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Photoperiodic Effects On The Avian Dietary LC50 With Bobwhite (Colinus Virginianus) And Mallards (Anus Platyrhynchos)

presented by

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PHOTOPERIODIC EFFECTS ON THE AVIAN DIETARY LC_{50} WITH BOBWHITE (*COLINUS VIRGINIANUS*) AND MALLARDS (*ANAS PLATYRHYNCHOS*)

by

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ABSTRACT

PHOTOPERIODIC EFFECTS ON THE AVIAN DIETARY LC_{50} WITH BOBWHITE (*COLINUS VIRGINIANUS*) AND MALLARDS (*ANAS PLATYRHYNCHOS*)

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Eight-day (five-day treatment and three-day recovery period) dietary LC_{50} trials were conducted with bobwhite (*Colinus virginianus*) and mallards (*Anas platyrhynchos*) using α-napthylthiourea (ANTU), fenthion, endrin, sodium secobarbital, and strychnine under 24 hours light:0 hours dark (24), 14 hours light:10 hours dark (14), and 14 hours light:10 hours dark with dimming and brightening of lights between light and dark periods to simulate dusk and dawn (14D), to determine the effect of photoperiod on the avian dietary LC_{50}. No significant differences in LC_{50} values between the three photoperiods could be established for a particular chemical in bobwhites or mallards. Mortality patterns and symptoms of toxicosis were generally similar between the 24, 14, and 14D photoperiods for each chemical. Photoperiod significantly affected feed consumption during the five-day treatment and three-day recovery periods. The 24-hour photoperiod resulted in significantly greater feed consumption than either of the two 14-hour photoperiods. Significant differences in feed consumption between the birds on the 14 and 14D photoperiods were less frequent than significant differences between feed consumption of birds

on the 24 and 14 or 24 and 14D photoperiods. Photoperiod also significantly affected body weights at day 0, 5, and 8 of the trial. The 24-hour photoperiod tended to produce significantly greater body weights than the 14-hour photoperiods.

INTRODUCTION

The Environmental Protection Agency has established testing guidelines for the registration of pesticides in the United States under the Federal Insecticide Rodenticide and Fungicide Act (FIFRA) (U.S. EPA, 1982) and for the determination of safety of industrial chemicals under the Toxic Substance Control Act (TSCA) (U.S. EPA, 1983). Portions of both acts deal with wildlife toxicity testing. At present, wildlife toxicity testing guidelines are not final and information required for a particular pesticide is determined on a case-by-case basis (Federal Register, 1978a). The oral LD_{50} and dietary LC_{50} are two tests frequently required early in the registration process, supplying basic lethality and species susceptibility data for a particular chemical. The avian dietary LC_{50} protocol, having no similar mammalian counterpart as does the LD_{50}, allows considerable variation in the methodology of experimentation, resulting in the potential for significant variations in test results and thus making it difficult for interpreting and categorizing a chemical's toxicity. One section of the proposed avian LC_{50} protocol allowing considerable experimental variation deals with photoperiod (Federal Register, 1978b).

Photoperiod, the relative length of light and dark periods, is one of many important factors which control the behavior and physiological state of most domestic and wild bird species (Morris, 1967; Van Tyne and Berger, 1976; Tucker and Ringer,

1982). Of particular significance to toxicity testing is the effect that light duration has on the bird's activity, feeding behavior, reproductive success, and the biochemical interactions which occur during these events.

Since in LC_{50} testing the quantity of toxic compound ingested is dependent on food consumption, the effects of photoperiod on feeding behavior is of critical concern. In studies with domestic chickens, increasing photoperiod lengths from 12 hours light:12 hours dark to 24 hours light:0 hours dark significantly altered the number of feedings, feeding duration, and feed consumption (Squibb and Collier, 1979). Overall, chickens reared under 24 hours light consumed significantly greater amounts of feed than chickens reared under diurnal lighting schemes. The birds reared under 24 hours light consistently (at short intervals) consumed small amounts of feed throughout most of the 24 hour period (Squibb and Collier, 1979), while birds raised on diurnal lighting schemes consumed large quantities of feed just prior to darkness and shortly after the onset of lighting (Dingle, 1971; Squibb and Collier, 1979). These differences in feeding patterns could possibly result in significant variations in toxicity. Birds which consume large quantities of treated feed at the initiation or cessation of the lighting periods would be exposed to greater doses of toxicants over a shorter period of time compared to birds consuming smaller quantities of feed over an extended period. Thus, chemicals which are more acutely toxic

and less persistent may be more toxic when administered to birds on diurnal lighting schemes. Conversely, less acutely toxic or more persistent chemicals may be more toxic when administered to birds under continuous light due to the overall increased feed consumption and increased chemical intake.

In addition to feeding behavior, significant increases in feeding efficiencies have been reported in chickens exposed to continuous light, resulting in increased early growth rates and heavier body weights (Squibb and Collier, 1979). This increase in feeding efficiency has been attributed to a continuous period of food processing, which is interrupted in birds exposed to alternate light:dark periods. Test birds housed under continuous light for the standard five-day pre-test acclimation period may show significantly greater body weights than diurnally acclimated birds at the onset of testing, making test results incompatable between studies using different lighting schemes.

Another potentially important effect of photoperiod on the avian LC_{50} is diurnal fluctuations in the level and activity of microsomal mixed function oxidase (MFO) enzymes. Fluctuating MFO enzyme activities may result in significant diurnal changes in the toxification-detoxification reactions producing oscilations in the levels of toxic compounds within the animal.

The purpose of this study was to assess the potential effects various lighting schemes have on dietary LC_{50} determinations in bobwhite (Colinus virginianus) and mallards (Anas

platyrhynchos). The study was funded by the Environmental Protection Agency on a contract basis in order to help develop a more uniform testing protocol for subacute dietary toxicity testing with avian wildlife species.

REVIEW OF LITERATURE

Regulation of Food Consumption

Sturkie (1976) states "Animals eat to satisfy energy requirements and volume receptors or to attain a state of fullness or satiety". The amount of feed consumed is dependent on many factors, some of which include photoperiod, activity, age, size, environmental temperature, reproductive stage, appearance and taste of food, and availability of water.

In mammals, it is generally recognized that the satiety and appetite centers are located in the ventromedial and lateral nucleus of the hypothalamus, respectively. Studies in various species of birds utilizing hypothalamic lesion techniques also indicate that the ventromedial nucleus and the lateral hypothalamus play an important role in the regulation of feed intake (Sturkie, 1976). Lesions in the ventromedial nucleus at the base of the third ventricle above the optic chiasma caused hyperphagia in chickens (Gallus domesticus) and white-throated sparrows (Zonotrichia albicollis) while lesions in the lateral hypothalamus just caudal to the ventromedial nucleus produced aphagia (Smith, 1969; Kuenzel, 1972).

The exact mechanism by which the hypothalamus controls feed intake has not been determined. Certain investigators

theorize that adipose tissue stores and energy requirement regulate appetite and feed intake. Mu et al. (1968) proposed that there is a "set point" for tissue fat concentration. If tissue lipid concentrations rise above this "set point", feed intake decreases and lipolysis is accelerated. Conversely, when the tissue lipid concentrations drop below the "set point" feed intake increases and lipogenesis and fat deposition are stimulated. When fat deposits approach or equal the "set point" an equilibrium forms stabilizing feed intake and body weight (Lepkovsky, 1973). Feed intake and indirectly the size of the fat droplets are regulated by the hypothalamus. Long term changes in eating behavior and fat deposits caused by hyperphagia or hypophagia are thought to be due to the development of new set points.

Lepkovsky (1973) supported the "set point" theory of feeding regulation by reporting that continued force feeding of chickens at twice their normal ad libitum intake and nutrient requirements made the birds obese. After the force feeding was terminated and the birds were allowed to feed ad libitum, they refused to eat for 7 to 10 days, lost weight, and depleted their abnormally high fat stores, theoretically to a level low enough (set point) to trigger the feeding center. Hill and Dansky (1954) also provided data which support energy requirements as a controlling factor in feed intake. When these authors varied the caloric content and nutrient density of feed, chickens altered their food consumption. Hill and Dansky also noted that the differences in energy intake

between birds of the same weight and egg laying performance could be attributed to other factors such as the volume of feed eaten or the stimulation of hypothetical volume receptors in the crop and esophagus.

Polin and Wolford (1973) conducted studies in chickens, the results of which contradict the "set point" hypothesis while supporting the theory that volume receptors are important in controlling the feeding behavior of birds. The authors fed groups of chickens ad libitum, ad libitum and force fed, and force fed 150 percent of the ad libitum group. The data showed a direct relationship between increasing blood lipids and adipose tissue with increasing feed intake, but failed to show any difference in feed consumption 2 to 3 days after the termination of force feeding even though there was considerable differences in fat deposits among the groups. Polin and Wolford concluded that their study provided evidence that volume receptors, which are influenced by rate of filling, capacity, and discharge of feed, control the feeding process and suggested that hormones or other factors such as energy requirements regulate the "set point" at which these receptors operate. They also pointed out that it is well known that the emptiness, fullness, or distention of the digestive tract in mammals influence food intake.

Squibb and Collier (1979) studied the individual eating patterns of broiler chicks from day one to 20 days of age under three different lighting schemes (12 hours light:12 hours dark (LD); continuous light (LL); and continuous dark

(DD)). The LL and LD lighting regimes were initiated on the first day after hatch while the DD regime started with a light dark period on day one after hatch, followed by a gradual decrease in light intensity until total darkness was reached on day 8. Previous studies showed that when chicks were immediately placed in total darkness upon hatching 75 percent of the birds died due to their inability to find food and water. As early as day 2 of the trial, the chicks on continuous lighting were eating roughly twice as many meals per day and spending approximately 40 percent more total time eating per 24 hour period than the LD chicks. Although the LL chicks consumed more meals and spent a greater amount of total time eating per 24 hour period early in the study, the LD chicks spent more time eating per meal. At day 3 the LD chicks ate for approximately 13 minutes per hour; the LL chicks ate 6 minutes per hour; and the DD chicks 11 minutes per hour. At day 11 the LD chicks had increased their hourly eating time to 25 minutes while the LL and DD chicks ate an average of 12 and 15 minutes per hour, respectively. The differences between the LL and LD lighting schemes in the number of meals and the time spent eating per meal continued and intensified throughout the study. During days 8 through 20, the birds on the continuous lighting schedule ate an average 1.6 times the number of meals than the birds on the diurnal photoperiod but spent approximately 50 percent less time eating per meal. The total time spent eating per 24 hour period was

greatest in the chicks under the diurnal lighting scheme at the completion of the study while the chicks reared in continuous light or dark spent roughly an equal amount of time eating per 24 hour period. Throughout the study, the LL and DD chicks ate each hour while the chicks on the diurnal photoperiod ate every hour during the 12 hour light period. The LD chicks did not attempt to feed at any hour during the 12 hours of darkness.

In this same study by Squibb and Collier (1979), at day 3, hourly feeding duration within a lighting regime remained relatively constant under all lighting schemes, except for a sharp surge during the hour the caretaker entered the room. Individual variability in minutes eating per hour at day 3 was greatest in the LD chicks. These birds were unable to anticipate "lights out" at this time, as the time spent feeding in the 2 hours prior to the beginning of the dark period was decreasing. By day 11, the LD chicks still showed a high degree of variability in hourly feeding time, but were able to anticipate lights out, as indicated by increasing time spent feeding during the four hours prior to darkness. At day 20, the chicks had developed a strong sense of timing in anticipation of lights out as shown by a very low individual variability in hourly feeding time and by a steep rise in the hourly feeding time just prior to the 12 hour dark period.

There was no significant difference in the total 20 day feed consumption between the LL and DD chicks but the LD chicks ate significantly less feed than either the LL or DD

chicks. Twenty day body weights of chicks on continuous light were significantly heavier than body weights of the LD chicks, while the DD chick's 20 day body weights were intermediate to the LL and LD birds. Efficiency of feed utilization was highest in the LL chicks, intermediate in the LD chicks, and lowest in the DD birds. Water to feed ratios were significantly lower in the DD group, but similar in the LL and LD birds.

An earlier study by Weaver and Siegel (1968), in which commercial broiler crosses were reared under continuous diurnal photoperiods, supports the feeding behavior conclusions of the Squibb and Collier study. Weaver and Siegel reported that the average percentage of birds feeding per hour during the light period was consistently lower under continuous light than that for the flock under the diurnal lighting regime. These researchers identified a highly significant time-light regime interaction in feed consumption in all photoperiods studied. Although the birds on the continuous light schedule showed feeding rhythms, the rhythms were less uniform and dramatic than those under the light-dark photoperiod, and produced no evidence of a day-night pattern. The birds on the diurnal lighting regime showed 2 large peaks in the percent of birds feeding; one just after the onset of light and the other just prior to darkness. These increases in percent feeding were due to the birds consuming large quantities of feed after the onset of lighting and prior to darkness, and showed that the chickens were able to condition themselves to the anticipated dark period.

Dose-Response Relationships

One of the major fundamental principles of pharmacology and toxicology is that of the relationship between the intensity of a response caused by a drug or xenobiotic and the dose administered. This dose-response relationship can be best summarized with three basic principles. (1) There are molecular or receptor sites in which the chemical reacts to produce a response. (2) The production and intensity of the response are related to the concentration of the chemical or toxic agent at the reactive site or receptor. (3) The concentration of the agent at the receptor site is related to the dose administered or exposure level. In addition, the intensity of the response elicited by the agent is thought to be proportional to the number of receptors occupied by the agent, with maximal response occurring when all receptors are occupied. This receptor occupancy is a function of drug concentration and its ability to react or combine with the receptor. The ability of a compound to react with a receptor or the drugs affinity for the receptor is generally considered to be constant (Klaassen and Doull, 1980).

In general, the toxic effects of a chemical in an organism are not produced unless the agent, its metabolites, or conversion products reach and react with receptor sites or macromolecules in concentrations and for a length of time to cause an alteration in normal function. Thus, the chemical's inherent toxicity, or its inherent ability to produce harm in an organism, and the degree of exposure of an organism to the

toxin are two important factors which determine toxicity. In addition, many factors contribute to the degree of inherent toxicity of a chemical and the exposure situation. Two important aspects of exposure which play a role in the development of toxicosis are the duration and frequency of exposure.

Many agents show quite different effects between acute and chronic exposure. Acute exposure to agents that are rapidly absorbed tends to produce immediate toxic effects, while chronic exposure to a toxin may produce both an acute effect, after each successive dose, and a long term effect due to the chronic presence of low levels of the compound. Fractionization of the dose can also reduce the intensity of the chemical's effect. Administering a specific quantity of chemical in two doses over a period of several hours may result in less than one half of the effect that would have been obtained had the same amount of chemical been given in a single dose. This "fractionization effect" occurs due to the compound being metabolized or excreted between successive doses, thus reducing the peak tissue or blood concentrations or by the ability of the organism to partially or fully reduce the injury caused by the first dose prior to the second. In certain incidences, the production of a toxic response can be totally dependent on the frequency rather than the duration of exposure (Klaassen and Doull, 1980).

The absorption or elimination of most drugs and xenobiotics follow expotential (first order) kinetics. A constant proportion of the compound administered is absorbed or

excreted per unit of time, since the chemical concentration within the organism usually does not saturate the mechanism responsible for absorption and excretion. In certain cases where the elimination process is restricted due to the limited quantity of carrier molecules, drug metabolizing enzymes, or cofactors in the metabolism pathway or the concentration of the toxic compound saturates the excretion mechanism, zero order elimination (constant quantity per unit time) kinetics results.

In first order kinetics an absorption or elimination rate constant (K) which expresses the fractional change per unit of time can be calculated. From this constant, a half-time, (.693/K) or the time required for 50 percent of the administered dose to be absorbed or excreted, can be determined. Both the rate constant (K) and the half-time are independent of drug concentration and dose. From these formulas, it can be determined that the effect of a single dose can be characterized by its latency, time to peak effect, time to peak concentration, magnitude of peak concentration, and the duration of effect. As the dose increases, the latency is reduced and the peak effect is increased without altering the time of peak effect. The duration of effect increases with increasing dosage but proportionally less than peak effect.

In first order kinetic models, 4 half-times are required for near complete elimination (94%) of a compound from the organism, and any dosing interval shorter than this results

in the accumulation of the compound. The accumulation of the compound continues during successive doses until the rate of elimination equals the rate of absorption. Once this equilibrium has been reached the body concentration becomes a function of the maintenance dose (dose/dose interval) or exposure rate and the half-time for elimination. Thus, if two dosing intervals are used for a compound but the total drug administered within a 24 hour period is equal between the two dosing schedules, the plateau levels of tissue stores would be equal, but the peak tissue concentrations would be different.

Light has been known to alter the metabolism and response to drugs in many animal models. In general, light can alter the organism's response to drugs or xenobiotics by two mechanisms; photochemical reactions or changes in the levels and activity of drug metabolizing enzymes. Photochemical reactions can be either deleterious or beneficial. Chemicals, such as selected sulfonamides, tetracyclines, nalidixic acid, sulfonylureas, thiazides, phenothiazines, and coal tars, when chronically ingested, result in photosensitivity after exposure to sunlight or artificial fluorescent light (Klaassen, 1980). Two types of photosensitive reactions have been observed; a phototoxic reaction which is characterized by the fundamental dose-response relationship and results from the production of a reactive chemical metabolite, and the photoallergic reaction which is an idiosyncratic response characterized by the promotion of a chemical reaction between the drug and subcutaneous proteins resulting in the formation of

a photoantigen (Klaassen, 1980). Conversely, photochemical reactions such as that observed in the phototherapy of premature infants with neonatal hyperbilirubinemia result in the formation of less toxic photometabolites. When infants with hyperbilirubinemia are exposed to blue or visible light, the radiation penetrates the skin photooxidizing bilirubin to a more hydrophilic product which can be easily eliminated by both biliary and urinary excretory pathways (Sanvordeker and Lambert, 1974). Similarly, alterations in drug metabolizing enzymes can be deleterious or beneficial depending on the nature of the chemical and its metabolites. Light affects the drug metabolizing enzyme system by promoting rhythmic changes in the activity and production of enzymes. In rat studies conducted by Joir et al. (1971) using female Long-Evan rats kept under 12 hours light:12 hours dark, liver microsomal drug metabolizing enzyme levels peaked during the dark phase of the diurnal photoperiod, then declined to minimum levels during the 12 hour light period. Nair and Casper (1969) reported similar diurnal fluctuations in hepatic drug metabolizing enzyme actitivy in male Sprague-Dawley rats raised under a 12 hour light:12 hour dark schedule. In addition to the diurnal photoperiod, Nair and Casper also studied the effects of continuous light and continuous darkness on the hepatic drug metabolizing enzymes of rats. Both of the continuous lighting schedules abolished the daily rhythms in enzyme activities. The rats raised in total darkness maintained significantly higher enzyme levels than the rats raised

under continuous light. When phenobarbital sleeping times were measured in these two groups of rats, the animals exposed to continuous light slept significantly longer than the rats on the dark schedule indicating light can indirectly alter the response to drugs by affecting drug metabolizing enzyme levels.

Chemicals

The chemicals used in this study were selected on the basis of their ability to affect the nervous system. The five chemicals represent agents that stimulate, inhibit, or have no effect on the nervous system.

ANTU

Synomyms: α-napthylthiourea; α-naphylthiocarbamide; Krysid

Description: A colorless to gray, odorless, bitter tasting, crystalline compound with a melting point of 198°C and solubilities of 0.06 g/100 ml water and 2.43 g/100 ml acetone.

Molecular weight: 202.27

Molecular formula: $C_{11}H_{10}N_2S$

Use, mode of action, and clinical signs: ANTU is a rodenticide that is highly specific for the adult Norway or brown rat (Rattus norvegicus) while being less toxic to other Rattus species and safe to most domestic animals. Of the domesticated animals, dogs, cats, and swine are the most susceptable.

ANTU is a fast acting toxicant which causes an increase in pulmonary capillary permeability leading to plural

effusions, pulmonary edema, and anoxia. Clinical signs include salivation, vomitation, diarrhea (general gastric irritation), dyspnea, coughing, tachycardia, and pulmonary rales. Post-mortem examinations reveal cyanosis, hydrothorax, inflammation of the trachea, bronchi, and gastrointestinal tract, and hyperemia of the kidneys and liver.

Chemical purity: 95%

Source: K and K Laboratories - ICN, 121 Express St., Plainview, NY.

Lot number: 45898A

Fenthion

Synonyms: Baytex, Baycid, ENTEX, Lebaycid, Mercaptophos

Description: A yellow, oily liquid with a slight odor of garlic, a boiling point of 87°C (commercial product boiling point 105°C) and a vapor pressure of 3.0×10^{-5} mmHg at 20°C. It is readily soluble in methanol, ethanol, ether, and other organic solvents. Its solubility in water is 5.5 mg/100 ml water.

Molecular weight: 278.34

Molecular formula: $C_{10}H_{15}O_3PS_2$

Use, mode of action, and clinical signs: Fenthion is an organophosphate insecticide which can be readily absorbed through the skin, mucous membranes, lungs, or digestive tract. Its toxicity results from irreversible binding to acetylcholinesterase causing acetylcholinesterase inhibition which

leads to overstimulation of the parasymphathetic nervous system. Clinical signs include lacrimation, salivation, tracheal congestion, gastrointestinal disturbances (hypermotility), muscle stimulation, convulsions, ataxia, immobility, and dyspnea. Post-mortem findings after acute exposure may be minimal and nonspecific while subacute or chronic exposures result in excessive secretions within the respiratory tract.

Chemical purity used: 94%

Source: Mobay Chemical Corp.; Agricultural Chemicals Division; P.O. Box 4913; 8400 Hawthorn Road; Kansas City, MO.

Batch number: 8030130

Endrin

Synonyms: Mendrin, Nendrin, Hexadrin, Compound 269

Description: A white crystalline solid or powder (technical powder tan) with a melting point of 226-230°C, a vapor pressure of 2.0×10^{-7} mmHg at 25°C and solubilities of 17 g/100 ml acetone, 13.8 g/100 ml benzene, and 7.1 g/100 ml hexane.

Molecular weight: 380.93

Molecular formula: $C_{12}H_8Cl_6O$

Use, mode of action, and clinical signs: Endrin is an organochlorine insecticide which was used mainly on field crops, particularly cotton. Its specific mechanism of action has yet to be established, but toxicity is thought to result primarily from the effects endrin and endrin metabolites have on the central nervous system. Chlorinated hydrocarbon insecticides in general act as diffuse stimulants or depressants

of the central nervous system. Clinical signs due to endrin toxicity are: convulsions, tremors, vomiting, abdominal distress, drowsiness, lethargy, and ataxia. Respiratory failure is considered to most common cause of death. The liver, being a secondary site of action, shows signs of fatty infiltration, hypertrophy, and proliferation of smooth endoplasmic reticulum.

Chemical purity used: 99%

Source: The Anspec Company, Inc.; P.O. Box 7730; 122 Enterprise Drive; Ann Arbor, MI.

Lot number: 5-171

Sodium secobarbital

Synonyms: Sodium Meballymal, Sodium Seconal, Sodium Bipinal, Immenoctal, Pramil, Quinalspan, Sebar, Sedutain

Description: A white, bitter tasting, hygroscopic powder with a melting point of 100°C. It is very soluble in water, soluble in alcohol, and practically insoluble in ether.

Molecular weight: 260.27

Molecular formula: $C_{12}H_{17}N_2NaO_3$

Use, mode of action, and clinical signs: Sodium secobarbital is a short acting barbituate. Barbituates in general act to reversibly depress the activity of all excitable tissue. Depression at sensitive CNS synapses may result from a presynaptic decrease in transmitter release or by enhancing the postsynaptic GABA responsive membranes. In the peripheral nervous system, barbituates depress the threshold of spinal

reflexes by diminishing the response of sympathetic ganglia to preganglionic stimulation. Respiratory complications result from the depression or elimination of the hypoxic and chromoreceptor drives controlling respiration. Barbituates also have various effects on the liver, kidneys, gastrointestinal tract, and skeletal muscles. Symptoms of poisoning include drowsiness, confusion, loss of coordination, lethargy, and ataxia. Death may result from cardiovascular collapse or respiratory arrest.

Chemical purity used: 99%

Source: Sigma Chemical Co.; P.O. Box 14508; St. Louis, MO.

Lot number: 059C0207

Strychnine

Synonyms: Mole death, Mouse-nots

Description: A colorless to white crystalline powder with a melting point of 270-280°C and solubilities of 1.56×10^{-6} g/100 ml water, 6.67×10^{-1} g/100 ml alcohol, and 5.56×10^{-1} g/ml benzene.

Molecular weight: 334.4

Molecular formula: $C_{21}H_{22}N_2O_2$

Use, mode of action, and clinical signs: Strychnine is an indole alkaloid used as a pesticide, ruminatoric, and stimulant. Its primary use as a pesticide is in bird and mammal control. Strychnine causes neural stimulation by antagonizing spinal and medullary post-synaptic inhibition,

preventing the moderating and controlling effects in normal reflexes. Early poisoning signs of nervousness, tenseness, and stiffness are followed by tetanic seizures, convulsions, muscle tremors, ataxia, and rigor. Convulsive seizures can be initiated by external stimuli such as light, sound, and touch (hypersensitivity). Clinical signs may appear within ten minutes of exposure and remain for up to two hours if untreated. Death usually results from anoxia brought about by convulsions.

Chemical purity used: 96%

Source: Pfaltz and Bauer, Inc.; Division of Aceto Chemical Co., Inc.; 375 Fairfield Ave.; Stamford, CT.

Lot number: 24763

PROCEDURE

Design and Chronology of Study

The study consisted of 30 individual LC_{50} tests conducted over an 11 month period from March 1982 through January 1983. The 30 tests were run in ten separate trials each consisting of three tests per trial (see Table 1 for chronology of trials). During each trial one of five chemicals (ANTU, fenthion, endrin, secobarbital, or strychnine) was tested in bobwhite or mallards. Individual tests within a trial were identical with the exception of photoperiod. The three photoperiods used were: 24 hours light:0 hours dark (24); 14 hours light:10 hours dark (14); and 14 hours light:10 hours dark with dimming and brightening of lights between light and dark

Table 1. Chronology of LC_{50} trials.

Chemical	Acclimation period	Test start	Period of chemical administration	Recovery period	Termination
Bobwhite					
ANTU	12/21/82-12/26/82	12/26/82	12/26/82-12/31/82	12/31/82- 1/ 3/83	1/ 3/83
Fenthion	3/27/82- 4/ 1/82	4/ 1/82	4/ 1/82- 4/ 6/82	4/ 6/82- 4/ 9/82	4/ 9/82
Endrin	4/10/82- 4/15/82	4/15/82	4/15/82- 4/20/82	4/20/82- 4/23/82	4/23/82
Secobarbital	1/ 1/83- 1/ 6/83	1/ 6/83	1/ 6/83- 1/11/83	1/11/83- 1/14/83	1/14/83
Strychnine	3/ 1/82- 3/ 6/82	3/ 6/82	3/ 6/82- 3/11/82	3/11/82- 3/14/82	3/14/82
Mallard					
ANTU	10/16/82-10/21/82	10/21/82	10/21/82-10/26/82	10/26/82-10/29/82	10/29/82
Fenthion	7/10/82- 7/15/82	7/15/82	7/15/82- 7/20/82	7/20/82- 7/23/82	7/23/82
Endrin	5/ 8/82- 5/13/82	5/13/82	5/13/82- 5/18/82	5/18/82- 5/21/82	5/21/82
Secobarbital	11/14/82-11/19/82	11/19/82	11/19/82-11/24/82	11/24/82-11/27/82	11/27/82
Strychnine	6/27/82- 7/ 1/82	7/ 1/82	7/ 1/82- 7/ 6/82	7/ 6/82- 7/ 9/82	7/ 9/82

periods to simulate dusk and dawn (14D). Eight treatments (two controls and six chemically-treated groups) of ten birds per treatment were used within each LC_{50} test.

Experimental Design

Photoperiods = 3 (24L:0D; 14L:10D; 14L:10D with dimming and brightening)

Chemicals/photo. = 5 (ANTU, fenthion, endrin, secobarbital, strychnine)

Species/chemical = 2 (bobwhite, mallard)

Treatments/chem. = 8 (2 controls, 6 treated diets)

Birds/treatment = 10

Range Finding - Preliminary Tests

A literature review of the chemicals used in this study indicated that dietary LC_{50} values for fenthion and endrin had been previously determined in bobwhite and mallards. The reported LC_{50} estimates for these two compounds (Heath et al., 1972) were used as the approximate median concentrations in the LC_{50} trials.

ANTU, secobarbital, and strychnine trial concentrations were determined, in part, through preliminary testing using three widely spaced dietary concentrations per chemical with ten birds per dietary concentration. The mortality results of these preliminary tests were used to set the median dietary concentrations and the concentration spacing for the LC_{50} trials (see Table 2 for preliminary test dietary concentrations and mortality).

Table 2. Percent mortality for bobwhites and mallards fed ANTU, secobarbital, and strychnine during preliminary range finding tests.

Chemical	Dietary concentration	Mortality (%)
Bobwhite		
ANTU	2,000[1]	10
	5,000	20
	10,000	30
Secobarbital	2,000	0
	5,000	0
	10,000	30
Strychnine	100	0
	375	20
	1,000	30
Mallard		
ANTU	2,000	30
	5,000	60
	10,000	50
Secobarbital	1,000	0
	3,000	10
	5,000	20
Strychnine	100	0
	375	30
	1,000	80

[1] Parts per million.

Facilities

All birds were housed in the Michigan State University Poultry Research and Teaching Center during the acclimation and testing period. The three rooms used, one for each photoperiod, were entirely enclosed and measured 2.95 m X 4.50 m X 2.54 m (W X L X H). Each room contained one Petersime 250-24 battery brooder in which the birds were maintained. The brooders were divided into six levels, each of which was separated into four equally sized sections measuring 34.3 cm X 99.4 cm X 24.1 cm (W X L X H). Only two levels (eight sections) with ten birds per section, allowing a minimum of 134.2 cm^2 floor space per bird, were used during each test. The galvanized steel wire mesh of the sides and floors measured 1.3 cm X 1.3 cm and 0.8 cm, respectively, for bobwhite and 1.3 cm X 2.4 cm and 1.7 cm X 1.7 cm, respectively, for mallards. Each section was provided with an individual feeder, measuring 5.7 cm X 61.0 cm X 1.9 cm (W X L X H) for bobwhite and 8.8 cm X 63.5 cm X 6.1 cm (W X L X H) for mallards, and waterer, one quart mason jar for bobwhite and 8.8 cm X 33.0 cm X 5.1 cm (W X L X H) trough for mallards. Mallard feeders and waterers were attached to the outside of each section while bobwhite feeders and waterers were placed within each section.

Photoperiod was controlled by Paragon model 41005-D automatic timers that were set for 24 hours light:0 hours dark or 14 hours light:10 hours dark. In addition, a voltage regulator constructed by the Department of Agricultural Engineering, Michigan State University, was used in one of the 14 hours

light rooms to create gradual dimming and brightening sequences of 17 min duration before dark and light periods. Light was provided by a 150 watt incandescent light bulb centered in the ceiling of each room. Light intensities for each room and battery level are given in Table 3 and 4.

A thermometer and humidity gauge were located on the wall inside each testing room. One 1,500 watt convection floor heater with variable temperature controls was used to heat each room when temperatures dropped below 21°C (70°F).

Animals

Bobwhite chicks were reared at Michigan State University from eggs collected from an established breeding population of second generation wild bobwhite. The original stock of birds was obtained from the Illinois State Game Farm, Mt. Vernon, IL. Upon hatching, all bobwhite were wing banded and placed in quarantine until five days of age.

All mallard ducklings were purchased from Whistling Wings Duck Farm, Hanover, IL. Prior to each trial, approximately 300 one-day old ducklings were shipped (shortly after hatching) and received at Michigan State University, Department of Animal Science within 36 hours, whereupon they were wing banded and placed in quarantine until five days of age.

During the quarantine periods, all birds (bobwhite and mallards) were observed and all deformed, sick, injured, or abnormal birds were removed from the flock. At five days of age all birds in apparent good health were transported to the

Table 3. Brooder and room light intensities[1].

Photoperiod	24[2]	14[3]	14D[4]
Room	69.9	69.9	69.9
Brooder upper level	32.3	32.3	32.2
Brooder lower level	30.1	30.1	30.1

[1] Light intensity in luxes.

[2] 24 hours light:0 hours dark.

[3] 14 hours light:10 hours dark.

[4] 14 hours light:10 hours dark with dimming and brightening between light and dark periods.

Table 4. Room light intensities[1] for the 14D[2] photoperiod during the brightening and dimming sequence.

Brightening sequence		Dimming sequence	
Time (min)	Intensity	Time (min)	Intensity
0	0	0	69.9 (maximum)
1	0	1	64.6
2	2.2	2	59.2
3	5.4	3	53.8
4	8.6	4	48.4
5	10.8	5	43.8
6	16.1	6	37.7
7	19.4	7	34.4
8	21.5	8	30.1
9	26.9	9	26.9
10	32.3	10	21.5
11	37.7	11	16.1
12	43.8	12	12.9
13	48.4	13	10.8
14	56.0	14	5.4
15	64.6	15	0 (minimum)
16	66.7	16	0
17	69.9 (maximum)	17	0

[1] Light intensity in luxes.

[2] 14 hours light:10 hours dark with brightening and dimming between dark and light periods.

testing facility and placed in their testing batteries for a five day acclimation period preceding each trial. At ten days of age the birds were randomly assigned to their testing sections. Prior to the assignment of birds to sections, any abnormally under-weight or over-weight birds were removed from the flock to increase flock uniformity and prevent bias caused by unequal body weights. All birds used for testing were ten days of age at the onset of testing. At trial terminations, all birds were killed by either cervical dislocation or chloroform asphyxiation.

Diets

All test diets were prepared by the Department of Animal Science, Michigan State University using a mash as the basal diet (Appendix A and B). Control diets consisted of basal mash plus any carriers used in the preparation of treated diets. Premixes treated with ANTU, endrin, secobarbital, or strychnine were prepared by adding the powdered form of these compounds to 1,500 grams of fine mash which had been filtered through a number 20 sieve. The entire 1,500 grams of mash and chemical were mixed and filtered through a number 20 sieve to prevent chemical clumping and obtain an equal distribution of the compound throughout the feed. The unfiltered feed which failed to pass through the sieve in obtaining 1,500 grams of fine mash, and any additional feed required to bring the premix to the desired concentration, were added to the chemical-fine mash mixture and tumbled for ten minutes on a mechanical tumbler. Treated diets used during the test were

prepared by dilutions of the premix diet. Specific quantities of premix and untreated feed were combined to reach the desired dietary concentration and tumbled for ten minutes to assure mixing. The fenthion premix was prepared by dissolving liquid Baytex in corn oil and adding this oil-chemical mixture to a specific quantity of mash. The corn oil content of the premix did not exceed one percent of the total feed-oil-chemical mixture. The premix was tumbled for ten minutes on a mechanical tumbler after the oil-chemical mixture had been thoroughly mixed manually into the mash. Fenthion test diets were prepared by the dilution method described for ANTU, endrin, secobarbital, and strychnine. Nominal dietary concentrations of all chemicals tested are listed in Table 5. Eight treatments per test were used in each trial; two controls plus six concentrations of the test chemical. Chemical concentrations within the six treated diets were geometrically spaced at intervals of 1.3X to 1.6X, depending on the chemical used. Feed and water were provided ad libitum throughout the quarantine, acclimation, and testing periods.

Observations of Record

Feed consumption, body weights, mortality, signs of intoxication, room and brooder temperatures, and relative humidity were recorded throughout the testing period. Average feed consumption and feed wastage per battery section (dietary concentration) were measured on days 2, 4, 5, 7, and 8 during

Table 5. Dietary concentrations (ppm) used during LC_{50} testing.

Chemical	Dietary treatment 1	2	3	4	5	6	7	8
Bobwhite								
ANTU	0.0[1]	0.0	2,000	2,800	3,920	5,488	7,683	10,756
Fenthion	0.0	0.0	10.9	15.3	21.4	30.0	42.0	58.8
Endrin	0.0	0.0	7.1	9.2	12.0	15.6	20.3	26.4
Secobarbital	0.0	0.0	2,000	2,800	3,920	5,488	7,683	10,756
Strychnine	0.0	0.0	586	938	1,500	2,400	3,840	6,144
Mallard								
ANTU	0.0	0.0	2,000	2,800	3,920	5,488	7,683	10,756
Fenthion	0.0	0.0	37	52	73	102	143	200
Endrin	0.0	0.0	10.4	13.5	17.5	22.8	29.6	38.4
Secobarbital	0.0	0.0	2,000	2,800	3,920	5,488	7,683	10,756
Strychnine	0.0	0.0	323	420	546	710	923	1,200

[1] Parts per million.

all bobwhite trials. Any significant amount of feed spilled into the litter pans beneath bobwhite sections was separated from feces and weighed. Mallard feed consumption was measured at identical times during the testing periods but feed wastage could not be accurately determined due to the difficulty in separating feed from feces and water which had collected in the litter pans. All birds were weighed at days 0, 5, and 8 or on their day of death. All weighings (feed consumption and body weights) within each trial were conducted at similar times of day in order to maintain consistency in weighing intervals.

Observations were made three times during the first day of exposure and twice daily thereafter until study termination. All signs of intoxication and the number of birds showing specific signs were recorded for each dietary concentration within a test. Band numbers and body weights of birds which died during the trial were also recorded at these times. Brooder temperature, room temperature, and room relative humidity were measured daily during the first observation period of each day.

Statistical Analysis

LC_{50} values with 95% confidence limits and the slope of the concentration response curves were determined for each test run using three different established methods; the probit analysis method described by Litchfield and Wilcoxon (1949), a log-probit analysis using a direct regression of dose on

mortality (Montgomery and Peck, 1982), and a log-probit analysis using an inverse prediction of the regression of mortality on dose (Gill, 1978). Statistical contrasts of LC_{50} values between photoperiods for a specific compound were accomplished by confidence limit comparisons. If the $LC_{50} \pm$ confidence limits of two photoperiods were separated (nonoverlapping) by > 10% of the smallest confidence limit, the LC_{50} values of the two photoperiods were considered significantly different at $\alpha \leq 0.05$ (Browne, 1979).

Initially, a two-way analysis of variance (photoperiod by dietary concentration), which compensates for unequal replication and missing blocks when appropriate, was run on all body weight and feed consumption data on a trial basis. The two-way analysis of variance on body weight was run at day 0, 5, and 8 of every trial, while the feed consumption analysis was run on the five-day treatment and three-day recovery periods. Feed consumption was measured three times during the five-day treatment period and twice during the three-day recovery period. Sampling units for body weights and feed consumption were individual bird weights and mean feed consumption per dietary concentration (expressed as grams/bird/day), respectively. Means of body weights and feed consumption for each of the photoperiods were compared by use of Tukey's all pairwise comparisons test, while means of body weights and feed consumption for each treatment were compared using Dunnett's two-tail t-test (Gill, 1978). The analysis of variance procedures used were run on the Michigan State University Cyber

750 Computer using the SPSS (Nie et al., 1975) and JENSTAT (Alvey et al., 1977) statistical packages.

RESULTS

Three trials resulted in insufficient mortality to properly determine LC_{50} values. The bobwhite-ANTU, bobwhite-secobarbital, and mallard-secobarbital trials failed to produce greater than 50% mortality at a dietary concentration exceeding 10,000 ppm, in at least two of the three photoperiods. Although insufficient data were obtained from these trials, LC_{50} values were calculated with the available information and are presented along with the other trials.

Litchfield and Wilcoxon Analysis

No significant differences in LC_{50} values between the three photoperiods could be established in bobwhite or mallards using the probit analysis method of Litchfield and Wilcoxon (Table 6). The photoperiod effects on LC_{50} determinations varied considerably among the two species and five chemicals tested. Bobwhite mortality on the 24 photoperiod produced lower LC_{50} values than either of the two 14-hour light photoperiods when chicks were exposed to fenthion, endrin, and strychnine, but produced higher and intermediate LC_{50} values compared to the LC_{50} values for the 14-hour photoperiods when chicks were exposed to ANTU and secobarbital, respectively (Tables 6 through 11). Mallard mortality under the 24 photoperiod resulted in higher LC_{50} values than the 14-hour photoperiods when ducklings were exposed to fenthion and endrin,

Table 6. LC_{50} values for ANTU, fenthion, endrin, secobarbital and strychnine in bobwhites and mallards calculated by the probit analysis method of Litchfield and Wilcoxon (1949).

Compound species	Age (days)	No. birds/ concentration	Photoperiod: 24 hours LC_{50} (95% C.L.)	Photoperiod: 14 hours LC_{50} (95% C.L.)	Photoperiod: 14 hours w/d[1] LC_{50} (95% C.L.)
ANTU[2]					
Bobwhite	10	10	28,000 (11,765-66,640)	8,200 (5,563-12,087)	15,000 (5,865-38,366)
Mallard	10	10	4,000 (1,786- 8,960)	3,400 (2,378- 4,862)	4,900 (3,490- 6,880)
Fenthion[3]					
Bobwhite	10	10	20.5 (16.8- 25.1)	23.7 (19.5-28.8)	21.0 (17.9-24.6)
Mallard	10	10	82 (61.7-109.1)	69.0 (48.5-98.2)	60.0 (45.1-79.8)
Endrin[4]					
Bobwhite	10	10	10.8 (8.2- 14.2)	10.8 (8.7-13.3)	13.6 (11.3-16.4)
Mallard	10	10	34.0 (24.6- 46.9)	29.6 (22.4-39.1)	29.6 (22.4-39.1)
Secobarbital[5]					
Bobwhite	10	10	16,300 (1,739-34,329)	12,800 (7,153- 22,904)	23,000 (10,892-48,567)
Mallard	10	10	10,600 (6,463-17,384)	98,000 (19,103-502,740)	7,683 (5,583-10,573)
Strychnine[6]					
Bobwhite	10	10	1,650 (1,284- 2,121)	1,700 (1,194-2,414)	1,700 (1,181-2,448)
Mallard	10	10	370 (284- 482)	270 (169- 430)	410 (320- 524)

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Chemical purity = 95%.

[3] Chemical purity = 94%.

[4] Chemical purity = 99%.

[5] Chemical purity = 99%.

[6] Chemical purity = 96%.

Table 7. Percent mortality and LC_{50} values for bobwhites fed dietary ANTU[1].

Dose (ppm)	n	Percent mortality 24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
2,000	10	10	0	30
2,800	10	10	20	0
3,920	10	10	20	40
5,488	10	20	20	10
7,683	10	10	50	50
10,756	10	40	60	20
LC_{50}[3]		28,000	8,200	15,000
95% C.L.		(11,765-66,640)	(5,563-12,087)	(5,865-38,366)
Slope		2,206	244	3,072
LC_{50}[4]		13,836	6,745	5,470
95% C.L.		(3,020-63,241)	(4,853- 9,376)	(2,805-10,715)
Slope		0.004	0.010	0.021
LC_{50}[5]		41,495	7,834	21,749
95% C.L.[6]		---	(2,051-∞)	---
Slope		471	121	333

[1] Chemical purity = 95%

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 8 . Percent mortality and LC_{50} values for bobwhites fed dietary fenthion[1].

Dose (ppm)	n	Percent mortality 24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
10.9	10	0	0	0
15.3	10	20	10	10
21.4	10	60	30	60
30.0	10	80	80	90
42.0	10	100	100	100
58.8	10	100	100	90
LC_{50}[3]		20.5	23.7	21.0
95% C.L.		(16.8-25.1)	(19.5-28.8)	(17.9-24.6)
Slope		0.25	0.22	0.12
LC_{50}[4]		21.2_a	22.2_b	24.0_c
95% C.L.		(21.1-21.3)	(21.9-22.5)	(23.0-25.1)
Slope		3.04	2.93	2.35
LC_{50}[5]		21.1	22.2	23.6
95% C.L.		(12.9-34.5)	(15.2-32.9)	(3.4-122.7)
Slope		0.31	0.31	0.48

[1] Chemical purity = 94%

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

Table 9 . Percent mortality and LC_{50} values for bobwhites fed dietary endrin[1].

Dose (ppm)	n	Percent mortality		
		24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0A	10	0	0	0
0B	10	0	0	0
7.1	10	30	30	0
9.2	10	40	40	30
12.0	10	100	40	40
15.6	10	70	80	50
20.3	10	80	100	90
26.4	10	100	100	90
LC_{50}[3]		10.8	10.8	13.6
95% C.L.		(8.2-14.2)	(8.7-13.3)	(11.3-16.4)
Slope		0.23	0.16	0.17
LC_{50}[4]		11.1_{ab}	10.4_{a}	14.8_{b}
95% C.L.		(7.6-16.4)	(8.9-12.2)	(14.0-15.6)
Slope		7.41	5.50	6.42
LC_{50}[5]		7.7	9.9	15.1
95% C.L.[6]		---	---	(6.1-44.9)
Slope		0.34	0.15	0.20

[1] Chemical purity = 99%

[2] Fourteen hours light:10 hours dark with dimming and brigntening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 10. Percent mortality and LC_{50} values for bobwhites fed dietary secobarbital[1].

Dose (ppm)	n	Percent mortality 24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	10[3]
0.0B	10	0	10[3]	0
2,000	10	0	10	0
2,800	10	0	0	0
3,920	10	20	20	0
5,488	10	0	100	10
7,683	10	0	50	10
10,756	10	40	30	30
LC_{50}[4]		16,300	12,800	23,000
95% C.L.		(7,739-34,329)	(7,153-22,904)	(10,892-48,567)
Slope		1,279	529	650
LC_{50}[5]		7,211	4,943	11,614
95% C.L.		(2,018-25,763)	(4,140- 5,902)	(5,420-24,831)
Slope		0.018	0.023	0.005
LC_{50}[6]		41,933	6,366	12,892
95% C.L.[7]		---	---	---
Slope		1,560	110	495

[1] Chemical purity = 99%

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] Percent mortality values used to compute LC_{50} values were adjusted to compensate for control mortality.

[4] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[6] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[7] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 11. Percent mortality and LC_{50} values for bobwhites fed dietary strychnine[1].

Dose (ppm)	n	Percent mortality 24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
586	10	0	0	10
938	10	10	20	10
1,500	10	20	40	50
2,400	10	100	70	60
3,840	10	100	100	100
6,144	10	100	100	100
LC_{50}[3]		1,650	1,700	1,700
95% C.L.		(1,284-2,121)	(1,197-2,414)	(1,181-2,448)
Slope		21.0	37.1	40.3
LC_{50}[4]		$1,309_a$	$1,549_b$	$1,371_a$
95% C.L.		(1,183-1,449)	(1,521-1,574)	(1,259-1,493)
Slope		0.051	0.028	0.025
LC_{50}[5]		3,122	1,552	1,337
95% C.L.[6]		-----	(674-3,673)	(14-23,659)
Slope		18.2	32.5	33.3

[1] Chemical purity = 96%.

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

however, mortality on the 24 photoperiod resulted in intermediate LC_{50} values to the 14-hour photoperiods when exposed to strychnine, ANTU, and secobarbital (Tables 6 and 12 through 16).

Similarly, mortality between the two 14-hour light photoperiods varied with species and chemicals (Table 6). Bobwhite LC_{50} values in the 14 photoperiod were lower than LC_{50} values of the 14D photoperiod when chicks were exposed to ANTU, endrin, or secobarbital (Tables 6, 7, 9, and 10). The LC_{50} values for bobwhite fed strychnine were equal for the two 14-hour photoperiods, while the 14 photoperiod produced a slightly higher LC_{50} value than the 14D photoperiod when the chicks were fed fenthion (Tables 6, 8, and 11). Mallard mortality resulted in higher LC_{50} values under the 14 photoperiod compared to the 14D photoperiod when ducklings were exposed to fenthion and secobarbital, but produced lower LC_{50} values under the 14D photoperiod when ducklings were exposed to ANTU and strychnine (Tables 6, 12, 13, 15, and 16). LC_{50} values for mallards between the two 14-hour photoperiods were equal for endrin (Tables 6 and 14).

In summary, the 24 photoperiod produced the highest LC_{50} values in three of the 10 trials while the 14 and 14D photoperiods produced the highest LD_{50} values in three and four of the trials, respectively. The lowest LC_{50} values between the three photoperiods showed a similar pattern of being equally distributed.

Table 12. Percent mortality and LC_{50} values for mallards fed dietary ANTU[1].

Dose (ppm)	n	Percent mortality		
		24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
2,000	10	27[3]	30	10
2,800	10	70	50	40
3,920	10	56[4]	40	40
5,488	10	70	60	70
7,683	10	50	90	60
10,756	10	80	90	80
LC_{50}[5]		4,000	3,400	4,900
95% C.L.		(1,786-8,960)	(2,378-4,862)	(3,490-6,880)
Slope		106	104	124
LC_{50}[6]		3,855	3,589	4,742
95% C.L.		(2,818-5,284)	(3,105-4,159)	(4,677-4,797)
Slope		0.0106	0.0086	0.0096
LC_{50}[7]		2,931	3,436	4,753
95% C.L.[8]		---	(1,161-7,586)	(1,879-12,388)
Slope		268	138	136

[1] Chemical purity = 95%

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] Mortality percentage based on 11 animals (n = 11).

[4] Mortality percentage based on nine animals (n = 9).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis described by Litchfield and Wilcoxon (1948).

[6] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[7] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[8] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 13. Percent mortality and LC_{50} values for mallards fed dietary fenthion[1].

Dose (ppm)	n	Percent mortality		
		24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
37	10	10	30	10
52	10	40	40	60
73	10	30	40	60
102	10	60	70	70
143	10	80	80	100
200	10	100	90	100
LC_{50}[3]		82	69	60
95% C.L.		(61.7-109.1)	(48.5- 98.2)	(45.1- 79.3)
Slope		1.67	2.09	1.12
LC_{50}[4]		74.5	68.5	61.0
95% C.L.		(66.8- 82.8)	(63.4- 74.1)	(51.3- 72.4)
Slope		0.46	0.38	0.78
LC_{50}[5]		71.4	67.6	57.9
95% C.L.[6]		(14.6-231.7)	(40.6-105.4)	(0 -310.0)
Slope		1.7	2.6	1.2

[1] Chemical Purity = 94%.

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 14. Percent mortality and LC_{50} values for mallards fed dietary endrin[1].

Dose (ppm)	n	Percent mortality		
		24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
10.4	10	0	0	10
13.5	10	10	20	0
17.5	10	30	10	10
22.8	10	10	40	30
29.6	10	40	50	50
38.4	10	60	60	70
LC_{50}[3]		34.0	29.6	29.6
95% C.L.		(24.6-46.9)	(22.4-39.1)	(22.4-39.1)
Slope		0.68	0.60	0.59
LC_{50}[4]		27.9	26.5	25.9
95% C.L.		(20.8-37.5)	(20.9-33.4)	(18.9-35.5)
Slope		2.72	3.09	3.66
LC_{50}[5]		31.9	29.4	31.5
95% C.L.[6]		(12.0-1.786)	(11.2-0.48)	----
Slope		0.4	0.3	0.4

[1] Chemical purity = 99%.

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 15. Percent mortality and LC_{50} values for mallards fed dietary secobarbital[1].

Dose (ppm)	n	Percent mortality 24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
2,000	10	0	0	10
2,800	10	0	0	10
3,920	10	20	20	10
5,488	10	20	0	10
7,683	10	50	10	50
10,756	10	40	20	70
LC_{50}[3]		10,600	98,000	7,683
95% C.L.		(6,463-17,384)	(19,103-502,740)	(5,583-10,573)
Slope		354	104,309	153
LC_{50}[4]		7,656	8,260	7,656
95% C.L.		(5,023-11,641)	(2,698- 25,351)	(4,932-11,885)
Slope		0.0098	0.0066	0.0080
LC_{50}[5]		8,959	20,502	9,354
95% C.L.[6]		---	---	---
Slope		121	531	209

[1] Chemical purity = 99%

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Table 16. Percent mortality and LC_{50} values for mallards fed dietary strychnine[1].

Dose (ppm)	n	Percent mortality		
		24 hrs. light	14 hrs. light	14 hrs. light w/d[2]
0.0A	10	0	0	0
0.0B	10	0	0	0
323	10	50	40	30
420	10	50	80	60
546	10	90	90	80
710	10	80	90	70
923	10	90	80	90
1,200	10	100	100	100
LC_{50}[3]		370	270	410
95% C.L.		(284-482)	(169-430)	(320-524)
Slope		5.97	8.43	7.13
LC_{50}[4]		432	424	460
95% C.L.		(319-585)	(285-628)	(367-577)
Slope		0.054	0.067	0.070
LC_{50}[5]		379	343	421
95% C.L.[6]		(18-927)	---	(69-955)
Slope		13.6	15.9	11.6

[1] Chemical purity = 96%

[2] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[3] LC_{50} values, 95% C.L., and Slopes were determined by the method described by Litchfield and Wilcoxon (1948).

[4] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using a direct regression of dose on mortality (Montgomery and Peck, 1982).

[5] LC_{50} values, 95% C.L., and Slopes were determined by a log-probit analysis using an inverse prediction of mortality on dose (Gill, 1978).

[6] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equaling zero.

Direct Regression of Dose on Mortality

Predicting LC_{50} values using the log-probit analysis with a direct regression of dose on mortality produced similar LC_{50} values to the Litchfield and Wilcoxon method in those trials in which sufficient mortality was obtained (Tables 7 through 17). The two analytical methods also produced similar frequencies in the relative toxicity (magnitude of LC_{50} values) of the chemicals between the three photoperiods. Although the LC_{50} values between the two methods were similar, the confidence limits for the LC_{50} values were more narrow for the direct regression of dose on mortality procedure (Tables 7 through 16), resulting in the determination of significant photoperiodic effects in the bobwhite-fenthion, bobwhite-endrin, and bobwhite-strychnine trials. In the bobwhite-fenthion trial all three LC_{50} values were significantly different ($P < 0.05$) between the photoperiods. The bobwhite-endrin trial resulted in the 14D photoperiod producing a significantly ($P < 0.05$) higher LC_{50} value than the 14 photoperiod while the bobwhite-strychnine trial resulted in the 14 photoperiod producing a significantly ($P < 0.05$) lower LC_{50} value than the 24 and 14D photoperiods.

Inverse Prediction of the Regression of Mortality on Dose

As was seen with the probit analysis of Litchfield and Wilcoxon, no significant differences in LC_{50} values between the three photoperiods could be established (Table 18 and 7 through 16). The 24 photoperiod resulted in the lowest toxicity (highest LC_{50} values) in 50% of the trials. The 14 and

Table 17 . LC_{50} values for ANTU, fenthion, endrin, secobarbital and strychnine in bobwhites and mallards calculated by log-probit analysis using a direct regression of dose on mortality.

Chemical species	Age (days)	No. birds/ concentration	Photoperiod: 24 hours LC_{50} (95% C.L.)	Photoperiod: 14 hours LC_{50} (95% C.L.)	Photoperiod: 14 hours w/d[1] LC_{50} (95% C.L.)
ANTU					
Bobwhite	10	10	13,836 (3,020-63,241)	6,745 (4,853-9,376)	5,470 (2,805-10,715)
Mallard	10	10	3,855 (2,818- 5,284)	3,589 (3,105-4,159)	4,742 (4,677- 4,797)
Fenthion					
Bobwhite	10	10	21.2[a] (21.1-21.3)	22.2[b] (21.9-22.5)	24.0[c] (23.0-25.1)
Mallard	10	10	74.5 (66.8-82.8)	68.5 (63.4-74.1)	61.0 (51.3-72.4)
Endrin					
Bobwhite	10	10	11.1[ab] (7.6-16.4)	10.4[a] (8.9-12.2)	14.8[b] (14.0-15.6)
Mallard	10	10	27.9 (20.8-37.5)	26.5 (20.9-33.4)	25.9 (18.9-35.5)
Secobarbital					
Bobwhite	10	10	7,211 (2,018-25,763)	4,943 (4,140- 5,902)	11,614 (5,420-24,831)
Mallard	10	10	7,656 (5,023-11,641)	8,260 (2,698-25,351)	7,656 (4,932-11,885)
Strychnine					
Bobwhite	10	10	1,309[a] (1,183-1,449)	1,549[b] (1,521-1,574)	1,371[a] (1,259-1,493)
Mallard	10	10	432 (319- 585)	424 (285- 628)	460 (367- 577)

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

Table 18. LC_{50} values for ANTU, fenthion, endrin, secobarbital and strychnine in bobwhites and mallards calculated by a log-profit analysis using an inverse prediction of a regression analysis of mortality on dose.

Chemical species	Age (days)	No. birds/ concentration	Photoperiod: 24 hours LC_{50} (95% C.L.)	Photoperiod: 14 hours LC_{50} (95% C.L.)	Photoperiod: 14 hours w/d[1] LC_{50} (95% C.L.)
ANTU					
Bobwhite	10	10	41,495 ( )[2]	7,834 (2,051- 00)	21,749 ( )
Mallard	10	10	2,931 ( )	3,436 (1,161-7,586)	4,753 (1,879-12,388)
Fenthion					
Bobwhite	10	10	21.2 (12.9- 34.5)	22.2 (15.2- 32.9)	23.6 (3.4-122.7)
Mallard	10	10	71.4 (14.6-231.7)	67.6 (40.6-105.4)	57.9 (0 -310.0)
Endrin					
Bobwhite	10	10	7.7 ( )	9.9 ( )	15.1 (6.1- 44.9)
Mallard	10	10	31.9 (12.0-1,786)	29.4 (11.2-408.3)	31.5 ( )
Secobarbital					
Bobwhite	10	10	41,933 ( )	6,366 ( )	12,892 ( )
Mallard	10	10	8,959 ( )	20,502 ( )	9,354 (2,767-00)
Strychnine					
Bobwhite	10	10	3,122 ( )	1,552 (673-3,673)	1,337 (14-23,659)
Mallard	10	10	379 (18-927)	343 ( )	421 (69- 955)

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Lack of confidence limits resulted from the inability to reject the hypothesis of slope equalling zero.

14D photoperiods produced the lowest toxicity values in 10 and 40% of the trials, respectively. The confidence limits on the LC_{50} values calculated by the inverse prediction of the regression of mortality on dose were greatly widened in comparison to the other two analytical methods used.

In comparing the variation ($\frac{LC_{50}\ max}{LC_{50}\ min}$) of LC_{50} values for a particular chemical and species over the three photoperiods tested, a range of 1.00:1.03 to 1.00:1.52, 1.00:1.08 to 1.00:1.42, and 1.00:1.09 to 1.00:2.34 was obtained for the Litchfield and Wilcoxon, direct regression, and inverse prediction methods, respectively. If the variation in LC_{50} values for a particular photoperiod, chemical, and species is contrasted by analytical method, a range of 1.00:1.03 to 1.00:2.39 is obtained. The average variations in LC_{50} values between photoperiods for a particular chemical and species were 27.6, 18.6, and 64.3% for the Litchfield and Wilcoxon, direct regression, and inverse prediction methods, respectively, while the average variation for individual LC_{50} determinations between analytical methods was 27.3%.

Mortality patterns of the three photoperiods for any particular chemical were similar regardless of the chemical's mechanism or speed of action. Secobarbital and strychnine caused high mortality during the first 24 hours of exposure followed by reduced rates of death in the remaining treatment period in both species and throughout all photoperiods (Tables 19 and 20). Conversely, the fenthion and endrin treatments resulted in minimal or no death during the first 24 hours of

Table 19. Mortality patterns of bobwhite and mallards during LC_{50} testing with secobarbital.

Dietary concentration (ppm)	Photoperiod																			
	24								14								14D							
	1[a]	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Bobwhite																								
2,000	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3,920	2[b]	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5,488	-	-	-	-	-	-	-	-	2	8	-	-	-	-	-	-	1	-	-	-	-	-	-	-
7,683	-	-	-	-	-	-	-	-	3	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-
10,756	2	2	-	-	-	-	-	-	1	1	-	-	1	-	-	-	1	1	-	-	1	-	-	-
Mallard																								
2,000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
2,800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
3,920	2	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-
5,488	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
7,683	4	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	4	-	-	1	-	-	-	-
10,756	1	-	2	-	1	-	-	-	1	-	-	-	1	-	-	-	6	-	-	1	-	-	-	-

[a] Day of trial.

[b] Number of birds that died.

Table 20. Mortality patterns of bobwhite and mallards during LC_{50} testing with strychnine.

Dietary concentration (ppm)	Photoperiod																							
	24								14								14D							
	1[a]	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Bobwhite																								
586	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1[b]	-	-	-	-	-
938	-	-	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	1	-	-	-	-	-
1,500	1	-	1	-	-	-	-	-	3	1	-	-	-	-	-	-	1	2	-	1	1	-	-	-
2,400	4	1	1	3	1	-	-	-	3	2	1	1	-	-	-	-	4	1	-	1	-	-	-	-
3,840	8	2	-	-	-	-	-	-	4	4	2	-	-	-	-	-	5	2	1	-	1	1	-	-
6,144	8	1	1	-	-	-	-	-	8	-	2	-	-	-	-	-	9	1	-	-	-	-	-	-
Mallard																								
323	1	2	-	1	-	1	-	-	3	-	-	1	-	-	-	-	-	1	1	1	-	-	-	-
420	1	-	2	1	1	-	-	-	5	-	1	2	-	-	-	-	2	2	-	1	1	-	-	-
546	2	2	2	3	-	-	-	-	5	-	1	2	1	-	-	-	1	1	1	3	1	1	-	-
710	4	2	1	1	-	-	-	-	6	-	1	2	-	-	-	-	2	2	2	1	-	-	-	-
923	6	1	-	1	1	-	-	-	5	1	2	-	-	-	-	-	3	2	1	2	1	-	-	-
1,200	4	1	4	-	1	-	-	-	8	2	-	-	-	-	-	-	4	2	3	1	-	-	-	-

[a] Day of trial.

[b] Number of birds that died.

treatment but, mortality sharply increased after three or four days of exposure (Tables 21 and 22). Also, as the dietary concentration of fenthion increased, the time required for the onset of mortality decreased. Thus, the higher dietary concentrations produced mortality during the second day of exposure while the lower dietary concentrations did not produce mortality until the third or fourth days of exposure. Mortality patterns due to ANTU ingestion were similar between photoperiods, but bobwhite and mallard reactions were somewhat different. The majority of bobwhite deaths occurred during day two or day four through six, while mallard mortality began during the first 24 hours of exposure and continued through day six at a relatively constant rate (Table 23).

In general, bobwhites were more sensitive than mallards to the more toxic chemicals fenthion and endrin, but showed less sensitivity than mallards to the less toxic chemicals ANTU, secobarbital, and strychnine (Table 6).

Clinical observations of the symptoms of toxicosis for each trial are reported in Table 24.

There were no significant differences in slopes between photoperiod for any of the trials. The slopes of the dose-mortality curves were relatively uniform between photoperiod for a particular trial with the exception of the three trials in which insufficient mortality was obtained for proper analysis. The maximum difference between the dose-response slopes for the three photoperiods of a partucilar trial was 2.17-fold.

Table 21. Mortality patterns of bobwhite and mallards during LC_{50} testing with Baytex.

Dietary concentration (ppm)	Photoperiod																							
	24								14								14D							
	1[a]	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Bobwhite																								
10.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15.3	-	-	-	-	2[b]	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-
21.4	-	-	-	2	4	-	-	-	-	-	1	1	1	-	-	-	-	-	2	2	2	-	-	-
30.0	-	2	3	3	-	-	-	-	-	-	1	2	3	2	-	-	-	-	4	1	1	3	-	-
42.0	-	3	-	3	4	-	-	-	-	-	5	2	3	-	-	-	-	2	8	-	-	-	-	-
58.0	-	5	3	2	-	-	-	-	-	3	6	1	-	-	-	-	-	3	4	2	-	-	-	-
Mallard																								
37	-	-	-	-	-	-	1	-	-	-	-	-	-	2	1	-	-	-	-	1	-	-	-	-
52	-	-	-	1	-	3	-	-	-	-	-	2	1	1	-	-	-	-	3	1	2	-	-	-
73	-	-	-	1	1	1	-	-	-	-	-	-	2	2	-	-	-	-	1	2	3	-	-	-
102	-	-	-	3	2	1	-	-	-	-	-	3	1	3	-	-	-	-	-	3	2	2	-	-
143	-	-	2	2	3	1	-	-	-	-	2	2	4	-	-	-	-	3	3	3	1	-	-	-
200	-	3	1	2	3	1	-	-	-	2	2	3	2	-	-	-	-	2	3	4	1	-	-	-

[a] Day of trial.

[b] Number of birds that died.

Table 22. Mortality patterns of bobwhite and mallards during LC_{50} testing with endrin.

Dietary concentration (ppm)	Photoperiod: 24								14								14D							
	1[a]	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Bobwhite																								
7.1	-	-	-	1[b]	2	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
9.2	-	-	-	1	1	2	-	-	-	-	-	1	1	2	-	-	-	-	-	-	2	1	-	-
12.0	-	-	3	4	3	-	-	-	-	-	1	-	1	2	-	-	-	-	-	-	3	1	-	-
15.6	-	-	2	1	2	2	-	-	-	-	2	2	4	-	-	-	-	-	3	2	-	-	-	-
20.3	-	-	4	2	1	1	-	-	1	-	5	3	1	-	-	-	-	-	6	2	1	-	-	-
26.4	-	-	6	-	2	1	1	-	-	-	5	5	-	-	-	-	-	-	6	2	1	-	-	-
Mallard																								
10.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
13.5	-	-	-	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
17.5	-	-	-	1	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
22.8	-	-	-	1	-	-	-	-	-	-	1	-	1	2	-	-	-	-	1	1	1	-	-	-
29.6	-	-	-	2	1	1	-	-	-	-	1	1	2	1	-	-	-	-	1	2	2	-	-	-
38.4	-	-	1	1	4	-	-	-	-	-	-	-	4	1	1	-	-	-	4	3	-	-	-	-

[a] Day of trial.

[b] Number of birds that died.

Table 23. Mortality patterns of bobwhite and mallards during LC_{50} testing with ANTU.

Dietary concentration (ppm)	Photoperiod																											
	24								14								14D											
	1[a]	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8				
Bobwhite																												
2,000	-	1[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-				
2,800	-	1	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-				
3,920	-	1	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	2	-	1	-				
5,488	-	2	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-	-	-	-				
7,683	-	1	-	-	-	-	-	-	-	2	-	-	2	1	-	-	-	2	-	-	3	-	-	-				
10,756	-	3	-	1	-	-	-	-	-	-	-	-	2	4	-	-	-	-	1	-	-	1	-	-				
Mallard																												
2,000	1	2	-	-	-	-	-	-	1	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-				
2,800	3	1	-	3	-	-	-	-	1	2	-	-	1	1	-	-	2	-	1	-	-	1	-	-				
3,920	2	-	-	2	1	-	-	-	-	-	1	2	-	1	-	-	2	-	1	1	-	-	-	-				
5,488	2	3	-	-	-	2	-	-	-	1	-	-	1	4	-	-	2	-	1	2	1	1	-	-				
7,683	3	-	1	-	1	-	-	-	1	3	-	-	5	-	-	-	1	-	-	2	1	2	-	-				
10,756	2	-	2	2	-	2	-	-	1	-	1	2	3	2	-	-	4	-	1	1	-	2	-	-				

[a] Day of trial.

[b] Number of birds that died.

Table 24 . Observed symptoms of toxicosis for bobwhite and mallard LC_{50} trials.

Chemcial	Species	
	Bobwhite	Mallard
ANTU	ataxic[1] lethargic prostrate wing drop withdrawal	ataxic lethargic withdrawal
Fenthion	ataxic frequent falling hyperactivity inability to stand inability to walk lack of coordination lethargic wing drop withdrawal	ataxic difficulty standing lack of coordination lethargy withdrawal
Endrin	ataxic lethargic tetanic seizures wing drop withdrawal	ataxic lack of coordination lethargic tetanic seizures withdrawal
Secobarbital	ataxic lack of coordination lethargic prostrate sleeping wing drop withdrawal	ataxic lack of coordination lethargic prostrate sleeping withdrawal
Strychnine	ataxic convulsions hyperacusis hyperexcitability lack of coordination tetanic seizures tremors	ataxic convulsions lack of coordination lethargic prostrate staggers tetanic seizures withdrawal

[1] Severe ataxia; inability to carry out voluntary movements.

In four of the ten trials there was a significant photoperiod effects on feed consumption during the five-day treatment period (Table 25). Bobwhite and mallard feed consumption were significantly ($P \leq 0.05$) greater under the 24 photoperiod than under the 14 photoperiod during exposure to endrin and secobarbital (Table 25). In addition, bobwhite exposed to secobarbital consumed significantly ($P \leq 0.05$) less feed during the treatment period on the 14D photoperiod than on the 24 photoperiod.

A nonstatistical comparison of mean feed consumption indicated that the birds under continuous light consumed a greater amount of feed during the treatment period in seven out of ten trials when contrasted to the feed consumption of birds under the 14 photoperiod and nine out of ten trials when compared to birds on the 14D photoperiod (Table 25).

Both bobwhite and mallard ANTU trials resulted in significant photoperiod effects on feed consumption for the three-day recovery period (Table 26). Bobwhite exposed to ANTU consumed significantly greater ($P \leq 0.05$) amounts of feed under the 24 photoperiod than under the 14D photoperiod during the recovery period (Table 26). Similarly, mallard food consumption during the three-day recovery period following exposure to ANTU was significantly greater ($P \leq 0.05$) under the 24 and 14 photoperiods than under the 14D photoperiod (Table 26). The only other significant photoperiod effect on feed consumption during the recovery period was in the mallard-strychnine trial. During this trial ducklings on the 24 and

Table 25. Mean feed consumption (grams/bird/day) by photoperiod of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine at day five of study.

Compound	Photoperiod: 24 hours n	24 hours $\bar{X} \pm$ S.E.	14 hours n	14 hours $\bar{X} \pm$ S.E.	14 hours w/d[1] n	14 hours w/d[1] $\bar{X} \pm$ S.E.
Bobwhite						
ANTU	24	$3.9^{2}_{a} \pm 0.19$	24	$3.5_{a} \pm 0.19$	24	$3.3_{a} \pm 0.19$
Fenthion	23	$2.4_{a} \pm 0.16$	22	$2.4_{a} \pm 0.16$	23	$2.2_{a} \pm 0.16$
Endrin	24	$2.4_{a} \pm 0.10$	24	$2.0_{b} \pm 0.10$	23	$2.2_{ab} \pm 0.10$
Secobarbital	24	$5.2_{a} \pm 0.17$	21	$4.4_{b} \pm 0.18$	22	$4.1_{b} \pm 0.18$
Strychnine	21	$3.9_{a} \pm 0.24$	22	$3.7_{a} \pm 0.23$	20	$4.4_{a} \pm 0.24$
Mallard						
ANTU	24	$17.7_{a} \pm 0.88$	24	$15.9_{a} \pm 0.88$	24	$14.7_{a} \pm 0.88$
Fenthion	24	$12.7_{a} \pm 0.64$	24	$13.2_{a} \pm 0.64$	24	$12.0_{a} \pm 0.64$
Endrin	24	$30.1_{a} \pm 1.80$	24	$23.1_{b} \pm 1.80$	24	$27.4_{ab} \pm 1.80$
Secobarbital	24	$25.2_{a} \pm 1.12$	24	$20.7_{b} \pm 1.12$	24	$23.4_{ab} \pm 1.12$
Strychnine	22	$14.9_{a} \pm 1.13$	22	$15.3_{a} \pm 1.13$	22	$14.4_{a} \pm 1.13$

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Any two means in a row with the same subscript are not significantly different ($P > 0.05$).

Table 26. Mean feed consumption (grams/bird/day) by photoperiod of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine (three day recovery period).

Species compound	Photoperiod 24 hours n	24 hours $\bar{X} \pm$ S.E.	14 hours n	14 hours $\bar{X} \pm$ S.E.	14 hours w/d[1] n	14 hours w/d[1] $\bar{X} \pm$ S.E.
Bobwhite						
ANTU	16	$5.8^{2}_{a} \pm 0.24$	16	$5.1_{ab} \pm 0.24$	16	$4.9_{b} \pm 0.24$
Fenthion	12	$4.9_{a} \pm 0.37$	12	$5.1_{a} \pm 0.37$	14	$4.6_{a} \pm 0.34$
Endrin	13	$4.6_{a} \pm 0.32$	12	$4.6_{a} \pm 0.34$	16	$4.0_{a} \pm 0.39$
Secobarbital	16	$6.9_{a} \pm 0.24$	14	$6.5_{a} \pm 0.26$	16	$6.1_{a} \pm 0.24$
Strychnine	10	$6.3_{a} \pm 0.27$	12	$6.5_{a} \pm 0.25$	12	$6.6_{a} \pm 0.25$
Mallard						
ANTU	16	$44.2_{a} \pm 3.09$	16	$45.9_{a} \pm 3.09$	16	$30.5_{b} \pm 3.09$
Fenthion	15	$33.1_{a} \pm 2.80$	16	$27.2_{a} \pm 2.71$	12	$36.2_{a} \pm 3.13$
Endrin	16	$50.5_{a} \pm 3.55$	16	$47.2_{a} \pm 3.55$	16	$46.1_{a} \pm 3.55$
Secobarbital	16	$34.1_{a} \pm 1.45$	16	$29.7_{a} \pm 1.45$	16	$34.3_{a} \pm 1.45$
Strychnine	14	$40.2_{a} \pm 1.23$	14	$42.4_{a} \pm 1.23$	14	$31.0_{b} \pm 1.23$

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Any two means in a row with the same subscript are not significantly different ($P > 0.05$).

14 photoperiods consumed significantly greater ($P \leq 0.05$) quantities of feed than ducklings on the 14D photoperiod (Table 26). Generally, there was no trend toward increased feed consumption in the 24 photoperiod during the recovery period. Only three trials resulted in continuous lighting having the highest feed consumption value of the three photoperiods during recovery (Table 26).

Significant differences ($P \leq 0.05$) in initial mean body weights between photoperiods occurred in three of the ten trials (Table 27). For the trial in which ANTU was administered to bobwhite, the chicks on the 24 photoperiod were significantly heavier at the initiation of the trial than chicks on the 14 or 14D photoperiods (Table 27). Also during the ANTU-bobwhite trial, chicks on the 14 photoperiod had significantly greater initial mean body weights than chicks on the 14D photoperiod. In the mallard trials, ducklings exposed to ANTU and the 14 photoperiod were significantly heavier at the study onset than ducklings exposed to ANTU and the 24 or 14D photoperiods (Table 27). Ducklings fed the fenthion treated diets and maintained on continuous lighting had significantly lower initial body weights than birds exposed to fenthion on the 14 photoperiod (Table 27).

The birds on the 24 photoperiod had the highest initial mean body weights of the three photoperiods in 50% of the trials run, while the 14 and 14D photoperiods produced the heaviest weights in 30 and 10% of the trials, respectively (Table 27).

Table 27 . Initial mean body weights (grams) by photoperiod of bobwhites and mallards fed dietary ANTU, fenthion,endrin, secobarbital, and strychnine.

Species compound	Photoperiod 24 hours n	$\bar{X} \pm$ S.E.	14 hours n	$\bar{X} \pm$ S.E.	14 hours w/d[1] n	$\bar{X} \pm$ S.E.
Bobwhite						
ANTU	80	$22.6^{2}_{a} \pm 0.24$	80	$21.1_{b} \pm 0.24$	80	$20.0_{c} \pm 0.24$
Fenthion	80	$17.9_{a} \pm 0.30$	80	$17.7_{a} \pm 0.30$	80	$17.9_{a} \pm 0.30$
Endrin	80	$16.8_{a} \pm 0.22$	80	$16.3_{a} \pm 0.22$	80	$16.1_{a} \pm 0.22$
Secobarbital	80	$19.0_{a} \pm 0.29$	80	$18.5_{a} \pm 0.29$	80	$18.6_{a} \pm 0.29$
Strychnine	80	$17.5_{a} \pm 0.26$	80	$17.7_{a} \pm 0.26$	80	$17.3_{a} \pm 0.26$
Mallard						
ANTU	80	$120_{a} \pm 2.1$	80	$127_{b} \pm 2.1$	80	$118_{a} \pm 2.1$
Fenthion	80	$77_{a} \pm 1.5$	80	$87_{b} \pm 1.5$	80	$82_{ab} \pm 1.5$
Endrin	80	$113_{a} \pm 1.8$	80	$110_{a} \pm 1.8$	80	$110_{a} \pm 1.8$
Secobarbital	80	$91_{a} \pm 1.9$	80	$92_{a} \pm 1.9$	80	$94_{a} \pm 1.9$
Strychnine	80	$92_{a} \pm 1.2$	80	$89_{a} \pm 1.2$	80	$90_{a} \pm 1.2$

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Any two means in a row with the same subscript are not significantly different ($P > 0.05$).

On day five of the LC_{50} studies, photoperiod caused a significant ($P \leq 0.05$) alteration in body weight in seven of the trials (Table 28). In all seven trials, the 24 photoperiod produced significantly greater mean body weights than one or both of the 14-hour lighting regimes. Bobwhite on the 24 photoperiod were significantly heavier at the end of the five-day treatment period than birds under the 14 and 14D photoperiods when exposed to ANTU, fenthion, endrin, or secobarbital (Table 28). In addition, bobwhite exposed to ANTU and the 14 photoperiod were significantly heavier than birds exposed to ANTU on the 14D photoperiod (Table 28). Mallards maintained on the 14D photoperiod had significantly lighter five-day body weights than ducklings exposed to the 24 and 14 photoperiod regimes during the ANTU and strychnine trials (Table 28). During the mallard-endrin trial, ducklings under the 14 photoperiod had significantly lower body weights than ducklings under the 24 and 14D photoperiods (Table 28).

Mean body weights at the study termination (day eight) of birds exposed to the 24 photoperiod were significantly ($P \leq 0.05$) greater than at least one of the 14 hour photoperiods in five of the ten trials (Table 29). Bobwhite fed ANTU, endrin, and secobarbital treated diets produced greater body weights at day eight under the 24 photoperiod than those under either of the 14-hour lighting regimes (Table 29). The 14D photoperiod also produced significantly lower body weights than the 14 photoperiod at day eight of the fenthion and secobarbital trial. Mallards fed ANTU showed significantly higher day

Table 28. Mean body weights (grams) by photoperiod of bobwhites and mallards fed dietary ANUT, fenthion, endrin, secobarbital, and strychnine at day five of study.

Species compound	Photoperiod 24 hours n	24 hours $\bar{X} \pm$ S.E.	14 hours n	14 hours $\bar{X} \pm$ S.E.	14 hours w/d[1] n	14 hours w/d[1] $\bar{X} \pm$ S.E.
Bobwhite						
ANTU	70	28.8_a[2] ± 0.45	67	26.3_b ± 0.46	67	24.4_c ± 0.46
Fenthion	44	24.5_a ± 0.52	59	22.1_b ± 0.45	48	21.3_b ± 0.49
Endrin	44	22.1_a ± 0.47	43	20.0_b ± 0.48	52	19.9_b ± 0.43
Secobarbital	74	30.8_a ± 0.58	58	27.7_b ± 0.57	74	26.0_b ± 0.58
Strychnine	47	25.9_a ± 0.61	47	26.3_a ± 0.61	49	25.5_a ± 0.60
Mallard						
ANTU	49	141_a ± 4.8	54	137_a ± 4.5	55	123_b ± 4.5
Fenthion	56	104_a ± 3.4	54	112_a ± 3.5	42	113_a ± 3.9
Endrin	65	168_a ± 4.3	67	149_b ± 4.3	63	166_a ± 4.4
Secobarbital	67	140_a ± 4.4	75	130_a ± 4.1	64	143_a ± 4.5
Strychnine	34	143_a ± 3.7	32	137_a ± 3.8	38	128_b ± 3.5

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Any two means in a row with the same subscript are not significantly different ($P > 0.05$).

Table 29. Mean body weights (grams) by photoperiod of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine at day eight of study.

Species compound	Photoperiod: 24 hours n	24 hours $\bar{X} \pm$ S.E.	14 hours n	14 hours $\bar{X} \pm$ S.E.	14 hours w/d[1] n	14 hours w/d[1] $\bar{X} \pm$ S.E.
Bobwhite						
ANTU	70	37.8_a[2] ± 0.57	64	34.3_b ± 0.60	64	32.4_b ± 0.60
Fenthion	44	32.5_a ± 0.68	48	29.8_b ± 0.65	45	26.4_c ± 0.67
Endrin	38	29.1_a ± 0.74	41	26.6_b ± 0.71	50	26.3_b ± 0.64
Secobarbital	74	40.9_a ± 0.62	58	37.2_b ± 0.70	74	34.7_c ± 0.62
Strychnine	48	32.9_a ± 0.83	47	34.2_a ± 0.84	47	33.5_a ± 0.84
Mallard						
ANTU	44	203_a ± 7.8	45	202_{ab} ± 7.8	50	176_b ± 7.4
Fenthion	48	155_a ± 5.2	45	148_a ± 5.4	40	157_a ± 5.7
Endrin	65	219_a ± 5.5	62	208_a ± 5.6	63	217_a ± 5.5
Secobarbital	67	167_{ab} ± 5.2	75	152_a ± 4.9	64	172_b ± 5.3
Strychnine	34	181_a ± 5.0	32	173_a ± 5.2	37	171_a ± 4.8

[1] Fourteen hours light:10 hours dark with dimming and brightening of lights between light and dark periods.

[2] Any two means in a row with the same subscript are not significantly different ($P > 0.05$).

eight body weights on the 24 photoperiod than on the 14D photoperiod, while ducklings exposed to secobarbital weighed significantly more on the 14D photoperiod than ducklings on the 14 photoperiod (Table 29).

Overall, eight-day body weights were heavier on the 24 photoperiod than on either the 14 or the 14D photoperiods in seven trials, while the 14 photoperiod produced heavier day eight body weights than the 14D photoperiod in seven trials (Table 29).

Treatment effects on feed consumption for bobwhite and mallards during the five-day exposure period were highly significant ($P \leq 0.001$) in all trials except the bobwhite-secobarbital trial. The trial in which bobwhite were fed secobarbital resulted in fairly uniform feed consumption throughout all treatments, while all other trials produced a general trend for decreasing feed consumption with increasing dietary concentrations (Table 30).

The treatment effect on feed consumption for the three-day recovery period did not result in the level of significance produced during the treatment period. For the recovery period, only four trials resulted in significant differences in feed consumption between levels (Table 31) indicating feed consumption of birds exposed to the various chemicals returned to control values in most cases. Of the four trials producing significant treatment effects, only seven diets resulted in significantly ($P \leq 0.05$) lower feed consumption than the control (Table 31).

Table 30. Mean feed consumption (grams/bird/day) by treatment of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine for the five-day treatment period.

Compound	Treatment[1] 0		1		2		3		4		5		6	
	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$
Bobwhite														
ANTU	18	5.2[2] ± 0.22	9	5.0 ± 0.31	9	4.3_a ± 0.31	9	3.0_a ± 0.31	9	2.8_a ± 0.31	9	2.2_a ± 0.31	9	1.1_a ± 0.31
Fenthion	18	4.2 ± 0.18	8	3.0_a ± 0.27	9	2.2_a ± 0.25	9	1.6_a ± 0.25	9	1.2_a ± 0.25	8	0.9_a ± 0.27	7	0.6_a ± 0.29
Endrin	18	3.7 ± 0.12	8	2.6_a ± 0.17	9	2.3_a ± 0.16	9	1.9_a ± 0.16	9	1.6_a ± 0.16	9	1.0_a ± 0.16	8	0.9_a ± 0.17
Secobarbital	16	4.7 ± 0.21	9	4.7 ± 0.28	9	4.9 ± 0.28	9	5.1 ± 0.28	6	4.5 ± 0.34	9	4.6 ± 0.28	9	3.8 ± 0.28
Strychnine	16	4.6 ± 0.27	9	4.9 ± 0.36	9	4.6 ± 0.36	9	4.1 ± 0.36	9	2.9_a ± 0.36	6	2.8_a ± 0.44	5	2.3_a ± 0.48
Mallard														
ANTU	18	37.3 ± 1.02	9	15.2 ± 1.44	9	14.6_a ± 1.44	9	9.6_a ± 1.44	9	6.0_a ± 1.44	9	5.1_a ± 1.44	9	3.8_a ± 1.44
Fenthion	18	28.1 ± 0.74	9	14.1_a ± 1.05	9	10.4_a ± 1.05	9	7.6_a ± 1.05	9	8.4_a ± 1.05	9	2.9_a ± 1.05	9	1.7_a ± 1.05
Endrin	18	35.4 ± 2.07	9	27.6 ± 2.93	9	27.8 ± 2.93	9	24.6_a ± 2.93	9	20.8_a ± 2.93	9	20.8_a ± 2.93	9	22.2_a ± 2.93
Secobarbital	18	31.9 ± 1.29	9	27.6 ± 1.82	9	26.8 ± 1.82	9	23.5_a ± 1.82	9	18.1_a ± 1.82	9	14.4_a ± 1.82	9	10.6_a ± 1.82
Strychnine	18	28.7 ± 1.25	9	14.2_a ± 1.76	9	12.3_a ± 1.76	8	8.5_a ± 1.87	9	7.6_a ± 1.76	7	7.4_a ± 2.00	6	6.1_a ± 2.16

[1] Treatments 0 through 6 refer to the eight dietary concentrations used during each trial. The treatments are in increasing dietary concentrations with 0 representing the control (the two control treatments were combined into one) and 6 representing the highest concentration used. For actual trial concentrations see Table 5.

[2] Means with subscripts are significantly different from controls ($\alpha \leq 0.05$).

Table 31. Mean feed consumption (grams/bird/day) by treatment of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine for the three-day recovery period.

Compound	Treatment[1] 0		1		2		3		4		5		6	
	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE
Bobwhite														
ANTU	12	6.1[2] ± 0.28	6	5.4 ± 0.40	6	6.0 ± 0.40	6	5.3 ± 0.40	6	4.6_a ± 0.40	6	4.4_a ± 0.40	6	4.0_a ± 0.40
Fenthion	12	5.2 ± 0.20	6	4.4 ± 0.29	6	5.0 ± 0.29	6	4.4 ± 0.29	6	4.4 ± 0.29	0	---	2	6.8 ± 0.51
Endrin	12	5.0 ± 0.34	6	4.4 ± 0.48	6	4.3 ± 0.48	4	4.6 ± 0.58	6	4.4 ± 0.48	4	4.5 ± 0.58	3	2.2_a ± 0.67
Secobarbital	12	6.4 ± 0.28	6	6.4 ± 0.39	6	6.6 ± 0.39	6	6.7 ± 0.39	4	6.4 ± 0.48	6	6.5 ± 0.39	6	6.5 ± 0.39
Strychnine	12	6.2 ± 0.25	6	6.6 ± 0.35	6	6.6 ± 0.35	6	6.9 ± 0.35	4	6.2 ± 0.43	0	---	0	---
Mallard														
ANTU	12	44.8 ± 3.57	6	43.3 ± 5.04	6	57.2 ± 5.04	6	37.1 ± 5.04	6	29.6 ± 5.04	6	30.3 ± 5.04	6	34.4 ± 5.04
Fenthion	12	36.8 ± 3.13	6	32.2 ± 4.42	6	38.3 ± 4.42	6	30.1 ± 4.42	6	28.9 ± 4.42	4	26.0 ± 5.42	3	14.7_a ± 6.26
Endrin	12	45.4 ± 5.80	6	46.0 ± 5.80	6	45.1 ± 5.80	6	46.4 ± 5.80	6	47.9 ± 5.80	6	54.2 ± 5.80	6	52.9 ± 5.80
Secobarbital	12	31.5 ± 2.36	6	30.5 ± 2.36	6	32.4 ± 2.36	6	29.9 ± 2.36	6	32.1 ± 2.36	6	38.7 ± 2.36	6	35.2 ± 2.36
Strychnine	12	40.1 ± 1.33	6	36.6 ± 1.88	6	41.8 ± 1.88	6	47.0_a ± 1.88	6	36.2 ± 1.88	6	23.1_a ± 1.88	0	---

[1] Treatments 0 through 6 refer to the eight dietary concentrations used during each trial. The treatments are in increasing dietary concentrations with 0 representing the control (the two control treatments were combined into one) and 6 representing the highest concentration used. For actual trial concentrations see Table 5.

[2] Means with subscripts are significantly different from control ($\alpha \leq 0.05$).

Significant differences in initial mean body weights between treatments occurred in the bobwhite-ANTU trial, mallard-ANTU trial, and mallard-secobarbital trial (Table 32). The LC_{50} tests in which bobwhite were exposed to ANTU resulted in the number three and four (3,920 and 5,488 ppm, respectively) treatment birds being significantly ($P \leq 0.05$) lower in initial body weights than the control birds (Table 32).

Conversely, in the mallard trial using ANTU, the control birds were significantly ($P \leq 0.05$) lower in initial body weights than the number one, two, three, and six treatment (2,000, 2,800, 3,920, and 10,756 ppm, respectively) birds (Table 32). The mallard-secobarbital trial started with the number four treatment (5,488 ppm) significantly ($P \leq 0.05$) lower in initial mean body weight than the control (Table 32).

All ten LC_{50} trials resulted in significant treatment effects on body weights at day five and day eight of the tests (Table 33 and 34), with most chemically-treated diets producing significantly lower body weights than the control diets.

Significant photoperiod-dietary concentration interaction occurred in initial, five-day, and eight-day body weights of bobwhite fed endrin and in feed consumption during the three-day recovery period of mallards fed strychnine. The interactions were attributed to inconsistent effects of dietary treatments across dietary concentrations and between photoperiods. Body weights and feed consumption were highly

Table 32. Initial mean body weights (grams) by treatment of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine.

Compound	Treatment[1] 0		1		2		3		4		5		6	
	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$
Bobwhite														
ANTU	60	21.9[2] ± 0.28	30	21.2 ± 0.39	30	21.1 ± 0.39	30	20.6_a ± 0.39	30	20.3_a ± 0.39	30	21.3 ± 0.39	30	21.4 ± 0.39
Fenthion	60	17.5 ± 0.34	30	17.6 ± 0.49	30	18.3 ± 0.49	30	18.0 ± 0.49	30	18.3 ± 0.49	30	18.1 ± 0.49	30	17.4 ± 0.49
Endrin	60	16.2 ± 0.25	30	16.4 ± 0.36	30	16.4 ± 0.36	30	16.4 ± 0.36	30	16.2 ± 0.36	30	16.4 ± 0.36	30	16.6 ± 0.36
Secobarbital	60	18.8 ± 0.33	30	19.9 ± 0.47	30	18.9 ± 0.47	30	18.0 ± 0.47	30	18.2 ± 0.47	30	18.3 ± 0.47	30	19.0 ± 0.47
Strychnine	60	17.4 ± 0.30	30	17.6 ± 0.43	30	17.4 ± 0.43	30	17.9 ± 0.43	30	17.5 ± 0.43	30	17.5 ± 0.43	30	17.0 ± 0.43
Mallard														
ANTU	60	113 ± 2.4	31	126_a ± 3.3	30	126_a ± 3.4	29	124_a ± 3.4	30	119 ± 3.4	30	123 ± 3.4	30	129_a ± 3.4
Fenthion	60	79 ± 1.7	30	84 ± 2.4	30	80 ± 2.4	30	84 ± 2.4	30	83 ± 2.4	30	84 ± 2.4	30	85 ± 2.4
Endrin	60	110 ± 2.1	30	113 ± 3.0	30	111 ± 3.0	30	110 ± 3.0	30	114 ± 3.0	30	110 ± 3.0	30	113 ± 3.0
Secobarbital	60	95 ± 2.2	30	96 ± 3.1	30	89 ± 3.1	30	91 ± 3.1	30	83_a ± 3.1	30	94 ± 3.1	30	96 ± 3.1
Strychnine	60	89 ± 1.4	30	90 ± 2.0	30	91 ± 2.0	30	89 ± 2.0	30	89 ± 2.0	30	90 ± 2.0	30	92 ± 2.0

[1] Treatments 0 through 6 refer to the eight dietary concentrations used during each trial. The treatments are in increasing dietary concentrations with 0 representing the control (the two control treatments were combined into one) and 6 representing the highest dietary concentration used. For actual trial concentrations see Table 5.

[2] Means with subscripts are significantly different from controls ($\alpha \leq 0.05$).

Table 33. Mean body weights (grams) by treatment of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine (day five of study).

Compound	Treatment[1] 0			1			2			3			4			5			6		
	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE
Bobwhite																					
ANTU	60	34.4[2]	± 0.49	26	30.0_a	± 0.74	27	28.3_a	± 0.72	25	24.6_a	± 0.75	26	20.5_a	± 0.74	19	17.8_a	± 0.86	21	15.7_a	± 0.82
Fenthion	60	27.4	± 0.44	30	24.4_a	± 0.63	26	19.0_a	± 0.67	16	17.4_a	± 0.86	10	16.0_a	± 1.08	0	---		1	13.7	± --
Endrin	60	24.1	± 0.40	24	19.4_a	± 0.64	24	19.9_a	± 0.64	13	16.7_a	± 0.87	12	14.4_a	± 0.90	4	14.8_a	± 1.57	2	14.4	± 2.22
Secobarbital	58	29.4	± 0.57	29	30.4	± 0.80	30	27.4	± 0.79	26	27.8	± 0.85	19	27.6	± 0.99	24	27.5	± 0.88	20	25.1_a	± 0.97
Strychnine	60	27.3	± 0.54	29	25.4	± 0.78	26	26.1	± 0.82	19	23.1_a	± 0.96	7	21.1_a	± 1.58	2	15.2_a	± 2.95	0	---	
Mallard																					
ANTU	60	193	± 4.3	25	119_a	± 6.7	16	111_a	± 8.3	17	93_a	± 8.1	17	79_a	± 8.1	12	81_a	± 9.6	11	77_a	±10.0
Fenthion	60	142	± 3.3	29	99_a	± 4.7	20	83_a	± 5.7	20	87_a	± 5.7	16	81_a	± 6.4	5	73_a	±11.4	2	74_a	±18.1
Endrin	60	200	± 4.5	29	163_a	± 6.5	27	164_a	± 6.7	25	144_a	± 7.0	24	126_a	± 7.1	17	119_a	± 8.4	13	117_a	± 9.7
Secobarbital	60	153	± 4.6	29	146	± 6.6	29	144	± 6.6	25	149	± 7.2	27	118_a	± 6.9	19	111_a	± 8.2	17	101_a	± 8.7
Strychnine	60	161	± 2.8	18	108_a	± 5.0	11	108_a	± 6.5	5	96_a	± 9.6	6	93_a	± 8.7	4	90_a	±10.7	0	---	

[1] Treatments 0 through 6 refer to the eight dietary concentrations used during each trial. The treatments are in increasing dietary concentrations with 0 representing the control (the two control treatments were combined into one) and 6 representing the highest concentration used. For actual trial concentrations see Table 5.

[2] Means with subscripts are significantly different from controls ($\alpha \leq 0.05$).

Table 34. Mean body weights (grams) by treatment of bobwhites and mallards fed dietary ANTU, fenthion, endrin, secobarbital, and strychnine (day eight of study).

	Treatment[1]																			
	0		1		2		3		4		5		6							
Compound	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE						
Bobwhite																				
ANTU	60	43.2[2] ± 0.62	26	37.7_a ± 0.94	27	36.5_a ± 0.92	23	32.4_a ± 1.00	25	28.7_a ± 0.96	19	24.9_a ± 1.10	18	23.3_a ± 1.13						
Fenthion	60	34.5 ± 0.58	30	26.7_a ± 0.82	26	26.4_a ± 0.89	15	24.3_a ± 1.16	5	22.9_a ± 2.02	0	---	1	16.1 ± --						
Endrin	60	30.6 ± 0.59	24	26.4_a ± 0.93	19	24.9_a ± 1.04	12	23.4_a ± 1.31	10	21.1_a ± 1.44	3	19.6_a ± 2.62	1	21.7 ± --						
Secobarbital	58	38.7 ± 0.70	29	39.9 ± 0.99	30	37.3 ± 0.98	26	36.8 ± 1.05	19	37.1 ± 1.23	24	36.8 ± 1.09	20	34.6_a ± 1.20						
Strychnine	60	34.9 ± 0.74	29	31.4_a ± 1.07	26	35.0 ± 1.13	19	31.8 ± 1.32	7	29.6 ± 2.17	0	---	0	---						
Mallard																				
ANTU	60	231 ± 6.7	24	187_a ± 10.7	14	186_a ± 14.0	16	153_a ± 13.1	10	149_a ± 16.5	10	132_a ± 16.5	5	119_a ± 23.4						
Fenthion	60	180 ± 4.6	25	140_a ± 7.2	16	139_a ± 9.0	17	122_a ± 8.7	10	127_a ± 11.4	4	102_a ± 18.0	1	102 ± --						
Endrin	60	240 ± 5.7	29	203_a ± 8.2	27	220 ± 8.5	25	199_a ± 8.6	22	192_a ± 9.4	16	200_a ± 11.0	11	201_a ± 13.3						
Secobarbital	60	178 ± 5.5	29	162 ± 7.9	29	164 ± 7.9	25	170 ± 8.5	27	147_a ± 8.2	19	152 ± 9.8	17	142_a ± 10.3						
Strychnine	60	188 ± 3.8	18	161_a ± 6.9	11	171 ± 8.9	4	151 ± 14.7	6	139_a ± 12.0	4	125_a ± 14.7	0	---						

[1] Treatments 0 through 6 refer to the eight dietary concentrations used during each trial. The treatments are in increasing dietary concentrations with 0 representing the control (the two control treatments were combined into one) and 6 representing the highest concentration used. For actual trial concentrations see Table 5.

[2] Means with subscripts are significantly different from controls ($\alpha \leq 0.05$).

irregular and did not follow the decreasing weights with increasing dietary concentration noted in all other studies.

Mean room temperature, brooder temperature, and room relative humidity are presented in Appendix C.

DISCUSSION

The avian subacute dietary LC_{50} is a fundamental parameter which may be required by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substance Control Act (TSCA) as a routine data point in evaluating the hazards of pesticides and other toxic chemicals when dietary exposure is the most probable route of intoxication. In addition to the subacute lethality and species susceptibility data, the LC_{50} provides a method for the comparisons of the short-term dietary toxicity of chemicals to a given species or between various species. Some authors have devised a toxicity ranking system, based on the LC_{50}, to categorize the inherent toxicity of compounds in an attempt to establish and predict the relative chemical toxicities by and within chemical classes (Heath et al., 1972). In addition, the slopes of the dose-response curves can be used to establish the rate of increase of mortality resulting from a proportional increase in exposure. The greater the slope, the more rapid is the increase in mortality and the lower the margin of safety.

When chemical contamination in the bird's natural diet can be established by analytical techniques, the degree of hazard may be predicted by comparing the concentration of the

chemical in the natural diet to the lethality of dietary concentrations in laboratory studies. When the contamination of natural diets cannot be determined, the potential lethality of the compound may be estimated by comparing the chemical in question with a chemical in which the field mortality is known for specific application rates. For example, if the LC_{50} for compound A and B are 10 and 20 ppm, respectively, and the field mortality of a species for compound B is known at various application rates or general residue levels, the potential mortality caused by compound A should be predictable. Certain authors use a similar analogy when they attempt to develop analytical methods of predicting lethality values, for a particular species, on the chemical and physical properties of toxic compounds (Rekker, 1980). Of key importance to the value of any classification or prediction scheme is the reliability and repeatability of the results.

There are several established and acceptable methods of analysis of the dose-mortality response. Three accepted and frequently used methods are the probit analysis of Litchfield and Wilcoxon (1948), the analysis of the regression of percent mortality on dose (Boyd, 1958; Gill, 1978), and the regression of dose on percent mortality (Boyd, 1965; Gill, 1978; Montgomery and Peck, 1982). Boyd (1972) states that values for the LD_{50} determined by one method have been found to be repeatedly significantly different from values calculated by another method with experiments run in his laboratory.

In looking at the analysis of data from this experiment using the Litchfield and Wilcoxon procedure, the variation between LC_{50} values of the three photoperiods in a particular trial, in which sufficient mortality was obtained to accurately calculate an LC_{50} value, was relatively low. The LC_{50} ratios between photoperiods for these trials ranged from 1.00:1.15 to 1.00:1.52, which was somewhat smaller than the range in LC_{50} values of intratrial replications (1.00:1.02 to 1.00:1.89) reported by Hill and Camardese (1981) in studies exposing Japanese quail (Coturnix coturnix japonica) to carbofuran, dicrotophos, and thionazin. LC_{50} variations between photoperiods were also considerably lower than the two-fold and four-fold variations in LC_{50} values of intertrial LC_{50} replications exposing bobwhite and mallards, respectively, to dieldrin (Heath et al., 1972). The variations between photoperiod for a specific chemical and species using the direct regression method ranged from 1.00:1.08 to 1.00:1.42 which was also considerably less than the intratrial replication of Hill and Camardese (1981) and the intertrial replication of Heath et al. (1972). The inverse prediction of the regression of mortality on dose resulted in greater photoperiodic variation 1.00:1.09 to 1.00:2.34 in LC_{50} values within a trial than the intratrial variation of Hill and Camardese but produced intermediate variations to the intertrial replications of Hill et al. (1975). The variation in LC_{50} values between analytical methods ranged from 1.00:1.03 to 1.00:2.39 and was also greater than, and intermediate to the intratrial

and intertrial variance, respectively, of Hill and Camardese (1981) and Hill et al. (1975). Although there were considerable variations in LC_{50} determinations between analytical methods, the LC_{50} values calculated in this study for endrin and fenthion were in agreement with values published in the literature with the exception of the LC_{50} of fenthion for mallards which was somewhat lower in this study than that published in the literature.

The slope of the dose-response curves for a particular trial also showed less variation between photoperiod than the variation of intra and intertrial replication reported by Hill and Camardese (1981). The maximum variation in slopes of the dose-response curves between photoperiods of a trial was 2.17-fold in comparison to the 2.64 and 4.30-fold variations obtained in intra and intertrial replications, respectively, of Hill and Camardese (1981).

Due to the considerable variation in photoperiod effects on mortality between the two species and five chemicals tested and the general inability to obtain significant differences in LC_{50} values between photoperiods, it must be concluded that photoperiod did not affect LC_{50} determinations in this study. Although feed consumption and body weights, especially during the five-day treatment period, proved on occasion to be significantly greater on the 24 photoperiod than the 14-hour photoperiods, the effects of increased chemical intake or altered feeding patterns were not strong enough to significantly affect mortality in these studies. Also, the significant differences in feed consumption and body weight between the two 14-hour

photoperiods were less frequent than the significant differences between the 24-hour and 14-hour photoperiods. Additional studies utilizing intratrial replication of photoperiod or alternate chemcials may prove otherwise, but based on this study and the data supplied by Heath et al. (1972), Hill et al. (1975), and Hill and Camardese (1981), it appears that intertrial variability of LC_{50} testing and analytical methods of analysis causes greater problems in evaluating and classifying chemical toxicities than does photoperiodic effects.

CONCLUSIONS

1. Photoperiod significantly affected feed consumption during the five-day treatment and three-day recovery periods. The 24-hour photoperiod tended to result in significantly greater feed consumption than either of the two 14-hour photoperiods. Significant differences in feed consumption between the 14 and 14D photoperiods were less frequent than significant differences between the 24 and 14 or 24 and 14D photoperiods.
2. Photoperiod significantly affected body weights at day 0, 5, and 8 of the trial. The 24-hour photoperiod tended to produce significantly greater body weights than the 14-hour photoperiods. Again, the frequency of significant differences between the 14-hour photoperiods was less than the frequency of significant differences between the 24-hour and 14-hour photoperiods.
3. Mortality patterns and symptoms of toxicosis were generally similar between the 24, 14, and 14D photoperiods.
4. Photoperiod did not significantly affect LC_{50}s.

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APPENDIX A

Composition of quail starter.

Ingredient	Parts per kg
Corn, #2 yellow	375.2
Soybean meal, dehulled (49% protein)	420
Dist. dried grains solubles, corn	40
Fish meal	40
Alfalfa meal, dehy. (17% protein)	50
Animal fat, stabl.	37.6
Dicalcium phosphate	20.0
Choline chloride (50%)	3.0
Methionine hydroxy analogue	0.7
Salt	3.5
Mineral mix[a]	5.0
Vitamin mix[b]	5.0

[a] Mineral mix: Supplies per kg diet: Cobalt, 50 mcg; Manganese, 55 mg; Magnesium, 500 mg; Iron, 80 mg; Copper, 4 mg; Zinc, 80 mg; Selenium, (from sodium selenite), 0.1 mg; Carrier (dist. dried sol., corn with 4% tallow) to 5.0 g.

[b] Vitamin mix: Supplies per kg diet: Vitamin A, 15,000 I.U.; Vitamin D_3, 1,500 I.C.U.; Vitamin E, 15 I.U.; Vitamin K (menadione sodium bisulfite complex), 2.7 mg; Thiamine, 6.0 mg; Riboflavin, 10.0 mg; Niacin, 100.0 mg; Pyridoxine, 10.0 mg; Biotin, 220 mcg; Folacin, 5.0 mg; Vitamin B_{12}, 11.0 mcg; Carrier (dist. dried sol., corn with 4% tallow) to 5.0 g.

APPENDIX B

Composition of duck starter.

Ingredient	Parts per kg
Corn, #2 yellow	503.1
Soybean meal (48% protein)	310
Alfalfa (17% protein)	50
Wheat bran	60
Corn oil, stabl.[a]	40
dl-methionine	0.9
Limestone	5
Dicalcium phosphate	22
Salt	3.0
Choline Cl_2, 50%	3.0
Vitamin mix[b]	3.0
Mineral mix[c]	0.5
Selenium mix[d]	0.5

[a] Ethoxyquin added at 125 mg/kg diet.

[b] Vitamin mix: Supplies per kg diet: Vitamin A, 15,000 I.U.; Vitamin D_3, 1,500 I.C.U.; Vitamin E, 15 I.U.; Vitamin K (menadione sodium bisulfite complex), 2.7 mg; Thiamine, 6.0 mg; Riboflavin, 10.0 mg; Niacin, 100.0 mg; Pyridoxine, 10.0 mg; Biotin, 220 mcg; Folacin, 5.0 mg; Vitamin B_{12}, 11.0 mcg; Carrier (dist. dried sol., corn with 4% tallow) to 3.0 g.

[c] Mineral mix: Supplies per kg diet: Cobalt, 50 mcg; Manganese, 55 mg; Magnesium, 500 mg; Iron, 80 mg; Copper, 4 mg; Zinc, 80 mg; Selenium, (from sodium selenite), 0.1 mg; Carrier (dist. dried sol., corn with 4% tallow) to 0.50 g.

[d] From Calcium Carbonate Co. at recommended levels.

APPENDIX C

Room temperature[1], brooder temperature[1], and room relative humidity[2] during LC_{50} testing.

Chemical	Photoperiod: 24 — Room temp	24 — Brooder temp	24 — Relative humidity	14 — Room temp	14 — Brooder temp	14 — Relative humidity	14D — Room temp	14D — Brooder temp	14D — Relative humidity
Bobwhite									
ANTU	76[3] ± 0.9	91 ± 0.4	39 ± 0.8	74 ± 1.2	91 ± 0.2	42 ± 1.3	75 ± 1.3	91 ± 0.2	37 ± 1.1
Fenthion	73 ± 0.5	91 ± 0.3	43 ± 0.9	73 ± 0.5	91 ± 0.3	37 ± 0.9	74 ± 0.4	91 ± 0.3	41 ± 0.9
Endrin	74 ± 0.3	92 ± 0.3	41 ± 1.0	73 ± 0.5	91 ± 0.6	43 ± 0.9	74 ± 0.4	91 ± 0.2	36 ± 1.3
Secobarbital	74 ± 0.7	91 ± 0.2	40 ± 0.6	76 ± 0.4	90 ± 0.2	36 ± 0.4	75 ± 1.4	91 ± 0.8	37 ± 1.5
Strychnine	72 ± 1.0	91 ± 0.3	44 ± 1.0	72 ± 0.6	91 ± 0.3	44 ± 1.2	72 ± 0.7	91 ± 0.2	44 ± 1.0
Mallard									
ANTU	74 ± 1.0	91 ± 0.3	56 ± 2.2	71 ± 1.6	91 ± 0.4	58 ± 1.4	71 ± 1.0	91 ± 0.4	43 ± 0.5
Fenthion	77 ± 1.7	90 ± 0.2	79 ± 1.2	77 ± 2.0	91 ± 0.2	73 ± 0.8	76 ± 1.8	91 ± 0.3	79 ± 0.9
Endrin	73 ± 0.8	90 ± 0.3	64 ± 1.4	72 ± 0.6	91 ± 0.4	62 ± 0.9	71 ± 0.5	90 ± 0.3	62 ± 1.0
Secobarbital	74 ± 1.2	90 ± 0.5	60 ± 1.0	77 ± 1.0	90 ± 0.4	55 ± 1.0	74 ± 0.6	90 ± 0.3	55 ± 1.2
Strychnine	71 ± 0.8	92 ± 0.5	73 ± 0.9	72 ± 0.6	91 ± 0.3	71 ± 0.7	72 ± 0.4	91 ± 0.3	72 ± 1.2

[1] Degrees fahrenheit.

[2] Percent.

[3] Mean ± standard error.