 10, or 25 ppm p,p'-DDT in their food for 64 days.

Model	F	P
Number of Eggs Laid		
Y = 19.83 - 0.05 PLASTOC[a] - 0.18 LIVRRET	2.76	0.0765
Y = 18.95 +0.002 LIVRPALM - 0.000007 PLASTOC	2.82	0.0707
Y = 10.19 + 0.06 BIRDWT	2.68	0.0449
Y = 26.95 - 0.16 PCTFAT - 0.09 LIVRRET	3.19	0.0332
Y = 20.37 - 1.91 PLASRET - 0.17 LIVRRET	2.82	0.0707
Y = 18.41 - 0.16 LIVRRET	5.60	0.0224
Y = 17.76 - 1.65 PPDDE	16.25	0.0002
Y = 17.83 - 1.16 ΣDDT	15.35	0.0003
Y = 17.97 - 1.22 RATIO	4.84	0.0331
Y = 10.84 + 0.06 BIRDWT - 0.18 PCTFAT - 0.03 PLASTOC - 0.02 PLASRET - 0.20 LIVRRET	2.15	0.0791
Y = 27.50 - 0.16 PCTFAT - 0.09 PLASTOC - 0.04 PLASRET - 0.25 LIVRRET	2.41	0.0646
Y = 18.95 - 0.00004 LIVRRET - 0.002 LIVRPALM	2.83	0.0700
Y = 18.95 - 0.002 LIVRRET - 0.0000001 PLASRET	2.83	0.0700
Y = 10.18 + 0.06 BIRDWT - 0.17 PCTFAT - 0.009 PLASRET - 0.19 LIVRRET	2.12	0.1957
Y = 28.00 - 0.16 PCTFAT - 3.14 PLASRET - 0.26 LIVRRET	3.40	0.0263
Y = 9.80 + 1.18 WETWT	3.89	0.0549
Y = -3.31 + 0.08 BIRDWT	3.25	0.0783
Y = -4.02 + 0.10 BIRDWT - 0.10 PCTFAT	2.46	0.0974
Y = 14.26 + 0.68 WETWT - 0.12 LIVRRET	3.16	0.0524
Y = 24.37 - 0.15 PCTFAT - 0.23 LIVRRET	4.83	0.0128
Y = 8.45 + 0.04 BIRDWT - 0.12 LIVRRET	3.07	0.0567
Y = 10.12 + 0.06 BIRDWT - 0.17 PCTFAT - 0.19 LIVRRET	3.70	0.0189
Y = 18.95 - 0.002 LIVRPALM	5.74	0.0209
Y = 18.95 +0.000005 WETWT - 0.002 LIVRPALM	2.82	0.0707
Y = 18.95 - 0.00002 PCTFAT - 0.002 LIVRPALM	2.83	0.0700
Y = 18.82 + 0.0002 BIRDWT - 0.002 LIVRPALM	2.82	0.0707

Table 6.9. Continued

Model	F	P
Number of Eggs Showing Embryonic Development		
Y = 3.69 + 1.26 WETWT	3.26	0.0778
Y = 11.93 - 1.19 PPDDE	4.32	0.0435
Y = 11.89 - 0.77 ΣDDT	3.02	0.0892
Y = 13.43 - 0.002 LIVRPALM	3.09	0.0857
Number of Eggs Hatching		
Y = 1.91 + 1.31 BIRDWT	3.44	0.0703
Y = 10.14 - 1.05 PPDDE	4.64	0.0368
Y = 12.06 - 0.002 LIVRPALM	3.65	0.0626

• ΣDDT = ΣDDT, PPDDE = liver p,p′-DDE concentrations, RATIO = liver DDE/DDT ratios, PLASTOC = plasma α-tocopherol concentrations, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

CHAPTER 7: GENERAL DISCUSSION

Compounds to be used as biomarkers must possess certain properties. The most important is that they allow for reliable prediction of the intended end point. For contaminant problems involving numerous wildlife species, a reliable biomarker also must provide consistent results across many species. Potential biomarkers should adequately reflect increasing exposure to a contaminant by showing a graded response. As endangered species become the focus of more contaminant studies, and public opinion moves toward less tolerance of killing animals during research, biomarkers must be readily available to reduce the need to remove animals from the wild. The most accessible tissue for nondestructive sampling is blood, making blood biomarkers of great interest. Adequate biomarkers should not be dependent on the route of exposure. Whether the contaminant is ingested, inhaled, or absorbed through the skin, the proposed biomarker should provide similar results at similar contaminant body burdens. A biomarker does not need to the sole predictor of the end point of interest but can act in conjunction with other characteristics to make accurate predictions. The biomarkers tested, vitellogenin, vitamin A, and vitamin E do not meet these requirements. While the results are not positive, they are reasonably convincing in eliminating these 3 blood components as useful biomarkers.

The results presented in Chapters 3, 5, and 6 show limited potential for the use of vitellogenin, vitamin A, and vitamin E as biomarkers of both contamination with the organochlorine pesticide, DDT and reproductive potential. Of the traits of biomarkers listed above, the only one that held for these 3 blood components was that they were accessible in the blood. They each failed to reliably predict reproductive success, to respond to greater DDT concentrations in both quail species across the doses of DDT tested or act in conjunction with another nondestructively sampled trait to predict reproductive success.

The results presented in Chapter 4 show that food addition and corn oil intubation are not equivalent methods of orally dosing northern bobwhites with DDT. Differences between contaminant accumulation based on the method of dosing could greatly affect the ability to compare conclusions from one study to those found in another or to apply the conclusions from a laboratory study with a field study.

As presented in Appendix D, pair management was shown to affect reproductive success. The choice of mating combinations, whether continuously paired, paired with the same mate during each mating opportunity, or paired with a different mate altered reproductive success. When males and females need to be held separately to provide them with different food, pairing females with different males during each mating session was found to be superior to pairing with the same male.

Both Japanese quail and northern bobwhites are indeterminate layers. Laying eggs for an indeterminate period has both advantages and poses problems for interpreting the results found here. Determinate layers, such as the ringed turtle doves used in various studies, would not be able to eliminate as much of their body burdens via eggs as could an indeterminate layer. The selection of a 'breeding period' for an indeterminate layer is necessarily artificial because the hens are likely to be in production for at least a portion of the pre-treatment period. The frequency of egg laying does not increase with mating, nor will it terminate with the removal of a mate. The major advantage of using an indeterminate layer is that a graded response is more readily apparent.

DDT CONTAMINATION

Both Japanese quail and northern bobwhite accumulated larger concentrations of DDT (shown as ΣDDT) in yolk than in livers (Tables 3.1 and 5.1), whereas livers showed a greater accumulation than brains (Table 4.1). The greater accumulation of DDT in yolks than in livers or brains suggests that DDT is deposited in yolks in a greater quantity than in other tissues. Denison et al. (1981) showed that DDT binds to a vitellogenin-like serum protein in mosquitofish (*Gambusia affinis*). Should DDT bind to vitellogenin while not preventing transport of vitellogenin into the yolk, this would provide an active transport mechanism for DDT's incorporation in egg yolks. Denison et al. also showed that DDT binds to a low molecular weight lipoprotein. The same membrane-bound receptor for vitellogenin also binds other lipoproteins (Steyer et al. 1990, Stifani et al. 1990). Active transport of lipoproteins would provide another route for accumulation of DDT in egg yolk.

The amount of DDT accumulated in livers of both quail species given 25 ppm dietary p,p'-DDT differed between the species. The Japanese quail showed greater mean accumulation after exposure for 64 d (82.3 ppm, Table 5.1) whereas the bobwhites showed less accumulation after exposure for 56 d (10.6 ppm, Table 4.1) or after exposure for 69 d (3.3 ppm, Table 6.1). The difference in liver accumulation of DDT between Japanese quail and bobwhites is probably a species difference. Mean fat concentrations in livers were numerically greater in the bobwhites in Chapter 6, than in the Japanese quail in Chapter 5, indicating that higher ΣDDT stores in livers in Japanese quail is not simply a result of greater liver fat stores. The slight difference in liver accumulation of DDT between the two bobwhite experiments may have been caused by a difference in age, a difference in protein content of the food, and/or a difference in reproductive status. The bobwhites with lower mean ΣDDT values received higher dietary protein (22% crude protein vs 15% crude protein), were younger ($<$ 6 mo vs $>$ 1 yr), and were laying eggs whereas the others were not. The

bobwhites in Chapter 6 were fed food with higher protein content because they refused to eat a new batch of the laying mash used in all other experiments.

In addition to differences in DDT contamination, differences in DDE/DDT ratios also occurred between species. The Japanese quail in Chapter 5 (Table 5.1) did not produce DDE/DDT ratios in livers greater than 1 for any dose group. The northern bobwhites in Chapter 6, however, produced ratios in livers of greater than 1 for the 10 ppm and 25 ppm groups. The bobwhites from Chapter 3 (Table 3.2) and Chapter 4 (Table 4.3) also produced ratios greater than 1.

The decreased accumulation and higher DDE/DDT ratios may be a key to the reduced susceptibility of bobwhites to eggshell thinning and their better reproductive performance, and the absence of reduced vitamin A concentrations in blood as compared to Japanese quail. Species better able to metabolize or eliminate a contaminant such as DDT could very likely exhibit fewer of its detrimental impacts.

The use of microsomal enzymes to monitor contaminant exposure has been proposed (Rattner et al. 1989). The use of microsomal enzymes to determine a species sensitivity to reproductive effects of exposure to an organochlorine contaminant also would be useful. However, unless a blood component could be found that would act as a biomarker for altered microsomal enzyme activity, enzyme activity would be difficult to assess without destructive sampling.

The results from Chapters 5 and 6 support the idea that DDE/DDT ratios are indicative of potential reproductive success. DDE/DDT ratios in bobwhites indicated that most of the dietary p,p′-DDT was metabolized, and the bobwhites did not show greatly diminished reproductive success. In contrast, Japanese quail were not as successful at metabolizing dietary p,p′-DDT, and they did exhibit significantly altered reproduction. The failure of Japanese quail to metabolize and eliminate p,p′-DDT and its metabolites probably led to the reduction of plasma concentrations of retinol. Brouwer and van den Berg (1986) showed that the binding of a polybrominated biphenyl to transthyretin destabilized the retinol binding

ability of the retinol-binding protein-transthyretin complex. Greater concentrations of DDT would increase the likelihood of the same effect occurring.

In Chapter 3, the relationships between DDE/DDT ratios and vitellogenin was investigated. No significant correlations were seen (Table 3.7) between vitellogenin and liver DDE/DDT ratios in bobwhites. In Chapter 5, liver DDE/DDT ratios showed significant negative correlations with plasma vitamin A in Japanese quail (Table 5.8), but not with plasma vitamin A or plasma vitamin E in bobwhites (Table 6.8). Plasma vitamin A showed significant negative correlations with both liver concentrations of p,p'-DDE and ΣDDT in Japanese quail, whereas plasma vitellogenin, vitamin A, and vitamin E did not do so in bobwhites. The failure of DDT accumulation or DDE/DDT ratios to strongly correlate with plasma measurements in bobwhites suggests that the reduced accumulation and/or higher DDE/DDT ratios prevent DDT from exhibiting its effects in bobwhites in comparison to Japanese quail.

PLASMA VITELLOGENIN, VITAMIN A, AND VITAMIN E AS BIOMARKERS

Vitellogenin, vitamin A, and vitamin E do not make acceptable biomarkers when measured in plasma. A problem with using many compounds as biomarkers in plasma is that if the site of action for that compound is not in plasma, interpreting the concentrations measured may be difficult. Biomarkers of reproduction are not likely to exhibit their effect in the plasma.

Vitamin A and vitamin E metabolism and physiological actions are complex and varied. A model for vitamin A turnover in rats was proposed by Lewis et al. (1990) (see Fig. 7.1) since the work presented here commenced. Their model demonstrates that plasma concentrations are only a small portion of the system of pathways involved in vitamin A metabolism.

Adequate plasma concentrations of vitamin A do not eliminate the possibility that a carrier protein or receptor protein involved at the site of biological activity is inhibited or blocked.

Robbins (1983) stated that acute vitamin A deficiencies in free-ranging populations are probably quite rare, but marginal deficiencies in conjunction with plant constituents (e.g. phytoestrogens) may be an important mechanism in controlling reproduction, especially in desert dwelling bird species. The dietary requirement of vitamin A for survival is less than that for reproduction. Nestler (1946) showed that dietary concentrations adequate for winter survival of bobwhites did not provide the necessary stores for spring reproduction. Lehmann (1953) was able to show a strong relationship between vitamin A liver stores, lush vegetation high in vitamin A precursors, and breeding success in bobwhites in Texas. Bobwhites will eat vegetative matter when available, thus suggesting that reproduction of a free-living population can be adversely affected by inadequate dietary vitamin A stores.

Both the Japanese quail and bobwhites were provided diets containing adequate vitamin A. Japanese quail experienced a reduction in plasma vitamin A and reproductive success after dosing with DDT at 25 ppm. However, plasma vitamin A was not important in the determination of reproductive success. The bobwhites, dosed with the same dietary concentrations of DDT failed to show marked reductions in either plasma vitamin A or reproductive success. Vitamin A may be indicative of DDT contamination in some sensitive species, such as Japanese quail, but not in others such as bobwhites.

Spear et al. (1989) found higher serum concentrations of vitamin A in ringed turtle dove females successfully producing eggs which hatched in comparison to those producing non-hatching eggs regardless of whether the females were exposed to a dioxin analog. The higher concentrations in some females exposed to a dioxin analog but not other females further supports the conclusion that vitamin A is not an adequate biomarker of reproduction.

Vitamin E is also available in diets of herbivorous/granivorous birds. No measurable concentrations of vitamin E were found in any Japanese quail plasma samples. No differences were found for plasma concentrations of vitamin E in bobwhites receiving different doses of DDT. It is difficult to determine the effects of DDT on vitamin E considering these results.

In contrast to vitamin A and vitamin E, vitellogenin has a single function. It is produced in the liver solely to be deposited in egg yolk. The simplicity of its physiological action would have made it a much better biomarker of reproduction. Vitellogenin is not available in the diet, preventing supplementation. The failure of plasma vitellogenin to be affected by dietary DDT in bobwhites provides very strong evidence that it is not a factor in reproductive failure caused by DDT contamination. The complexity of vitamin A and vitamin E physiology and their availability in the diet, prevent the conclusion being made that they are not involved with reproductive failure in birds caused by DDT, but the failure to find a strong relationship suggests that plasma concentrations do not reflect or help interpret any possible role.

FUTURE RESEARCH

Constraints are necessarily placed on all experiments. One trade-off is between the number of experimental animals, and the amount of blood that can be sampled, especially when repeated measurements are desired. Larger body size would provide greater blood volumes, but also would increase the cost of feeding and increase the space requirements, reducing the sample size.

Many other constraints and difficulties also must be considered. It is difficult to synchronize breeding cycles to ensure that all birds are at the same point in the dose schedule when they initiate egg production. In indeterminate layers, more eggs being laid during a pretreatment period would allow females to eliminate a greater portion of their body burden. Dosing both sexes eases handling requirements, but also reduces the ability to determine mechanisms for reduced reproductive success.

Two results presented here suggest that future work on blood biomarkers should pursue blood components derived from alterations in microsomal enzyme activities. The first is that liver morphological characteristics and reproductive success for both Japanese quail and

northern bobwhite were correlated, and the second is that liver DDE/DDT ratios showed marked differences between the two species after similar exposures to dietary DDT. Also, it would be worthwhile to investigate different dose regimens between species that would produce similar concentrations in livers to see if accumulation rather than degree of exposure is more important.

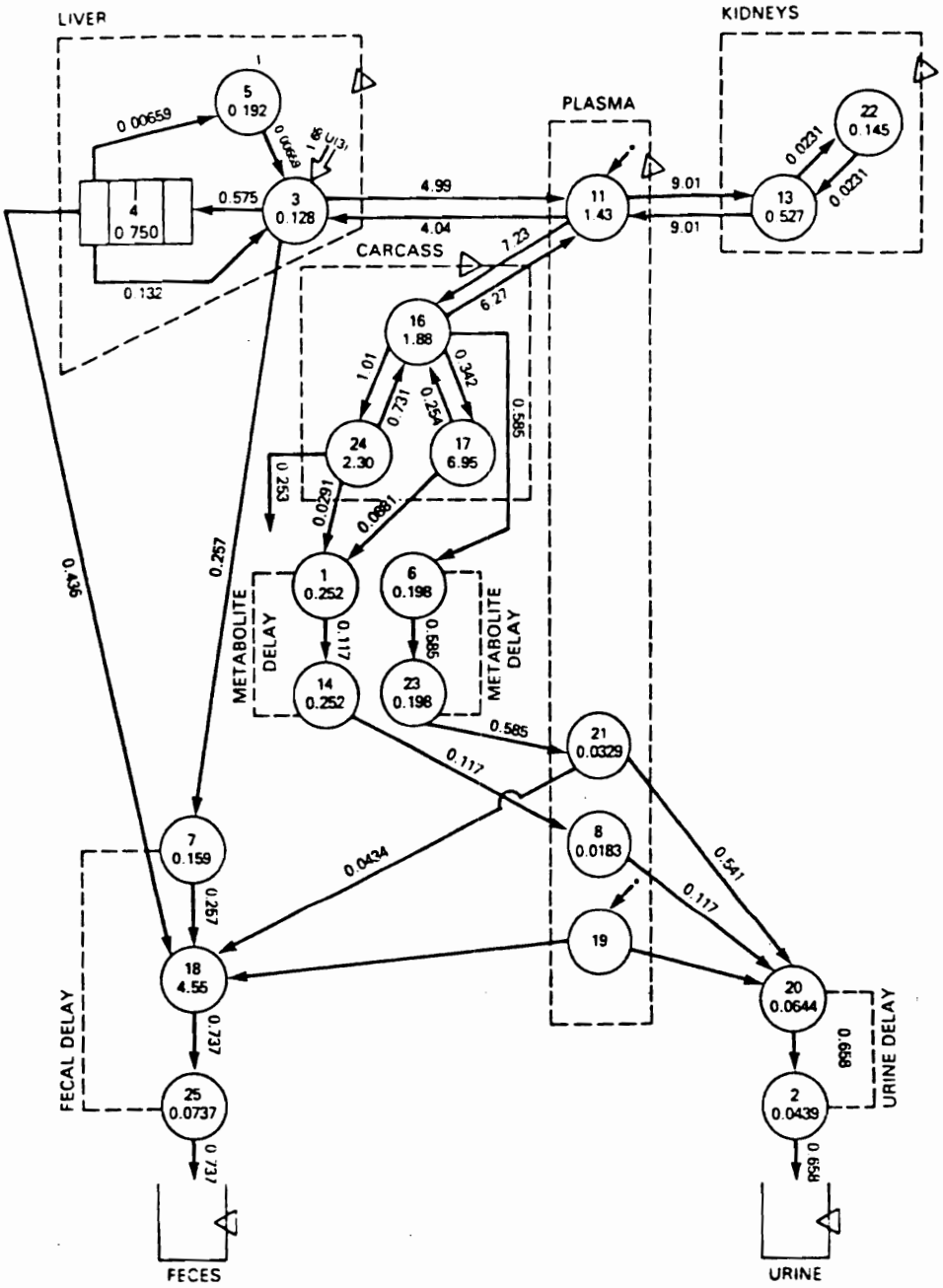

Figure 7.1. Model for vitamin A turnover in the rat. (Courtesy of Lewis et al. 1990)

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Appendix A.

Table A.1. Total numbers of eggs laid by individual female northern bobwhite from Chapter 3 after receiving 0, 1, 10, or 25 ppm p,p′-DDT intubated in corn oil.

Group	Bird	Number Laid
Control	1	2
	2	5
	3	0
	4	0
	5	5
	6	34
	7	1
	8	14
	9	36
	10	25
	11	11
	12	22
1 ppm	1	33
	2	1
	3	20
	4	31
	5	23
	6	21
	7	0
	8	11
	9	18
	10	21
	11	25
	12	13
10 ppm	1	13
	2	0
	3	0
	4	25
	5	20
	6	23
	7	23
	8	14
	9	15
	10	8
	11	12
	12	23

Table A.1. Continued.

Group	Bird	Number Laid
25 ppm	1	22
	2	22
	3	15
	4	34
	5	13
	6	20

Appendix B.

Table B.1. Total numbers of eggs laid, settable, showing embryonic development, and hatching by individual female Japanese quail from Chapter 5 after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 64 days.

Group	Bird	Number Laid	Number Settable	Number Developing	Number Hatching
Control	1	32	23	19	8
	2	37	35	20	6
	3	36	33	22	0
	4	24	22	10	3
	5	37	36	14	0
	6	35	35	29	14
	7	42	42	26	10
	8	42	42	28	18
	9	42	41	32	14
	10	40	38	8	0
1 ppm	1	13	1	0	0
	2	27	24	10	2
	3	27	22	13	4
	4	22	22	16	7
	5	38	37	19	8
	6	30	21	7	0
	7	32	27	16	3
	8	36	33	10	1
	9	29	13	6	0
	10	39	38	32	12
	11	35	33	14	0
10 ppm	1	34	33	20	13
	2	32	29	23	7
	3	21	14	3	0
	4	39	15	13	2
	5	39	37	32	8
	6	35	34	27	14
	7	42	42	29	11
	8	37	36	23	7
	9	38	39	35	12
	10	27	26	14	3
	11	37	34	19	6

Table B.1. Continued.

Group	Bird	Number Laid	Number Settable	Number Developing	Number Hatching
25 ppm	1	14	9	1	0
	2	42	42	32	13
	3	30	28	5	0
	4	38	30	9	0
	5	28	25	19	10
	6	35	32	14	4
	7	16	6	0	0
	8	38	5	2	0
	9	39	36	19	4
	10	32	13	15	1
	11	32	31	25	5
	12	40	39	15	5

Table B.2. Spearman rank correlation coefficients relating the number of Japanese quail eggs laid, number of settable eggs, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, liver weights and body weights for the control group (n = 10).

Treat	LΣDDT[a]	LPPDDE	LRATIO	YΣDDT	YPPDDE	YRATIO	PLASRET	LIVRRET	LIVRPALM	DRYWT	WETWT	PCTFAT	BIRDWT
Number Laid	-0.159 (0.6618)[b]	-0.085 (0.8146)	-0.071 (0.0239)	0.244 (0.4971)	0.354 (0.3161)	0.061 (0.8671)	0.244 (0.4971)	-0.689 (0.0274)	0.206 (0.5678)	0.317 (0.3720)	0.012 (0.9733)	0.567 (0.0873)	-0.326 (0.3377)
Number Settable	-0.213 (0.5538)	-0.055 (0.8803)	-0.738 (0.0149)	0.335 (0.3435)	0.476 (0.1647)	0.073 (0.8408)	0.183 (0.6130)	-0.729 (0.0169)	0.179 (0.6215)	0.262 (0.4643)	-0.012 (0.9733)	0.640 (0.0461)	-0.329 (0.3529)
Number Develop.	-0.067 (0.8548)	-0.006 (0.9867)	0.139 (0.7009)	-0.091 (0.8028)	-0.030 (0.9338)	0.127 (0.7261)	-0.042 (0.9074)	-0.433 (0.2111)	0.669 (0.0343)	-0.442 (0.2004)	-0.636 (0.0479)	0.358 (0.3104)	-0.394 (0.2600)
Number Hatching	-0.086 (0.8129)	-0.080 (0.8261)	0.062 (0.8659)	0.142 (0.6905)	0.246 (0.4929)	0.246 (0.4929)	0.154 (0.6713)	-0.085 (0.8147)	0.312 (0.3801)	-0.111 (0.7606)	-0.345 (0.3294)	0.585 (0.0759)	-0.012 (0.9731)

[a] LΣDDT = liver ΣDDT, LPPDDE = liver p,p′-DDE concentrations, LRATIO = liver DDE/DDT ratios, YΣDDT = yolk ΣDDT, YPPDDE = yolk p,p′-DDE concentrations, YRATIO = yolk DDE/DDT ratios, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, DRYWT = liver dry weights, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

[b] Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.6. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the control group.

	YPPDDE	LΣDDT	YΣDDT	PLASRET	LIVRRET	LIVRPALM	YRATIO	LRATIO	PERCFAT	DRYWT	WETWT	BIRDWT
LPPDDE	-0.236 (0.5109)*	0.442 (0.2004)	-0.370 (0.2931)	0.176 (0.6272)	0.032 (0.9294)	0.294 (0.4103)	0.042 (0.9074)	0.164 (0.6515)	0.006 (0.9867)	-0.467 (0.1739)	-0.503 (0.1383)	-0.079 (0.8287)
YPPDDE		-0.079 (0.8287)	0.952 (0.0001)	0.273 (0.4458)	-0.175 (0.6296)	-0.150 (0.6787)	0.406 (0.2443)	-0.382 (0.2763)	0.297 (0.4047)	0.418 (0.2291)	0.442 (0.2004)	0.127 (0.7261)
LΣDDT			0.079 (0.8287)	0.430 (0.2145)	0.472 (0.1685)	0.225 (0.5314)	-0.503 (0.1383)	0.406 (0.2443)	0.152 (0.6761)	-0.055 (0.8810)	-0.248 (0.4888)	0.224 (0.5334)
YΣDDT				0.297 (0.4047)	-0.045 (0.9012)	-0.137 (0.7068)	0.224 (0.5334)	-0.248 (0.4888)	0.248 (0.4888)	0.467 (0.1739)	0.479 (0.1615)	0.188 (0.6032)
PLASRET					-0.278 (0.4367)	0.355 (0.3141)	-0.261 (0.4671)	-0.467 (0.1739)	0.503 (0.1383)	0.430 (0.2145)	0.067 (0.8548)	-0.055 (0.8810)
LIVRRET						-0.350 (0.3220)	-0.149 (0.6818)	0.562 (0.0905)	-0.149 (0.6818)	0.187 (0.6040)	0.213 (0.5540)	0.705 (0.0229)
LIVRPALM							-0.027 (0.9403)	0.355 (0.3141)	0.294 (0.4103)	-0.498 (0.1426)	-0.724 (0.0180)	-0.246 (0.4936)
YRATIO								-0.079 (0.8287)	-0.297 (0.4047)	-0.006 (0.9867)	0.236 (0.5109)	0.212 (0.2263)
LRATIO									-0.224 (0.5334)	-0.576 (0.0816)	-0.503 (0.1383)	0.273 (0.4458)
PERCFAT										0.212 (0.5563)	-0.248 (0.4888)	0.055 (0.8810)
DRYWT											0.855 (0.0016)	0.503 (0.1383)
WETWT												0.418 (0.2291)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.7. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the 1 ppm treatment group.

	YPPDDE	LΣDDT	YΣDDT	PLASRET	LIVRRET	LIVRPALM	YRATIO	LRATIO	PERCFAT	DRYWT	WETWT	BIRDWT
LPPDDE	0.309 (0.3848)*	0.718 (0.0128)	-0.067 (0.8548)	-0.127 (0.7092)	-0.474 (0.1410)	0.533 (0.0913)	0.309 (0.3848)	0.500 (0.1173)	0.118 (0.7293)	0.118 (0.7293)	0.027 (0.9366)	0.627 (0.0388)
YPPDDE		-0.030 (0.9338)	0.588 (0.0739)	-0.079 (0.8287)	-0.758 (0.0111)	0.322 (0.3639)	0.673 (0.0330)	-0.164 (0.6515)	0.030 (0.9338)	-0.212 (0.5563)	-0.382 (0.2763)	-0.006 (0.9867)
LΣDDT			-0.394 (0.2600)	-0.009 (0.9788)	-0.137 (0.6882)	0.191 (0.5730)	-0.006 (0.9867)	0.082 (0.8110)	0.564 (0.0710)	0.436 (0.1797)	0.273 (0.4171)	0.609 (0.0467)
YΣDDT				-0.139 (0.7009)	-0.253 (0.4813)	0.079 (0.8282)	0.200 (0.5796)	-0.164 (0.6515)	-0.212 (0.5563)	-0.406 (0.2443)	-0.358 (0.3104)	-0.564 (0.0897)
PLASRET					0.158 (0.6428)	0.091 (0.7899)	0.224 (0.5334)	-0.445 (0.1697)	0.409 (0.2115)	0.545 (0.0827)	0.318 (0.3403)	0.200 (0.5554)
LIVRRET						-0.037 (0.9141)	-0.546 (0.1023)	-0.358 (0.2797)	0.021 (0.9510)	0.042 (0.9021)	0.126 (0.7112)	-0.021 (0.9510)
LIVRPALM							0.723 (0.0687)	0.064 (0.8522)	-0.223 (0.5094)	-0.132 (0.6986)	-0.118 (0.7287)	0.351 (0.2902)
YRATIO								-0.006 (0.9867)	-0.188 (0.6032)	-0.152 (0.6761)	-0.248 (0.4888)	0.055 (0.8810)
LRATIO									-0.464 (0.1509)	-0.391 (0.2345)	-0.282 (0.4011)	0.064 (0.8525)
PERCFAT										0.763 (0.0062)	0.418 (0.2006)	0.382 (0.2466)
DRYWT											0.873 (0.0005)	0.364 (0.2716)
WETWT												0.173 (0.6115)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.8. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the 10 ppm treatment group.

	YPPDDE	LΣDDT	YΣDDT	PLASRET	LIVRRET	LIVRPALM	YRATIO	LRATIO	PERCFAT	DRYWT	WETWT	BIRDWT
LPPDDE	0.645 (0.0320)*	0.845 (0.0010)	0.564 (0.0710)	-0.118 (0.7293)	0.183 (0.5893)	0.051 (0.8812)	0.336 (0.3118)	-0.182 (0.5926)	0.782 (0.0075)	0.518 (0.1025)	0.345 (0.2847)	-0.064 (0.8525)
YPPDDE		0.536 (0.0890)	0.636 (0.0353)	0.218 (0.5192)	-0.064 (0.8507)	-0.326 (0.3284)	0.536 (0.0890)	-0.073 (0.8317)	0.636 (0.0479)	0.436 (0.1797)	0.355 (0.2847)	-0.118 (0.7293)
LΣDDT			0.700 (0.0165)	0.036 (0.9155)	0.436 (0.1798)	0.200 (0.5553)	0.000 (1.0000)	-0.445 (0.1697)	0.903 (0.0003)	0.618 (0.0426)	0.427 (0.1899)	-0.045 (0.8944)
YΣDDT				0.591 (0.0556)	0.337 (0.3108)	-0.386 (0.2408)	-0.209 (0.5372)	-0.473 (0.1420)	0.770 (0.0092)	0.873 (0.0005)	0.818 (0.0021)	0.300 (0.3701)
PLASRET					-0.094 (0.7830)	-0.754 (0.0074)	-0.473 (0.1420)	-0.473 (0.1420)	0.321 (0.3655)	0.527 (0.0956)	0.700 (0.0165)	0.400 (0.2229)
LIVRRET						0.271 (0.4194)	-0.124 (0.7166)	-0.258 (0.4441)	0.369 (0.2944)	0.436 (0.1798)	0.327 (0.3261)	-0.119 (0.7276)
LIVRPALM							0.060 (0.8598)	0.270 (0.4223)	-0.013 (0.9726)	-0.442 (0.1735)	-0.633 (0.0367)	-0.526 (0.0967)
YRATIO								0.518 (0.1025)	-0.055 (0.8810)	-0.209 (0.5372)	-0.273 (0.4171)	-0.473 (0.1420)
LRATIO									-0.455 (0.1869)	-0.455 (0.1601)	-0.473 (0.1420)	-0.427 (0.1899)
PERCFAT										0.745 (0.0133)	0.600 (0.0667)	0.030 (0.9338)
DRYWT											0.955 (0.0001)	0.527 (0.0956)
WETWT												0.600 (0.0510)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.9. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the 25 ppm treatment group.

	YPPDDE	LΣDDT	YΣDDT	PLASRET	LIVRRET	LIVRPALM	YRATIO	LRATIO	PERCFAT	DRYWT	WETWT	BIRDWT
LPPDDE	0.091 (0.8028)*	0.923 (0.0001)	-0.139 (0.7009)	0.190 (0.5539)	-0.398 (0.2005)	-0.508 (0.0921)	-0.127 (0.7261)	0.364 (0.2453)	0.902 (0.0001)	0.762 (0.0040)	0.720 (0.0082)	0.084 (0.7954)
YPPDDE		0.042 (0.9074)	0.394 (0.2600)	0.790 (0.0065)	0.259 (0.4691)	-0.350 (0.3208)	0.297 (0.4047)	0.067 (0.8548)	0.055 (0.8810)	0.248 (0.4888)	0.200 (0.5796)	-0.261 (0.4671)
LΣDDT			-0.030 (0.9338)	0.211 (0.5098)	-0.367 (0.2413)	-0.516 (0.0860)	-0.139 (0.7009)	0.014 (0.9656)	0.909 (0.0001)	0.909 (0.0001)	0.867 (0.0003)	0.336 (0.2861)
YΣDDT				0.644 (0.0443)	0.041 (0.9105)	0.142 (0.6962)	-0.612 (0.0600)	-0.273 (0.4458)	-0.212 (0.5563)	0.200 (0.5796)	0.285 (0.4250)	0.333 (0.3466)
PLASRET					0.181 (0.5743)	-0.080 (0.8057)	-0.103 (0.7763)	-0.190 (0.5539)	0.261 (0.4134)	0.359 (0.2516)	0.282 (0.3751)	-0.120 (0.7109)
LIVRRET						0.362 (0.2477)	0.027 (0.9403)	-0.172 (0.5939)	-0.234 (0.4643)	-0.406 (0.1910)	-0.452 (0.1399)	0.226 (0.4797)
LIVRPALM							-0.306 (0.3903)	-0.058 (0.8573)	-0.399 (0.1983)	-0.449 (0.1428)	-0.558 (0.0597)	0.137 (0.6705)
YRATIO								0.115 (0.7514)	-0.030 (0.9338)	-0.067 (0.8548)	-0.127 (0.7261)	-0.382 (0.2763)
LRATIO									0.126 (0.6967)	-0.112 (0.7292)	-0.112 (0.7292)	-0.378 (0.2262)
PERCFAT										0.776 (0.0030)	0.664 (0.0185)	0.105 (0.7456)
DRYWT											0.958 (0.0001)	0.371 (0.2356
WETWT												0.378 (0.2262)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.3. Spearman rank correlation coefficients relating the number of Japanese quail eggs laid, number of settable eggs, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, liver weights and body weights for the 1 ppm group (n = 11).

Treat	LΣDDT[a]	LPPDDE	LRATIO	YΣDDT	YPPDDE	YRATIO	PLASRET	LIVRRET	LIVRPALM	DRYWT	WETWT	PCTFAT	BIRDWT
Number Laid	-0.446 (0.1686)[b]	-0.396 (0.2275)	-0.478 (0.1367)	0.673 (0.0330)	0.467 (0.1739)	0.358 (0.3104)	0.282 (0.4000)	-0.185 (0.5867)	-0.016 (0.9628)	-0.118 (0.7287)	-0.269 (0.4242)	0.014 (0.9682)	-0.733 (0.0102)
Number Settable	-0.434 (0.1825)	-0.393 (0.2322)	-0.507 (0.1116)	0.687 (0.0282)	0.505 (0.1369)	0.377 (0.2830)	0.274 (0.4149)	-0.185 (0.5858)	0.007 (0.9840)	-0.128 (0.7079)	-0.279 (0.4069)	0.023 (0.9469)	-0.562 (0.0722)
Number Develop.	-0.446 (0.1691)	-0.533 (0.0911)	0.372 (0.2593)	0.554 (0.0966)	0.265 (0.4600)	0.105 (0.7736)	0.382 (0.2468)	0.000 (1.0000)	-0.309 (0.3556)	-0.253 (0.4531)	-0.460 (0.1548)	0.041 (0.9038)	-0.534 (0.0905)
Number Hatching	-0.270 (0.4223)	-0.326 (0.3284)	-0.340 (0.3069)	0.569 (0.0860)	0.400 (0.2518)	0.119 (0.7437)	0.372 (0.2597)	0.070 (0.8378)	-0.079 (0.8168)	-0.530 (0.0933)	-0.735 (0.0100)	-0.051 (0.8812)	-0.233 (0.4912)

[a] LΣDDT = liver ΣDDT, LPPDDE = liver p,p′-DDE concentrations, LRATIO = liver DDE/DDT ratios, YΣDDT = yolk ΣDDT, YPPDDE = yolk p,p′-DDE concentrations, YRATIO = yolk DDE/DDT ratios, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, DRYWT = liver dry weights, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

[b] Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.4. Spearman rank correlation coefficients relating the number of Japanese quail eggs laid, number of settable eggs, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, liver weights and body weights for the 10 ppm group (n = 12).

Treat	LΣDDT[a]	LPPDDE	LRATIO	YΣDDT	YPPDDE	YRATIO	PLASRET	LIVRRET	LIVRPALM	DRYWT	WETWT	PCTFAT	BIRDWT
Number Laid	0.415 (0.2049)[b]	0.301 (0.3689)	-0.656 (0.0284)	0.223 (0.5094)	-0.023 (0.9470)	-0.337 (0.3107)	0.196 (0.5637)	-0.017 (0.9595)	-0.079 (0.8168)	0.447 (0.1686)	0.378 (0.2515)	0.309 (0.3848)	0.425 (0.1930)
Number Settable	0.405 (0.2160)	0.296 (0.3766)	-0.624 (0.0401)	0.278 (0.4080)	-0.068 (0.8418)	-0.410 (0.2104)	0.201 (0.5545)	0.015 (0.9653)	-0.015 (0.7588)	0.515 (0.1051)	0.456 (0.1591)	0.328 (0.3544)	0.752 (0.0076)
Number Develop.	0.228 (0.5005)	0.178 (0.6012)	-0.383 (0.2454)	0.173 (0.6107)	-0.100 (0.7694)	-0.255 (0.4490)	-0.100 (0.7694)	0.079 (0.8163)	-0.026 (0.9403)	0.465 (0.1498)	0.374 (0.2578)	0.091 (0.8022)	0.756 (0.0071)
Number Hatching	-0.114 (0.7382)	0.000 (1.0000)	-0.260 (0.4395)	-0.233 (0.4907)	-0.269 (0.4231)	-0.146 (0.6681)	-0.187 (0.5815)	-0.169 (0.6187)	-0.049 (0.8861)	0.018 (0.9575)	-0.046 (0.8939)	-0.256 (0.4751)	0.574 (0.0648)

[a] LΣDDT = liver ΣDDT, LPPDDE = liver p,p′-DDE concentrations. LRATIO = liver DDE/DDT ratios, YΣDDT = yolk ΣDDT, YPPDDE = yolk p,p′-DDE concentrations, YRATIO = yolk DDE/DDT ratios, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, DRYWT = liver dry weights, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

[b] Values in parentheses are p-values corresponding to the correlation coefficients.

Table B.5. Spearman rank correlation coefficients relating the number of Japanese quail eggs laid, number of settable eggs, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, liver weights and body weights for the 25 ppm group (n = 12).

Treat	LΣDDT[a]	LPPDDE	LRATIO	YΣDDT	YPPDDE	YRATIO	PLASRET	LIVRRET	LIVRPALM	DRYWT	WETWT	PCTFAT	BIRDWT
Number Laid	-0.151 (0.6403)[b]	-0.214 (0.5049)	-0.158 (0.6247)	0.212 (0.5563)	0.273 (0.4458)	0.624 (0.0537)	0.003 (0.9914)	-0.321 (0.3101)	-0.290 (0.3611)	0.102 (0.7534)	0.119 (0.7124)	-0.109 (0.7369)	-0.235 (0.4620)
Number Settable	-0.112 (0.7292)	-0.168 (0.6021)	-0.112 (0.7292)	-0.297 (0.4047)	0.321 (0.3655)	0.685 (0.0289)	-0.028 (0.9312)	-0.296 (0.3497)	-0.316 (0.3167)	0.133 (0.6806)	0.133 (0.6806)	-0.063 (0.8459)	-0.476 (0.1182)
Number Develop.	-0.554 (0.0614)	-0.463 (0.1294)	0.168 (0.6008)	-0.463 (0.1774)	-0.043 (0.9068)	0.561 (0.0916)	-0.036 (0.9138)	-0.188 (0.5589)	0.217 (0.4979)	-0.386 (0.2153)	-0.442 (0.1501)	0.372 (0.2338)	-0.621 (0.0311)
Number Hatching	-0.388 (0.2829)	-0.276 (0.3851)	0.149 (0.6441)	-0.363 (0.3024)	-0.258 (0.4708)	0.338 (0.3387)	-0.153 (0.6360)	-0.510 (0.0900)	0.199 (0.5356)	0.018 (0.5800)	-0.046 (0.4889)	-0.256 (0.4744)	-0.502 (0.0961)

[a] LΣDDT = liver ΣDDT, LPPDDE = liver p,p'-DDE concentrations, LRATIO = liver DDE/DDT ratios, YΣDDT = yolk ΣDDT, YPPDDE = yolk p,p'-DDE concentrations, YRATIO = yolk DDE/DDT ratios, PLASRET = plasma retinol concentrations, LIVRRET = liver retinol concentrations, LIVRPALM = liver retinyl palmitate concentrations, DRYWT = liver dry weights, WETWT = liver wet weights, PCTFAT = percent liver fat, BIRDWT = female body weight.

[b] Values in parentheses are p-values corresponding to the correlation coefficients.

Appendix C.

Table C.1. Total numbers of eggs laid, settable, showing embryonic development, and hatching by individual female northern bobwhite from Chapter 6 after receiving food containing 0, 1, 10, or 25 ppm p,p′-DDT for 69 days.

Group	Bird	Number Laid	Number Settable	Number Developing	Number Hatching
Control	1	19	19	2	2
	2	6	6	4	4
	3	20	20	20	17
	4	16	16	15	12
	5	18	18	15	14
	6	18	18	9	8
	7	20	20	20	20
	8	16	16	13	11
	9	18	18	18	15
	10	8	8	8	7
	11	13	13	13	11
1 ppm	1	20	20	18	15
	2	21	21	20	19
	3	15	15	13	10
	4	20	20	0	0
	5	14	14	13	11
	6	19	19	15	10
	7	18	18	0	0
	8	18	18	17	15
	9	16	16	0	0
	10	8	8	8	7
	11	19	19	19	17
	12	9	8	8	8
10 ppm	1	10	10	10	7
	2	20	20	0	0
	3	9	9	7	4
	4	21	21	2	2
	5	21	21	19	18
	6	18	18	18	13
	7	19	19	1	1
	8	21	21	16	14
	9	1	1	0	0
	10	12	12	11	11
	11	20	20	0	0
	12	18	18	18	18

Table C.1. Continued.

Group	Bird	Number Laid	Number Settable	Number Developing	Number Hatching
25 ppm	1	20	20	17	12
	2	17	17	10	9
	3	7	7	5	4
	4	19	19	19	17
	5	7	7	7	4
	6	1	0	0	0
	7	14	14	14	11
	8	13	13	13	12
	9	20	20	0	0
	10	8	8	6	3
	11	13	13	13	11

Table C.2. Spearman rank correlation coefficients relating the number of northern bobwhite eggs laid, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, vitamin E concentrations, liver weights, and body weights for the control group.

Treatment	PPDDE	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
Number Laid	-0.309 (0.3555)*	-0.452 (0.1632)	0.189 (0.5779)	-0.456 (0.1584)	-0.622 (0.0409)	-0.005 (0.9893)	0.521 (0.1005)	0.226 (0.5043)	0.547 (0.0814)	0.046 (0.8930)
Number Developing	-0.618 (0.0428)	-0.723 (0.0119)	0.114 (0.7376)	-0.508 (0.1106)	-0.654 (0.0289)	-0.247 (0.4638)	0.014 (0.9680)	0.060 (0.8620)	0.250 (0.4584)	-0.211 (0.5344)
Number Hatching	-0.642 (0.0331)	-0.706 (0.0152)	0.150 (0.6590)	-0.519 (0.1016)	-0.674 (0.0229)	-0.246 (0.4659)	-0.023 (0.9470)	0.068 (0.8418)	0.276 (0.4109)	-0.187 (0.5824)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.3. Spearman rank correlation coefficients relating the number of northern bobwhite eggs laid, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, vitamin E concentrations, liver weights, and body weights for the 1 ppm group.

Treatment	PPDDE	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
Number Laid	-0.056 (0.8622)*	-0.081 (0.8028)	0.070 (0.8281)	-0.134 (0.6790)	-0.141 (0.6630)	-0.251 (0.4846)	0.178 (0.6004)	0.102 (0.7526)	0.552 (0.0609)	0.250 (0.4341)
Number Developing	0.431 (0.1617)	0.046 (0.8873)	-0.194 (0.5450)	-0.205 (0.5228)	-0.332 (0.2915)	0.264 (0.4614)	0.585 (0.0585)	0.000 (1.0000)	0.523 (0.0810)	0.208 (0.5155)
Number Hatching	0.406 (0.1899)	0.007 (0.9826)	-0.269 (0.3987)	-0.124 (0.7017)	-0.332 (0.2915)	0.321 (0.3657)	0.520 (0.1014)	-0.088 (0.7848)	0.459 (0.1330)	0.173 (0.5905)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.4. Spearman rank correlation coefficients relating the number of northern bobwhite eggs laid, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, vitamin E concentrations, liver weights, and body weights for the 10 ppm group.

Treatment	PPDDE	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
Number Laid	-0.527 (0.0786)*	-0.661 (0.0193)	0.435 (0.1814)	-0.399 (0.1985)	-0.403 (0.1941)	-0.453 (0.1616)	-0.510 (0.1087)	0.332 (0.2915)	0.746 (0.0054)	0.625 (0.0296)
Number Developing	0.134 (0.6779)	0.109 (0.7351)	-0.055 (0.8720)	-0.176 (0.5835)	-0.159 (0.6222)	0.028 (0.9358)	-0.279 (0.4069)	0.325 (0.3034)	-0.004 (0.9913)	0.247 (0.4691)
Number Hatching	0.166 (0.6066)	0.148 (0.6459)	-0.124 (0.7161)	-0.187 (0.5607)	-0.134 (0.6779)	0.087 (0.7984)	0.333 (0.3165)	0.367 (0.2408)	0.021 (0.9479)	0.247 (0.4391)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.5. Spearman rank correlation coefficients relating the number of northern bobwhite eggs laid, number of eggs showing embryonic development, and number of eggs hatching to DDT, vitamin A concentrations, vitamin E concentrations, liver weights, and body weights for the 25 ppm group.

Treatment	PPDDE	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
Number Laid	-0.677 (0.0220)*	-0.641 (0.0337)	-0.201 (0.5527)	-0.114 (0.7376)	-0.169 (0.6186)	0.348 (0.3251)	0.000 (1.0000)	-0.256 (0.4468)	0.261 (0.4384)	0.458 (0.1569)
Number Developing	-0.703 (0.0158)	-0.690 (0.0189)	-0.082 (0.8101)	-0.132 (0.6979)	-0.315 (0.3453)	-0.232 (0.5195)	-0.009 (0.9787)	-0.457 (0.1580)	0.352 (0.2890)	0.644 (0.0325)
Number Hatching	-0.564 (0.0706)	-0.532 (0.0920)	-0.193 (0.5703)	-0.037 (0.9147)	-0.335 (0.3141)	-0.190 (0.5998)	-0.087 (0.7989)	-0.284 (0.3966)	0.358 (0.2800)	0.757 (0.0070)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.6. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the control group.

	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
PPDDE	0.774 (0.0053)*	-0.164 (0.6307)	0.345 (0.2981)	0.582 (0.0604)	-0.127 (0.7092)	0.273 (0.4171)	0.145 (0.6696)	-0.210 (0.5363)	0.036 (0.9155)
ΣDDT		-0.009 (0.9788)	0.336 (0.3118)	0.518 (0.1025)	-0.009 (0.9788)	-0.173 (0.6115)	-0.027 (0.9366)	-0.128 (0.7086)	-0.091 (0.7904)
PLASRET			-0.418 (0.2006)	-0.545 (0.0827)	0.273 (0.4171)	-0.100 (0.7699)	0.145 (0.6696)	0.419 (0.1994)	0.036 (0.9155)
LIVRRET				0.927 (0.0001)	-0.418 (0.2006)	0.282 (0.4011)	-0.755 (0.0073)	-0.843 (0.0011)	-0.318 (0.3403)
LIVRPALM					-0.364 (0.2716)	0.173 (0.6115)	-0.518 (0.1025)	-0.788 (0.0040)	-0.173 (0.6115)
PLASTOC						-0.245 (0.4669)	0.345 (0.2981)	0.282 (0.4000)	0.045 (0.8944)
RATIO							-0.182 (0.5926)	-0.091 (0.7899)	-0.064 (0.8525)
PCTFAT								0.724 (0.0117)	0.655 (0.0289)
WETWT									0.442 (0.1736)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.7. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the 1 ppm treatment group.

	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
PPDDE	0.762 (0.0040)*	-0.252 (0.4299)	-0.154 (0.6331)	-0.112 (0.7292)	0.176 (0.6272)	0.573 (0.0655)	0.510 (0.0899)	0.217 (0.4986)	0.385 (0.2170)
ΣDDT		-0.063 (0.8459)	0.343 (0.2756)	0.084 (0.7954)	-0.164 (0.6515)	0.173 (0.6115)	0.741 (0.0058)	-0.126 (0.6967)	0.371 (0.2356)
PLASRET			-0.399 (0.1993)	-0.189 (0.5567)	-0.091 (0.8028)	-0.109 (0.7495)	0.434 (0.1591)	0.378 (0.2262)	0.105 (0.7456)
LIVRRET				0.706 (0.0102)	-0.127 (0.7261)	0.264 (0.4334)	-0.601 (0.0386)	-0.399 (0.1993)	-0.336 (0.2861)
LIVRPALM					-0.539 (0.1076)	0.155 (0.6500)	-0.161 (0.6175)	-0.587 (0.0446)	-0.329 (0.2969)
PLASTOC						0.250 (0.5165)	-0.261 (0.4671)	0.345 (0.3282)	-0.261 (0.4671)
RATIO							0.136 (0.6893)	0.355 (0.2847)	-0.164 (0.6307)
PCTFAT								0.322 (0.3079)	0.259 (0.4168)
WETWT									0.301 (0.3423)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.8. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the 10 ppm treatment group.

	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
PPDDE	0.839 (0.0006)*	-0.136 (0.6893)	0.273 (0.3911)	0.664 (0.0185)	0.782 (0.0045)	0.418 (0.2006)	0.217 (0.4986)	-0.559 (0.0586)	-0.098 (0.7621)
ΣDDT		-0.164 (0.6307)	0.545 (0.0666)	0.559 (0.0586)	0.591 (0.0556)	0.109 (0.7495)	-0.133 (0.6806)	-0.734 (0.0065)	-0.315 (0.3191)
PLASRET			0.182 (0.5926)	0.027 (0.9366)	-0.200 (0.5554)	-0.067 (0.8548)	-0.182 (0.5926)	0.191 (0.5739)	0.455 (0.1601)
LIVRRET				0.671 (0.0168)	-0.164 (0.6307)	0.282 (0.4011)	-0.594 (0.0415)	-0.643 (0.0240)	-0.483 (0.1121)
LIVRPALM					0.309 (0.3550)	0.700 (0.0165)	-0.007 (0.9828)	-0.678 (0.0153)	-0.455 (0.1377)
PLASTOC						0.285 (0.4250)	0.200 (0.5554)	-0.118 (0.7293)	0.209 (0.5372)
RATIO							-0.127 (0.7092)	-0.373 (0.2589)	-0.236 (0.4841)
PCTFAT								0.182 (0.5717)	0.196 (0.5419)
WETWT									0.762 (0.0040)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Table C.9. Spearman rank correlation coefficients relating different measurements of DDT contamination, vitamin A, liver weights and body weights for the 25 ppm treatment group.

	ΣDDT	PLASRET	LIVRRET	LIVRPALM	PLASTOC	RATIO	PCTFAT	WETWT	BIRDWT
PPDDE	0.982 (0.0001)*	0.064 (0.8525)	-0.364 (0.2716)	0.445 (0.1697)	-0.176 (0.6272)	-0.255 (0.4500)	0.727 (0.0112)	-0.545 (0.0827)	-0.418 (0.2006)
ΣDDT		0.073 (0.8317)	-0.500 (0.1173)	0.318 (0.3403)	-0.139 (0.7009)	-0.382 (0.2466)	0.818 (0.0021)	-0.436 (0.1797)	-0.309 (0.3550)
PLASRET			-0.182 (0.5926)	0.273 (0.4171)	-0.430 (0.2145)	-0.427 (0.1899)	0.209 (0.5372)	-0.327 (0.3259)	-0.036 (0.9155)
LIVRRET				0.036 (0.9155)	-0.115 (0.7514)	0.791 (0.0037)	-0.800 (0.0031)	-0.009 (0.9788)	-0.364 (0.2716)
LIVRPALM					0.055 (0.8810)	0.082 (0.8110)	0.155 (0.6500)	-0.836 (0.0013)	-0.555 (0.0767)
PLASTOC						0.018 (0.9602)	-0.152 (0.6761)	0.285 (0.4250)	-0.261 (0.4671)
RATIO							-0.691 (0.0186)	-0.082 (0.8110)	-0.400 (0.2229)
PCTFAT								-0.300 (0.3701)	0.127 (0.7092)
WETWT									0.382 (0.2466)

* Values in parentheses are p-values corresponding to the correlation coefficients.

Appendix D. EFFECTS OF HANDLING AND PAIR MANAGEMENT ON JAPANESE QUAIL

INTRODUCTION

During both nutritional and toxicological studies, it is often of interest to expose only one sex to a treatment to determine the effects of the treatment on the reproductive function of that sex only. When the treatment is being provided via food or water, sexes must be held separately, except for mating. Such a design is practical only if viable offspring can be produced after periodic, relatively brief mating periods. The approach is applicable to birds as they are able to store spermatozoa for several days (Van Krey 1990) In northern bobwhites (*Colinus virginianus*), for example, fertility was still 50% 9 d after mating (Schom and Abbot 1974). Japanese quail (*Coturnix coturnix*) and many other gallinaceous species will behave promiscuously under certain circumstances (Johnsgard 1973, Cromp and Simmons 1980) making pairing with numerous mates feasible under laboratory conditions. Gallinaceous species, by delaying incubation until the clutch is completed, are convenient for many

laboratory studies because it is easy to foster egglaying over long periods without interuption by broodiness.

Studies in confined gallinaceous species have shown pair management to alter egg production or eggshell quality. Japanese quail exhibited reduced egg production when males were introduced to previously unmated females (Siopes and Wilson 1977) Japanese quail females showed no change in egg production during a period of their being moved to males' cages daily or every other day, but, during the following week, when transfer to the males' cages had ceased, the mated females laid fewer eggs than unmated control females (Gerken et al. 1988). After being moved from group pens to single cages, ISA Brown (*Gallus domesticus*) hens laid a lower proportion of eggs with normal eggshells (Hughes et al. 1986). Mills et al. (1987) found that ISA Warren (*Gallus domesticus*) hens caged singly laid fewer eggs with abnormal eggshells than hens penned in groups of 3 or 4. Egg production was highest for Medium White turkey (*Meleagris gallopavo*) hens mated naturally, but paired only for 15 min, than for females physically isolated or visually, aurally, and physically isolated from males (Jones and Leighton 1987). The presence of a male, either physically, or by visual and/or aural exposure, also may have reduced broodiness.

Indeterminately laying species, such as Japanese quail, generally mate frequently during the laying period. The present series of experiments addressed several questions concerning the effects of handling, pairing, and frequency of pairing on Japanese quail reproduction. Will females exposed temporarily to males produce as many young as those exposed continuously? Will handling involved with daily pairing and separation of pairs daily hamper reproductive outcome? Does familiarity with a mate enhance or inhibit reproduction? If not paired continuously, does the interval between pairing affect reproductive outcome? To address these questions, pairs were left together continuously, paired daily, or paired every third day. In different experiments, either the males or females were moved to each others' cages to test for differences in reproductive outcome. In some treatment groups, females were paired with different males during each mating period.

METHODS

Three experiments were performed during this study. Age of both females and male quail was 3 - 4 mo at the beginning of the first experiment. During the first experiment, 48 female quail were randomly assigned to one of 4 treatment groups. Individual females were paired continuously with the same male (CP group). Individual females were maintained in cages separate from their mates but transferred to their mates' cages for 90 min daily (P1SM group). Individual females were maintained in cages separate from the males, and moved to the cage of one of a group of males' for 90 min daily (P1DM group). Each female in this third group was paired with the same male only twice with an interval of 12 d. Individuals of the final group of females were paired with the same male but on every third day (P3SM) for a total of 8 pairings. This experiment lasted 24 d.

The second experiment used the same group assignments. A 7 d interval was provided between experiments. This interval was to allow fertility to diminish substantially. The difference between the first and second experiments was that the males were moved to the females' cages.

Before the third experiment, the groups were re-randomized. Forty-four females were available for this experiment, and the experiment lasted 24 d. Two groups of females were continuously paired with their mates. One group was left unhandled throughout the experiment (CP). In the other group (CPH), the females were caught and returned to the cage at the start and at the end of the 90 min mating period (CPH) to simulate the handling involved with moving birds between cages. Individuals of the other groups were paired on every third day. One of these groups of females was paired with the same mate each time (P3SM). The other group of females was paired with a new male each time (P3DM). Females were introduced to males' cages.

All quail (males and females) were kept in the animal room in the Department of Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University. Quail

were held in standard quail laying cages with a sloping floor measuring 20.3 x 30.5 cm in the back, 25.4 x 30.5 cm in the front, and 50.8 cm long. Males and females were not visually or aurally isolated from each other. The light cycle was maintained at 15L:9D. Laying mash (15% crude protein) and water were provided *ad libitum* to males and females during all experiments.

Eggs were collected daily (day 1), marked with date and female number and all settable eggs were placed in a poultry incubator set at 37-38 C and approximately 55% relative humidity and automatically turned every 2 h for 16 d. Settable eggs were those with fully calcified and intact shells. On day 16, eggs were transferred to a stationary hatching incubator at 38 C and approximately 70% relative humidity. Hatching occurred on days 17 - 20.

All data were analyzed using SAS® statistical package (SAS, Institute 1985a,b) to perform analyses of variance and Duncan's multiple range tests comparing the total number of eggs laid, the number of settable eggs, the number of eggs showing embryo development, and the number of eggs hatching. All that did not hatch were cracked open to check for evidence of embryonic development. Eggs that hatched were those that produced live chicks. Eggs whose chicks did not fully extricate themselves from the shell were not considered to have hatched.

RESULTS

Not all females laid eggs every day in any experiment, but some individuals did. Females laid up to 24 eggs during the 24 d experimental period. In Experiment 1, all groups performed similarly for number of eggs produced, and number of settable eggs (Table D.1). The CP and P1DM groups had significantly ($P = 0.05$) more eggs showing embryonic development than the P1SM and P3SM groups. The only groups to differ significantly for

hatchability were the CP and P3SM groups ($P = 0.10$) with the CP group having more eggs hatch.

In Experiment 2 (Table D.2), none of the groups differed significantly for numbers of eggs laid, settable eggs, or eggs hatching. The CP and P1DM groups differed significantly from the P3SM group for number of eggs showing embryonic development. The P1SM group did not differ significantly from any of the other groups regarding number of eggs showing embryonic development.

In Experiment 3 (Table D.3), as in the first two experiments, the treatments did not affect the number of eggs laid. The CP, CPH and P3DM groups all differed significantly from the P3SM group for number of settable eggs ($P = 0.08$) and number of eggs showing embryonic development ($P = 0.01$). The CP and P3DM groups both differed significantly from the P3SM group ($P = 0.10$) regarding number of eggs hatching, but the CPH group did not differ significantly from any of the other groups.

DISCUSSION

Comparisons should not be made among Experiments 1-3 because of slightly varying experimental conditions. Within experiments comparisons can all refer to the CP group as a control group because all experiments had CP groups and because CP groups resemble conditions of many avian reproductive experiments. Within each experiment, two patterns are apparent.

The first pattern involves the characteristics measured. No apparent effect exists between any treatment and the number or external quality of the eggs laid, except in Experiment 3 where number of settable eggs was reduced in the P3SM group. Number of eggs showing embryonic development and number of eggs hatching were both reduced,

particularly in P1SM and P3SM groups as compared to CP groups. Experiment 2, where number of eggs hatching failed to change, was an exception to this pattern.

The second pattern involves a comparison among treatment groups within each experiment. In Experiments 1 and 2, the P1DM females performed similarly to the CP females when considering the number of eggs showing embryonic development. The P1SM and P3SM groups also performed similarly regarding number of eggs showing embryonic development. If the number of eggs showing embryonic development accurately reflects fertility, then results from Experiments 1 and 2 indicate that if paired with the same male, females produce similar numbers of fertile eggs whether mated daily or every third day. However, temporary pairing of females with a different male during each mating session produces a higher fertility rate closely resembling that of females continuously paired with a single male.

Compton et al. (1978) found that spermatozoa are released from uterovaginal sperm-host glands in the reverse order of which they entered. In a promiscuous species, it would of greater advantage for a male to copulate with unfamiliar females than with familiar females. If evolutionary selection has favored such behavior, the males in the groups visited by different females may have been more persistent in their attempts to mate. The males in the CP likely had sufficient access to sufficiently inseminate the females to ensure adequate fertility.

In Experiment 3, two groups (CPH and P3DM) performed comparably to the CP group regarding embryonic development. These observations reinforce the conclusion that reducing frequency of mating opportunity, to every third day, does not diminish production of eggs showing embryonic development. However, the diminished success of the P3SM group suggests that a mating regime involving mating with different males can compensate for short, infrequent mating opportunities. Access to numerous males may reflect the natural situation for a promiscuous breeder.

In most experiments, however, hatchability, not fertility is of primary interest. A slightly different pattern emerges for hatchability than for embryonic development in Experiments 1 and 2. Only the CP and P3SM groups were significantly different in Experiment 1, and no

significant differences existed in Experiment 2. The results from Experiment 3 tend to support the conclusion that pair management has an effect. In Experiment 3, however, the P3DM group performed better than both the CP and CPH groups. Again, this supports the conclusion that exposure to a different male during each mating period is superior to mating with the same male each time.

To determine the effects of moving either males or females between cages, comparisons must be made within Experiments 1 and 2 between CP and all other groups. The number of eggs produced showing embryonic development by CP groups was similar in both experiments, however the other three groups produced similar numbers or slightly fewer eggs showing embryonic development compared to the CP group in Experiment 2 than in Experiment 1. The percent decrease in number of eggs hatching seen between the CP group and the P1SM, P1DM, and P3SM groups was greater for all three of these groups in Experiment 1 than in Experiment 2. The results of Experiment 3 would seem to discount handling the females as having a major effect on their reproduction. Regardless of the sex being moved, the differences were not great. The greatest difference between experiments was that the males appeared to better accept the females into their cages than vice versa.

CONCLUSIONS

In a nutritional or toxicological study in which reproducing male and female birds must be maintained on different diets, it is better to pair the females with different males during each mating session. This tactic may only be be useful for species that are known to exhibit promiscuous behavior in the wild. An interval of up to three days between matings will not reduce fertility or hatchability. It did not matter whether males or females were moved between cages in so far as fertility or hatchability were concerned. However, the males appeared more receptive of females being introduced into their cages than vice versa.

Table D.1. Reproductive success of Japanese quail from Experiment 1 for the continuously paired group (CP), group paired daily with the same male (P1SM), group paired daily with a different male (P1DM), and the group paired every third day with the same male (P3SM). Females were moved to males' cages of P1SM, P1DM, and P3SM groups. Mean (±S.E.) values and analysis of variance F-values with df = 3 are reported.

	CP	P1SM	P1DM	P3SM	F	P
No. eggs[a]	20.92 ± 0.79	19.25 ± 1.10	21.58 ± 0.50	20.42 ± 0.78	1.45	0.24
No. set	20.42 ± 0.80	18.42 ± 1.17	20.92 ± 0.58	20.00 ± 0.93	1.45	0.24
No. dev.	19.33 ± 0.85[b]	15.50 ± 1.30[c]	19.50 ± 0.62[b]	15.58 ± 1.63[c]	3.68	0.02
No. hatched	10.33 ± 1.18[d]	7.58 ± 0.99[d,e]	8.67 ± 1.09[d,e]	6.42 ± 1.24[e]	2.18	0.10

[a] No. eggs = total number of eggs laid during the experiment by individual hens
No. set = number of eggs laid with intact shells
No. dev. = number of eggs showing embryonic developing, including those eggs that hatched
No. hatched = number of eggs producing live chicks

[b,c] Row means followed by different letters are significantly different according to Duncan's multiple range test. Alpha for Duncan's test was chosen to be 0.05.

[d,e] Row means followed by different letters are significantly different according to Duncan's multiple range test. Alpha for Duncan's test was chosen to be 0.10.

Table D.2. Reproductive success of Japanese quail from Experiment 2 for the continuously paired group (CP), group paired daily with the same male (P1SM), group paired daily with a different male (P1DM), and the group paired every third day with the same male (P3SM). Males were moved to the females' cages of P1SM, P1DM, and P3SM groups. Mean (±S.E.) values and analysis of variance results with df = 3 are reported.

	CP	P1SM	P1DM	P3SM	F	P
No. eggs[a]	21.58±0.58	21.09±0.62	20.92±0.84	20.67±0.70	0.32	0.81
No. set	20.75±0.54	19.18±0.99	20.42±0.86	20.17±0.79	0.68	0.57
No. dev.	18.75±0.86[b]	15.80±1.24[b,c]	17.92±0.98[b]	13.17±2.21[c]	3.09	0.04
No. hatched	13.92±0.96	11.60±0.99	13.00±1.50	11.00±2.00	0.91	0.44

[a] No. eggs = total number of eggs laid during the experiment by individual hens
No. set = number of eggs laid with intact shells
No. dev. = number of eggs showing embryonic developing, including those eggs that hatched
No. hatched = number of eggs producing live chicks

[b,c] Row means followed by different letters are significantly different according to Duncan's multiple range test. Alpha for Duncan's test was chosen to be 0.05.

Table D.3. Reproductive success of Japanese quail from Experiment 3 for the continuously paired group (CP), continuously paired and handled group (CPH), group paired every third day with the same male (P3SM), and the group paired every third day with a different male (P3DM). Females were moved to males' cages of P3SM and P3DM groups. Mean (±S.E.) values and analysis of variance results with df = 3 are reported.

	CP	CPH	P3SM	P3DM	F	P
No. eggs[a]	22.00±0.62	21.54±0.82	20.27±0.68	21.64±0.54	1.25	0.30
No. set	21.45±0.65[d]	21.00±0.73[d]	19.00±0.94[e]	21.27±0.54[d]	2.42	0.08
No. dev.	18.73±1.02[b]	17.18±1.56[b]	12.00±1.76[c]	17.54±1.41[b]	4.18	0.01
No. hatched	9.10±1.71[d]	7.36±0.90[d,e]	5.11±1.06[e]	9.00±1.02[d]	2.24	0.10

[a] No. eggs = total number of eggs laid during the experiment by individual hens
No. set = number of eggs laid with intact shells
No. dev. = number of eggs showing embryonic developing, including those eggs that hatched
No. hatched = number of eggs producing live chicks

[b,c] Row means followed by different letters are significantly different according to Duncan's multiple range test. Alpha for Duncan's test was chosen to be 0.05.

[d,e] Row means followed by different letters are significantly different according to Duncan's multiple range test. Alpha for Duncan's test was chosen to be 0.10.

Vita

Joseph P. Sullivan was born to James M. and Evelyn L. Sullivan on January 24, 1960. He graduated form Benet Academy in Lisle, IL in 1978. In 1982, he received a B.A. in Biology from Ripon College, Ripon, WI. In 1986, he received a M.S. in Biology/Ecology from Utah State University, Logan, UT. In September of 1986, he enrolled in the Ph.D. program in the Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University.

Joe is a member of Beta Beta Beta, Gamma Sigma Delta, and Laurel Society. He is also a member of Colonial Waterbird Society, Sigma--The Research Society, Society of Environmental Toxicology and Chemistry, Virginia Academy of Science, The Wildlife Society, Wildlife Disease Association, and Wilson Ornithological Society. While at VPI & SU, he was elected president of the Virginia Tech Student Chapter of The Wildlife Society and as Chair of the Department of Fisheries and Wildlife Graduate Student Advisory Committee. In 1990, he received the A.B. Massey Award in recognition of superior performance and professionalism while a graduate student.

After completion of his dissertation, Joe began work as a research toxicologist for American Cyanamid in Princeton, NJ.