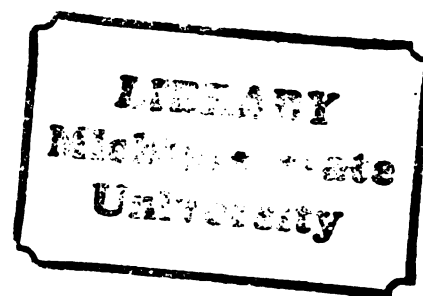


THESIS



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SUBACUTE AND CHRONIC DIETARY TOXICITY OF
ETHALFLURALIN TO MALLARDS AND BOBWHITES

presented by

William J. Breslin

has been accepted towards fulfillment
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M.S. degree in Animal Science

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SUBACUTE AND CHRONIC DIETARY TOXICITY OF
ETHALFLURALIN TO MALLARDS AND BOBWHITES

by

William J. Breslin

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

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ABSTRACT

SUBACUTE AND CHRONIC DIETARY TOXICITY OF ETHALFLURALIN TO MALLARDS AND BOBWHITES

Ethalfluralin was evaluated for its subacute and chronic reproductive dietary toxicity to mallards (Anas platyrhynchos) and bobwhites (Colinus virginianus).

Mallards and bobwhites fed ethalfluralin in the diet at 30, 100, 300, 1,000, 3,000, or 10,000 ppm for two and three week periods, respectively, showed no signs of intoxication, morbidity, increased mortality, or weight loss. Feed consumption of mallards fed ethalfluralin up to 10,000 ppm in the diet was not significantly reduced. Bobwhites on 10,000 ppm ethalflurlin diets significantly decreased feed consumption one week after the administration of treated feed, but feed consumption returned to normal the following week.

Administration of ethalfluralin to mallards and bobwhites at 100, 300, or 1,000 ppm for 28 and 24 week periods, respectively, did not significantly affect behavior, mortality of adults, egg production, fertility, embryo survival, hatchability, egg shell thickness, or duckling and chick survivability. Ethalfluralin did not produce pathological tissue alterations when fed at these concentrations.

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INTRODUCTION

Ethalfluralin is a experimental dinitroaniline selective herbicide for the pre-emergent control of certain annual grasses and broadleaf weeds. It was developed by Eli Lilly and Company in the late 1960's as a variation of the substituted 2,6-dinitroaniline herbicide trifluralin. Ethalfluralin was synthesized in an attempt to develop a product with shorter soil residual properties and improved control of black nightshade (Solanum nigrum).

In fulfillment of one of the Environmental Protection Agency's requirements for registering pesticides in the United States, Eli Lilly and Company awarded a contract to Michigan State University for the purpose of testing the effects of ethalfluralin on chronic avian reproduction. The two test species used were the bobwhite (Colinus virginianus) and the mallard (Anas platyrhynchos).

Under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) the Environmental Protection Agency is required to publish guidelines stating the kinds of information that will be required to support the registration of a pesticide (Federal Register, 1975a). This required information can be categorized into four broad sections: product performances, label development, chemistry, and hazard evaluation (Federal Register, 1975b). At present these guidelines, which are published in the Federal Register, are not final, and information required on a specific pesticide

is determined on a case-by-case basis (Federal Register, 1975b).

The chronic avian reproduction test is required if any of the following conditions exist: (1) "The pesticide or any of its major metabolites or degradation products is persistent in the environment to the extent that toxic amounts in avian feed could be expected to become present under normal pesticide use"; (2) "The pesticide, or any of its major metabolites or degradation products, is stored or accumulated in plant or animal tissues"; (3) "The pesticide product is intended for use under conditions where birds may be subjected to repeated or continued exposure to the pesticide or any of its major metabolites or degradation products"; and (4) "Any other test information which indicates that reproduction of birds may be adversely affected by a pesticide or by product use" (Federal Register, 1978). Condition three may exist with normal use of ethalfluralin. This herbicide has the potential to be used in a way birds may be subjected to repeated or continuous exposure.

Bobwhites and mallards were selected as test animals because they are endemic to the continental United States, represent upland and aquatic forms of avian wildlife, and are readily available for toxicological testing.

LITERATURE REVIEW

The majority of literature available on the dinitro-anilines is directed towards trifluralin. Since ethalfluralin is still in the experimental stage of development and little

literature has been released, this review will focus on the closely related substituted 2,6-dinitroaniline herbicides in general. Data on ethalfluralin will be presented when available.

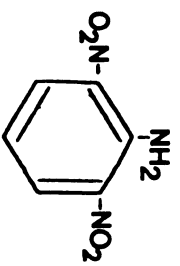
Physical and Chemical Properties

Ethalfluralin (N-ethyl-N-(2 methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine) is produced by Eli Lilly and Company (Elanco) under the product name Solalan[®]. Its molecular formula is $C_{13}H_{14}F_3N_3O_4$, has a molecular weight of 333.3 g/M, and a specific gravity of 1.32 g/ml (see Figure 1 for the structural formula). Ethulfluralin is a yellow-orange crystalline solid with a melting point of 55-56°C and a vapor pressure of 8.2×10^{-5} mmHg at 25°C. Its thermal decomposition temperature is 256°C. The solubility in water at pH 7 and 25°C is 0.3 ppm. The chemical is readily soluble (> 500 mg/ml) in organic solvents such as acetone, acetonitrile, benzene, chloroform, methylene chloride, and xylene. Its solubility in methanol is 80-100 mg/ml with a partition coefficient of 5.11. This herbicide is susceptible to decomposition from ultraviolet light (Elanco, 1977, 1979).

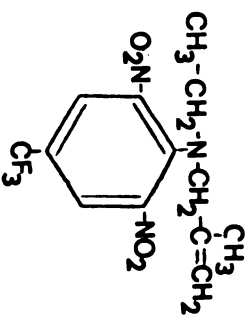
Table 1 lists the physical and chemical properties of three major dinitroaniline herbicides. Most of the dinitroaniline herbicides, especially the substituted 2,6-dinitroanilines, have very similar physical and chemical properties.

The first member in the class of herbicides labeled as substituted 2,6-dinitroanilines was introduced by Lilly

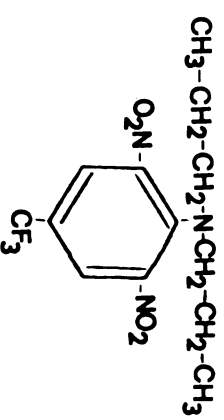
Figure 1. Structure of 2,6-dinitroaniline, ethalfluralin, and trifluralin.



2,6-DINITROANILINE



ETHYLFLURALIN



TRIFLURALIN

Table 1. Chemical and physical properties of the dinitroaniline herbicides ethalfluralin, trifluralin, and benefin.

	Ethalfluralin			Trifluralin		Benefin	
	N-ethyl-n-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzen-amine			α,α-trifluoro-2,6-dinitric-n,n-dipropyl-p-toluidine		N-butyl-n-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine	
Trade name	Sonalan			Treflan		Balan	
Empirical formula	C ₁₃ H ₁₄ F ₃ N ₃ O ₄			C ₁₃ H ₁₆ F ₃ N ₃ O ₄		C ₁₃ H ₁₆ E ₃ N ₃ O ₄	
Physical state	Yellow crystalline solid			Orange crystalline solid		Yellow crystalline solid	
Melting point	55-57°C			48-49°C		65-66°C	
Vapor pressure	8.2 x 10 ⁻⁵ mmHg at 25°C			19.9 x 10 ⁻⁵ mmHg at 25°C		3.9 x 10 ⁻⁵ mmHg at 25°C	
Partition coefficient	5.11			5.07		5.25	
Solubility in water (ph 7 and 25°C)	0.3 ppm			0.3 ppm		0.1 ppm	
Organic solvent							
Solubility (25°C) mg/ml							
Acetone	>500			>500		>500	
Acetonitrile	>500			>500		>250	
Benzene	>500			>500		>500	
Chloroform	>500			>500		>500	
Hexane	80-100			>500		55-70	
Xylene	>500			>500		>500	
Methanol	80-100			125-250		33-55	
Stability in ultraviolet light	Susceptible to decomposition			Susceptible to decomposition		Susceptible to decomposition	
Stability to hydrolysis (0.5 ppm in 10% aqueous MeOH, 25°C)	pH 3 = 3.8 weeks pH 6 = 2.0 pH 9 = 2.0			pH 3 = 3.0 pH 6 = 2.0 pH 9 = 1.2		---	
Approximate half-life							
Thermal decomposition	256°C			275°C		---	

Research Laboratories in 1960 (Alder et al., 1960). There are now at least 14 dinitroanilines on the market or in product development. The development started with the discovery of 2,6-dinitroaniline (Figure 1), an intermediate in the production of other herbicides. 2,6-dinitroaniline was tested and found to have good non-specific herbicidal activity. Further investigations showed that the two nitro groups on the ring had a major role in the herbicide's activity. When the nitro groups were in the two and six positions the activity was enhanced. Substituting groups on the amine portion of the molecule also contributed to the activity of the herbicides. When R1 and R2 were both alkyls the products were more specific pre-emergence herbicides. Alkyls with three to eight carbons gave the best activity (Parka et al., 1976).

The most recent major discovery in the development of the 2,6-dinitroanilines came with the addition of a CF₃ group in the fourth position of the ring. Adding CF₃ gave many of the dinitroanilines maximal activity (Parka et al., 1976).

Efficacy

Ethalfluralin has been shown to control many weed species as they germinate, with little or no damage to crops for which the herbicide was intended [soybeans (Glycine max), peanut (Arachis hypogaea), cotton (Gossypium Sp.), peas (Pisum Sp.), edible beans, and cucurbits]. Table 2 lists the annual grasses and broadleaf weed for which ethalfluralin provides commercially acceptable control (Elanco, 1977). In

Table 2. Annual grasses and broadleaf weeds for which ethalfluralin provides commercially acceptable control.

Annual grasses		Annual broadleaf weeds	
Common name	Scientific name	Common name	Scientific name
Annual bluegrass	(<u>Poa annua</u>)	Carpetweed	(<u>Mollugo verticillate</u>)
Barnyard grass	(<u>Echinochloa crus-galli</u>)	Common chickweed	(<u>Stellaria media</u>)
Brome grasses	(<u>Bromus sp.</u>)	Common purslane	(<u>Portulaca oleracea</u>)
Crab grasses	(<u>Digitaria sp.</u>)	Conical catchfly	(<u>Silene conica</u>)
Foxtails	(<u>Setaria sp.</u>)	Florida pusley	(<u>Richardia scabra</u>)
Goose grass	(<u>Eleusine indica</u>)	Goosefoot	(<u>Shenopodium hybridum</u>)
Italian rye grass	(<u>Lolium multiflorum</u>)	Henbit	(<u>Lamium amplexicaule</u>)
Johnson grass	(<u>Sorghum halepense</u>)	Knotweed	(<u>Polygonum gviculare</u>)
Junglerice	(<u>Echinochloa colonum</u>)	Kochia	(<u>Kochia scoparia</u>)
Paniciums	(<u>Panicum sp.</u>)	Lambsquarters	(<u>Chenopodium album</u>)
Sandbar	(<u>Cenchrus incertus</u>)	Nightshades	(<u>Solanum sp.</u>)
Sprangletop	(<u>Leptochlog filiformis</u>)	Pigweeds	(<u>Amaranthus sp.</u>)
Stink grass	(<u>Eragrostis cilianensis</u>)	Russian thistles	(<u>Salsob kali</u>)
Wild oat	(<u>Avena fatua</u>)		

cucurbit studies Humphreys et al. (1978) reported excellent control of large crabgrass (Digitaria sanguinalis), goosegrass (Eleusine indica), foxtail millet (Setario italica), and red-root pigweed (Amaranthos retroflexos) on soils ranging from sands of south and middle Florida to sandy clay loam in Texas, silt loam in Mississippi, and loams sands of Georgia and North Carolina at rates of 0.5 to 1.25 lbs/A, the recommended rate of application. All cucurbits showed good tolerance at these rates with no significant reduction in yields over the control. Other studies by Murphy and Marrow (1978) and Ilnicki et al. (1977) on potatoes (Solanum tuberosum) and Kenof (Hibiscus cannabinus) reported that ethalfluralin gave good weed control on a variety of weeds with little or no damage to the crop. Humphreys et al. (1978) reported that ethalfluralin, at higher rates than recommended (1.0 to 1.75 lbs/A) for most weeds, gave improved control of black nightshade as compared to other dinitroaniline herbicides.

Mode of Action

The dinitroanilines are classified as mitotic poisons. It is widely recognized that the major morphological effect of these herbicides in plants is swelling of the root tip. This swelling can be attributed to the inhibition or cessation of cell division with a continuation of radial expansion (Bayer et al., 1967). These herbicides interrupt both cortical and spindle microtubules in root cells (Parka and Soper, 1977).

The mitotic process is interrupted in metaphase, anaphase, and telophase, those phases which require the presence of microtubules. When microtubules are absent, the cells begin normal division with prophase, but fail to align along the metaphase plate, resulting in aberrant mitotic figures. When microtubules are present prior to cell division the mitotic process is arrested at the stage that had been reached when the disappearance had occurred (Parka and Soper, 1977).

The dinitroaniline's effect on chromosomes resembles the effects produced by colchicine, a known cortical and spindle microtubule inhibitor. While these herbicides may act similar to colchicine in plants their actions are different in animals. In vitro polymerization studies with pig brain microtubules showed that trifluralin, orizaline and propham, all dinitroanilines, did not inhibit polymerization of microtubular protein while colchicine did. Colchicine binds to a different site on the tubulin molecule than the herbicides (Bartels and Hilton, 1973).

Moreland et al. (1972) reported the effects of 12 substituted 2,6-dinitroaniline herbicides in isolated spinach (Spinacia oleracca) chloroplasts, and mungbean (Phascolus ourcus) mitochondria. Both photoreduction and coupled photophosphorylation were inhibited in chloroplasts by all the herbicides. In mitochondria, 11 of the dinitroanilines inhibited phosphorylating electron transport with malate as a substrate. Most were also strong inhibitors of NADH and succinate oxidation. Moreland et al. demonstrated that if

these herbicides became incorporated into the organelles, photosynthesis and respiration were inhibited. Since plants depend biochemically and physiologically on oxidation and phosphorylation for energy, inhibition of these pathways may also be a major mechanism of the dinitroaniline's phytotoxicity.

Some dinitroanilines are also known to inhibit the proteolytic enzymes, phytase and dipeptidase. The combined reduction of these enzymes is sufficient to influence seedling growth (Parka and Soper, 1977).

Absorption, Translocation and Metabolism in Plants

It is currently thought that the major site of absorption of the dinitroaniline herbicides is through the shoot of monocots and the hypocotyl of dicots (Nishimoto and Warren, 1971). Other studies conducted on grain crops have supported these areas as the site of uptake (Parka and Soper, 1977).

The translocation of the dinitroanilines is concentrated in the root areas of the plant, although some acropetal and basipetal movement has been reported when the herbicides were applied in a topical fashion (Kecherside et al., 1969). When soybeans and cotton were treated with ^{14}C -trifluralin the majority of radioactivity was found on the root surface and in the root walls of the xylem vessels. There was little movement out of the roots. Further examination found that the majority of radioactivity was absorbed or bound to the root epidermis or cuticle. Research on peanuts, tobacco (Nicotiana glauca) and tomato (Lycopersicon esculentum) supports the conclusion

that the dinitroanilines are either absorbed by or absorbed to the roots with minimal translocation into the stems and leaves (Probst et al., 1967).

Plant metabolism studies with trifluralin and nitralin indicate that the major residues are the parent compound. Identifiable metabolites represent 5% or less of the recovered compounds (Colab et al., 1967). These plant metabolites have also been found in soil. It has not been determined if the plants are absorbing the metabolites from the soil or if they have been transformed from the parent compound within the plant.

Persistence

Under most field conditions ethalfluralin and the other dinitroanilines will degrade in one season, allowing rotational crops to be seeded four to five months after application without toxic effects (Elanco, 1979). The persistence of these herbicides is greatly affected by the soil temperature and ion exchange capacity.

Temperature may increase or decrease the persistence of the herbicides in the soil by altering volatilization rates. High temperatures will cause rapid volatilization of most dinitroanilines. In hot climates this increased loss reduces the waiting period before rotational crops can be safely planted. In contrast, colder soils, such as those found in Saskatchewan, may take in excess of one growing season before soil herbicide levels drop low enough to allow safe planting of rotational crops (Rahman and Ashford, 1973).

The dinitroanilines are quickly and strongly absorbed to soil particles. Soils that are high in organic matter and clay have a higher ion exchange capacity and absorb the herbicides more readily. High ion exchange capacity soils bind the dinitroanilines tightly, increasing their persistence while rendering them ineffective in weed control. Ethalfluralin is not recommended for soils with an organic matter content above 10% (Elanco, 1977).

Degradation

The major mechanisms of degradation and removal of most dinitroanilines from the soil are volatilization, photochemical instability, and microbial decomposition. These three processes are influenced by soil moisture, temperature, texture, and rate of application.

Most dinitroanilines are considered to be slightly volatile. This is the major route of loss from the soil. Parochetti and Hein (1973) reported a 24.5% and 12.5% loss of trifluralin and benefin, respectively, over a three hour period from a Lakeland loam soil. Trifluralin and benefin, which have a similar vapor pressure to ethalfluralin, showed an increase in volatilization when moisture levels were increased from air-dry to saturation. These authors also reported a significant increase in volatilization when the soil temperature was increased from 30°C to 40°C. Kennedy and Talbert (1977) reported a 34%, 25%, and 18% loss of profluralin, benefin, and trifluralin, respectively, over a 24-hour period, due to volatilization from a Toloka silt loam.

Delayed soil incorporation of several dinitroanilines (nitralin, butralin, dinitramine, fluchloralin, penaxalin, trifluralin, profluralin, benefin, isopropalin, and orizalin) for up to three days caused a dramatic increase in herbicide loss. Only orizalin concentrations were not greatly reduced over the three-day period (Kennedy and Talbert, 1977). Orizalin has a very low vapor pressure (1×10^{-7} mmHg at 25°C). Without soil incorporation, especially in moist warm soils, most of the dinitroanilines will volatilize, reducing their concentration and effectiveness. Thus, when the herbicides are used as post-emergence (surface applied), the recommended application rates are increased to assure that adequate amounts remain in the soil.

The dinitroanilines are susceptible to photodecomposition. Kennedy and Talbert (1976) reported oryzalin, nitralin, butralin, dinitramine, fluchloralin, penoxalin, trifluralin, profluralin, benefin, and isopropalin underwent some degree of photodecomposition under ultraviolet light over a 24-hour period. When comparing treated thin-layer soil plates to treated unsoiled glass plates, the soil greatly reduced photodecomposition. In another study, Wright and Warren (1965) also reported reduced photodecomposition when a dinitroaniline was applied to soil. Trifluralin on glass completely lost its biological activity when exposed to six hours of sunlight. This reduced degradation has been attributed to the soil's nonuniform surface. Unlike the glass plate's smooth surface, which exposes most of the herbicide to light, the soil allows

the herbicide to penetrate the exposed surface and escape the light's effect.

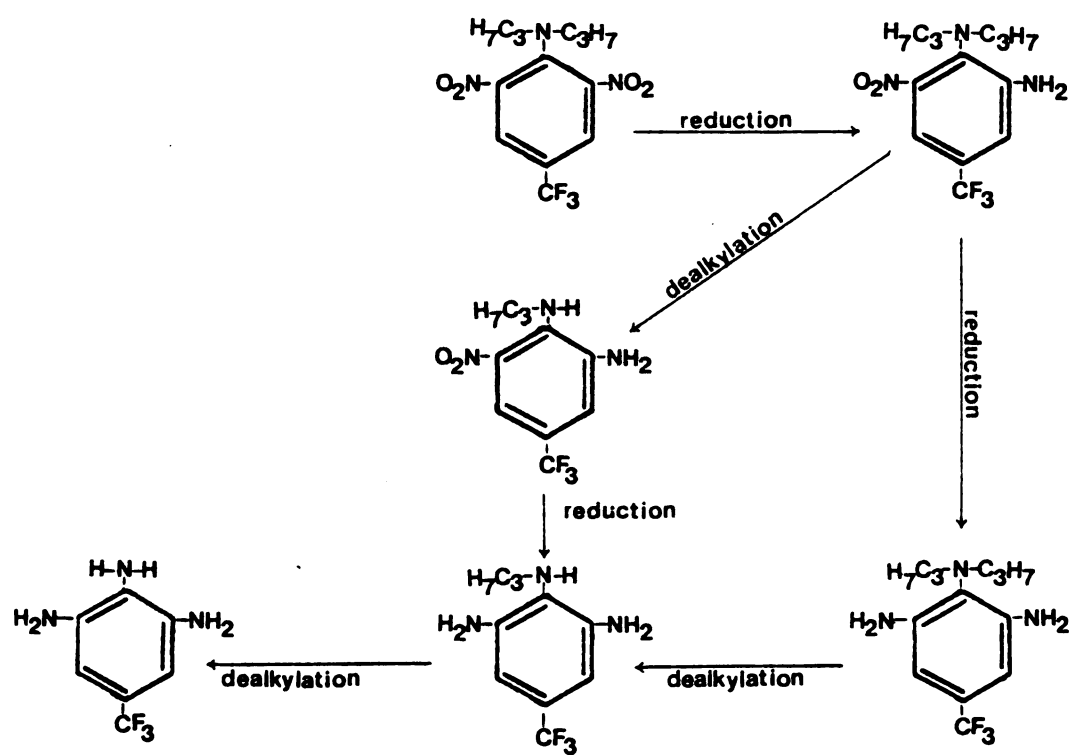
Parechetti and Dec (1978) studied the decomposition of eleven dinitroanilines in soil exposed to unfiltered sunlight for 7 days. Photodecomposition was noted for all of the herbicides tested. The losses ranged from 8.2% for isopropalin to 73.3% for dinitramine. The average was 25.8%.

The major photochemical reactions appeared to involve oxidative dealkylation, nitro reduction, and cyclization (Letis and Crosby, 1974; Plimmer and Klingebiel, 1974; and Newsom and Woods, 1973).

The biological breakdown of the dinitroanilines accounts for only a fraction of the loss in soils. Messersmith et al. (1971) reported that the conversion of ^{14}C -trifluralin to $^{14}\text{CO}_2$ was only 3 to 5% of the loss of ^{14}C trifluralin activity. Probst et al. (1967) used autoclaved and nonautoclaved soil to determine if microorganisms played an important role in the breakdown of trifluralin. Their results showed that the nonautoclaved soil degraded trifluralin more rapidly than did the autoclaved soil. Although they failed to find a buildup of any specific organism, Hamdi and Tewifik (1969) isolated some species of bacteria Pseudomonas sp. capable of degrading trifluralin.

Helling (1976) stated that dinitroanilines degrade in soil via oxidation and reductive pathways, involving oxidative dealkylation of carbon chains, reduction of the nitro groups, and oxidative cyclization (see Figure 2 for the postulated major pathways of degradation of trifluralin).

Figure 2. Degradation pathways of trifluralin.

Trifluralin

Leaching and Water Movement

Since the dinitroanilines are strongly absorbed to soil and have low water solubilities, they are among the least mobile herbicides. Many studies indicate a relatively minute amount of leaching occurs. Gagnon and Hamilton (1973) reported no detectable leaching of butralin, dinitramine, nitralin, profluralin, and trifluralin on sandy loam plots during a one-year study in which 145 cm of rain and irrigation water entered the soil. Trifluralin was found to be immobile in fourteen soils ranging from sand loam to silty clay (Helling, 1971).

The degree of leaching can be affected by soil moisture levels. Koren (1972) showed that leaching of trifluralin and oryzalin increased when the soil was initially moist rather than dry. This may be due to a reduction in soil-herbicide binding. Increasing the water content of the soil can reduce the availability of binding sites by acting as a barrier or competitor to the herbicide. In either case, moist or dry, leaching was minute.

Runoff water from various crop fields have also shown little or no movement of the dinitroanilines. Davis and Rahn (1970) found no trifluralin in surface water from a 500-acre lima bean field on a loamy sand soil when applied at 0.75 lbs/A. In addition the researchers found no trifluralin in a drainage ditch next to a 20-acre soybean field that had been treated.

Sheets et al. (1973) studied the movement of trifluralin in surface runoff water and found that less than 1% of the

total herbicide applied could be recovered. The highest concentration detected in the water was 24 ppm. The filtered sediments of the runoff contained an average of 84% of the detectable herbicide, indicating most of the herbicide was carried from the field soil-bound.

Toxicity

Technical grade ethalfluralin and the dinitroanilines, as a group, are not hazardous to mammals and birds but are toxic to fish and other aquatic organisms (Elanco, 1979). Acute toxicity data (Table 3) indicate that both ethalfluralin and trifluralin are slightly or probably non-toxic to mice, rats, dogs, cats, rabbits, chickens, bobwhite, and mallards. Subacute, dermal, and ocular data (Table 3) also indicate ethalfluralin is relatively non-toxic to rats, bobwhite, and mallards. Commercial herbicides containing ethalfluralin or trifluralin produce greater toxicities in mammals and birds. This increased sensitivity can be attributed to the solvent vehicles (Elanco, 1977; 1979).

Unlike mammals and birds, aquatic organisms are very sensitive to the dinitroanilines. Low concentrations of ethalfluralin are toxic to fish (Table 3). Although most dinitroanilines are extremely toxic to fish they do not represent a threat unless dumped directly into a water source or spilled in quantities large enough to overcome the soil's binding capabilities. Parka and Worth (1965) reported that using trifluralin under field conditions and recommended

Table 3. Acute and subacute toxicity of ethalfluralin and trifluralin¹.

	Ethalfluralin	Trifluralin
<u>LD₀</u>		
Rat	>10,000 ²	>10,000
Bobwhite	>2,000	---
Dog	>200	>2,000
Cat	>200	---
Rabbit	---	>2,000
Rabbit (dermal)	>2,000	---
Chicken	---	>2,000
<u>LD₅₀</u>		
Mouse	>10,000	>5,000
Rat	>10,000	>10,000
Mallard	---	>2,000
Bluegill	0.032	0.047
Rainbow trout	0.32	0.01-0.09
Goldfish	0.26	---
<u>LC₅₀</u>		
Rat	>5,000	---
Bobwhite	>5,000	---
Mallard	>5,000	---
Daphnia	---	0.56
Rabbit (ocular)	Slight irritant	Slight irritant

¹ Elanco, 1979.² Parts per million.

application rates, 2 inches of soil from 45.6 acres of Princeton fine sand would have to be moved into a one-acre pond with an average depth of three feet to produce the same affect as an LC₅₀ on bluegills (Lepomis macrochirus).

Emmerson and Anderson (1966) reported that orally administered ¹⁴C-trifluralin was excreted during the first 24 hours after dosing. About 80% of the activity was recovered in the feces while the remaining 20% was removed through the urine. Of the total amount excreted in the feces, 8% was recovered as unchanged trifluralin. The major metabolite in the feces was a compound resulting from the reduction of one nitro group into an amine, a metabolite similar to one that occurs in the soil. The authors suggested that even though trifluralin was quickly absorbed into fats it had a low order of absorption from the gut. Bile excretion accounted for only 11-18% of the activity. Also, similar structurally related compounds such as halogenated nitrobenzenes are known to be poorly absorbed from the gut when administered orally. Ten urinary metabolites were found but only three could be isolated and identified. One metabolite was formed by the complete dealkylation of trifluralin. The other two were formed by the reduction of one nitro group and/or the removal of both propyl groups (dealkylation).

Emmerson and Anderson (1966) reported that the fate of trifluralin in the dog was similar to that of the rat.

When dairy cows were administered oral doses of ¹⁴C-trifluralin, 99% of the ingested activity was recovered after

a six-day period, 18% in the urine and 81% in the feces. After six-days, no radioactivity could be found in the blood or milk (Golab et al., 1969). Golab et al. (1970) also reported similar results when using ^{14}C -benefin.

EXPERIMENT 1. The effects of ethalfluralin on feed palatability in the mallard and bobwhite.

PROCEDURE

Chronology of Study

Adult mallards and bobwhite were obtained in mid May, 1979 and were immediately placed in quarantine for one week. After this period, both species went through a two-week acclimation period. During the acclimation period the birds were in their appropriate pens and were fed an untreated diet (control).

The mallard study began on June 5, 1979 and continued through July 3, 1979. From June 5 through June 18, 1979 the birds were fed untreated diets. On June 19, ethalfluralin-treated feed was administered for the remaining two-week period.

The bobwhite study began on June 4, 1979 and continued through July 10, 1979. From June 4 through June 18, 1979 the quial were fed untreated diets. On June 19, the birds received ethalfluralin-treated diets for the remaining three-week period.

Animals

Male mallards were obtained from Whistling Wings Hatchery, Hanover, Illinois. The birds were leg banded and randomly assigned, three to a pen.

Male bobwhites were obtained from Michigan State University Poultry Science Research and Teaching Center in East Lansing, Michigan. The birds were wing banded and randomly assigned, one animal per pen.

All birds selected for the study appeared to be in good health.

Diets and Treatment Level

The test diets were prepared by Lilly Research Laboratories in Greenfield, Indiana, using Lilly Mash (see Appendix A for composition and analysis). The dietary levels mixed were 0.001%, 0.003%, 0.010%, 0.030%, 0.100%, 0.300%, and 1.000% ethalfluralin. Random assignments of dietary ethalfluralin levels to pens and cages was accomplished with the use of a random numbers table. Two mallard pens and six quail cages were assigned to each dietary level.

Facilities

All birds were housed at the Michigan State University Poultry Research and Teaching Center. The rooms were entirely enclosed. Each pen and cage was numbered and labeled with dietary level and bird identification numbers. Each pen and cage also had its own individually identifiable feed container and scoop so that feed consumption could be accurately measured. Photoperiods were controlled by an automatic timer (Paragon model 41005-0).

The mallard rooms measured 11.0 m x 12.2 m x 2.4 m (W x L x H). There were three rows of six pens suspended from the ceiling by chains. Each pen measured 1.5 m x 1.6 m x 0.7 m (W x L x H) with no top. Pens were constructed of plastic coated 2.5 cm x 2.5 cm steel mesh wire.

The bobwhite rooms measured 5.0 m x 9.3 m x 2.4 m (W x L x H). Two groups of cages hung from the ceiling, each

consisting of 15 cages aligned back to back. The dimensions of each cage were as follows: 40.0 cm x 43.0 cm x 44.5 cm (W x L x H). The size of the mesh of the wire caging was 2.6 cm x 9.8 cm and the flooring was 1.3 cm x 1.3 cm. The flooring was plastic coated wire.

Testing

From the beginning of the tests, feed consumption was measured weekly throughout the duration of the study. Body weights were measured at day 0, 7, 14, 21, and 28 for the mallards and day 0, 7, 14, 21, 28, and 35 for the bobwhite. Mortality, morbidity, behavior, and any signs of intoxication were recorded daily.

Statistical Analysis

Body weight and food consumption were evaluated with the use of a Dunnett's two-tailed test (Gill, 1978). Sampling units were individual pens.

RESULTS

Body Weights

Body weights of mallards fed ethalfluralin at 0.003%, 0.010%, 0.030%, 0.100%, 0.300%, and 1.000% of the diet were not significantly different from those of the control birds throughout the length of the experiment (Table 4). Body weights between all dietary treatments at any given time were fairly uniform. The largest difference in weights between concentrations was 11% which occurred in the second week prior to the administration of treated feed. There was a

Table 4. Mean body weights (g $\pm$ S.E.) of mallards on palatability test fed ethalfluralin.

Dietary level of ethalfluralin %	Pre-treatment		Treatment	
	Initial weight	Week 1	Week 2	Week 3
				Week 4
0.000	1211 $\pm$ 36 _a ¹	1264 $\pm$ 20 _a	1323 $\pm$ 18 _a	1358 $\pm$ 18 _a
0.003	1250 $\pm$ 20 _a	1332 $\pm$ 30 _a	1396 $\pm$ 2.0 _a	1424 $\pm$ 12 _a
0.010	1182 $\pm$ 16 _a	1223 $\pm$ 20 _a	1294 $\pm$ 27 _a	1331 $\pm$ 39 _a
0.030	1244 $\pm$ 0.0 _a	1308 $\pm$ 2.0 _a	1366 $\pm$ 6.0 _a	1392 $\pm$ 12 _a
0.100	1279 $\pm$ 2.0 _a	1314 $\pm$ 7.0 _a	1402 $\pm$ 9.0 _a	1411 $\pm$ 1.0 _a
0.300	1256 $\pm$ 24 _a	1319 $\pm$ 25 _a	1362 $\pm$ 3.0 _a	1370 $\pm$ 11 _a
1.000	1226 $\pm$ 28 _a	1268 $\pm$ 31 _a	1345 $\pm$ 20 _a	1356 $\pm$ 10 _a
				1383 $\pm$ 1.0 _a

¹ Means in the same column with the same subscript are not significantly different from the control (P $\geq$ 0.05).

trend at all dietary levels towards increasing body weights over the four-week test period. All concentrations had greater body weights at termination than at the onset of the experiment. Average body weights did not decrease after the introduction of treated feed at any treatment concentration.

Body weights of bobwhite fed ethalfluralin were also not significantly different from the control birds (Table 5). There was a trend for decreasing body weight as dietary concentration of ethalfluralin increased, but the trend was present prior to the administration of treated feed and is considered to be a random occurrence. Body weights within treatments showed an increasing trend over the five-week duration of the study. Only the birds that received ethalfluralin at 1.000% decreased in weight one week after the administration of treated feed. The weight loss was not maintained and the birds gained in the following week. Final body weights for all dietary levels were higher than the original weights.

Food Consumption

There was no significant difference in food consumption of either mallards fed the various concentrations of ethalfluralin (Table 6). No uniform trend in mallard food consumption could be established. Drakes fed dietary concentrations of ethalfluralin at 0.000%, 0.003%, and 0.010% increased or maintained their consumption rates during the first week the treated diets were introduced. Mallards fed

Table 5. Mean body weights ($g \pm S.E.$) of bobwhites on palatability test fed ethalfluralin.

Dietary concentration of ethal- fluralin (%)	Initial weight	Pre-treatment			Treatment		
		Week 1	Week 2	Week 3	Week 4	Week 5	
0.000	199 ± 5.9^1_a	$201 \pm 6.0_a$	$201 \pm 8.0_a$	$202 \pm 7.5_a$	$205 \pm 7.8_a$	$210 \pm 7.6_a$	
0.003	$200 \pm 2.7_a$	$202 \pm 4.0_a$	$200 \pm 4.6_a$	$201 \pm 4.1_a$	$201 \pm 4.8_a$	$205 \pm 4.9_a$	
0.010	$189 \pm 5.4_a$	$195 \pm 5.3_a$	$195 \pm 5.1_a$	$198 \pm 5.8_a$	$198 \pm 7.0_a$	$205 \pm 6.7_a$	
0.030	$187 \pm 6.2_a$	$190 \pm 7.0_a$	$190 \pm 7.0_a$	$192 \pm 7.2_a$	$193 \pm 7.6_a$	$196 \pm 8.0_a$	
0.100	$187 \pm 2.6_a$	$192 \pm 3.3_a$	$193 \pm 3.8_a$	$194 \pm 4.0_a$	$195 \pm 4.4_a$	$200 \pm 4.2_a$	
0.300	$189 \pm 3.5_a$	$188 \pm 4.6_a$	$186 \pm 4.1_a$	$189 \pm 4.4_a$	$189 \pm 4.4_a$	$191 \pm 4.4_a$	
1.000	$183 \pm 4.9_a$	$187 \pm 3.9_a$	$187 \pm 4.3_a$	$180 \pm 6.2_a$	$181 \pm 6.1_a$	$186 \pm 3.7_a$	

¹ Means in the same column with the same subscript are not significantly different from the control ($P \geq 0.05$).

Table 6. Feed consumption (grams/bird/day, $\bar{X} \pm \text{S.E.}$) of mallards on palatability test fed ethalfluralin.

Dietary level of ethalfluralin %	Pre-treatment			Treatment	
	Week 1	Week 2		Week 3	Week 4
0.000	125 $\pm$ 0.0 ¹ _a	144 $\pm$ 3.9 _a		152 $\pm$ 15.2 _a	139 $\pm$ 15.6 _a
0.003	130 $\pm$ 8.1 _a	133 $\pm$ 4.2 _a		133 $\pm$ 1.4 _a	110 $\pm$ 4.2 _a
0.010	108 $\pm$ 8.5 _a	115 $\pm$ 16.3 _a		116 $\pm$ 9.2 _a	100 $\pm$ 5.3 _a
0.030	144 $\pm$ 11.3 _a	136 $\pm$ 14.1 _a		124 $\pm$ 4.9 _a	110 $\pm$ 0.7 _a
0.100	131 $\pm$ 18.4 _a	146 $\pm$ 27.2 _a		128 $\pm$ 19.4 _a	132 $\pm$ 24.7 _a
0.300	123 $\pm$ 7.1 _a	153 $\pm$ 15.2 _a		135 $\pm$ 10.6 _a	125 $\pm$ 2.8 _a
1.000	120 $\pm$ 8.5 _a	133 $\pm$ 1.4 _a		132 $\pm$ 1.8 _a	136 $\pm$ 6.72

¹ Means in the same column with the same subscript are not significantly different from the control ($P \geq 0.05$).

dietary concentrations of 0.030%, 0.100%, 0.300%, and 1.000%, ethalfluralin decreased their consumption during this period. Final mallard feed consumption values for most concentrations, including the controls, declined from the previous week but were not significantly different from their initial values. Treating the feed with ethalfluralin at these levels did not significantly decrease mallard feed consumption at any time during the study.

Similarly, there was no significant difference in the bobwhite's initial or final feed consumption values (Table 7). However, birds fed ethalfluralin at 1.000% had a significantly lower consumption rate than the controls one week after the administration of the treated diets (Figure 3). Feed consumption for this dietary concentration increased in the following weeks but never achieved pre-treatment concentrations. Food consumption between and within levels varied and little uniform trends could be established. All groups of birds, including the controls, either maintained or decreased their consumption after the onset of the treatment phase. Final feed consumption rates were lower than the initial rates in dietary concentrations of 0.003%, 0.030%, 0.100%, and 1.000% while the 0.000%, 0.010%, and 0.300% concentrations had final consumption rates greater than their initial rates.

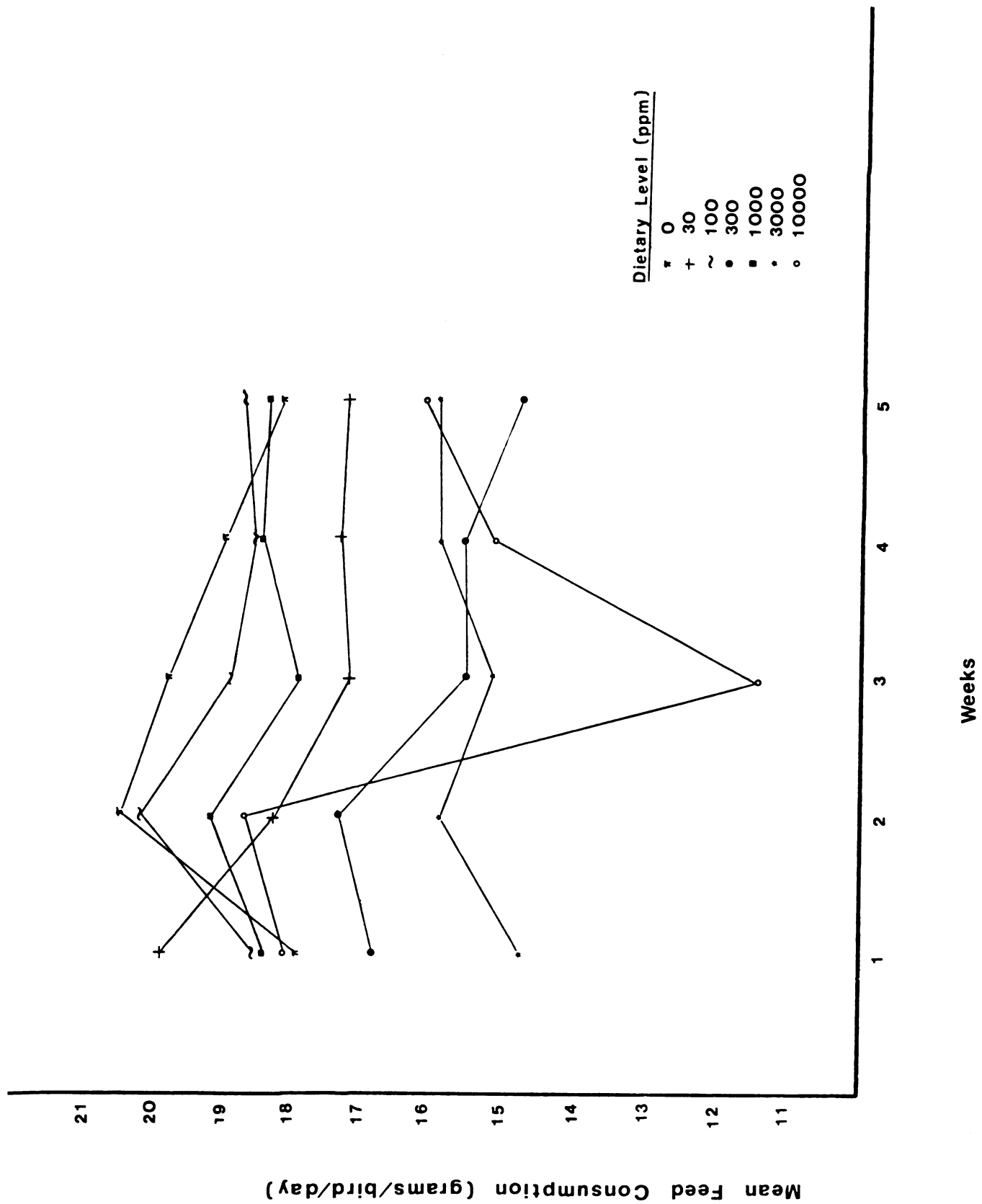
Mallard and bobwhite dietary consumption of ethalfluralin was correlated to the concentration of the diet. Dietary concentrations were increased roughly 300% over the previous lower dose. Since feed consumption was not effected by the

Table 7. Feed consumption (grams/bird/day, $\bar{X} \pm \text{S.E.}$) of bobwhite on palatability test fed ethalfluralin.

Dietary level of ethalfluraline %	n	Pre-treatment			Treatment		
		Week 1	Week 2	Week 3	Week 4	Week 5	
0.000	6	18.0 $\pm$ 0.97 ¹ _a	20.5 $\pm$ 0.91 _a	19.8 $\pm$ 2.00 _a	19.0 $\pm$ 1.21 _a	18.2 $\pm$ 1.03 _a	
0.003	6	19.9 $\pm$ 1.04 _a	18.3 $\pm$ 0.95 _a	17.2 $\pm$ 0.83 _a	17.4 $\pm$ 0.99 _a	17.3 $\pm$ 0.45 _a	
0.010	6	18.6 $\pm$ 0.90 _a	20.2 $\pm$ 1.30 _a	18.8 $\pm$ 1.18 _a	18.6 $\pm$ 1.10 _a	18.8 $\pm$ 1.27 _a	
0.030	6	16.9 $\pm$ 1.43 _a	17.4 $\pm$ 1.80 _a	15.6 $\pm$ 1.45 _a	15.6 $\pm$ 1.45 _a	14.8 $\pm$ 0.81 _a	
0.100	6	18.5 $\pm$ 0.57 _a	19.2 $\pm$ 0.73 _a	18.0 $\pm$ 0.30 _a	18.5 $\pm$ 0.84 _a	18.4 $\pm$ 0.83 _a	
0.300	6	14.8 $\pm$ 2.13 _a	16.0 $\pm$ 1.37 _a	15.2 $\pm$ 0.96 _a	16.0 $\pm$ 0.81 _a	16.0 $\pm$ 0.65 _a	
1.000	6	18.2 $\pm$ 1.49 _a	18.7 $\pm$ 1.51 _a	11.6 $\pm$ 2.17 _b	15.2 $\pm$ 1.41 _a	16.2 $\pm$ 0.92 _a	

¹ Means with the same subscript are not significantly different from the control ($P \geq 0.05$).

Figure 3. Mean feed consumption of bobwhite on palatability test fed ethalfluralin at various concentrations.



herbicide, ethalfluralin intake also increased 300% from one dose level to the next highest level (Table 8). Mallard and bobwhite consumed a maximum of 1,365 and 163 grams/bird/day, respectively.

Mortality

No mortality occurred in the mallard or bobwhites studied over the 28 or 35 day test period, respectively. No observable signs of intoxication or morbidity were observed in any animal in either study throughout the duration of the treatment period. All animals on examination appeared to be in good health at the study termination.

DISCUSSION

Comparing pesticide levels in feed tolerated by birds in the laboratory to those levels found or expected in the environment can be useful in estimating the potential hazard of pesticides to birds (Kenaga, 1973). Low levels of highly toxic pesticides such as endrin produce toxicity and death in laboratory animals (Omer, 1970; Hill et al., 1975). One could reason that similar or higher levels of this same chemical contaminant, found in the environment on feedstuff specific to an organism, would also produce toxicities to those organisms consuming the contaminated feed. This relationship has been shown to occur in a variety of animals (Prince et al., 1971; Murphy, 1980; Hornshaw, 1981). Conversely, high levels of other pesticides such as trifluralin are relatively nontoxic to laboratory animals (mammals

Table 8. Mallard and bobwhite dietary consumption of ethalfluralin (mg/bird/day) and (mg/kg/bird/day).

Dietary level of ethalfluralin %	n	mg/bird/day			mg/kg/bird/day		
		Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
<u>Mallard</u>							
0.003	2	4 + 0.1 ¹	3 + 0.1		3 + 0.1	2 + 0.1	
0.010	2	12 + 1.3	10 + 0.8		8 + 0.9	7 + 0.5	
0.030	2	37 + 2.1	33 + 0.3		29 + 1.7	24 + 0.3	
0.100	2	128 + 27.5	132 + 35.0		92 + 20.0	92 + 23.7	
0.300	2	408 + 45.0	375 + 12.0		302 + 31.2	268 + 11.7	
1.000	2	1,315 + 25.0	1,365 + 94.8		966 + 32.2	991 + 62.6	
<u>Bobwhite</u>							
0.003	6	1 + 0.0	1 + 0.0	1 + 0.0	2 + 0.1	3 + 0.2	3 + 0.1
0.010	6	2 + 0.1	2 + 0.1	2 + 0.2	10 + 0.5	10 + 0.4	9 + 0.7
0.030	6	5 + 0.5	5 + 0.5	4 + 0.2	24 + 2.4	24 + 2.2	23 + 0.7
0.100	6	18 + 0.4	19 + 0.8	19 + 0.9	93 + 1.9	95 + 4.7	93 + 4.8
0.300	6	46 + 3.2	48 + 2.8	48 + 2.1	244 + 16.3	254 + 14.7	254 + 16.8
1.000	6	115 + 23.8	151 + 14.7	163 + 9.6	629 + 133.6	840 + 78.1	882 + 68.0

¹ $\bar{X} \pm \text{S.E.}$

and birds) resulting in few reported incidences of poisoning due to environmental exposure (Worth and Anderson, 1965).

Ethalfluralin, which is similar structurally, physically, and chemically to trifluralin, was shown to cause little or no observable signs of toxicity to mallards and bobwhite in these subacute studies. Overall behavior, body weight, and food consumption values for these two species were similar to those reported by Flaga (1980) and Jones (1977).

Increasing body weight trends in the mallard and bobwhite over the duration of the studies can be attributed to normal growth and development of the animals. All dietary levels for both the mallard and the bobwhite, with the exception of the bobwhite on the 10,000 ppm ethalfluralin diet, continued to show an increase in body weight after the administration of treated feed, suggesting that ethalfluralin had no detrimental effect on body growth. Feed consumption in the mallard and bobwhite showed no trend with increasing dietary levels of ethalfluralin, indicating ethalfluralin was tolerated at the concentrations administered. The only sign of feed rejection was with bobwhites on the 10,000 ppm ethalfluralin diet. These birds temporarily reduced their feed intake resulting in a small, short term loss of body weight.

Environmental levels of ethalfluralin should be predictable when considering the data available on the herbicide frequency and rate of application, rate of degradation, and solubilities. Recommended application rates of 1.5 gallons per acre of Sonalan[®] would result in approximately 0.505

grams of technical grade ethalfluralin per square meter of soil and vegetation (surface applied). With volatilization rates ranging from four to five percent in a three-hour period (Parochetti et al., 1976) to 50 percent in a 24-hour period (Helling, 1975) for trifluralin, which volatilizes at a similar rate to ethalfluralin, the amount of ethalfluralin expected to be remaining in the field would be reduced significantly in a short period of time. Photodecomposition would further reduce the residue levels on exposed plant and soil surfaces. Kenaga (1973) states that field residue levels of most pesticides decline rapidly with most levels falling below 1 ppm within one week after application. Even the more persistent chlorinated hydrocarbons have been reported to drop below 10 ppm one week after application. In addition, residue studies with mammals report that similar dinitroanilines do not readily accumulate in animal tissues, and are completely excreted seven days after exposure.

Considering the high doses of ethalfluralin tolerated during past acute and subacute studies with mallards and bobwhite, and this palatability study, it is unlikely that any acute environmental exposure due to normal use of this pesticide would produce an immediate threat to these species. The mallard and bobwhite are capable of ingesting 1,365 mg/bird/day (mallard and 163 mg/bird/day (bobwhite) of ethalfluralin for up to a two and three week period, respectively, without any apparent detrimental effects. If concentrations in the environment should rise above 10,000 ppm, such as in a spill, data presented indicates that the

bobwhite will reject feed when high levels of ethalfluralin are present. Although the mallards did not reject feed at the 10,000 ppm level, ethalfluralin did not produce toxicity. In addition, high levels of ethalfluralin in the environment would be short lived due to the herbicides physical and chemical properties.

EXPERIMENT 2. The effects of ethalfluralin on mallard and bobwhite reproduction.

PROCEDURE

Chronology of Study

The mallards used for this study were received on August 23, 1979 and were immediately placed into quarantine until September 3, 1979. Bobwhites were received on August 29, 1979 and placed in quarantine until September 4, 1979. Both species were then allowed a two-week acclimation period in which the birds were placed in their appropriate testing cages and were fed untreated basal diets. The mallards and bobwhites first received their assigned dietary level of ethalfluralin on September 18 and 19, respectively, which was the beginning of the pre-production phase. The pre-production phase lasted eight weeks, at which time the photoperiods were increased from seven hours light:17 hours dark to 16 hours light:eight hours dark. This phase of increased photoperiod, the production phase, lasted throughout the remainder of the study. The adult terminal kill for mallards and bobwhites was on March 26, and March 5, 1980, respectively. The mallard egg collection period started on January 4, 1980 and ended on March 13, 1980. The bobwhite egg collection period was from December 17, 1979 through March 2, 1980. The final groups of ducklings and chicks hatched from eggs provided by the treated breeders were terminated on April 24, 1980 and May 1, 1980, respectively.

Animals

Mallards were purchased from the Max McGraw Foundation, Elgin, Illinois. The birds were hatched on June 19, 1979 and were received at the test facility on August 23, 1979. The birds were 13 weeks old at the start of the pre-production phase of the study.

Bobwhites were purchased from Barrett's Quail Farm, Houston, Texas. The quail were hatched on May 26, 1979 and were received at the test facility on August 29, 1979. The birds were 16.5 weeks old at the start of the pre-production phase.

All birds of both species appeared to be in good health upon arrival. All birds were randomly assigned to cages, two males and five females per mallard pen and one male and one female per quail cage. Random assignment of dietary concentration to cage was accomplished by the use of a random numbers table.

Diets

The test diets were prepared by Lilly Research Laboratory, Greenfield, Indiana, using Lilly Mash (see Appendix A through I for composition and analysis). The dietary concentrations mixed for adult birds were 0.00% (control), 0.01%, 0.03%, and 0.10% of ethalfluralin. Before the rations were shipped to the test facility, samples were removed for determination of the presence of the test chemical at the desired concentration. When the initial lots were prepared, homogeneity of mix and eight week stability in feed were determined.

Facilities

All birds were housed at the Michigan State University Poultry Science Research and Teaching Center. All rooms were entirely enclosed. Photoperiod was controlled by an automatic timer that was appropriately set for the current phase of the study. Electric and gas heaters were used to maintain a temperature of 10°C.

Mallard rooms measured 11.0 m x 12.2 m x 2.4 m (W x L x H). There were three rows of six pens that were suspended from the ceiling by chains. Each pen measured 1.47 m x 1.55 m x 0.70 m with no top. The pens were constructed of plastic coated 1" x 1" steel mesh wire. The floor of each pen was sloped downward from the center to the outer sides allowing the eggs to roll from the center of the pen towards the aisles facilitating egg collection.

Bobwhite rooms measured 5.0 m x 9.3 m x 2.4 m (W x L x H). Two groups of cages hung from the ceiling, each consisting of two rows of 15 cages aligned back to back. The dimensions of each cage were as follows: 40 cm wide x 43 cm long x 45 cm deep. The size of the mesh wire caging equaled 2.6 x 9.8 cm and the flooring was 1.27 cm x 1.27 cm. The flooring wire was plastic coated. The floor of all cages sloped toward the aisles which allowed the eggs to roll down to the collecting area.

Each pen and cage was provided with an individual feeder and watering cup. Pens and cages were numbered and labeled with study number, dietary level, and individual bird numbers.

Individually identifiable feed containers and scoops were used for each pen or cage.

Testing

Beginning with the initiation of the test, feed consumption was measured biweekly throughout the duration of the study. Body weights were measured at 0, 2, 4, 6, and 8 weeks and at termination. In order to avoid any adverse effects on egg production, body weights were not measured during the production phase.

Egg production, mortality, morbidity, behavior, and any observable clinical signs of intoxication were recorded daily. All birds that died during the study were subjected to gross necropsy. If any abnormalities were noted, samples were taken for histopathology. All birds surviving to study termination were killed by CO₂ asphyxiation and necropsied. At this date, tissues were collected for histopathology from at least five animals of each sex per treatment level. The following tissues were taken: kidney, liver, heart, lung, spleen, pancreas, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, colon, testes, ovary, magnum, shell gland, pectoral muscle, leg muscle, thymus, sciatic nerve, and skin.

Egg Collection, Storage, and Incubation

Egg collection began when approximately 50% egg production was reached. Prior to this time, all eggs laid were discarded. Once 50% production was achieved eggs were collected daily and each was marked in pencil with the

corresponding cage number and date. Eggs that were soft-shelled or lacking shells were recorded and discarded. Eggs were stored at 15.6°C (14.4-16.7°C) until one week's collection had accumulated. At that time, all eggs were candled and those with cracks were recorded and discarded. The remaining eggs were organized according to pen number and were then placed in an incubator (Jamesway, single stage, Model 242) for 23 days. The dry and wet bulb temperature readings were 37.5°C (37.2-37.8°C) and 30°C (29.4-30.6°C), respectively. On day 14 of incubation, mallard eggs were candled to determine the number of fertile and infertile eggs, and the number of early dead embryos. The eggs were candled again on day 21 to check for embryo deaths that had occurred beyond day 14. On day 23 the eggs were transferred to pedigree baskets in which all eggs were separated according to pen number. The eggs were then placed in the hatchery incubator (Jamesway, single stage, Model 252) which had dry and wet bulb temperature readings of 37.2°C (36.9-37.5°C) and 31.7°C (31.1-32.2°C), respectively. On day 27, all pipped (dead and live), unpipped (dead and live), and hatched ducklings were recorded. All hatchlings were individually wing banded and recorded according to parent stock number. The hatchlings were placed in cardboard chick boxes and were transported the five mile distance to the MSU Poultry Research and Teaching Center. Precautions were taken to prevent exposure of the ducklings to cold temperatures. The ducklings were moved quickly from the incubation room to the vehicle and

and from the vehicle to Building 3 of the research facility. The vehicle was warmed prior to transportation. At the research facility, the ducklings were placed in Petersime 250-24 battery brooders according to dietary level of parent stock. Temperatures were maintained at 35°C (34.4-35.5°C) and the ducklings were given both feed (starter diet) and water ad libitum. The ducklings were observed daily for two weeks and all mortality was recorded. At the end of the two-week period, all surviving ducklings were killed by exposure to chloroform. Bobwhite eggs and chicks were handled identically, except for duration of incubation and candling dates. Bobwhite eggs were candled on day 11 and day 18. The eggs were transferred into the hatcher on day 21 and the hatchlings removed on day 24.

Eggshell Thickness

On Wednesday of every other week, eggs were collected for determination of eggshell thickness. The eggs were marked with collection date and pen number. The eggs were cracked in half, contents removed, and shells washed with tap water to remove the albumin. The shells were then left to air dry at room temperature 21.1°C (20.0-22.2°C) on paper for at least 48 hours. Eggshell thickness was measured using an Ames Thickness Gauge (model 25ME). Four equidistant points around the shell circumference were approximated, and thickness was measured (shell plus eggshell membranes) to the nearest 0.01 mm. From these four data points, an average was calculated.

Statistical Analysis

The following parameters were measured and recorded throughout the study: body weight, food consumption, number eggs laid/hen/day, percent eggs cracked, number of eggs set, percent eggs fertile, number live embryos at the second candling, number live pipped, number dead pipped, number dead not pipped, percent hatched, percent hatchlings surviving 14 days, and eggshell thickness. Treatment means were compared by use of Dunnett's two-tail test (Gill, 1978).

Adjustments were made in survivability whenever dead ducklings were found without wing bands. In such cases, the numbers of bandless, dead ducklings were apportioned among the stock pen numbers within their treatment concentration for that particular hatch number.

Adult mortality was analysed by the chi squared procedure (Gill, 1978).

RESULTS

Body Weight

Ethalfuralin at dietary concentrations of 0.0%, 0.010%, 0.030% and 0.10% had no effect on mallard body weight (Table 9). All groups of birds increased in mean body weight in the first two-week period immediately following the administration of treated diets, and continued to increase in weight until the onset of the production phase. Mallard weights in all groups were lower at the study termination than at the onset of the production phase, but remained higher than their initial weights. Male body weights were consistently higher than female weights.

Table 9. Mean body weights of adult mallards fed ethalfluralin.

Dietary level	Pre-treatment				Treatment			
	Week -2		Week 0		Week 2		Week 4	
	M	F	M	F	M	F	M	F
<u>0 ppm</u>								
$\bar{X}$	967	1146	984	1118	1023	1188	1037	1183
SE	13.4	66.3	15.6	38.4	18.1	46.4	12.5	58.0
N	8	19	8	19	8	19	8	19
<u>100 ppm</u>								
$\bar{X}$	975	1163	944	1159	983	1219	1034	1219
SE	29.4	11.3	11.0	31.3	18.4	27.4	38.4	55.3
N	7	15	7	15	7	15	7	15
<u>300 ppm</u>								
$\bar{X}$	943	1155	949	1165	997	1222	1014	1252
SE	9.0	31.7	15.4	30.8	17.3	32.7	21.4	41.0
N	6	18	6	18	6	18	6	18
<u>1000 ppm</u>								
$\bar{X}$	1012	1177	1000	1209	1045	1221	1080	1252
SE	25.7	31.7	5.6	56.7	9.4	32.2	19.8	29.3
N	6	18	6	18	6	10	6	14

Table 9. (con't).

Dietary level	Treatment					
	Week 6		Week 8		Termination	
	M	F	M	F	M	F
<u>0 ppm</u>						
$\bar{X}$	1077	1215	1112	1246	1099	1245
SE	21.2	65.4	29.2	63.4	11.4	56.7
N	8	19	8	19	8	19
<u>100 ppm</u>						
$\bar{X}$	1074	1223	1114	1267	1090	1262
SE	25.4	30.0	31.5	43.5	51.5	26.6
N	7	15	7	15	7	15
<u>300 ppm</u>						
$\bar{X}$	1071	1286	1107	1334	1096	1225
SE	25.1	48.4	31.8	54.1	33.6	67.7
N	6	18	6	18	6	18
<u>1000 ppm</u>						
$\bar{X}$	1128	1303	1177	1324	1145	1244
SE	16.4	35.2	25.1	29.1	42.1	45.5
N	6	18	6	18	6	18

Similarly, ethalfluralin had no effect on the body weights of bobwhite (Table 10). No significant difference could be established when comparing the weights of the control birds with the weights of the birds on treated diets. Birds in all treatment levels maintained or increased in body weight from the onset of the study until the beginning of the production phase. Terminal body weights for female birds continued to show an increase over the previous measurement while most males had a reduced body weight at termination. Only the control males continued to increase in weight through the final measurement.

Feed Consumption

No significant difference in feed consumption between control mallards and any treatment level mallards occurred throughout the 30-week experiment (Table 11). Consumption in all concentrations increased during the first four weeks after the pre-treatment phase. During weeks five and six consumption dropped in all concentrations from the previous week but not below the initial pre-treatment phase. Feed consumption again increased during the seventh and eighth weeks but then sharply declined during weeks 9 and 10, the start of the production phase when the photoperiod was increased to stimulate reproduction. Following this decline consumption increased steadily in all levels for an eight-week period. During the remaining weeks feed consumption in all groups tended to level, then decline, until the termination of the study. Variation between treatments was relatively small

Table 10. Mean body weights of adult bobwhite fed ethalfluralin.

Dietary level	Pre-treatment				Treatment			
	Week -2		Week 0		Week 2		Week 4	
	M	F	M	F	M	F	M	F
<u>0 ppm</u>								
$\bar{X}$	173	173	187	188	196	196	200	201
SE	4.5	4.3	5.6	5.0	6.3	4.0	6.2	4.4
N	15	15	15	15	15	15	15	15
<u>100 ppm</u>								
$\bar{X}$	172	181	187	192	194	198	196	199
SE	5.1	5.7	4.5	6.3	4.7	7.0	5.4	6.6
N	13	13	13	13	13	13	13	13
<u>300 ppm</u>								
$\bar{X}$	171	171	181	182	190	189	191	193
SE	5.4	4.3	5.5	4.9	4.3	5.9	4.6	6.9
N	12	12	12	12	12	12	12	12
<u>1000 ppm</u>								
$\bar{X}$	169	171	180	186	186	189	192	193
SE	5.6	6.5	6.1	5.9	6.7	7.9	7.0	7.5
N	12	12	12	12	12	12	12	12

Table 10. (con't).

Dietary level	Treatment					
	Week 6		Week 8		Termination	
	M	F	M	F	M	F
<u>0 ppm</u>						
$\bar{X}$	208	211	210	213	211	241
SE	5.7	5.6	6.7	5.5	6.5	7.1
N	15	15	15	15	15	15
<u>100 ppm</u>						
$\bar{X}$	201	207	204	210	202	241
SE	5.0	7.3	5.3	7.2	7.1	7.4
N	13	13	13	13	13	13
<u>300 ppm</u>						
$\bar{X}$	198	199	202	202	197	224
SE	5.1	5.9	5.2	6.5	4.8	8.6
N	12	12	12	12	12	12
<u>1000 ppm</u>						
$\bar{X}$	201	200	204	200	203	226
SE	6.9	8.7	6.9	8.8	7.6	10.2
N	12	12	12	12	12	12

Table 11. Feed consumption of adult mallards fed ethalfluralin.

Dietary level	Pre-treatment		Treatment						
	Week -2	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	
<u>0.00%</u>									
$\bar{X}$	108.6 ¹	130	128	120	139	91	134	144	
SE	7.2	15.1	14.0	8.4	7.8	8.5	12.2	7.7	
N	4	4	4	4	4	4	4	4	
<u>0.01%</u>									
$\bar{X}$	122.7 ²	142 _a	145 _a	139 _a	138 _a	92 _a	146 _a	154 _a	
SE	1.3	5.5	15.8	5.2	8.7	6.2	1.3	6.7	
N	3 ³	3	3	3	3	3	3	3	
<u>0.03%</u>									
$\bar{X}$	104.1 _a	133 _a	135 _a	122 _a	131 _a	96 _a	136 _a	146 _a	
SE	4.1	3.0	7.6	4.3	6.3	15.6	8.3	5.3	
N	4	4	4	4	4	4	4	4	
<u>0.10%</u>									
$\bar{X}$	126.6 _a	151 _a	153 _a	140 _a	144 _a	92 _a	141 _a	149 _a	
SE	4.2	8.6	8.7	9.6	11.1	9.6	12.0	9.1	
N	4	4	4	4	4	4	4	4	

¹ Grams/bird/day.² Means with subscript "a" are not significantly different from their respective controls ($P > 0.05$).³ Unequal replication is the result of elimination of one pen, both males died.

Table 11. (con't).

Dietary level	Treatment						
	Week 16	Week 18	Week 20	Week 22	Week 24	Week 26	Week 28
<u>0.00%</u>							
$\bar{X}$	170	202	205	199	183	166	184
SE	11.6	10.6	14.0	19.9	17.4	14.2	15.1
N	4	4	4	4	4	4	4
<u>0.01%</u>							
$\bar{X}$	178 _a	218 _a	218 _a	212 _a	202 _a	180 _a	196 _a
SE	8.0	7.2	13.7	10.5	17.4	20.3	17.7
N	3	3	3	3	3	3	3
<u>0.03%</u>							
$\bar{X}$	194 _a	236 _a	238 _a	227 _a	219 _a	192 _a	188 _a
SE	16.7	18.5	19.7	15.3	16.2	17.8	18.2
N	4	4	4	4	4	4	4
<u>0.10%</u>							
$\bar{X}$	169 _a	216 _a	210 _a	219 _a	194 _a	178 _a	191 _a
SE	14.5	8.6	6.7	8.4	10.9	4.5	7.7
N	4	4	4	4	4	4	4

with no group of birds consuming greater than 20% more feed than another. All levels followed very similar increasing and decreasing trends over time.

Feed consumption of bobwhites fed the treated diets was not significantly different than the control throughout the duration of the experiment (Table 12). The biweekly measurements varied, but this variation was consistent across all treatment levels, including control. All treatment groups showed a trend for increasing food consumption throughout the study. This increase can be attributed to the nutritional needs for normal growth and development of the birds as evident by the increases in body weight. All feed consumption values are estimates; wastage was not measured.

Reproduction

Feeding ethalfluralin in the diet had no detectable effect on the reproductive performance of the mallard or bobwhite (Table 13 and 14) breeders. No significant difference between the controls and any treatment level could be established for the following parameters: eggs per bird per day, percent of eggs fertile, 14-day embryo survival (mallard), 21-day embryo survival (mallard), 11-day embryo survival (bobwhite), 18-day embryo survival (bobwhite), percent of eggs pipped alive, percent of eggs pipped dead, percent of eggs unpipped alive, percent of eggs unpipped dead, percent of eggs hatching, and eggshell thickness.

Similarly, no significant difference in duckling or chick survivability occurred between the control and any treatment level during the test period.

Table 12. Feed consumption of adult bobwhites fed ethalfluralin.

Dietary level	Pre-treatment Week 2	Treatment						
		Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14
<u>0.00%</u>								
$\bar{X}$	15.0 ¹	17.5	17.3	18.8	20.0	20.4	21.9	23.3
SE	0.27	0.42	0.32	0.36	0.39	0.53	0.42	0.58
N	15	15	15	15	15	15	15	15
<u>0.01%</u>								
$\bar{X}$	14.6 ²	17.1 ^a	16.6 ^a	16.9 ^a	19.0 ^a	19.7 ^a	21.5 ^a	23.0 ^a
SE	0.37	0.51	0.31	0.40	0.50	0.59	0.52	0.56
N	13	13	13	13	13	13	13	13
<u>0.03%</u>								
$\bar{X}$	14.7 ^a	17.6 ^a	16.4 ^a	17.3 ^a	19.5 ^a	20.8 ^a	21.4 ^a	22.8 ^a
SE	0.33	0.38	0.40	0.47	0.49	0.80	0.87	0.88
N	12	12	12	12	12	12	12	12
<u>0.10%</u>								
$\bar{X}$	14.5 ^a	16.6 ^a	16.5 ^a	17.8 ^a	19.0 ^a	19.9 ^a	21.3 ^a	22.7 ^a
SE	0.41	0.36	0.34	0.31	0.30	0.45	0.71	0.89
N	12	12	12	12	12	12	12	12

¹ Grams/bird/day.² Means with subscript "a" are not significantly different from their respective control (P > 0.05).

Table 12. (con't).

Dietary level	Treatment				
	Week 16	Week 18	Week 20	Week 22	Week 24
<u>0.00%</u>					
$\bar{X}$	23.8	25.9	26.2	28.0	27.4
SE	1.04	0.58	0.72	0.85	0.78
N	15	15	15	15	15
<u>0.01%</u>					
$\bar{X}$	24.8 ^a	26.4 ^a	26.6 ^a	27.7 ^a	28.3 ^a
SE	0.57	0.91	1.24	1.24	1.16
N	13	13	13	13	13
<u>0.03%</u>					
$\bar{X}$	24.1 ^a	25.1 ^a	25.9 ^a	28.1 ^a	28.3 ^a
SE	0.74	0.81	1.28	1.49	1.04
N	12	12	12	12	12
<u>0.10%</u>					
$\bar{X}$	23.7 ^a	25.5 ^a	26.6 ^a	28.2 ^a	27.2 ^a
SE	1.22	1.32	1.28	1.35	1.32
N	12	12	12	12	12

Table 13. The effects of feeding ethalfluralin on reproductive parameters in mallards.

Dietary concentration of ethalfluralin	E/F/D ¹	Eggs set per hen	Percent fertile ²	14 day embryo survival ³	21 day embryo survival ⁴	Percent pipped alive ⁵
<u>0.00%</u>						
$\bar{X}$	0.39 ⁶ _a	27 _a	88 _a	96 _a	93 _a	2.2 _a
SE	0.060	4.2	3.6	1.7	3.8	0.22
N	4	4	4	4	4	4
<u>0.01%</u>						
$\bar{X}$	0.35 _a	25 _a	86 _a	98 _a	96 _a	3.3 _a
SE	0.044	3.1	3.5	0.7	1.6	1.96
N	3*	3	3	3	3	3
<u>0.03%</u>						
$\bar{X}$	0.61 _a	43 _a	86 _a	97 _a	95 _a	5.2 _a
SE	0.050	3.5	5.2	0.7	0.9	0.74
N	4	4	4	4	4	4
<u>0.10%</u>						
$\bar{X}$	0.38 _a	27 _a	74 _a	97 _a	96 _a	6.2 _a
SE	0.073	5.1	9.0	2.2	2.1	3.38
N	4	4	4	4	4	4

¹ Eggs/female/day.

² No. eggs fertile x 100/no. eggs set.

³ No. embryos alive at day 14 x 100/no. eggs fertile.

⁴ No. embryos alive at day 21 x 100/no. eggs fertile.

⁵ No. of eggs pipped alive x 100/no. eggs fertile.

⁶ Means in the same column with the same subscript are not significantly different from the control ($P \geq 0.05$).

* Unequal replication is the result of the elimination of pens due to bird deaths.

Table 13. (con't).

Dietary concentration of ethalfluralin	Percent pipped dead ⁷	Percent unpipped alive ⁸	Percent unpipped dead ⁹	Per- cent hatch ¹⁰	Percent surviv- ability	Egg shell thickness (mm)
<u>0.00%</u>						
$\bar{X}$	4.0 _a	0.5 _a	17 _a	70 _a	88 _a	0.45 _a
SE	1.62	0.25	1.3	3.7	4.5	0.002
N	4	4	4	4	4	4
<u>0.01%</u>						
$\bar{X}$	3.7 _a	1.0 _a	23 _a	66 _a	93 _a	0.43 _a
SE	1.36	0.47	3.6	5.6	2.2	0.014
N	3	3	3	3	3	3
<u>0.03%</u>						
$\bar{X}$	4.0 _a	0.8 _a	25 _a	61 _a	96 _a	0.44 _a
SE	0.35	0.65	2.2	0.7	0.4	0.002
N	4	4	4	4	4	4
<u>0.10%</u>						
$\bar{X}$	2.0 _a	0.0 _a	26 _a	61 _a	84 _a	0.44 _a
SE	0.61	0.00	4.6	6.3	1.9	0.004
N	4	4	4	4	4	4

⁷ No. of eggs pipped dead x 100/no. eggs fertile.

⁸ No. of eggs unpipped alive x 100/no. eggs fertile.

⁹ No. of eggs unpipped dead x 100/no. eggs fertile.

¹⁰ No. of eggs hatched x 100/no. eggs fertile.

¹¹ No. of ducklings surviving 14 days x 100/no. ducklings hatched.

Table 14. The effects of feeding ethalfluralin on reproductive parameters in adult bobwhite.

Dietary concentration of ethalfluralin	E/F/D ¹	Eggs set per hen	Per-cent fer-tile ²	Per-cent sur-vival ³	Per-cent sur-vival ⁴	Percent pipped alive ⁵
<u>0.00%</u>						
$\bar{X}$	0.48 ⁶ _a	30 _a	63 _a	81 _a	77 _a	28 _a
SE	0.070	19.2	8.7	8.8	8.5	5.3
N	15	15	15	15	15	15
<u>0.01%</u>						
$\bar{X}$	0.52 _a	32 _a	70 _a	90 _a	87 _a	40 _a
SE	0.062	15.6	7.4	4.9	4.9	4.0
N	13*	13	13	13	13	13
<u>0.03%</u>						
$\bar{X}$	0.44 _a	35 _a	76 _a	92 _a	89 _a	39 _a
SE	0.062	13.7	6.8	3.5	4.0	6.1
N	12	10	10	10	10	10
<u>0.10%</u>						
$\bar{X}$	0.29 _a	18 _a	62 _a	80 _a	80 _a	36 _a
SE	0.068	14.0	8.3	6.2	6.1	6.6
N	12	11	11	10	10	10

¹ Eggs/female/day.² No. eggs fertile x 100/no. eggs set.³ No. embryos alive at day 11 x 100/no. eggs fertile.⁴ No. embryos alive at day 18 x 100/no. eggs fertile.⁵ No. of eggs pipped alive x 100/no. eggs fertile.⁶ Means in the same column with the same subscript are not significantly different from the control ($P > 0.05$).

* Unequal replication is the result of the elimination of pens due to bird deaths and disqualification (i.e. 0% because 0 fertile).

Table 14. (con't).

Dietary concentration of ethalfluralin	Per-cent pipped dead ⁷	Per-cent unpipped alive ⁸	Per-cent unpipped dead ⁹	Per-cent hatch ¹⁰	Percent sur-vivabi-lity ¹¹	Egg shell thick-ness (mm)
<u>0.00%</u>						
$\bar{X}$	0.53 _a	3.9 _a	11.9 _a	34 _a	64 _a	0.23 _a
SE	0.399	2.28	5.74	7.8	7.9	0.005
N	15	15	15	15	9	14
<u>0.01%</u>						
$\bar{X}$	0.92 _a	5.3 _a	2.8 _a	38 _a	62 _a	0.23 _a
SE	0.810	2.18	1.86	6.5	6.1	0.005
N	13	13	13	13	11	12
<u>0.03%</u>						
$\bar{X}$	0.80 _a	1.1 _a	3.3 _a	43 _a	70 _a	0.23 _a
SE	0.506	1.04	1.78	4.4	5.4	0.007
N	10	10	10	10	9	7
<u>0.10%</u>						
$\bar{X}$	1.90 _a	7.4 _a	1.8 _a	33 _a	68 _a	0.22 _a
SE	0.754	3.22	0.91	8.4	11.9	0.011
N	10	10	10	10	9	6

⁷ No. of eggs pipped dead x 100/no. eggs fertile.

⁸ No. of eggs unpipped alive x 100/no. eggs fertile.

⁹ No. of eggs unpipped dead x 100/no. eggs fertile.

¹⁰ No. of eggs hatched x 100/no. eggs fertile.

¹¹ No. of chicks surviving 14 days x 100/no. chicks hatched.

Clinical Signs of Toxicity

No clinical signs of toxicity could be directly attributed to feeding ethalfluralin to adult mallards or bobwhite at 0.01%, 0.03%, or 0.10% in the diet. The only clinical signs observed in mallards were feather loss, ataxia, and limping. Feather loss and ataxia occurred equally in all treatment groups including the control. These two signs were attributed to increasing the photoperiod which induced excessive aggression, primarily among the drakes. Ataxia occurred prior to death in all cases. Limping was attributed to injuries caused by the caging. No other abnormalities were observed in the mallards.

Abnormalities observed in the quail were limping, swollen legs, ataxia, and weight loss. Limping and swollen legs were again attributed to injuries caused by the wire caging. The loss of weight and ataxia that was observed in one bird was believed to be the result of starvation. The cause of starvation was attributed to ulcerative enteritis found at necropsy.

Mortality

Thirteen mallards died during the course of the study: one female from the control diet, three males and one female from the 0.01% level, two males and two females from the 0.03% level, and two males and two females from the 0.10% level (Table 15). No mortality occurred prior to increasing the photoperiod. Following the increased photoperiod, the two males in each pen became excessively aggressive resulting in the deaths of seven males. Excessive breeding and aggression between females caused the loss of six female birds. Tissue

Table 15. Interim deaths for mallards fed ethalfluralin.

Dietary concentration	Sex	Date of death	Cause of death
0.00%	F	February 6	General trauma
0.01%	F	February 6	General trauma
0.01%	M	January 20	General trauma
0.01%	M	January 21	Spleen amyloidosis
0.01%	M	January 12	General trauma
0.03%	M	November 22	General trauma
0.03%	F	December 30	General trauma
0.03%	F	January 10	General trauma
0.03%	M	January 31	General trauma
0.10%	M	November 25	General trauma
0.10%	M	January 2	General trauma
0.10%	F	January 31	General trauma
0.10%	F	January 3	General trauma

samples were taken for histopathology from all animals; one male fed the 0.01% diet showed signs of splenic amyloidosis.

Eight bobwhite died during the course of the study (Table 16): one male and one female from the 0.01% diet, two males and one female from the 0.03% diet, and one male and two females from the 0.10% diet. The causes of death were accidental strangulation in two males, ulcerative enteritis in two females, emaciation in one male, impacted oviduct in one female, fractured tibia in one female, and an unknown cause in one male. No distinct signs of chemical toxicity were observed.

Terminal Necropsy and Histopathology

Terminal necropsy and histopathology indicated that very little substantive tissue alterations occurred in either species. Mallard abnormalities included magnum or shell gland obstruction in one female fed the control diet and one female fed the 0.10% diet, thymic candidiasis in one female on the 0.01% diet and hepatic microgranulomas in one female, ulcerative enteritis of the jejunum in a 0.01% female, crop mycotic ingluvitis accompanied by emaciation and mild intestinal enteritis in a 0.01% male, focal hemosiderosis of the heart and duodenum in a 0.03% male and air sacculitis in a 0.10% female. No consistent grossly abnormal observations or pathological alterations could be attributed to feeding ethalfluralin in the diet.

Table 16. Interim deaths for bobwhite fed ethalfluralin.

Dietary concentration	Sex	Date of death		Cause of death
0.01%	M	September	9	Accidental strangulation
0.01%	F	November	8	Ulcerative enteritis
0.03%	F	February	14	Fractured tibia
0.03%	M	February	26	Emaciation
0.03%	M	January	12	Unknown
0.10%	M	October	4	Accidental strangulation
0.10%	F	October	22	Ulcerated enteritis
0.10%	F	February	18	Impacted oviduct

DISCUSSION

Of the 13 mallards which died during the course of the study, general trauma accounted for 12 deaths. The trauma was induced by aggressive behavior associated with the normal activities of reproduction. No mallards died prior to the onset of the reproductive phase. Because of the abnormally high concentration of breeding birds held together without a means of escape, normal conflicts which usually end in one duck fleeing resulted in prolonged aggression and death. Competition between males resulted in seven dead drakes while excessive treading, rape, and female to female aggression resulted in six female deaths. Similar behavior and mortality were reported by Hochstein and Ringer (1982), Flaga (1980), and Jones (1977) in reproductive feeding trials with mallards.

The single interm death caused by splenic amyloidosis which occurred in a male on the 0.01% ethalfluralin diet could not be attributed to feeding ethalfluralin. No mallards or bobwhite on the 0.03 or 0.10% ethalfluralin diet showed similar signs of amyloidosis.

Although all eight deaths in the bobwhite groups occurred on the ethalfluralin treated diets, no significant difference in mortality between the control and any other treatment level could be established. None of the deaths were considered to be chemically induced. The two incidences of strangulation were caused by the birds becoming caught between the cage bottom and the forward side of the cage, which allows the eggs

to roll out into the collecting area. One incident of a fractured tibia was also attributed to injuries caused by the caging. The two cases of ulcerative enteritis were not considered abnormal in a study of this size. Bobwhite are known to be extremely susceptible to the bacterium (Clostridium colinum), which causes this disease, when kept in captivity (Schwartz, 1977). Impaction of the oviduct occurs with muscle paralysis or partial twisting of the oviduct, (Schwartz, 1977) which is commonly found in large populations of laying hens. The cause of emaciation could not be determined.

Pre-reproductive body weights for mallards and bobwhite were similar to weights reported by Jones (1977), Flaga (1981), and Hochstein and Ringer (1982) in similar reproductive studies. Trends for increasing body weights over the pre-reproductive phase occurred in both species. Hochstein and Ringer and Flaga also showed increasing weights over time while Jones reported a trend for slightly lower body weights in mallards over the same period. Body weights for male and female mallards did not continue to increase over the reproductive phase. Terminal weights were similar or lower than the final pre-reproductive weights. This decrease is in contrast to the body weights of reproductively active mallards reported by Jones (1977) and Hochstein and Ringer (1982). Hochstein and Ringer, and Jones reported average increases of 140 and 90 grams, respectively, during the reproductive phase, while the mallards of this study declined in weight an average of 50 grams over a similar period. Bobwhite weights at the

study termination were consistent with those reported by Flaga (1981) and Hochstein and Ringer (1982).

Feed consumption values for mallards and bobwhite were similar to figures reported by Jones (1977), Flaga (1981), and Hochstein and Ringer (1982). Both species showed trends for increased consumption over time which corresponded to increases in body weights. A sharp decline (33%) in mallard feed consumption occurred during the tenth week of the study. This reduction corresponded to the increase in photoperiod which stimulated aggressive behavior between males. Once dominance was established in the pens, feed consumption rates returned to normal.

No significant difference in reproductive parameters occurred between any treatment groups. Total number of eggs laid per bird per day for the mallards and bobwhite ranged from 25 to 43 and from 18 to 35, respectively. Mallard egg production in all dietary levels was in general agreement with results reported by Jones (1977), Federal Register (1978), and Flaga (1982). Bobwhite egg production with the exception of the 1000 ppm group, was also in agreement with these data sources. Bobwhite fed the 1000 ppm diet averaged only 18 eggs per bird over the 10 week laying period. The poor performance of this group was due to three females failing to produce eggs. Most of the remaining birds in the 1000 ppm group produced at rates equivalent to birds on the 300, 100, and 0 ppm diet.

Fertility for mallards and bobwhite was consistent with data reported by Jones (1977), Flaga (1981), and Hochstein and

Ringer (1982). Fertility on control bobwhites and mallards fed 1000 ppm diets was lower than that reported as typical for these species. Embryo survival and the number of eggs pipped were also within the range provided by these sources.

Hatchability for mallards and bobwhite was consistent with results reported by Flaga (1981). Hatchability values reported in the Federal Register (1978) for the bobwhite were higher than what was obtained in this study, while Hochstein and Ringer (1982) reported mallard hatchability below mallards on ethalfluralin.

Mallard 14 day survival was lower in the control and 1000 ppm group than results reported by Hochstein and Ringer (1982), Federal Register (1978), and Jones (1977). All bobwhite groups had lower survival rates than reported by these sources. Lower survival rates in the bobwhite and possibly in the mallards may have been attributed to cold exposure. The birds were transported in cold weather from the hatchery to the testing facility. Although precautions were taken to prevent chill, the birds may have been exposed to the cold for short periods. The one-day old bobwhites' small volume to surface area ratio and inability to thermoregulate leaves it susceptible to rapid cooling. Young birds incapable of thermoregulation show decreased survivability when their body temperatures drop too low from cold exposure (Ringer, 1981).

Eggshell thickness for both species was consistent with data published by Hochstein and Ringer (1982), Flaga (1981), and Jones (1977). Mallard eggshell thickness was considerably

greater than the values reported in the Federal Register (1978).

No significant chemically induced pathological alterations occurred in the mallards or bobwhite. A few inflammatory, bacterial, and fungal lesions occurred in both species, but were not considered abnormal for a population of this magnitude. One mallard showed signs of splenic amyloidosis. Amyloidosis is characteristic of tuberculosis, osteomyelitis, and carcinoma (Thomas, 1981). None of these diseases were diagnosed in this case.

CONCLUSION

Ethalfuralin did not produce toxicity when fed to mallards and bobwhite at concentrations up to 1000 ppm in the diet. Ethalfuralin caused no observable chemically induced alterations in normal behavior, reproductive behavior, reproductive function or performance, tissue function, or tissue morphology. Adult survivability, food consumption, body weight, egg production, and fertility were not altered significantly by ethalfuralin. Similarly, eggshell thickness, embryo survivability, hatchability, and 14 day chick and duckling survivability were not significantly effected by this herbicide.

LITERATURE CITED

- Alder, E.F., W.L. Weight, and Q.F. Soper, 1960. Control of seedling grasses in turf with diphenylacetonitrile and a substituted dinitroaniline. Proc. N. Cent. Weed Contr. Conf. 17:23-24.
- Barrentine, W.L. and G.F. Warren, 1971. Shoot zone uptake and translocation of soil applied herbicides. Weed Sci. 19:156-161.
- Bartels, P.G. and J.L. Hilton, 1973. Comparison of trifluralin, oryzalin, pronaminide, propham, and colchicine treatments on microtubules. Pest. Biochem. Physiol. 3:462-472.
- Bayer, D.E., C.L. Foy, T.E. Mallroy, and E.G. Culter, 1967. Morphological and histological effects of trifluralin on root development. Am. J. Bot. 54:945-952.
- Davis, W.A. and E.M. Rahn, 1970. Atrazine, trifluralin, and bromacil in surface water from selected agriculture and industrial sites. Proc. Northeast Weed Contr. Conf. 24:283.
- Elanco, 1977. Technical report on Sonalan[®] a selective, soil-incorporated, preemergence herbicide. Lilly Research Laboratories, a division of Eli Lilly and Co., Indianapolis Indiana 46206. pp. 4.
- Elanco, 1979. *Herbicide Handbook of the Weed Science Society of America*. Ed. W.R. Mulleson, 4th Edition. pp. 200-203.
- Emmerson, J.L. and R.C. Anderson, 1966. Metabolism of trifluralin in the rat and dog. Toxicol. Appl. Pharmacol. 9(1):84-97.
- Federal Register, July 3, 1975a. U.S. Environmental Protection Agency. Vol. 40, No. 129. pp. 28281-28284.
- Federal Register, July 3, 1975b. U.S. Environmental Protection Agency. Vol. 40, No. 129. pp. 28260-28261.
- Federal Register, July 10, 1978. U.S. Environmental Protection Agency. Vol. 43, No. 132. pp. 29729-29731.
- Flaga, C., 1981. The effect of dietary administration of fluridone on selected reproductive parameters of bobwhites and mallards. M.S. Thesis, Michigan State University.
- Gagnon, S.A. and K.C. Hamilton, 1973. Persistence of various dinitroanilines under irrigated and desert fallow conditions. Proc. W. Soc. Weed Sci. 26:24-25.

- Gill, J.L., 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Vol. 1. Iowa State University Press, Ames, Iowa. pp. 409.
- Golab, T., R.J. Herberg, S.J. Parka, and J.B. Tepe, 1967. Metabolism of carbon-14 trifluralin in carrots. J. Agric. Food Chem. 15:638-641.
- Golab, T., R.J. Herberg, E.W. Day, A.P. Raun, F.J. Helzer, and G.W. Probst, 1969. Fate of carbon-14 trifluralin in artificial rumen fluid and in ruminant animals. J. Agric. Food Chem. 17(3):576-580.
- Golab, T., R.J. Herberg, J.V. Gramlich, A.P. Raun, and G.W. Probst, 1970. Rate of benefin in soils, plants, artificial rumen fluid and the ruminant animal. J. Agric. Food Chem. 18:838-844.
- Hamdi, Y.A. and M.S. Tewifik, 1969. Decomposition of the herbicide trifluralin by a pseudomonad. Acta. Microbiol. Pol. Ser. B. 1(2):83-84.
- Helling, C.S., 1971. Pesticide mobility in soils. III. Influence of soil properties. Soil. Sci. Soc. Am. Proc. 35:743-748.
- Helling, C.S., 1976. Dinitroaniline herbicides in soils. J. Environ. Qual. 5:1-15.
- Hill, E.F., R.G. Heath, J.W. Spann, and J.W. Williams, 1975. Lethal dietary toxicities of environmental pollutants to birds. U.S.D.I., U.S. Fish and Wildlife Service, Special Scientific Report-Wildlife No. 191, Washington, D.C. p. 61.
- Hochstein, J.R. and R.K. Ringer, 1982. One generation reproductive study in mallards and bobwhite with Lilly compound 48828. Research Report to Eli Lilly and Co., pp. 56.
- Hornshaw, T.C., 1981. Renewed use of underutilized species of Great Lakes fish for animal feed. M.S. Thesis, Michigan State University.
- Humphreys, W.H., D.A. Addison, R.D. Hicks, K.E. McNeill, J. F. Nicholson, L.B. Rowland, and H.L. Webster, 1978. Ethalfluralin for weed control in cucurbits. Proc. South Weed Sci. Soc. 31:159-166.
- Ilnicki, R.D., J.R. Justin, and R.W. Michieka, 1977. The effects of some dinitroaniline herbicides on kenaf. Proc. Northeast Weed Sci. Soc. 31:93-97.

- Jones, R.E., Jr., 1977. Toxicity of diisoprophylmethylphosphonate and dicyclopentadiene on the mallard (Anas platyrhynchos). M.S. Thesis, Michigan State University.
- Kecherside, M.D., T.E. Boswell, and M.G. Merkle, 1969. Uptake and translocation of substituted aniline herbicides in peanut seedlings. *Agron. J.* 61:185-187.
- Kenaga, E.E., 1973. Factors to be considered in the evaluation of the toxicity of pesticides to birds in their environment. *Env. Qual. Safe.* 2:166-181.
- Kennedy, J.M. and R.E. Talbert, 1976. Comparative persistence of dinitroanilines on the soil surface. *Proc. South Weed Sci. Soc.* 27:321.
- Kennedy, J.M. and R.E. Talbert, 1977. Comparative persistence of dinitroaniline type herbicides on the soil surface. *Weed Sci.* 25(5):373-381.
- Koren, E., 1972. Leaching of trifluorolol and oryzalin in soil with three surfactants. *Weed Sci.* 20:230-232.
- Leitis, E. and D.G. Crosby, 1974. Photodecomposition of trifluralin. *J. Agric. Food Chem.* 22:842-848.
- Messersmith, C.G., O.C. Burnside, and T.L. Lavy, 1971. Biological and nonbiological dissipation of trifluralin in soil. *Weed Sci.* 19:285-290.
- Moreland, D.E., F.S. Farmer, and G.G. Hussy, 1972. Inhibition of photosynthesis and respiration by substituted 2,6-dinitroaniline herbicides.
- Murphy, H.J. and L.S. Morrow, 1978. An evaluation of several herbicides for weed control in Katahid potatoes. *Proc. Northeast. Weed Sci. Soc.* 32:157-160.
- Murphy, S.D., 1980. "Pesticides". In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*. Ed. Doull, J., C.D. Klaassen, and M.O. Amdur. 2nd edition. MacMillan Publishing Co., Inc., pp. 778.
- Newsom, H.C. and W.G. Woods, 1973. Photolysis of the herbicide dinitramine (N^3, N^3 -diethyl-2,4-dinitro-6-trifluoromethyl-m-phenyl-enediamine). *J. Agric. Food Chem.* 21:598-601.
- Nishimoto, R.K. and G.F. Warren, 1971. Shoot zone uptake and translocation of soil applied herbicides. *Weed Sci.* 19:156-161.
- Omer, V.V. St., 1970. Chronic and acute toxicity of the chlorinated hydrocarbon insecticides to mammals and birds. *Can. Vet. J.* 11(11):215-226.

- Parka, S.J. and H.M. Worth, 1965. The effects of trifluralin to fish. Proc. South. Weed Conf. 18:469-474.
- Parka, S.J., Q.F. Soper, and J.R. Beck, 1976. History of development and physiology of dinitroaniline herbicides. Presented as an invitational paper at the Weed Science Society of America Meeting, Denver, Colorado.
- Parka, S.J. and Q.F. Soper, 1977. The physiology and mode of action of the dinitroaniline herbicides. Weed Sci. 25: 79-87.
- Parochetti, J.V. and E.R. Hein, 1973. Volatility and photodecomposition of trifluralin, benefin, and nitralin. Weed Sci. 21:469-473.
- Parochetti, J.V., G.W. Dec, Jr., and G.W. Burt, 1976. Volatility of eleven dinitroaniline herbicides. Weed Sci. 24(6):529-532.
- Parochetti, J.V. and G.W. Dec, 1978. Photodecomposition of eleven dinitroaniline herbicides. Weed Sci. 26:153-156.
- Plimmer, J.K. and V.I. Klingebiel, 1974. Photodecomposition of N-sec-butyl-4-tect-butyl-2,6-dinitroaniline. J. Agric. Food Chem. 22:689-693.
- Prince, M.A., R.D. Radeleff, S.E. Kunz, and R.E. Everett, 1971. Toxicity of soil application of dursban to bobwhite quail. Tex. Agric. Exp. Stn. Prog. Rep. 3000. pp. 1-3.
- Probst, G.W., T. Golab, R.J. Herberg, F.J. Holzen, S.J. Parka, C Van der Scans, and J.B. Tepe, 1967. Fate of trifluralin in soils and plants. J. Agric. Food Chem. 15:592-599.
- Rahman, A. and R. Ashford, 1973. Persistence of trifluralin under field conditions in Saskatchewan. Can. J. Plant Sci. 53(2):421-423.
- Ringer, R.K., 1981. Personal Communication.
- Sheets, T.J., J.R. Bradley, Jr., and M.D. Jackson, 1973. Movement of trifluralin in surface water. Proc. South. Weed Sci. Soc. 26:376.
- Schwartz, L.D., 1977. *Poultry Health Handbook*. 2nd edition. College of Agriculture, The Pennsylvania State University. pp. 221.
- Thomas, C.L., 1981. *Taber's Cyclopedic Medical Dictionary*. 14th edition. F.A. Davis Co., Philadelphia, PA. pp. 1818.

- Worth, H.M. and R.C. Anderson, 1965. The toxicity of trifluralin, Treflan[®], an herbicide to mammals and chickens. Proc. Southern Weed Conf. 18:711-712.
- Worth, H.M., 1968. The toxicologic evaluation of benifin and trifluralin. Ind. Med. Surg. 37:545.
- Wright, W.L. and G.F. Warren, 1965. Photochemical decomposition of trifluralin. Weed Sci. 13:319-331.

APPENDIX A

Composition of duck breeder-layer diet.

Ingredients	Percent	lbs/ton
Corn, yellow ground	62.42	1248.4
Soybean meal, solvent extracted, dehulled (49%)	19.58	391.6
Fish meal	2.00	40.0
Meat and bone meal	4.00	80.0
Oat groats, rolled	2.50	50.0
Beef tallow	0.93	18.6
Dicalcium phosphate, feed grade	0.65	13.0
Limestone	6.82	136.4
Trace mineral premix TK-01 (1.02) ¹	0.10	2.0
Salt	0.30	6.0
Vitamin premix TK-01 (1.03) ²	0.50	10.0
Methionine hydroxy analog	<u>0.20</u>	<u>4.0</u>
	100.00	2000.0

¹ Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron, and 1 mg of iodine per kg of complete feed.

² Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of pantothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B₁₂, 100 mcg of biotin, and 125 mg of ethoxyquin per kg of complete feed.

APPENDIX B

Nutritional analysis of duck breeder-layer diet.

Protein, %	18.50	Vitamin K, mg/kg ²	0.7
Met. energy, Kcal/kg	2893	Choline, mg/kg	1804
Me/P ratio	(71.1)	Niacin, mg.kg	85.2
Rat, %	3.80	Pantothenic acid mg/kg	10.9
Fiber, %	2.58	Vitamin B ₆ , mg/kg	7.0
Ash, %	10.97	Riboflavin, mg/kg	5.6
Calcium, %	3.25	Thiamine, mg/kg	2.9
Phosphorus, %	0.66	Folic acid, mcg/kg	1082
Available phosphorus, %	0.46	Vitamin B ₁₂ , mcg/kg	107
Manganese, mg/kg	89.0	Biotin, mcg/kg	228
Iron, mg/kg	105.7	Arginine, %	1.29 (6.97) ¹
Copper, mg/kg	9.7	Lysine, %	0.97 (5.24)
Zinc, mg/kg	76.6	Glycine, %	1.10 (5.94)
Selenium, mcg/kg	107	Methionine, %	0.48 (2.59)
Magnesium, mg/kg	2172	Cystine, %	0.26 (1.41)
Potassium, mg/kg	6706	Total sulfur amino acids	0.74 (4.00)
Sodium, mg/kg	1821	Tryptophan, %	0.23 (1.24)
Iodine, mg/kg	1	Linoleic acid, %	1.24
Vitamin A, IU/kg	5060		
Vitamin E, mg/kg	56.2		
Vitamin D, ICU/kg	900		

¹ Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

² As supplied from vitamin premix: content of other dietary ingredients not included.

APPENDIX C

Composition of quail breeder-layer diet.

Ingredient	Percent	lbs/750
Corn, yellow ground	49.37	370.28
Soybean meal, solvent extracted dehulled (49%)	35.67	267.52
Fish meal, Menhaden	2.00	15.00
Corn distillers dried solubles	4.00	30.00
Beef tallow	1.00	7.50
Dicalcium phosphate, feed grade	2.88	21.60
Calcium carbonate (limestone)	3.88	29.10
Vitamin premix TK-01 (1.03) ¹	0.50	3.75
Trace mineral premix TK-01 (1.02) ²	0.10	0.75
Salt	0.30	2.25
Methionine hydroxy analog	<u>0.30</u>	<u>2.25</u>
	100.00	750.00

¹ Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of panthothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B₁₂, 100 mcg of biotin, and 125 mg of ethoxyquin per kg of complete feed.

² Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron, and 1 mg of iodine per kg of complete feed.

APPENDIX D

Nutritional analysis of quail breeder-layer diet.

Protein, %	24.00	Vitamin K, mg/kg	0.7
Met. energy, Kcal/kg	2803	Choline, mg/kg	2139
ME/P ratio	116.79 (53.09)	Niacin, mg/kg	97
Fat, %	3.32	Pantothenic acid, mg/kg	12.6
Fiber, %	2.68	Vitamin B ₆ , mg/kg	7.5
Ash, %	9.80	Riboflavin, mg/kg	6.2
Calcium, %	2.30	Thiamine, mg/kg	2.7
Phosphorus, %	1.00	Folic acid, mg/kg	1.6
Avail. phosphorus, %	0.72	Vitamin B ₁₂ , mg/kg	103
Manganese, mg/kg	93	Biotin, mcg/kg	301
Iron, mg/kg	116	Arginine, %	1.717 (7.15)
Cooper, mg/kg	18	Lysine, %	1.380 (5.75)
Zinc, mg/kg	84	Glycine, %	1.248 (5.20)
Selenium, mcg/kg	121	Methionine, %	0.629 (2.62)
Magnesium, mg/kg	2104	Cystine, %	0.353 (1.47)
Potassium, %	0.95	Total sulfur amino acids	0.982 (4.09)
Sodium, mg/kg	1626	Tryptophan, %	0.337 (1.40)
Iodine, mg/kg	1	Linoleic acid, %	1.22
Vitamin A, IU/kg	4629		
Vitamin D, ICU/kg	900		
Vitamin E, mg/kg	55.2		

¹ Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

APPENDIX E

Composition of duck starter diet.

Ingredients	Percent	lbs/ton
Corn, yellow ground	60.43	1208.6
Soybean meal, solvent extracted, dehulled (49%)	27.09	541.8
Fish meal	2.00	40.0
Meat and bone meal	4.00	80.0
Oat groats, rolled	2.50	50.0
Beef tallow	1.90	38.0
Dicalcium phosphate, feed grade	0.64	12.8
Limestone	0.34	6.8
Trace mineral premix TK-01 (1.02) ¹	0.10	2.0
Salt	0.30	6.0
Vitamin premix TK-01 (1.03) ²	0.50	10.0
Methionine hydroxy analog	<u>0.20</u>	<u>4.0</u>
	100.00	2000.0

¹ Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron, and 1 mg of iodine per kg of complete feed.

² Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of pantothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B₁₂, 100 mcg of biotin, and 125 mg of ethoxyquin per kg of complete feed.

APPENDIX F

Analysis of duck starter diet.

Protein, %	22.01	Vitamin K, mg/kg ¹	0.7
Met. energy, Kcal/kg	3079	Choline, mg/kg	1932
ME/P ratio	139.9 (63.6)	Niacin, mg/kg	89.7
Fat, %	4.74	Pantothenic acid, mg/kg	11.8
Fiber, %	2.74	Vitamin B ₆ , mg/kg	7.5
Ash, %	5.19	Riboflavin, mg/kg	5.8
Calcium, %	0.80	Thiamine, mg/kg	2.8
Phosphorus, %	0.70	Folic acid, mcg/kg	1345
Avail. phosphorus, %	0.46	Vitamin B ₁₂ , mcg/kg	107
Manganese, mg/kg	87.0	Biotin, mcg/kg	250
Iron, mg/kg	99.7	Arginine, % ²	1.56 (7.09)
Copper, mg/kg	12.3	Lysine, %	1.21 (5.50)
Zinc, mg/kg	78.9	Glycine, %	1.30 (5.91)
Selenium, mcg/kg	112	Methionine, %	0.53 (2.41)
Magnesium, mg/kg	2149	Cystine, %	0.32 (1.45)
Potassium, mg/kg	8120	Total sulfur amino acid	0.85 (3.86)
Sodium, mg/kg	1820	Tryptophan, %	0.29 (1.32)
Iodine, mg/kg	1	Linoleic acid, %	1.22
Vitamin A, IU/kg	4994		
Vitamin E, mg/kg	56.1		
Vitamin D, ICU/kg	900		

¹ As supplied from vitamin premix; content of other dietary ingredients not included.

² Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

APPENDIX G

Composition of quail starter.

Ingredient	Percent	lbs/160
Corn, yellow ground	46.52	348.90
Soybean meal, solvent extracted dehulled (49%)	41.26	309.45
Fish meal, Menhaden	4.00	30.00
Corn distillers dried solubles	4.00	30.00
Dried whey	2.00	15.00
Dicalcium phosphate, feed grade	0.39	2.92
Calcium carbonate (limestone)	0.73	5.48
Vitamin premix TK-01 (1.03) ¹	0.50	3.75
Trace mineral premix TK-01 (1.02) ²	0.10	0.75
Salt	0.20	1.50
Methionine hydroxy analog	<u>0.30</u>	<u>2.25</u>
	100.00	750.00

¹ Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of pantothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B₁₂, 100 mcg of biotin, and 125 mg of ethoxyquin per kg of complete feed.

² Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron, and 1 mg of iodine per kg of complete feed.

APPENDIX H

Nutritional analysis of quail starter.

Protein, %	27.99	Vitamin K, mg/kg	0.7
Met. energy, Kcal/kg	2861	Choline, mg/kg	2330
ME/P ratio	102.22 (46.46)	Niacin, mg/kg	102
Fat, %	2.46	Pantothenic acid, mg/kg	14.3
Fiber, %	2.79	Vitamin B ₆ , mg/kg	7.8
Ash, %	5.54	Riboflavin, mg/kg	6.9
Calcium, %	0.70	Thiamine, mg/kg	2.8
Phosphorus, %	0.65	Folic acid, mg/kg	1.8
Avail. phosphorus, %	0.34	Vitamin B ₁₂ , mcg/kg	107
Manganese, mg/kg	95	Biotin, mcg/kg	324
Iron, mg/kg	123	Arginine, %	1.991 (7.11)
Copper, mg/kg	20	Lysine, %	1.675 (5.98)
Zinc, mg/kg	89	Glycine, %	1.488 (5.32)
Selenium, mcg/kg	168	Methionine, %	0.710 (2.54)
Magnesium, mg/kg	2146	Cystine, %	0.413 (1.48)
Potassium, %	1.13	Total sulfur amino acids, %	1.124 (4.02)
Sodium, mg/kg	1629	Tryptophan, %	0.169 (0.60)
Iodine, mg/kg	1	Linoleic acid, %	1.17
Vitamin A, IU/kg	4535		
Vitamin D, ICU/kg	900		
Vitamin E, mg/kg	54.8		

¹ Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

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