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The toxicity of droppings from encapsulated Rabon-fed poultry of larvae of the little house fly, Fannia canicularis (L.).

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THE TOXICITY OF DROPPINGS FROM ENCAPSULATED RABON-FED POULTRY

TO LARVAE OF THE LITTLE HOUSE FLY,

FANNIA CANICULARIS (L.)

A Thesis Presented

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Insect pests have been the scourge of man since the advent of recorded history. They attack his crops and stored products; they parasitize him and his domestic animals; they transmit organisms responsible for disease; and they annoy man wherever they are present.

Chemicals have been our chief weapons in combating insects. The chlorinated hydrocarbons, in particular, have been effective control agents for nearly 30 years; however, their persistance and subsequent contamination of our environment raises questions as to how long their use can be continued.

A new organophosphate, Rabon[1], has shown promise as a broad spectrum insecticide although it is relatively safe to warm-blooded animals. This chemical has been used effectively against phytophagous insects (Young et al., 1966). animal ectoparasites (Pogoff et al., 1968), and manure-breeding flies (Treece, 1964).

One manure-breeding fly of particular importance in New England is the lesser house fly, Fannia canicularis (L.). A pest of poultry, the lesser house fly causes irritability among the hens. Also in areas adjacent to poultry houses it has become a nuisance to man. In areas where high density human populations come in contact with this fly, the probability of Fannia spp. causing myiasis is increased. James (1947) reported that the little house fly was an accidental cause of both intestinal and urinary myiasis in man.

[1] 2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate is a product of Shell Chemical Company.

Present control recommendations suggest residual applications of ronnel or dimethoate to fly resting areas in poultry houses every four weeks during the summer months (Wheeler, 1968). However, most poultry-men find the present recommendations economically unfeasible because of the time and expense involved in the pesticide applications. Recent investigations on the toxicity to fly larvae of manure from insecticide-fed hens appear to offer a possible control program.

Dennis (1969) reported that manure from Rabon-fed white leghorn chickens gave almost 100 per cent mortality to larvae of _Fannia canicularis_ (L.) at a dosage of 375 ppm. of wettable powder mixed with the mash. However, Yadava (1969) detected some residue accumulation of Rabon in the fatty tissues of Rabon-fed white leghorn hens. Unless a method can be found to decrease the amount of pesticide residue in the tissues of insecticide-fed hens, this promising means of control may not pass the rigid residue tolerances set up by the United States Food and Drug Administration.

Recently, however, new formulations of encapsulated Rabon have been developed with the hope that the encapsulated insecticide can be mixed with feed and fed to poultry. If the manure from these hens is toxic to larvae of the lesser house fly, and there is less insecticide left in the hens, an effective and potentially feasible control may be near at hand.

There are no published data on the toxicity of manure from encap-sulated Rabon-fed hens. Therefore, this investigation was undertaken to: (1) determine the levels of encapsulated Rabon needed to give

control of the larvae of <u>Fannia canicularis</u> (L.); (2) determine the persistance of the chemical in the manure with regard to control of the larvae of this fly; (3) compare the efficacy of three different formulations of encapsulated Rabon; and (4) observe any changes in behavior or performance of hens fed encapsulated Rabon.

LITERATURE REVIEW

Rabon, or Gardona, or SD-8447 as it was originally code-named, has gained wide recognition as a broad-spectrum insecticide with low mammalian toxicity. Its efficacy against poultry pests and other insects on and around livestock is especially important to this study of little house fly control. The effects of Rabon on many different kinds of insect pests have been reported. The more pertinent of these are summarized below, with special emphasis on the control of dipterous species. All dosages are given in terms of active ingredients, and all were prepared from a 75 per cent wettable powder, unless otherwise stated.

Rabon Toxicity to Dipterous Larvae

Brady and LaBrecque (1966) tested 16 compounds as larvicides against the house fly, Musca domestica L. Compound SD-8447 when added to poultry manure at the rate of one gram per square meter, gave control about equal to that of the dimethoate standard. Reducations in larval house fly populations averaged 99 per cent at one day post-treatment, 74 per cent at three to four days, and zero per cent at five to eight days. None of the 16 compounds tested gave adequate control during the fifth to eighth days after treatment.

Drummond, Ernst et al. (1967b) fed 21 different insecticides to beef cattle. The manure from these cattle was collected and its toxicity to larvae of the house fly, Musca domestica L., and the horn fly,

Haematobia irritans (L.), was determined. Of the 21 insecticides
tested, only SD-8447 and bromophos, both used at 10 milligrams per
kilogram per day, were effective. All other treatments failed to give
acceptable control. Eleven different insecticides were fed to dairy
cattle for 10 days by the same authors. This manure was collected and
infested with larvae of the house fly, Musca domestica L., and the
horn fly, Haematobia irritans (L.). During the 10 days of treatment,
SD-8447 at 10 milligrams per kilogram per day gave nearly 100 per cent
larval control, and at two days post-treatment gave 100 per cent
larval mortality. No other insecticide tested gave as good control
of both species of fly.

Balsbaugh et al. (1970) made biweekly ultra-low-volume applications
of 94 per cent Rabon at 12 ounces per acre on a cattle ranch. Rabon,
at this rate, gave 81 per cent control of the horn fly, Haematobia irri-
tans (L.) at one day post-treatment, and 31.3 per cent control seven
days after application.

Sherman et al. (1967) administered SD-8447 mixed with feed at eight
concentrations to white leghorn chickens. The toxicities of the resulting
manure samples to larvae of four different species of manure breeding
flies were determined. The highest concentration (800 ppm.) of SD-8447
gave 100, 86, 97, and 91 per cent mortalities of Musca domestica L.,
Fannia pusio (Wiedemann), Chrysomya megacephala (F.) and Para-
sarcophaga argyrostoma (Robineau-Desvoidy), respectively, within
three days after first instar larvae had been placed on the treated

manure. Ten ppm. of SD-8447, added directly to the manure, produced larval mortalities of 100, 86, 83, and 75 per cent respectively.

Axtell (1968) tested 12 insecticides against larvae of the house fly, Musca domestica L., and its mite parasite, Macrocheles muscae-domesticae (Scapoli). A 0.5 per cent and a 1.0 per cent spray of SD-8447, prepared from a 2 pound per gallon emulsifiable concentrate, gave 87 and 93 per cent control, respectively, of the mite 14 days post-treatment. However, both treatments prevented an increase in the number of fly larvae up to seven days post-treatment.

Bailey et al. (1968) applied SD-8447 to the surface of poultry droppings at the rate of two grams per square meter to test its effi-cacy against larvae of the house fly, Musca domestica L. Counts made at one, five, seven and nine days post treatment gave respective reductions in larval populations of 99.6, 92.0, 84.2 and 47.9 per cent, as compared with the pretreatment counts.

Treece (1964) fed SD-8447 to Jersey and Holstein cattle at a rate of 0.5 milligrams per kilogram per day for four or five consecutive days. Rectal samples of feces were collected and infested with first instar larvae of the face fly, Musca autumnalis DeGeer. Post-treatment counts on days one, two, three, and four gave 100 per cent larval mortalities.

Mount et al. (1967) evaluated 19 compounds against adults and larvae of the stable fly, Stomoxys calcitrans (L.). Compound SD-8447 applied to the CSMA larval medium at the rate of 1.95 ppm. resulted in 90 per cent mortality to treated third instar fly larvae. Of the 19 relatively safe insecticides tested, SD-8447 was the most effective larvicide.

Drummond (1967) tested 14 insecticides to determine their systemic effectiveness for the control of the common cattle grub, _Hypoderma lineatum_ ('de Villers), and the northern' cattle grub, _Hypoderma bovis_ (L.). Compound SD-8447 was one of the only two insecticides tested which gave better than 90 per cent grub mortality when used either as a pour-on of a 16 per cent emulsion, or as a 5.0 per cent wettable powder spray. Compound SD-8447 was considered lower in mammalian toxicity by a factor of 10 than some materials then in use to control cattle grubs (Rogoff _et al._, 1968).

Rogoff _et al._ (1968) applied SD-8447 to Hereford calves by the pour-on method for cattle grub control. At 30 grams per animal, SD-8447 gave 41 and 60 per cent grub control on two groups of calves, although preliminary tests had shown 89 to 100 per cent control. The treated cattle demonstrated no symptoms of poisoning, changes in weight, or blood cholinesterase depression during the tests.

Dennis (1969) fed Rabon as a 75 per cent wettable powder mixed in the mash to white leghorn chickens at five dosages. The manure was collected and infested with larvae of the lesser house fly, _Fannia canicularis_ (L.). Greater than 95 per cent control was quite consistently obtained with dosages of 300 ppm. or above.

Rabon Toxicity to Adult Flies

Mathis and Schoof (1965) tested SD-8447 against adult house flies, _Musca domestica_ L., in two dairies by applying a suspension at one gram per square meter as a residual spray. In one case this gave six weeks

of excellent control, followed by seven weeks of satisfactory control. In the second dairy the same dosage provided adequate control for only one week. Retreatment at double the original dosage gave six additional weeks of satisfactory control.

Brady, Meifert et al. (1966) reported on tests in which an emulsion of SD-8447 applied at one gram per square meter gave variable control of house fly adults. Post-treatment surveys indicated 97 to 74 per cent control at one day, 60 to 6 per cent at three to four days, and 14 to zero per cent after seven or eight days.

LaBrecque (1967) made wind tunnel tests in evaluating 119 insecticides as contact sprays against a susceptible and a resistant strain of the house fly, Musca domestica L. Compound SD-8447 was one of 61 compounds which were more effective than the two standards, ronnel and malathion. Tested at five concentrations (2.5 per cent down to 0.1 per cent), the LC_{50} for Rabon against the susceptible Orlando strain was found to be 0.06 per cent, while against the Cradson P Strain (DDT, coumaphos, malathion, and parathion resistant) the LC_{50} was 0.30 per cent.

Hansens et al. (1967) tested a one per cent spray of SD-8447, prepared from a 75 per cent wettable powder, on house fly resting areas in two barns. Over a three-year period in one barn, and a two-year period in the other, SD-8447 gave six to ten weeks of satisfactory control. In a follow-up Hansens et al. (1968) reported that residual applications of Rabon at a rate of 15.4 micrograms per square centimeter gave 20 to 57 per cent mortalities of adult house flies.

Mathis et al. (1968) chemically treated all potential resting spots for adult house flies at nine dairies over a three-year period. Gardona (another trade name for SD-8447), applied as a 5.0 per cent emulsion at the rate of two grams actual per square meter, gave variable control for zero to 14 weeks.

Seawright et al. (1968) tested five insecticides to control face fly adults, Musca autumnalis DeGeer, on beef cattle in shed-protected dust bags set up in 24 pastures. These self-applicating devices applied roughly seven grams of a 5.0 per cent SD-8447 dust per head per day. Weekly post-treatment counts indicated a mean reduction in face fly adults of 73.6 per cent. In this case the other insecticides tested gave comparable results.

Morgan and Blume (1966) subjected 24- and 72-hour-old horn fly adults, Haematobia irritans (L.) to residual treatments of 0.1, 0.5, and 1.0 per cent SD-8447 for periods of one to six minutes. All three concentrations gave 90 per cent mortality within 24 hours, regardless of fly age, even at the shortest exposure.

Oehler et al. (1969) treated dairy cattle with the minimum amount of Gardona needed to control the horn fly, Haematobia irritans (L.). The milk from cows treated with one milliliter of a 0.5 or 1.0 per cent Gardona spray, prepared from a 75 per cent wettable powder, was analyzed for pesticide residues over a four-week period. No residues of Gardona were ever detected in milk from the treated cows.

Wilson (1968) coated the insides of quart-size glass jars with 50 milliliters of a 0.125 SD-8447 solution each. Striped horse fly adults, Tabanus lineola F., were exposed within the treated jars for varying

periods of time. Average 24-hour mortalities for two, five, ten, twenty, and thirty minute exposure periods were 25, 25, 15, 25, and 50 per cent respectively.

Morgan (1967) developed an electro-chemical device for control of the horn fly, Haematobia irritans (L.). Utilizing ultraviolet light as an attractant, and a cotton curtain impregnated with a 5.0 per cent solution of Rabon as toxicant, this attractant-toxicant device was reportedly quite satisfactory in reducing adult horn fly populations.

Rabon Toxicity to Non-dipterous Ectoparasites

Drummond, Whetstone et al. (1967a) compared two formulations of SD-8447 against the Lone Star tick, Amblyomma americanum (L.) on cattle. One-tenth per cent concentrations of SD-8447 as wettable powder and emulsifiable concentrate formulations gave 97 and 100 per cent control, respectively, at one day post-treatment. Wettable powder formulations gave no control at one week post-treatment, whereas 0.1 and 0.5 per cent emulsions gave 10 and 50 per cent control, respectively, at that time.

Drummond et al. (1968) compared the effectiveness of Rabon as a spray and as a dip to control the cattle tick, Boophilus annulatus (Say), and the Southern cattle tick, Boophilus microplus (Canestrini). A concentration of 0.45 per cent Rabon emulsion, prepared from a two pound per gallon EC, gave 99 per cent control of Boophilus annulatus (Say), and 100 per cent control of Boophilus microplus (Canestrini). When the cattle were sprayed with 0.1 per cent Rabon suspension prepared from a 75 per cent wettable powder, 96.7 and 99.8 per cent controls were afforded

against the respective species. Dipping in freshly charged vats containing 0.25 per cent Rabon suspension controlled both species of ticks satisfactorily, but after six weeks of aging and moderate use the control was less pronounced.

Drummond, Whetstone et al. (1967b) tested 26 insecticides against the cattle ear tick, Otobius megnini (Duges). As a 0.5 per cent spray, prepared from a two pound per gallon EC, SD-8447 gave better than 95 per cent control of this tick at one week post-treatment. However, at four weeks post-treatment this concentration provided only 66 per cent control.

Hoffman and Hogan (1967) applied a 5.0 per cent SD-8447 dust to poultry litter at the rate of 50 grams per square meter. Post-treatment counts up to 34 days indicated that at this rate SD-8447 killed 90 per cent of three species of chicken lice: the chicken body louse, Menacanthus stramineus (Nitzsch), the wing louse, Menopon gallinae (L.), and the shaft louse, Lipeurus caponia (L.).

Nelson et al. (1969) compared the efficacy of six different formulations of SD-8447 for the control of the Northern fowl mite, Ornithonysus sylviarum (Canestrini and Fanzago). Three formulations, a 3.0 per cent dust, a 0.5 per cent suspension, and a 0.5 per cent emulsion, each combined with .05 per cent dichlorvos, proved to be highly effective as compared with the 0.5 per cent standard carbaryl suspension. Neither egg production, nor feed consumption were significantly affected by these treatments.

Furman and Lee (1968) treated white leghorn chickens with a 0.5 per cent suspension of Rabon wettable powder at the rate of one gallon per 100 birds. Nearly 100 per cent control of the Northern fowl mite, _Ornithonyssus sylviarum_ (Canestrini and Fanzago), was obtained by this treatment.

Rabon Toxicity to Pests of Plants

Young and Bowman (1966) in tests on the fall armyworm, _Spodoptera frugiperda_ (Smith), and the corn earworm, _Heliothis zea_ (Boddie), found that leaf discs dipped in an emulsion of SD-8447 at a concentration of .0625 pounds actual per twenty-five gallons of water gave better control of both species than the standard p,p'-DDT. In the field SD-8447 applied at one pound actual per acre was as effective as the p,p'-DDT standard against both the fall armyworm and the corn earworm. Janes and Greene (1969) found that Gardona at 0.75 pounds per acre gave 98 per cent control of the fall armyworm, and that at only 0.5 pounds per acre it gave 98 per cent control of the corn earworm.

Other workers have obtained good to excellent control of the corn earworm with this insecticide. Klostermeyer (1968) applied Gardona to field corn at two pounds per acre. At this rate it was about equivalent to the carbaryl standard of two pounds per acre. Harrison (1968) obtained 80.7 per cent control of corn earworm with Gardona at two pounds per acre. McMillan et al. (1968) mixed 1.8 milligrams of SD-8447 with 0.5 grams of a corn earworm larval feeding-arrestant. Applied on seedlings 20 days after planting, this treatment gave 90 per cent control

of larvae after 48 hours. This mixture produced significantly higher larval mortalities than either the feeding-arrestant or SD-8447 used alone at the same rates. Keaster (1969) showed that a three per cent emulsion used so as to give 1.5 pounds per acre gave 97.5 per cent control of the corn earworm. Finally, Anderson and Nakakihara (1968) found that Gardona as a two per cent or five per cent dust applied at 0.5 pounds actual toxicant per acre gave 87 and 93 per cent larval mortalities, respectively, of the corn earworm in tests over a one-year period.

Harding et al. (1968) field-tested a Gardona suspension against first generation European corn borer, Ostrinia nubilalis (Hubner). Applied at 0.75 pounds per acre it proved as effective as one pound of DDT per acre in producing 70 per cent corn borer control.

Compound SD-8447 also has proved effective against the tobacco hornworm, Manduca sexta (Johannson). Rabb and Guthrie (1964) reported that at one pound per acre it gave promising control of this insect. Dominick (1968) showed that SD-8447 gave seven days of 100 per cent control of both tobacco hornworm and the tomato hornworm, Manduca guinquemaculata (Haworth), when applied at one pound per acre.

Stored product insects have also been controlled with SD-8447. Strong and Sbur (1965) obtained 100 per cent control of the rice weevil, Sitophilus oryzae (L.), and the granary weevil, Sitophilus granarius (L.), with a suspension of SD-8447 applied to the grain surface at 2.5 ppm. Also they completely controlled the confused flour beetle,

Tribolium _confusum_ (L.) when it was applied in the same way at 15 ppm.

Forty-eight insecticides were subsequently tested against the Angoumais grain moth, _Sitotroga_ _cerelella_ (Olivier), the confused flour beetle, _Tribolium_ _confusum_ (L.), the rice weevil, _Sitophilus_ _oryzae_ (L.), the granary weevil, _Sitophilus_ _granarius_ (L.), and the saw-toothed grain beetle, _Oryzaephilus_ _surinamensis_ (Linne). Strong and Sbur (1968) found that Gardona, applied to the grain surface as a 0.1 per cent suspension, gave 100, 100, 8, 100, and 68 per cent control, respectively, of the pests listed. More recently Strong (1970) reported Gardona effective against both the confused flour beetle and the red flour beetle, _Tribolium_ _castaneum_ (Herbst).

Control of two different species of "webworms" has been obtained with SD-8447. Buffan _et_ _al_. (1969) found that at one pound per acre it gave 40 per cent control of the fall webworm, _Hyphantria_ _cunea_ (Drury) at 10 days post-treatment. Nordin and Appleby (1969) used Gardona (two pounds per gallon EC) at one quart to 100 gallons, and obtained 100 per cent control of the juniper webworm, _Dichomeris_ _marginella_ (F.), up to two weeks post-treatment.

Compound SD-8447 has given promising control of several tree pests. Campbell (1968) demonstrated the potential value of Gardona against a number of pests of Scotch pine Christmas trees. Doane (1966) found SD-8447 to be promising against the gypsy moth, _Porthetria_ _dispar_ (L.). Against the lesser peach tree borer, _Synanthedon_ _pictipes_ (Grote and Robinson), Bobb (1969) found that Gardona gave 81.7 per cent control

at one week post-treatment when applied to peach trees at one pound
per 100 gallons. Boulanger (1965) applied SD-8447 at 12 ounces per
100 gallons and obtained 98.7 per cent control of the oyster-shell
scale, Lepidosaphes ulmi (L.) when it was in the crawler stage.

Compound SD-8447 also has shown promise of being at least useful
against the following pests: the pickleworm, Diaphania nitidalis
(Stoll), Waites and Habeck (1968); the red-banded leaf roller,
Argyrotaenia velutinana (Walker), Cox (1966) and Asquith (1970); the
rice water weevil, Lissorhoptrus oryzophilus Kuschel, Grigarick and
Beards (1965); the tobacco flea beetle, Epitrix hirtipennis (Mel-
sheimer), Tappan (1965); and finally the Mexican bean beetle, Epilachna
varivestis Mulsant, the Potato leafhopper, Empoasca fabae (Harris), and
plant bugs (Lygus species), Judge et al. (1970).

Anderson and Atkins (1966) tested SD-8447 on a 16-acre plot at the
rate of 16 ounces per five gallons of water per acre to determine its
effect on honey bees. A pretreatment survey showed 165 dead bees,
whereas at one day post-treatment 445 dead bees were found in six traps.
During the same period 30 field counts indicated that field visits by
bees declined from 626 to 96. Anderson, Atkins et al. (1968) concluded
that Gardona at 16 ounces per acre caused no severe bee losses at the
colonies, but that it did reduce field visits up to two days post-treat-
ment. Also when tested at 16 ounces per acre his pretreatment counts
showed 32 dead bees per day per colony, compared with 79 dead three days
post-treatment.

Inconclusive or less promising control with SD-8447 has been obtained of the green peach aphid, _Myzus persicae_ (Sulzer), Thurston (1965); citrus red mites, _Phyllocoptruta oleivora_ (Ashmead) and _Aculus pelekassi_ (Keifer), Reed et al. (1967); the potato tuberworm, _Phthorimaea operculella_ (Zeller), Shaney et al. (1967); the alfalfa weevil _Hypera postica_ (Gyllenhal), Dorsey (1966); the cabbage looper, _Trichoplusia ni_ (Hubner) and _Pseudoplusia includens_ (Walker), Chalfant (1969); a weevil, _Brachyrhinus cribricollis_ (Gyllenhal), Wene (1969); and a tortricid leaf roller, _Platynota sultana_ (L.), Ota (1969).

Based on an examination of the literature, Rabon appears to be fairly effective against at least some species representing several insect orders. Diptera, both larvae and adults, appear to be, in general, highly susceptible. Against Lepidoptera the results are variable, although it does appear promising against many species. Also, the results on Coleoptera have been good in many cases. Excellent control has been obtained on various species of Mallophaga, particularly poultry pests. Against Heteroptera the results are variable, and no general conclusion can be reached as to the effectiveness of Rabon against the group as a whole. Several groups, including the Orthoptera, Homoptera and Acarina, appear to be quite tolerant of Rabon, and tests on representatives of these groups have generally been either inconclusive or failures.

PROPERTIES OF RABON

The physical and chemical properties of any toxicant determine
its applicability, its effectiveness, its mode of action, and its
safety. It is therefore necessary to review the properties of Rabon
to gain a better perspective of its potential value as an insecticide.

The information below on the physical, chemical, and biological
properties of Rabon was taken from a Shell Technical Bulletin entitled,
"Summary of Basic Data for Technical Rabon Insecticide," unless other-
wise indicated.

Rabon, known also as Gardona, and formerly as SD-8447, has a
chemical structure of 2-chloro-1-(2,4,5, trichlorophenyl) vinyl di-
methyl phosphate. The empirical formula of Rabon is $C_{10}H_9O_4Cl_4P$.
The structural formula of Rabon is as follows:
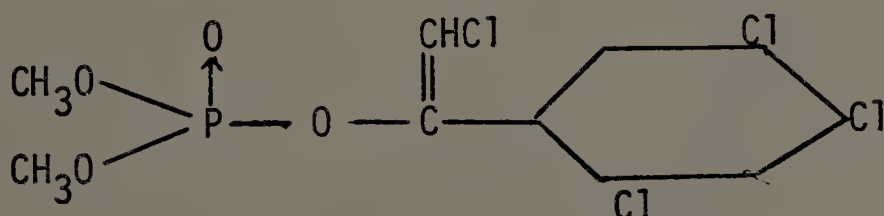
Technical Rabon is a tan to brown solid with a molecular weight of
365.98 (principal constituent) and a melting range of 93-98°C. Its
solubilities in several solvents are listed below:

Solvent	% wt. at 0°C
Chloroform	40
Methylene Chloride	40
Acetone	<20
Xylene	8
- -	
Water (24°C)	15 ppm

Rabon decomposes slowly at 250°F. Its hydrolytic half-life is 37 hours at pH 9.1, and 7200 hours at pH 1.1. Under a sunlamp at pH 1.1, 75 per cent by weight was recovered by bioassay after four hours. (Whetstone et al., 1966)

Technical Rabon is prepared in 30 minutes by the addition of 23 grams (0.49 moles) of trimethyl phosphite to 50 grams (0.17 moles) of pentachloroacetophenone at 30° to 50°C. This reaction mixture is heated at 110°C for 30 minutes, and then cooled in about 100 milliliters of ether. Upon cooling, the solid is filtered and washed with pentane. A dry weight of 49 grams technical Rabon has been obtained by this synthesis which represents a 79 per cent yield. (Whetstone et al. 1966)

A Shell Company Chemical Bulletin reports that Rabon is relatively nontoxic to mammals, with an oral LD_{50} for white rats of 4000-5000 mg/kg. Its acute dermal LD_{50} for rabbits is greater than 5000 mg/kg. Acute oral feeding studies indicate that Rabon is relatively nontoxic to both fish and wildlife.

Several investigations have indicated that Rabon is a relatively safe insecticide. Whetstone et al. (1966) found Rabon to be highly toxic to several species of insects, but at the same time quite safe to laboratory animals. Similarly, Yadava (1969) reported that Rabon-fed chickens contained very little residue in their body tissues. Ivey et al. (1968) found only small residue accumulations in the fat of dairy cattle fed daily doses of Gardona, and these small accumulations were eliminated in 21 days or less. Also, Ivey et al. (1969)

found only small amounts of Gardona in the tissues of white leghorn chickens kept in houses treated with 45 grams of Gardona (on an unmentioned surface area).

CULTURING METHODS

Our stock culture of the lesser house fly, _Fannia canicularis_ (L.) was obtained from Leonard's Poultry Farm in North Amherst in May, 1968. Adult flies were collected singly in pint-size paper containers as they rested on the poultry house walls. The flies were cultured for over a year before tests were initiated, at which time it was felt that the population was fairly homogeneous.

The fly colony was housed in newly constructed 2' x 2' x 2' wood and screen cages. Reasonably constant environmental conditions were important, and fluctuations sometimes caused serious problems. At first the cages were constantly illuminated with artificial light, plus natural light during the day. However, during the fall of 1969, egg production ceased completely, suggesting a possible photoperiodic effect. Illumination with only artificial light resulted in reinitiation of oviposition within a few days. Temperature variations at times affected the fly colony. A constant temperature of 70°F was about ideal for producing active, healthy flies with average life spans of about six weeks. Conversely, temperatures greater than 80°F produced significant mortalities, and on one occasion resulted in complete mortality of over 20,000 flies. Cage density also played an important role in culture vitality. It was found that optimum cage density approximated 1500 flies in a 2' x 2' x 2' cage. Under favorable conditions and at this density, the population lived longer, produced more eggs, and was generally more active than populations caged at either higher or lower densities.

Initially the adults were fed molasses and canned evaporated milk, both diluted 20:1 with water as recommended by Steve (1959) and Eversole (1965). Because high adult mortality was sometimes obtained at these low concentrations, both milk and molasses were later diluted 1:1 with water, with much better results. The molasses and milk were poured into separate three-ounce paper cups, into each of which a crumpled paper towel had been placed. This towel absorbed the molasses or milk solution, and allowed the flies easy access to food.

Three major problems arose with the feeding procedure described above. First, a great number of flies were trapped and killed in the sticky food. Second, mold growth became a problem in both the molasses and milk solutions, making daily changes of food necessary. Lastly, fatulence was observed in both male and female flies. Histological investigation revealed that this was due to a swelling of the gut, and removal of the molasses solution remedied the swelling of the abdomen. Because of these problems, the culture was ultimately reared on a 1:1:1 mixture of sugar, powdered milk and powdered egg yolk, with a copious supply of free water available from wicks on a separate container. This diet alleviated all the problems associated with adult feeding.

Two different kinds of oviposition media were tried. A Chemical Specialties Manufacturers Association (CSMA) larval house fly medium was fermented as suggested by Steve (1959) and Eversole (1965). The fermented CSMA was placed in three-ounce paper cups, and two containers were

placed in each fly cage. Oviposition was extremely poor with this
material. Therefore, it was decided to utilize the natural oviposition
medium, poultry manure, which was moistened and packed into three-
ounce paper cups to fill them about half way. Two such cups were placed
in each cage, and left there until they were removed 12 hours later.

Only about the top half-inch of manure was removed from the ovi-
position cups because it contained over 90 per cent of the eggs present.
This layer of manure containing the eggs was next placed in a petri-dish
and incubated at about 80°F and 95 per cent relative humidity for 24 to
36 hours, by which time more than 90 per cent of the eggs usually had
hatched. The resulting first instar larvae were then ready for transfer
to the larval rearing medium used in the tests.

Two different larval rearing media were used. As recommended by
Steve (1959a), about one and one-half pints of CSMA medium were mixed
with 350 milliliters of water in a number 10 can. An additional 50
milliliters of molasses were mixed in as a larval nutrient. To this
medium approximately 500 first instar larvae of the lesser house fly
were added. However, the heat of fermentation killed many larvae, with
temperatures going as high as 150°F in this medium. Addition of water
reduced fermentation, but made the medium too moist for ideal larval
growth. Because of the problems involved with CSMA, a change was made
to the natural larval medium, poultry manure.

To prepare the second medium, one pint of poultry manure was placed
in a number 10 can. Approximately twenty-five milliliters of water were
added when necessary. This made a suitable rearing medium for 500

larvae. The larvae grew much faster and to a larger size in the natural
medium, thus shortening the life cycle by several days. Within seven
days after first instar larvae had been seeded on the manure, the larvae
migrated to the top layer of the manure to pupate.

Several methods were tried for separating the mature larvae from
the larval medium. Washing the medium through a strainer was ineffective
because very little of the CSMA would pass through. Separation with a
Berlese funnel was attempted, but high larval mortalities were obtained
by this method. Another method allowed the mature larvae to crawl
between strips of corrugated cardboard. However, this was a slow and
labor-consuming process. Finally, separation of pupae from the rearing
medium by flotation in a dish of water was not successful because only
30 to 40 per cent of the pupae floated, even when salt was added to the
water. Finally, it was decided to simply allow the larvae to pupate in
the rearing cans, which were placed in the rearing cages when the adults
began to emerge.

Because the pupae were allowed to develop in the rearing medium, a
large population of the predaceous mite, Macrocheles muscaedomesticae
(Scapoli), developed by the end of the first season. Steve (1959b)
reported that this mite attacked eggs and first instar larvae of the
little house fly, and Eversole (1965) reported that it attacked and
killed little house fly adults. Our observations on the activity of
this mite showed that large numbers of eggs were destroyed by it, and
that as the mite population increased a large percentage of the adult

flies also became parasitized. As many as five adult mites were observed feeding on a single little house fly adult at one time. Chemical control of the mite, <u>Macrocheles muscaedomesticae</u> (Scapoli) proved unsatisfactory. Treatment of the larval medium with Kelthane at 10 ppm. controlled the mites, but gave unacceptable larval mortality, whereas lower dosages did not control the mites. Since the mite was a major problem, the mites were hand picked from all newly laid eggs, in both the larval rearing cans and the adult cages. Although this was a time-consuming process, eventually mite-free rearing cans were obtained, and within a month, all mites had been eliminated from the culture. Subsequent mite outbreaks occurred, but this method of physical separation of the fly eggs from mite infestation always alleviated the problem quickly.

During the summer of 1969 some late instar larvae were attacked by a small hymenopterous parasite, <u>Pachycrepoideus vendemniae</u> (Rond.). This outbreak came on quickly and was not detected until the parasite had caused greater than 90 per cent mortality in our rearing cans. Further problems with this parasite were eliminated by substituting a fine mesh nylon for the cheesecloth used to cover the larval rearing cans. Access to the larvae was thus denied to the adult parasites.

RESULTS AND DISCUSSION

Three experiments, each involving a different formulation of
encapsulated Rabon, were run to determine the toxicity of manure from
encapsulated Rabon-fed hens to larvae of the lesser house fly, _Fannia_
canicularis (L.). These were supplied by the Shell Chemical Company
under the following codes: (1) 93 per cent Rabon, Wallace and Tiernan,
No. 620-169; (2) 60 per cent Rabon, AC 360, Lot 2, AC 407; and (3) 52
per cent Rabon, #230-97, Code CS-52.

Larval survivals from the 50 larvae per replicate tested for the
different formulations are presented in Tables 1a, 3a, and 5a. Conver-
sions of these data to corrected larval mortalities by the use of
Abbott's formula are presented in Tables 1b, 3b, and 5b. Results from
the first day's manure collections were not indicative of the dosage
fed, probably because individual hens varied in the amount of time
needed to pass the toxicant through their digestive systems. Results
of tests with manure from 52 and 60 per cent encapsulated Rabon
feedings showed significantly higher survival (lower mortality) in
manure collected the first day after initiation of feeding than the
subsequent samples tested.

In the tests reported in Tables 1 to 6, no attempt was made to
test the manure from the same birds in all the replicates at a parti-
cular dosage. Eight birds were fed each dosage, and in many instances
the manure from a particular bird was not sufficient for the three
replicates of a bioassay. For this reason the manure sample at a

survival. If the larvae were not counted until the eighth day, migration from the containers occurred so that accurate counts could not be obtained.

On the seventh day the samples were removed from the incubator. Each individual sample was placed in a strainer and washed under tap water. By this procedure the manure was washed away so as to leave only the mature larvae in the strainer. This method afforded a quick and accurate means of determining larval survival.

weighed out into half-pint paper containers. About 20 milliliters of water were added to each 75 gram manure sample, except in those cases where the manure was judged to be sufficiently moist for larval survival. The manure was then thoroughly mixed to insure uniformity. In the persistance tests, the manure samples were incubated at 80°F until tests were run.

When the first instar larvae were ready to be transferred to the manure samples, the procedures of Eversole (1965) and Dennis (1969) were used. A minuten insect pin was driven into the blunt end of a wooden match stick. First instar larvae would adhere to the minuten pin, especially if it had been moistened in water. The larvae were then transferred by this simple device to the test manure. Although up to 10 larvae could be picked up at one time, it was decided never to transfer more than four at a time to the manure. In this way counting errors were minimized. Care was taken not to contaminate the pin by touching the manure, but any such contamination was nulified by the fact that separate pins were used for each insecticide dosage.

When 50 larvae had been transferred from the incubation dish to the test manure, the uncovered half-pint container was placed in an incubator held at 80°F and 95 per cent relative humidity. This was done for all replicates and all dosages. Usually two days of collected manure were tested simultaneously. This involved the counting of 1200 larvae, which could usually be accomplished in four hours.

The containers were kept in the incubator for seven days, at which time it was extremely important to make the counts to determine larval

Rabon-fed hens, it was decided to keep the birds on the insecticide diet for 14 days. During these 14 days the above-mentioned procedure for weighing mash consumption and measuring water consumption was maintained. This was done with all three groups of experimental hens.

Twenty-four hours after the initiation of feeding encapsulated Rabon, and each day thereafter, the manure from each bird was carefully scraped into individual half-pint containers from Kraft paper placed underneath the cages. The 30 containers for each day, one for each bird, were labeled and placed in a freezer where they remained until the bioassays could be run.

The bioassays were initiated when the stock culture was built up to approximately 20,000 adult flies. This population was needed to insure an adequate supply of eggs to produce the first instar larvae needed for the tests.

Thirty-six hours prior to initiating a bioassay, manure for oviposition was placed in the culture cages. Twelve hours later the newly-laid eggs were collected and placed in a petri dish. Care was taken to remove the eggs from the oviposition manure so that very little manure was transferred to the petri dish. The eggs were then placed in an incubator kept at 80°F and 95 per cent relative humidity, where the eggs hatched within 24 hours.

While the eggs were being incubated, the methods of Eversole (1965) and Dennis (1969) were followed in preparing the bioassay. One day's test manure was unfrozen by placing the half-pint containers at 80°F for eight hours. Three replicates of 75 grams of manure at each dosage were

At first individual bird weights were taken daily (white leghorn chickens), but it was found that daily weighing had an unsettling effect on the birds. Therefore the second group, the Rhode Island red hens, were weighed only every third day, and the last group, the salmon sex link hens were weighed only on each seventh day. By eliminating daily weighings, the behavior pattern of the birds was not disturbed; irritability and nervousness were decreased; and the birds remained calmer.

Previous work by Dennis (1969) indicated that an oral dose of 375 ppm. of actual Rabon, gave nearly 100 per cent mortality of first instar larvae of the lesser house fly, Fannia canicularis (L.), seeded on the manure from the treated hens. Since information on the toxicity and chemical persistance of Rabon was desired, a dosage range of 100 to 800 ppm. was chosen. This range enabled tests to be made for both initial mortality and toxicant persistance when manure from treated birds was held at room temperature for varying periods prior to testing.

The 30 birds were divided into four groups (six birds in the control, and eight in each of the other three groups) by random selection. Four levels of encapsulated Rabon (zero, 100, 400, and 800 ppm.), were then incorporated into the mash. The insecticide was added to the mash by placing the desired amount in a gallon jar in which 1500 grams of laying mash had already been added. The jars were rotated on a motor-driven roller for two hours for mixing. To facilitate thorough mixing, adhesive tape was placed on the inside of the jars to increase the friction between the glass and the mash.

Since co-workers planned work on the manure from encapsulated

MATERIALS AND METHODS

Three separate experiments, each involving a different formulation
of encapsulated Rabon, were conducted on white leghorn chickens, Rhode
Island red hens, and salmon sex link hens, respectively. Since the
numbers of birds, the mode of insecticide application in the feed mash,
and the handling of the birds were the same in all three experiments,
only the procedure for the white leghorns will be explained in detail.
Except for the smaller dosages fed to the salmon sex link hens, all
procedures were exactly the same for all three groups.

Thirty young female white leghorn chickens of uniform age and weight
were obtained from the University farm. They were placed in separate wire
cages immediately upon receipt, and allowed to adjust to their new environ-
ment. Except for daily feeding and changing of water, the birds remained
undisturbed for a period of three weeks, by which time all had initiated
egg production.

At this time daily measurements of feed consumption, water consumption,
and bird weights were made for each individual bird, and this was continued
throughout the experiment. Daily aliquots of 250 grams of laying mash
were weighed out for each bird, and 250 milliliters of fresh water were
supplied daily. After 24 hours the weight of uneaten mash and volume of
remaining water were taken, and these portions of food and water were
discarded. A new measured supply of food and water was then given to each
hen. It was noticed that in the course of feeding some mash was spilled;
however, this loss was judged as unavoidable and was probably about
equal for all birds.

particular dosage frequently was not obtained entirely from the same bird.

In the persistance tests a bioassay with three replicates of 50 larvae each was run on manure which had been aged at room temperature for the time period indicated. Lack of sufficient manure prevented daily bioassays on all dosages through the 49th day of aging. Like-wise, once a particular dosage showed survival equal to the control, testing ceased. Lack of manure and lowered control survival in aged manure made testing beyond the 49th day impractical.

The results obtained with manure from 93 per cent encapsulated Rabon feedings are presented in Tables 1a and 1b. Manure collected on the first day after initiation of feeding gave fairly good control at 100 ppm. (77.4 per cent corrected mortality), and this level of control was maintained or exceeded throughout the 14-day feeding period. The two higher dosages tested (400 and 800 ppm.) gave 100 per cent control of larvae seeded on the manure collected during the first day of Rabon feeding.

Investigations on the persistance of the toxicant in manure from the 93 per cent Rabon-fed hens revealed that by the sixth day during which the manure was held at room temperature, a dosage of 100 ppm. mixed into the feed gave no larval mortality when compared to the control group. However, at the same time (six days) 100 per cent mortality was still afforded by both the 400 and 800 ppm. dosages. Continued persistance tests revealed that on day 21 of aging at room

32

Table 1a. Little house fly larvae surviving after seven days in manure collected from experimental birds fed with 93% encapsulated Rabon.

Rabon level in mash (actual)	Days in treatment period:						
	2	4	6	8	10	12	14
0 ppm (control)	39	43	47	31	43	47	47
	44	38	36	46	39	29	50
	43	46	43	45	44	40	48
100 ppm	15	1	12	9	0	2	8
	11	10	0	7	7	10	10
	3	16	13	11	11	11	9
400 ppm	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
800 ppm	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0

Table 1b. Corrected average per cent mortalities of little house fly larvae grown in manure from 93% encapsulated Rabon-fed hens.[1]

Rabon level in mash (actual)	Days in treatment period:						
	2	4	6	8	10	12	14
0 ppm (control)	0	0	0	0	0	0	0
100 ppm	77.4	78.8	78.6	77.8	85.7	80.5	81.4
400 ppm	100	100	100	100	100	100	100
800 ppm	100	100	100	100	100	100	100

[1] Larval mortalities from Table 1a corrected by Abbott's formula.

Table 2. Persistance of toxicant in manure from 93% encapsulated Rabon-fed hens to larvae of the little house fly, as bioassayed by larval survival after seven days in the treated manure.

Rabon level in mash (actual)	Number of days manure was aged at room temperature before larval exposure:												
	1	2	3	4	5	6	7	14	21	28	35	42	49
0 ppm (control)	47	41	45	43	43	50	44	43	48	37	41	37	28
	41	43	46	50	44	45	45	46	44	46	22	29	30
	49	44	49	39	45	45	40	30	41	38	40	30	35
100 ppm	9	11	10	13	19	44	-	-	-	-	-	-	-
	0	5	13	17	30	47	-	-	-	-	-	-	-
	2	9	6	10	36	41	-	-	-	-	-	-	-
400 ppm	-	-	-	-	-	-	0	0	0	49	-	-	-
	-	-	-	-	-	-	0	0	16	45	-	-	-
	-	-	-	-	-	-	0	0	0	44	-	-	-
800 ppm	-	-	-	-	-	-	0	0	0	0	0	0	40
	-	-	-	-	-	-	0	0	0	0	0	0	41
	-	-	-	-	-	-	0	0	0	0	0	0	12

34

Figure 1. Larval survival of Fannia canicularis (L.) on manure collected from control hens and hens treated with three different levels of encapsulated 93 per cent Rabon, when the manure was "aged" at room temperatures for varying periods of time before testing. Each point represents the average survival in three replicates of 50 larvae each.

temperature one replicate at 400 ppm. showed 16 of 50 larvae surviving, while 100 per cent mortality was still obtained in the other two replicates. By the 28th day, however, survival was equal to the control at all dosages. Undoubtedly some survival would have occurred between days 21 to 28; however, bioassays could not be run on manure from these days.

Results from tests of the toxicity of manure from 60 per cent encapsulated Rabon-fed Rhode Island red hens are presented in Tables 3a, 3b, and 4. On manure collected during the second day after initiation of feeding, larval mortalities were quite low in two of the three replicates of the 100 ppm. dosage. However, by day four no survival was obtained at 100 ppm. The same trend held true for both the 400 and 800 ppm. dosages. Some survival was recorded for the higher dosages on the second day, but by the fourth day no survival was observed.

Persistance tests indicated that by the seventh day of storage at room temperature, the manure from the 60 per cent encapsulated Rabon-fed hens (100 ppm. dosage) and the untreated controls were comparable with regard to number of larvae surviving. In manure from the 400 ppm. dosage, aging continued until the 28th day before any survival was observed. Again, as with the 93 per cent Rabon formulation, larval survival was first noted on the 49th day of aging where the feeding level was 800 ppm.

The dosages for the 52 per cent Rabon feeding experiment were lowered to 0, 50, and 100 ppm. (four birds were fed 800 ppm. for persistance tests).

Table 3a. Little house fly surviving after seven days in manure collected from experimental birds fed with 60% encapsulated Rabon.

Rabon level in mash (actual)	Days in treatment period:						
	2	4	6	8	10	12	14
0 ppm (control)	50	46	44	48	46	44	42
	50	47	44	43	38	47	48
	48	38	47	44	41	45	41
100 ppm	44	0	0	0	0	0	0
	47	0	0	0	0	0	0
	9	0	0	0	0	0	0
400 ppm	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	6	0	0	0	0	0	0
800 ppm	1	0	0	0	0	0	0
	3	0	0	0	0	0	0
	0	0	0	0	0	0	0

Table 3b. Corrected average per cent mortalities of little house fly larvae grown in manure from 60% encapsulated Rabon-fed hens.[1]

Rabon level in mash (actual)	Days in treatment period:						
	2	4	6	8	10	12	14
0 ppm (control)	0	0	0	0	0	0	0
100 ppm	66.7	100	100	100	100	100	100
400 ppm	95.9	100	100	100	100	100	100
800 ppm	97.0	100	100	100	100	100	100

[1] Larval mortalities from Table 3a corrected by Abbott's formula.

38

Table 4. Persistance of toxicant in manure from 60% encapsulated Rabon-fed hens to larvae of the little house fly, as bioassayed by larval survival after seven days in the treated manure.

Rabon level in mash (actual)	Number of days manure was aged at room temperature before larval exposure:												
	1	2	3	4	5	6	7	14	21	28	35	42	49
o ppm (control)	41	47	44	41	45	38	39	43	40	41	36	31	30
	45	40	42	47	48	48	44	47	38	44	36	34	26
	43	46	48	44	42	46	48	40	41	40	39	35	33
100 ppm	0	0	0	0	0	11	39	-	-	-	-	-	-
	0	0	0	0	3	8	41	-	-	-	-	-	-
	0	0	0	0	0	25	40	-	-	-	-	-	-
400 ppm	-	-	-	-	-	-	0	0	0	40	-	-	-
	-	-	-	-	-	-	0	0	0	40	-	-	-
	-	-	-	-	-	-	0	0	0.	37	-	-	-
800 ppm	-	-	-	-	-	-	0	0	0	0	3	0	11
	-	-	-	-	-	-	0	0	0	0	0	0	15
	-	-	-	-	-	-	0	0	0	0	0	0	0

39

Figure 2. Larval survival of Fannia canicularis (L.) on manure collected from control hens and hens treated with three different levels of encapsulated 60 per cent Rabon, when the manure was "aged" at room temperatures for varying periods of time before testing. Each point represents the average survival in three replicates of 50 larvae each.

Tables 5a and 5b show that salmon sex link hens fed 52 per cent
encapsulated Rabon mixed in mash also produced manure toxic to larvae
of the lesser house fly. At a dosage of 50 ppm., a range of 68.2 to
81.6 per cent control was obtained from the fourth day to the fourteenth
day after initiation of feeding. Some survival was recorded at 100 ppm.,
although this dosage gave 95.5 to 100 per cent mortalities from the
fourth day onward.

Persistance tests indicated that the toxicant broke down faster at
room temperature in the manure from the 52 per cent Rabon-fed hens than
in manure from birds fed the other formulations. Survival at 50 ppm.
was equal to that of the control manure by the the third day, and like-
wise the manure from the 100 ppm. dosage was equal to the control by
the fifth day. Even the 800 ppm. manure showed larval survival on the
35th day in two of the three replicates, and was equal to the controls
by the 42nd day.

In comparing the three Rabon formulations, it is apparent that
there were no marked differences in larval toxicities between the 93
and 60 per cent encapsulated Rabon. However, both were superior in
performance to the 52 per cent Rabon formulation under the conditions
of our experiments.

During the experiments regular measurements were taken on feed
intake, water consumption, and weights of each bird in every experiment.

The white leghorn hens fed 93 per cent encapsulated Rabon showed
significant differences in feed consumption with respect to treatment

Table 5a. Little house fly larvae surviving after seven days in manure collected from experimental birds fed with 52% encapsulated Rabon.

Rabon level in mash (actual)	Days in treatment period:						
	2	4	6	8	10	12	14
0 ppm (control)	45	42	45	46	43	43	47
	45	39	44	49	41	44	43
	46	47	47	39	40	44	42
50 ppm	46	21	18	12	12	11	10
	8	12	8	13	3	5	6
	20	22	16	5	16	8	11
100 ppm	30	2	0	1	0	1	0
	7	0	0	5	2	3	0
	5	0	0	0	0	0	0

Table 5b. Corrected average per cent mortalities of little house fly larvae grown in manure from 52% encapsulated Rabon-fed hens.[1]

Rabon level in mash (actual)	Days in treatment period:						
	2	4	6	8	10	12	14
0 ppm (control)	0	0	0	0	0	0	0
50 ppm	46.2	68.2	69.2	77.5	74.6	81.6	79.5
100 ppm	69.2	98.8	100	95.5	98.8	96.6	100

[1] Larval mortalities from Table 5a corrected by Abbott's formula.

Table 6. Persistance of toxicant in manure from 52% encapsulated Rabon-fed hens to larvae of the little house fly as bioassayed by larval survival after seven days in the treated manure.

Rabon level in mash (actual)	Number of days manure was aged at room temperature before larval exposure:											
	1	2	3	4	5	6	7	14	21	28	35	42
0 ppm (control)	40	44	29	44	46	44	41	41	45	38	43	34
	43	46	43	41	45	43	46	47	43	39	37	30
	45	41	46	46	45	40	43	40	33	44	31	39
50 ppm	16	37	43	-	-	-	-	-	-	-	-	-
	8	31	45	-	-	-	-	-	-	-	-	-
	5	35	44	-	-	-	-	-	-	-	-	-
100 ppm	5	0	5	37	43	-	-	-	-	-	-	-
	0	3	7	40	41	-	-	-	-	-	-	-
	0	1	11	38	46	-	-	-	-	-	-	-
800 ppm	-	-	-	-	-	-	0	0	0	0	19	38
	-	-	-	-	-	-	0	0	0	0	15	34
	-	-	-	-	-	-	0	0	0	0	0	24

44

Per Cent Larval Survival

100 — 90 — 80 — 70 — 60 — 50 — 40 — 30 — 20 — 10 —

Control

800 ppm

100 ppm

50 ppm

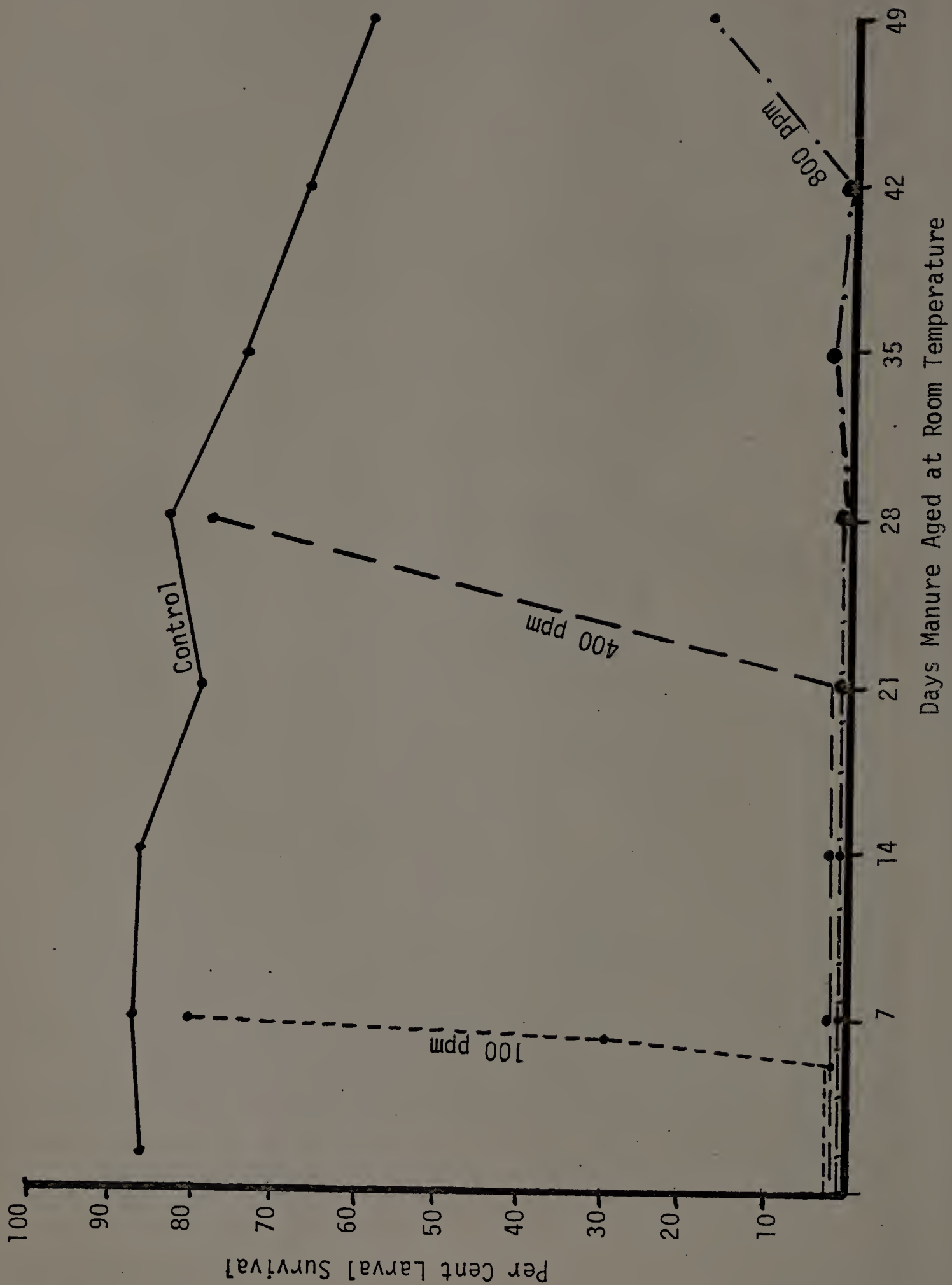7   14   21   28   35   42   49

Days Manure Aged
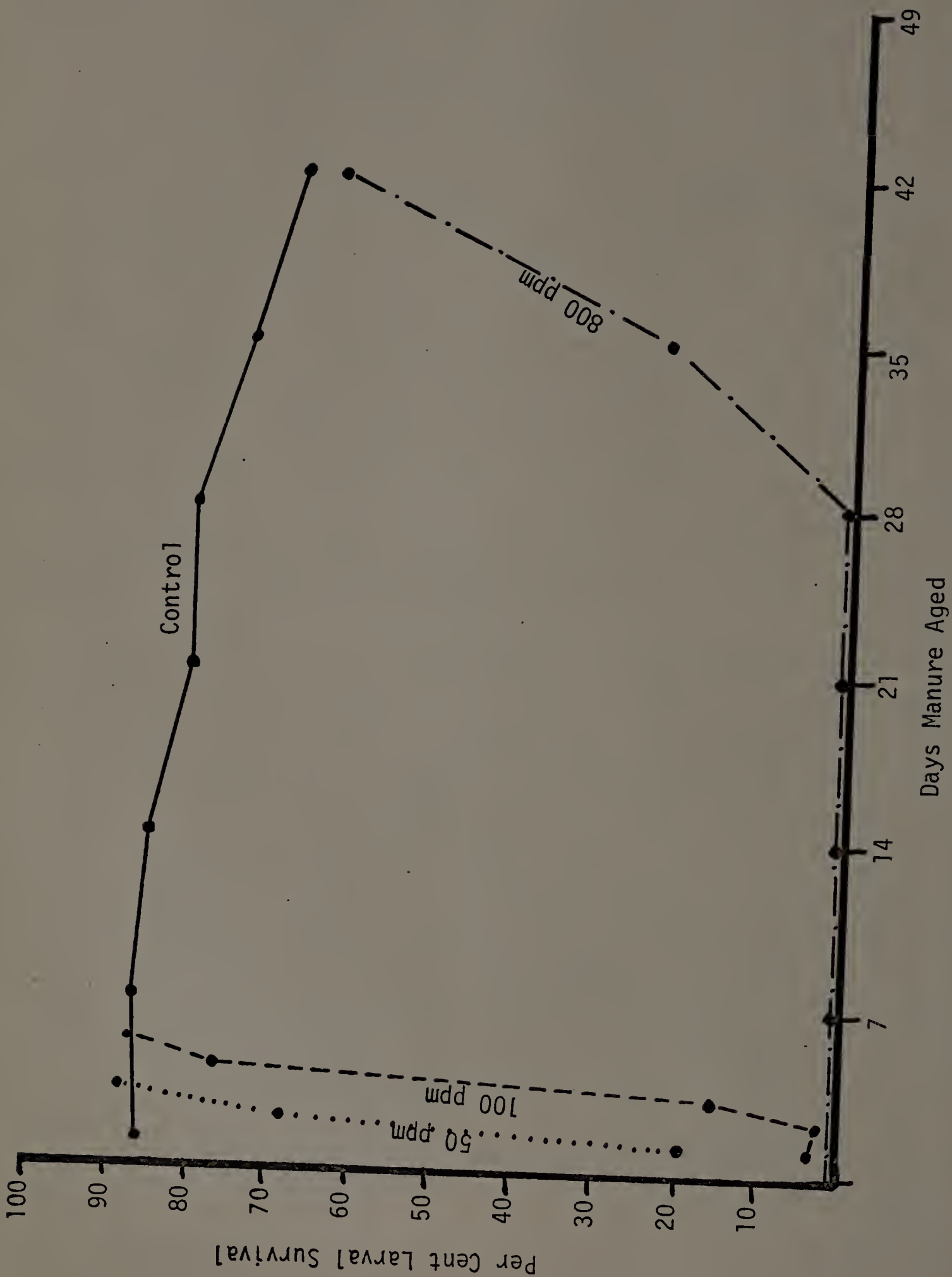
Figure 3. Larval survival of Fannia canicularis (L.) on manure collected from control hens and hens treated with three different levels of encapsulated 52 per cent Rabon, when the manure was "aged" at room temperatures for varying periods of time before testing. Each point represents the average survival in three replicates of 50 larvae each.

dosage. The control, 400, and 800 ppm. groups were fairly uniform in total feed consumed during the treatment period (Table 7). However, the 800 ppm. group consumed only about half as much water as the birds in the other three groups (Table 8). The average bird weights were significantly different (Table 9), but these were closely correlated with feed consumption.

Although significant differences were found in feed intake, water consumption, and average bird weights between treatments, no cause or effect should be inferred from the data. On the contrary, there appear to be no trends, and the differences noted may well be due solely to individual bird differences.

Similar measurements were made on the Rhode Island red hens fed 60 per cent encapsulated Rabon. Again feed and water consumption, when statistically analyzed, were found to be significantly different between treatments (Tables 10, 11). Feed consumption was high at 100 and 800 ppm. and was fairly low for the control and 400 ppm. dosages. Total water intake was very high in the 100 and 400 ppm. groups, but much lower for the control and 800 ppm. groups. Final average weights again followed feed consumption very closely, since the 100 and 800 ppm. groups were significantly different from the control and 400 ppm. dosage birds (Table 12). However, inferences should not be made as to cause and effect relationships between treatments.

The analyses of the results from the tests on 52 per cent Rabon-fed salmon sex link hens are less conclusive. While no significant weight differences were found between treatments (Table 15), analyses did show

Table 7. Average water consumption in milliliters of white leghorn hens fed 93% encapsulated
Rabon during a 14-day test period, starting June 17.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	204	164	167	172	139	218	167	150	184	146	180	182	147	157
100 ppm	8	198	196	187	164	168	208	208	155	176	126	168	130	169	146
400 ppm	8	155	168	173	179	126	203	183	151	208	155	178	162	171	194
800 ppm	8	151	140	138	162	161	189	149	124	148	116	147	128	139	135

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	3	7248.2	2416.1	9.03*
Days	13	15710.0	1475.1	5.51*
Error	39	10437.6	267.6	
Total	55	33395.8		

Results of Duncan's multiple range test

Value of p (.05)	2	3	4
SSR	2.86	3.01	3.10
LSR	12.46	13.12	13.51

Dosage (ppm.)	800	100	400	0
Mean (milliliters)	144.78	169.78	171.36	171.85

Table 8. Average feed consumption in grams of white leghorn hens fed 93% encapsulated Rabon during a 14-day test period, starting June 17.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	100	107	107	135	95	107	99	102	109	103	108	100	102	106
100 ppm	8	93	96	94	97	83	82	82	87	81	89	81	75	80	75
400 ppm	8	92	93	107	113	105	101	95	93	101	97	106	101	100	100
800 ppm	8	94	90	94	106	103	105	104	104	114	105	103	91	104	106

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	3	3326.4	1108.8	25.43*
Days	13	1223.0	94.1	2.15*
Error	39	1699.1	43.6	
Total	55	6248.5		

Results of Duncan's multiple range test

Value of p (.05)	2	3	4
SSR	2.86	3.01	3.10
LSR	5.03	5.29	5.45

Dosage (ppm.)	100	400	800	0
Mean (milliliters)	85.35	100.28	101.64	105.71

Table 9. Average weights in grams of white leghorn hens fed 93% encapsulated Rabon during
a 14-day test period, starting June 17.

Treatment Number (ppm of actual Rabon)	of birds	Days in treatment period:														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	1502	1540	1537	1534	1556	1523	1553	1573	1599	1572	1578	1594	1585	1575	1550
100 ppm	8	1512	1532	1536	1544	1534	1534	1543	1545	1535	1524	1512	1579	1555	1695	1512
400 ppm	8	1533	1533	1547	1568	1580	1586	1567	1569	1583	1587	1603	1626	1621	1613	1600
800 ppm	8	1500	1499	1515	1523	1547	1558	1577	1567	1561	1555	1557	1569	1543	1538	1681

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	3	10,388	3462.7	3.50*
Days	14	34,848	2489.0	2.51*
Error	42	41,587	990.2	
Total	59	86,823		

Results of Duncan's multiple range test

Value of p (.05)	2	3	4
SSR	2.86	3.01	3.10
LSD	23.22	24.44	25.12

Dosage (ppm.)	100	800	400	0
Mean (milliliters)	1546.13	1552.06	1581.06	1558.06

Table 10. Average water consumption in milliliters of Rhode Island red hens fed 60% encapsulated Rabon during a 14-day test period.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	201	172	193	206	196	172	216	162	240	181	188	198	161	189
100 ppm	8	215	209	213	208	230	220	202	209	229	184	204	215	220	220
400 ppm	8	206	206	210	213	220	219	209	207	221	194	206	213	189	190
800 ppm	8	198	213	219	170	203	169	175	172	179	184	214	213	214	173

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	3	4891.3	1630.4	6.48*
Days	13	4365.2	335.8	1.33 ns
Error	39	9813.2	215.6	
Total	55	19069.7		

Results of Duncan's multiple range test

Value of p (.05)	2	3	4
SSR	2.86	3.01	3.10
LSR	12.55	13.21	13.60

Dosage (ppm.)	0	800	400	100
Mean (milliliters)	191.07	192.57	207.36	212.71

Table 11. Average feed consumption in grams of Rhode Island red hens fed 60% encapsulated Rabon during a 14-day test period.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	93	108	107	104	84	90	97	111	96	115	90	109	100	109	104
100 ppm	8	89	115	100	103	95	104	120	128	103	121	111	114	110	118	118
400 ppm	8	97	97	104	108	105	109	113	112	91	102	102	112	111	101	113
800 ppm	8	96	96	120	103	110	113	108	129	101	116	117	122	124	122	112

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	3	1165.6	388.5	7.84**
Days	14	2810.1	200.7	4.05**
Error	42	2081.9	49.6	
Total	59	6057.6		

Results of Duncan's multiple range test

Value of p (.05)	2	3	4
SSR	2.86	3.01	3.10
LSR	5.17	5.44	5.61

Dosage (ppm.)	0	400	100	800
Mean (milliliters)	94.46	105.13	109.93	112.6

Table 12. Average weights in grams of Rhode Island red hens fed 60% encapsulated Rabon during a 12-day test period.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:					
		0	1	3	6	9	12
0 ppm (control)	6	2036	2053	2035	1981	1995	2006
100 ppm	8	2106	2103	2103	2103	2071	2059
400 ppm	8	1990	2016	2005	2012	1986	1978
800 ppm	8	2083	2106	2107	2081	2111	2131

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	3	40521.5	13507.2	36.55*
Days	5	7117.7	1423.5	3.85*
Error	15	8542.8	569.5	
Total	23	56181.9		

Results of Duncan's multiple range test

Value of p (.05)	2	3	4
SSR	2.86	3.01	3.10
LSR	27.85	29.31	30.19

Dosage (ppm.)	400	0	100	800
Mean (milliliters)	1997.83	2017.66	2090.83	2103.16

that both feed and water consumption were significantly different between treatments (Tables 13 and 14).

Only one fairly consistent trend was observed in all three experiments. With one exception, an increased Rabon dosage was accompanied by a decrease in water consumption (Tables 7, 10 and 13). Feed consumption appeared to be closely related to initial bird weights, which in these experiments were not equal for all groups. Generally speaking, the heavier birds consumed the most feed.

In none of the experiments were any symptoms of poisoning ever noticed, even at the 800 ppm dosage. Only one bird demonstrated any noticeable abnormality. A white leghorn hen fed a dosage of 100 ppm (93 per cent encapsulated Rabon) became partially paralyzed during the feeding experiment; consequently her feed and water consumption were lowered. Thus the total feed and water consumption for the group of birds fed 100 ppm Rabon in this experiment were reduced (Tables 7 and 8). This explains, at least in part, the reduced feed consumption of the hens on the 100 ppm dosage of 93 per cent encapsulated Rabon in this experiment (Table 8).

Table 13. Average water consumption in milliliters of salmon sex link hens fed 52% encapsulated Rabon during a 14-day test period.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	243	231	223	235	247	239	237	232	238	250	237	243	238	238
50 ppm	8	200	204	248	206	221	222	199	155	215	242	221	235	238	225
100 ppm	8	211	205	171	206	204	185	219	178	167	246	204	250	231	218

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	2	7109.3	3554.7	11.75*
Days	13	8420.1	647.7	2.14*
Error	26	7866.7	302.6	
Total	41	23396.1		

Results of Duncan's multiple range test

Value of p (.05)	2	3
SSR	2.86	3.01
LSR	13.27	13.96

Dosage (ppm.)	100	50	0
Mean (milliliters)	206.5	216.5	237.5

Table 14. Average feed consumption in grams of salmon sex link hens fed 52% encapsulated Rabon during a 14-day test period.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 ppm (control)	6	96	112	110	105	88	93	95	88	105	92	73	94	93	103
50 ppm	8	93	99	95	94	92	87	100	81	88	105	78	95	92	106
100 ppm	8	110	119	117	110	108	104	107	93	113	118	79	108	103	104

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	2	1391.1	695.6	23.50*
Days	13	2860.3	220.0	7.43*
Error	26	769.6	29.6	
Total	41	5021.0		

Results of Duncan's multiple range test

Value of p (.05)	2	3
SSR	2.86	3.01
LSR	4.15	4.36

Dosage (ppm.)	50	100	0
Mean (milliliters)	93.2	96.2	106.6

Table 15. Average weights in grams of salmon sex link hens fed 52% encapsulated Rabon during a 14-day test period.

Treatment (ppm of actual Rabon)	Number of birds	Days in treatment period:			
		1	7	14	
0 ppm (control)	6	2079	2022	1991	
50 ppm	8	2096	2028	2000	
100 ppm	8	2030	2038	2024	

Analysis of variance

Source of variation	df	Sum of squares	Mean square	F value
Treatments	2	227.6	113.8	0.16 ns
Days	2	6124.3	3062.1	4.32 ns
Error	4	2833.7	708.4	
Total	8	9185.6		

SUMMARY AND CONCLUSIONS

1. Three different formulations of encapsulated Rabon were fed to hens in three separate experiments at levels as low as 50 ppm. and as high as 800 ppm., (mixed with their mash) for 14-day periods.

2. The manure from the hens fed encapsulated Rabon was tested for its toxicity to larvae of the little house fly, _Fannia canicularis_ (L.), during the feeding period, and also after varying periods of storage (up to 49 days) at room temperature.

3. Feed consumption, water consumption, body weights and general behavior of the 90 hens used in the three experiments were determined and recorded during the feeding period.

4. Starting with manure collected on the fourth day after feeding started, all three Rabon formulations at 100 ppm. gave 78 to 100 per cent control of little house fly larvae in the laboratory, whereas the 400 and 800 ppm. dosages resulted in 100 per cent mortalities.

5. Some of the manure from Rabon-fed hens was held at room temperature for varying periods of time to check on the longevity of the Rabon residues. The toxicities of manure from the 100 ppm. dosages markedly declined within five to seven days, whereas manure from birds fed at 400 ppm. and especially at 800 ppm. retained marked toxicities for at least 35 days of storage.

6. Mash consumption varied significantly among individuals and treatments (groups receiving the same formulation); however, there were no consistent trends nor were there visible reactions which might indicate a degree of unpalatability.

7. Water consumption also varied significantly; in this case the general trend was for water consumption to drop as the Rabon dosage increased.

8. Body weights varied significantly between treatments and, as might be expected, showed a direct correlation with food intake.

9. There were no visible symptoms or behavioral differences between the birds in the different groups (treatments) in any of the experiments. However, the birds fed the 93 per cent encapsulation produced markedly more watery droppings at the 400 and 800 ppm. levels than the control and 100 ppm. groups; this trend did not hold in the other two experiments where different Rabon formulations were fed.

10. The results obtained in this study, when compared with the findings of Dennis (1969), suggest that encapsulated Rabon may be considerably more toxic to Fannia canicularis (L.) larvae in the manure than equal dosages of powdered Rabon fed to hens.

11. These laboratory findings suggest that encapsulated Rabon might be incorporated into poultry mash at perhaps 100 ppm. to prevent lesser house fly larvae from breeding in the resulting manure. The persistance tests, in fact, indicate that Rabon-treated mash might be

effective, even if it were fed only during alternate weeks through the fly breeding season.

12. More work should be done on the storage in, and adverse effects (if any) of Rabon in poultry body tissues and in eggs. Also, field trials should be carried out to determine how well these laboratory findings hold up under practical conditions.

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