THE EFFICACY OF THREE CANDIDATE ANTHELMINTICS AGAINST ADULTS AND PRE-ADULTS OF *HETERAKIS GALLINARUM* AND *ASCARIDIA GALLI* OF CHICKENS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES AND FIGURES

INTRODUCTION

Until recent years subclinical parasitism has been ignored and believed to be relatively unimportant. However, studies such as that of Todd and Hansen (1951) have shown that in addition to the clinically obvious damages incurred by the host in reaction to parasitic forms, depression of weight is also a very important factor. When the decrease in weight is converted into dollars it emphasizes the subtle activity of parasites which is robbing the farmer of much profit as he cannot market the animal at the youngest age possible with the least amount of food intake.

In domestic fowl, Heterakis gallinarum and Ascaridia galli are responsible for clinical and subclinical damages. Ascaridia galli (Schrank, 1788), family Heterakidae, commonly known as the large roundworm of poultry, is one of the most common nematode parasites of chickens in the United States. Herms and Beach (1916), Ackert and Herrick (1928), and Todd and Hansen (1951) noted the unthriftiness of poultry during the larval phases of this parasite. There was a slowing down of muscular and bone development, loss of blood, and increased susceptibility to bacterial infections.

Heterakis gallinarum (Schrank, 1788) Madson 1949, family Heterakidae, commonly known as the cecal worm, is economically important as it appears to be associated with the blackhead organism Histomonas meleagridis. Histomoniasis usually is not severe in the chicken; however, the disease organism can be

transmitted from chickens to turkeys via the heterakid egg. In turkeys the disease is severe and usually fatal (Biester, 1952).

The primary purpose of the present day agriculturalist in dealing with parasitisms should be to keep the parasitic population at such a level that gross clinical damages and depression of weight does not occur. Present methods employed are good management and the use of drug therapy. Neither can be entirely successful by itself, but a combination of the two can be very effective (Jones, 1957).

Due to increased awareness of the detrimental role of parasitism, there is a continual search for anthelmintics which will prove more effective than those presently available. Drug manufacturers are seeking drugs of high efficacy and low toxicity. Such drugs should be easy to administer, preferably as a one-dose treatment, and should be economical (Jones, 1957).

The Food and Drug Administration of the Department of Health, Education and Welfare requires industrialists to prove efficacy of products beyond any doubt and the research reported here is an adjunct to such proof. The objective of this study was to test the toxicity and efficacy of Bayer 9001T, Bayer 9002, and Bayer 2353 against Ascaridia galli and Heterakis gallinarum infections in poultry.

LITERATURE REVIEW

Since 1899, when Paul Ehrlich began the work that established the principles of modern chemotherapy (Albert, 1960), numerous compounds have been tested for their chemotherapeutic

value. Herms and Beach (1916) pioneered the testing of therapeutic agents against roundworm in poultry. They found flocks infected with roundworms to be unthrifty, and tested the anthelmintic value of various materials, viz. powdered areca nut, powdered pomegranate root bark, turpentine, gasoline, iron sulfate and tobacco. They found finely chopped tobacco stems soaked in water for two hours, mixed in mash, and fed for two days were 100 per cent effective against roundworms.

Since this early work many compounds have been tested. Among them are nicotine sulfate, carbon tetrachloride, tetrachlorethylene, iodine, garlic, n-butylidene chloride, pyrethrum, antimonyl potassium tartrate, nicarbazine, dithiocarbamates, hygromycin, piperazine, phenothiazine and organic phosphates (Plate I). Freeborn (1923) found nicotine sulfate given to birds was toxic; however when mixed with Lloyd's alkaloid reagent at a rate of 6.6 cc. to 16 grams of reagent and given in a dose of 350 mg., it would eliminate Ascaridia galli but not Heterakis gallinarum. Bleecker and Smith (1933) found a mixture of nicotine sulfate (40 per cent) and 16 grams of Lloyd's alkaloid reagent (Pulvules 142, Lilly) in a dose of 350-400 mg. was 72.2 per cent efficient for removal of A. galli. Roberts (1937) found nicotine sulfate made according to Freeborn's formula was only 25 per cent effective against A. galli. He concluded that the drug was inefficient and unsafe for use.

In 1929 Graham and Ackert conducted studies using carbon tetrachloride. Three hundred chickens from 9-10½ weeks of

age were used in four experiments. They were treated with 4-10 cc. of carbon tetrachloride per kilogram of body weight four weeks after infection. Dosages of 10 cc. per kilogram were highly toxic to the chickens whereas a dosage of 4 cc. per kilogram was 100 per cent efficient in removing Ascaridia and showed low toxicity. Roberts (1937) found carbon tetrachloride highly effective against A. galli at 0.75 mg. per pound of body weight. He recommended that the dosages not exceed 2 ml. per pound of body weight. Mohan (1954) gave CCl_4 at the level of 0.75-1 cc. per pound of body weight to 30 birds after 18 hours fasting and reported a 98 per cent to 99 per cent efficacy against A. galli.

Tetrachloroethylene was tested by Schingman (1926) at levels of 1 cc. to 5 cc. per bird. He found 1 cc. per bird to be highly effective in removing A. galli, and a dosage of 5 cc. per bird not toxic. In later studies (1929) he found doses of less than 1 cc. per bird were ineffective against this roundworm. Roberts (1937) found tetrachloroethylene was highly efficient in most birds but its action inconsistent leading to the conclusion that it was unreliable as an anthelmintic.

Chandler (1924) found a castor oil solution, iodine crystals (1 gram per ounce), highly effective as a vermicide against ascarids and heterakids in poultry. Stafseth and Thompson (1932) treated 29 birds with Chandler's iodine vermicide and found nearly 100 per cent efficacy, and Bleecker and Smith (1933) found the compound was 75 per cent effective in a 36-bird test.

Other chemicals have been tested for effectiveness as anthelmintic for poultry. Wright, Bozicevich, Underwood and Schaffer (1933) tested N-butylidene chloride on 21 birds infected with A. galli and H. gallinarum. It was 98.4 per cent effective against A. galli and 4.7 per cent against H. gallinarum. Rebrassier (1934) treated 30 adult chickens with pyrethrum in dosages of 200 mg. containing 0.8 per cent Pyrethrin I and obtained an efficacy of 95.14 per cent against A. galli. Roberts (1937) found no efficacy from 750 mg. of Pyrethrin I after 17 hours fasting. Roberts (1937) tested several other drugs, including oil of turpentine, oil of chenopodium and copper sulfate, against A. galli. One milliliter of oil of turpentine in 3 ml. of castor oil was 53.3 per cent efficient. Two milliliters of oil of turpentine was toxic to the chickens, but 92.9 per cent efficacy was obtained. He found 0.4 ml. of oil of chenopodium, followed by 3 ml. of castor oil, to be 100 per cent efficient, but it had a high toxicity; therefore, he did not recommend the drug. Copper sulfate at a rate of 1 gram per 4 ml. of water was highly toxic; however, combining copper sulfate with nicotine gave 97.5 per cent efficacy in 8 birds, but toxicity was still high.

Many of the early compounds tested were only partially effective or were entirely ineffective against Heterakis gallinarum. In the early 1940's phenothiazine was found to be highly useful as an antinematodal drug. It was found to be highly effective against the cecal worm of poultry, Heterakis gallinarum.

McCullogh and Nicholson (1940) conducted tests with phenothiazine using dosages of 0.05 grams to 1 gram per bird given either in food or capsule to 12 naturally infected hens. Efficacy was 80-100 per cent against H. gallinarum. Tests done on 14 birds treated with similar dosages for 3-7 days showed the compound to be 97-100 per cent efficient. No toxic effect was found up to 25 mg. per bird.

In an attempt to find an anthelmintic effective against both H. gallinarum and A. galli, Allen et. al. (1942) gave tablets containing 33 parts nicotine-bentonite, which had proven effective against A. galli, with 66 parts phenothiazine, an anthelmintic for H. gallinarum. This mixture was 83.7 per cent effective against a total of 1012 Heterakis and removed 131 Ascaridia giving an efficacy of 96.2 per cent. In 1944 (b) Harwood and Guthrie reported that a tablet of phenothiazine-nicotine-bentonite weighing 1.33± 0.29 grams was effective in removing A. galli and H. gallinarum in adult birds.

Oliver et. al. (1946) found a 75:1 medicated mash phenothiazine mixture removed all but one cecal worm of 655 present. The birds were fasted 16 hours and then allowed access to the mash for 7½ hours. Total consumption of phenothiazine was 0.46 to 0.91 grams.

A mixture of phenothiazine and prickly ash bark (Xanthroxylum Clavo-Herculis) was tested by Harwood and Guthrie (1944 a). This mixture removed 7.3 per cent of 136 A. galli and 95.7 per cent of 645 H. gallinarum. They concluded that this mixture did not improve the overall efficacy of phenothiazine.

Jaquette and Wehr (1949) found that phenothiazine and nicotine-bentonite (44 g. mash, 40 per cent nicotine-sulfate, 151 g. phenothiazine, and 287 g. bentonite) removed 97.7 per cent of ascarids and 97.7 per cent of cecal worms present in 35 naturally infected birds.

During World War II a piperazine derivative, diethycarbamizine, was proved to be highly effective against filariasis in man and dog. Since that time piperazine salts have been highly successful as antinematodal drugs (Jones, 1957). It was found to be particularly effective against A. galli in poultry.

In 1955 Bradley studied the anthelmintic properties of piperazine citrate in two large broiler flocks. He obtained good results against A. galli when he treated one flock (7 weeks old) at the rate of 20 cc. of piperazine per gallon of water for 60 hours, and another flock at the rate of 15 cc. of piperazine per gallon of water for 24 hours.

Shumard and Eveleth (1956) achieved almost complete control of A. galli in artificially infected birds using 2000 or 4000 mg. of piperazine citrate per gallon water.

Horton-Smith and Long (1956) found piperazine adipate and piperazine citrate effective against adult A. galli when administered in a single dose; dosages varied from 100-500 mg. per kilogram body weight. Considerable numbers of larvae were removed; however, the effectiveness of the compounds on intervillar migrating larvae was undetermined.

Piperazine carbodithoic given to 300 artificially infected birds by Shumard (1957) showed 90 per cent effectiveness

against A. galli. Doses higher than 125 mg. per kilogram failed to remove H. gallinarum.

Worley et. al. (1957) found that piperazine dihydrochloride when given at a rate of 25 mg. per day on day 7 and 14 showed evidence of killing tissue phase larvae.

Alicata (1958) found piperazine citrate administered at a rate of 1,500-2,000 mg. per kilogram to be 75 per cent or more effective for the removal of 2-17 day-old larvae. Complete elimination of adult parasites was achieved when piperazine citrate was administered at a rate of 200-300 mg. per kilogram.

Phenothiazine and piperazine have been the most reliable anthelmintics developed for poultry to date. Their toxicity is low and efficacy high.

In recent years additional compounds have been tested in hopes of finding a drug which will be effective against all stages of the life cycle and will have a broad spectrum of action against all parasites. So far compounds thus tested do not fill these requirements.

Gupta and Mukherjii (1956) treated 16 birds, which were naturally infected with Ascaridia sp., Heterakis sp., and Capillaria sp., with garlic juice (5 cc. for 5 days) and garlic bulbs. They concluded that garlic bulbs and juice were equally effective. Capillaria sp. was most susceptible, followed by Heterakis sp. and Ascaridia sp. respectively. Nicarbazine was tested by Cuckler et. al. in 1956. It was highly effective against A. galli but showed poor efficacy against H. gallinarum. Knapp and Hansen (1960) found carbon

disulfide given at a dose rate of 0.6 ml. per kilogram body weight was 100 per cent effective against A. galli; however, weight losses from the drug were large. It was concluded that carbon disulfide was not a desirable anthelmintic for chickens. Leiper (1958) found N-butyl-N-phenyldithiocarbamate, given at a rate of 72 mg. per kilogram, to be 100 per cent effective against A. galli, but ineffective against H. gallinarum.

Methyridine, 2-(2-methoxyethyl) pyridine, has been tested by Thienpont et. al. (1963). Diluted methyridine, administered subcutaneously at the rate of 200 milligram per kilogram, removed adult A. galli but did not affect H. gallinarum. Hassan (1963) reported that methyridine given subcutaneously was markedly effective against Ascaridia, Heterakis and Capillaria if given at a rate of 0.25 ml. per bird.

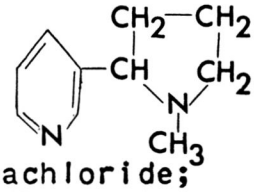
Numerous organic phosphorous compounds have been tested for insecticidal and anthelmintic properties. Since 1956, when Trolene (Dow ET-57, Dow ET-14, Korlan, Ronnel, O,O-dimethyl, O-2,4,5-trichlorophenyl phosphorothioate) was marketed as a systemically active drug against migrating stages of warbles in cattle, many compounds have been tested for antiparasitic activity. Since Linquist's study (1956) on the insecticidal value of Trolene against cattle grubs, most of these studies have been concerned with parasites of cattle, sheep, and horses; and to date very little is recorded in the literature concerning their value as anthelmintics in poultry. Organic phosphates have been used to control ectoparasites of poultry (Reid, 1956), (Furman, 1956), and (Knapp, 1960) and to control

EXPLANATION OF PLATE I

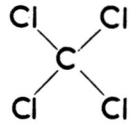
The structural formulae and information concerning some anthelmintics previously tested against *Ascaridia galli* and *Heterakis gallinarum* in poultry.

PLATE I

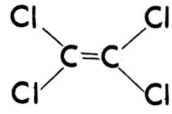
Nicotine:
L-3-(1-methyl-2-pyrrolidyl) pyridine: L-1-methyl-2 (3-pyridyl) pyrrolidine; pyridyl-N-methyl pyrrolidine.



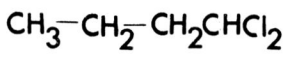
Carbon Tetrachloride;
Perchlormethane; tetrachloromethane.



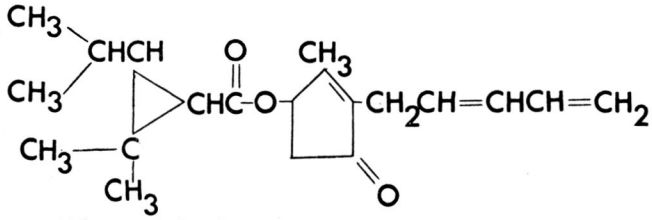
Tetrachloroethylene:
Tetrachloroethene; perchloroethylene.



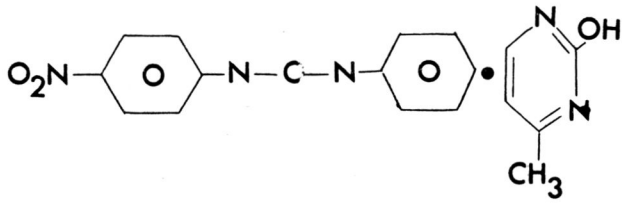
N-butylidene chloride:



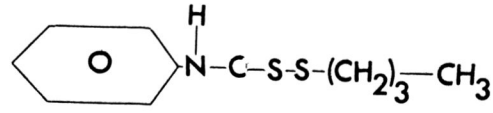
Pyrethrin I:



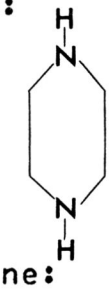
Nicarbizin:



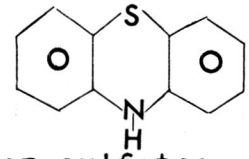
N-butyl-N-phenyl-dithiocarbamate:



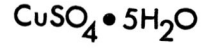
Piperazine:



Phenothizine:
Phenthiazine, thiodiphenylamine, dibenzo-1, 4-thiazine.

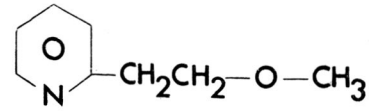


Copper sulfate:

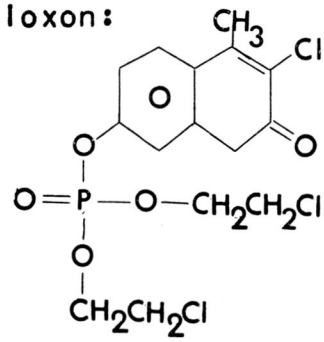


Carbon Disulfide:

Methyridine:
2-(2-methoxyethyl) pyridine.



Haloxon:



capillariasis in poultry (Clark, 1963). An efficacy of 99.8 per cent was achieved with Haloxan (Plate I) when 100 mg. per kilogram and 75 mg. per kilogram were administered for two days. Harbour (1963) also found that Haloxon showed good promise against capillarids in poultry. Given in dosage rates of 25 or 50 mg. per kilogram body weight (three doses in the feed) or 110, 165 and 220 ppm. in the feed, Bayer 9002 was found to be very effective against Capillaria (Bayer data sheet).

The present study was done to evaluate the suitability of two organo-phosphates, Bayer 9001 T (Chemical formula is restricted information) and Bayer 9002 (N-hydroxynapthalimide diethyl phosphate); the efficacy and toxicity of these compounds were two factors considered. In addition, Bayer 2353 (2', 5-dichloro-4' - nitrosalicylanilide) which is not an organic phosphate was evaluated. These compounds were tested against Ascaridia galli adults, pre-adults, and larvae and Heterakis gallinarum adults and pre-adults.

MATERIALS AND METHODS

All chickens used in this study were obtained from commercial hatcheries as day-old birds and were vaccinated against Newcastle disease. Both White Rocks and White Leghorns were used in the experimental work. These chickens were raised in electric brooders, and were fed commercial ration and water ad libitum during the experimental procedure.

At fourteen days of age the birds were banded, weighed and divided into groups of equal weight according to the

Gardiner-Wehr method (1950). Some of the birds were infected with embryonated eggs of either Ascaridia galli or Heterakis gallinarum; others were retained as controls.

Collection and Recovery Methods

Ascaridia galli and H. gallinarum were collected by a modification of the hydraulic method of Ackert and Nolf (1929). Entrails were obtained from a local processing plant. A. galli was collected by flushing the contents of the intestine from the duodenum to the cecum by means of water pressure. In the collecting of H. gallinarum, the cecae were removed and a slit was made in the blind extremity; the contents were then flushed out by water pressure into a sieve. Nematodes which were recovered by this procedure were kept at 7-9° C. in 0.85 per cent saline solution to which was added one drop of 1:1000 merthiolate solution per 10 cc. of saline.

After exposure of hosts to the embryonated eggs, A. galli and H. gallinarum were allowed to reach maturity (40-50 days post-exposure); the chickens were treated with an anthelmintic according to the design of each individual experiment. The dropping pan of each cage was lined with heavy black plastic so that the feces could be easily seen and collected for 3-7 days after treatment. In order to collect eliminated A. galli, the plastic was removed each day and placed on a flushing pan devised by Hansen and Foxx (See Fig. 1). The feces were collected in buckets and allowed to soak for a short time and then were washed through 20 and 10 mesh sieves. The worms collected were stored in Ward's fixative.

EXPLANATION OF FIGURE 1

Device used for collecting A. galli from dropping pans. Measurements are the following:

Aluminum: 36" in length
30" in width
6" in height
3" at mouth rolled edge

Wooden stand: 29" at maximum slant
18½" at minimum slant


D2484

Heterakis gallinarum were recovered from feces by collecting the cecal droppings. Droppings were collected daily for 3-7 days post treatment and were soaked in saline (0.85 per cent) solution. This solution was then shaken vigorously and poured through a 20 mesh screen. The material on the screen was washed into a black photographic pan and was examined under strong light and with a binocular dissecting microscope.

At the end of each post-treatment period any worms remaining within the intestine were recovered. The intestinal contents from the gizzard to the cecum were flushed with water into quart jars. The contents of each jar were poured through a 20 mesh screen and were examined for worms. All worms found in each bird were placed in Ward's fixative in individual containers containing the wing band for identification.

Heterakis gallinarum were recovered from the cecae by an adaption of the method of Larson (1957). The cecal contents were flushed into pint jars and were allowed to soak overnight. The jars were shaken vigorously to break up the cecal material. The contents of the jars were placed in Pilsner glasses and were allowed to settle 10-20 minutes. The surface fluid was then siphoned off and more water was added until the solution was fairly clear. Finally, the 10 cc. aliquot of the clear fluid remaining after siphoning was examined under a binocular dissecting microscope. The worms recovered were placed in Ward's fixative associated with their proper host by the wing band.

Culture Methods

The eggs of both nematodes were cultured using an adaption of the method of Hansen, Olson and Ackert (1954); Hansen, Terhaar and Turner (1956) and Larson (1957). Depending upon the type of culture desired, either several adult A. galli or H. gallinarum were placed in a mortar and were finely ground with a pestle. Artificial digestive juice (1.0 per cent pepsin and 0.5 per cent hydrochloric acid) was poured over the macerated worms and was allowed to soak 4-5 minutes. The mixture was poured through an 80 mesh screen into clean, oil free Petri dishes which had been rinsed with distilled water. The cuticula and debris remained on the screen. The screen was washed several times with distilled water in order to flush through as many eggs as possible. The eggs were allowed to settle in the Petri dish for 5-10 minutes and then those which had not adherred to the bottom were poured into another clean Petri dish. Distilled water was poured over the first dish and any loosened eggs were allowed to settle. This procedure was repeated until there was a thin even layer of eggs adherring to the bottom of the Petri dish. Each dish containing adherring eggs was washed several times with distilled water to free them from artificial digestive juice.

All egg cultures were incubated 30° C. to 33° C. for 14 days. Two methods were used to inhibit growth of mold; either a drop of 1:1000 merthiolate solution (Larson, 1957) or a 0.2 per cent formalin solution was placed in the Petri dish.

Method of Exposure to Eggs

Usually the birds were exposed at 14 days of age to 100 ± 10 A. galli or H. gallinarum eggs. These were administered by means of a calibrated micropipette inserted into the crop. The following variation in determining the egg dose technique by Hansen et. al. (1956) was used. The culture solution was poured off and 5-10 ml. of 1.25 M sucrose solution were poured into the dish. The eggs were scraped from the bottom of the Petri dish with a rubber tipped spatula. The sugar-egg solution was poured into a small bottle which had been treated with Desicoat (Beckman), a hydrophobic surface agent, to prevent eggs from sticking to the glass surface. Small glass beads were placed in the bottom to help mixing. Three drops of sugar-egg solution were placed on a glass slide and the number of embryonated eggs were counted in each drop. An average of three drops was taken, and then the micropipette was calibrated to deliver the desired number of eggs, generally 100 ± 10.

In Experiment I, Trials 1 and 3, and Experiment II, Trial 1, 14-day-old White Rocks were given 100 ± 10 embryonated A. galli eggs. In Experiment I, Trial 2, 28-day-old White Rocks were given 100 ± 10 embryonated eggs. The exposure of one group at a later age was due to a conflict in schedule. In Experiment II, Trial 2, 3-day-old White Rocks were given 100 ± 10 embryonated A. galli eggs. Since hydrocortisone could not be used in conjunction with Bayer 9001 T to increase worm burden and because a preliminary experiment

showed an increased worm burden if the birds were infected at an extremely early age, it was decided to expose 3-day-old birds. According to studies by Ackert et. al. (1934), the lighter breeds of fowl have higher A. galli worm burdens, thus in subsequent studies White Leghorns were used. White Leghorns were given 100 ± 10 embryonated H. gallinarum eggs in Experiment IV, Trial 1. White Leghorns were also used in Experiment V, Trials 1 and 2. They were given 100 ± 10 embryonated A. galli eggs. In Experiment VI only 75 ± 10 H. gallinarum eggs were given since in an earlier attempt 98 per cent of the birds infected had contracted blackhead; therefore, it was determined that decreasing the number of ova would decrease the liklihood of blackhead.

Administration of Anthelmintics

Bayer 9001 T, 2353, and 9002 (Table I) were supplied by Chemagro Corporation, Kansas City, Missouri. In Experiments I, II, III and IV the efficacy and toxicity of Bayer 9001 T was tested against A. galli and H. gallinarum. This compound was supplied as a fine yellow pellet which was insoluble in water. The calculated dosages were weighed on the Metler balance and were placed in gelatin capsules (size 1). Administration of the capsules was accomplished by placing a piece of polished glass tubing into the bird's crop and dropping the capsule through the tubing lumen into the crop. Dosage levels for Bayer 9001 T were based on daily feed consumption. One per cent (688 mg.), 0.5 per cent (344 mg.) and 0.2 per cent (138 mg.) of the daily feed consumption of a 40-day-old bird was

determined on the basis of information in Cassel.

The dosage levels of Bayer 9001 T given in Experiment I, Trials 1 and 2, and Experiment IV, Trial 1, were 688 mg. per one day, 344 mg. per 2 days, and 138 mg. per 5 days. In Experiment I, Trial 3, birds receiving hydrocortisone weighed one-half the weight of the normal birds and probably consumed the amount of food of 20-day-old birds; therefore, dosages were based on the corresponding food consumption as given in Cassel. Hormone-receiving birds were given the following doses of Bayer 9001 T: 313 mg. per 1 day, 156 mg. per 2 consecutive days, and 62 mg. per 5 consecutive days according to the design of the experiment. The birds not receiving hormone were given the same amounts as in Experiment I, Trials 1 and 2.

In Experiment II, Trial 1, 3-week-old chicks were given the same dose as the birds receiving hormone in Experiment I, Trials 1 and 2.

In Experiment II, Trial 1, 3-week-old chicks were given the same dose as birds receiving hormone in Experiment I, Trial 3. In Trial 2 of Experiment II the dose level was found in the following manner. The average weight of all experimental birds was 105 g.; the number of milligrams necessary for a dose of 1200 mg. per kg. of body weight was then determined. Thus one-half and one-fifth the total dose was also given according to the experimental design. The levels were 126 mg. per 1 day, 63 mg. per 2 days and 25 mg. per 5 days.

In studies concerning lethal dosage of Bayer 9001 T, more accuracy was desired; therefore, the dosage levels were determined by using the following formula.

(wt. of the bird in kg.) x (desired dose in mg./kg.) = number mg. given

Experiment IV and V were done to test the efficacy of Bayer 2353 and 9002 against H. gallinarum and A. galli. These compounds were in powder form. It was decided that greater speed and better accuracy in administration could be achieved by suspending the compound in water. Because the compounds were not water soluble (Table I) they were kept in suspension by means of a magnetic stirring device. The appropriate amounts were pipetted by means of a pro-pipette and were placed directly into the bird's crop.

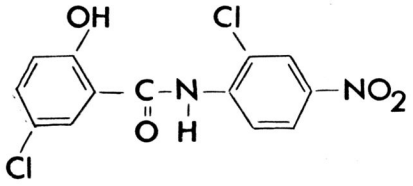
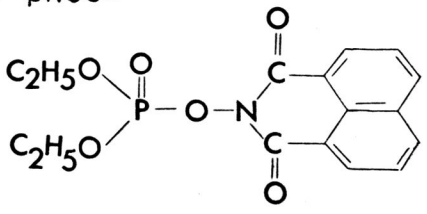
The dosages were determined in the following manner. The compound was suspended so that 40 mg. per ml. could be obtained, i.e. two grams in 50 ml. The factor by which each individual's weight was multiplied in order to find the number of milliliters necessary for individual bird dosages was determined in the following manner.

$$\frac{\text{number of mg./kg. of bird}}{\text{40 mg./ml.}} = \text{ml./kg. of bird}$$

The ml. per kg. of bird was multiplied by the number of kg. of bird.

TABLE I

STRUCTURAL FORMULAE AND INFORMATION CONCERNING
CHEMAGRO COMPOUNDS USED IN EXPERIMENTAL WORK

Compound	Chemical Name	Chemical Formula	Physical and Chemical Properties
Bayer 2353	2',5-dichloro-4'-nitrosalicylanilide		Apearance: White, odorless, almost tasteless powder. Molecular Weight: 327.1 Melting point: 222°C- 224° C. Solubility: Soluble in ethanol and acetone. Insoluble in water, aromatic and aliphatic hydrocarbons. Stability: Good stability to heat, alkali, and acid. Requires concentrated alkali or acid to saponify.
Bayer 9002	N-Hydroxynaphthalimide diethyl phosphate.		Appearance: Tan crystalline powder. Molecular Weight: 349 Melting point: 174° C.-179° C. Solubility: Difficulty, soluble in most organic solvents. Slightly soluble in acetonitrile. Soluble in methylene chloride. Stability: Subject to hydrolysis under alkaline conditions.
Bayer 9001 T	organo-phosphate		*

* Due to application pending for patent, Chemagro is unable to release any information concerning this compound.

The Bayer data sheet containing information about Bayer 2353 (a taenicide) suggested 400 mg. per kg. of body weight as the proper dosage for treatment of tapeworms; therefore, this dosage was used as a basis for testing the compound against A. galli and H. gallinarum. Four hundred mg. per kg. were given over a period of days as well as in one dose. Therefore in Experiment IV, Trial 2, the following dosages were given to one group: day one--50 mg. per kg., day two--100 mg. per kg., day three--100 mg. per kg. and day four--150 mg. per kg. In Experiment V, Trial 2, 100 mg. per kg. were given over a four-day period, thus total dosage was 400 mg. per kg.

Bayer 9002 was administered in the same manner as Bayer 2353. The dosage levels were as follows: 50 mg. per kg. for one day, 25 mg. per kg. for one day, 16 mg. per kg. for each of three days. This dosage level was chosen because the Bayer data sheet suggested 25 or 50 mg. per kg. body weight for control of Capillaria.

Before administration of the drug, the birds were weighed and the weight was recorded; the dose was given according to this weight. At the end of each experiment the birds were again weighed and then killed. In experiments involving Bayer 9001 T and 9002, necropsy examinations were conducted by a pathologist from the Department of Pathology, Parasitology, and Public Health, Kansas State University. Any gross pathological findings were recorded and, in addition, during the toxicity studies, rectal temperature was recorded periodically and any symptoms of poisoning noted during the experimental period.

Administration of Hydrocortisone

In Experiment I, Trial 3, and Experiment II, Trial 1, each bird received 0.625 mg. of hydrocortisone every 3 days for a total of 3 to 5 injections. The hormone was administered subcutaneously to coincide with the period of intervillar migration of A. galli since Johnson (1963) found hydrocortisone increased the worm burden of A. galli in chickens.

Laparotomy

In Experiment V, laparotomies were performed 10 days post-exposure to determine the presence of histomoniasis. This procedure was necessary since birds suffering from histomoniasis spontaneously lose H. gallinarum infections when the cecal core is formed. The birds were anesthetized with ether and a one-half inch incision was made in the lower right abdominal area. The cecae were examined for a hard core or inflammation, symptoms of the presence of the histomoniasis organism. The incision was closed with suture clamps. Birds having histomoniasis were not used in efficacy experiments.

RESULTS

Experiment I

Trial 1. The objective was to test the efficacy of Bayer 9001 T against Ascaridia galli adults and pre-adults according to the design given in Table II. Ninety-six of 144, 14-day-old White Rocks were infected with A. galli (Groups 1-9 and 13-15). Groups 10-12 and 16-18 were uninfected controls.

Weights were recorded at time of first treatment, after final treatment and at the end of the experimental period. All groups gained weight during the treatment period with the exception of Group 11 (Table II). The failure of Group 11 to gain weight is difficult to explain when all groups were fed and watered alike. However, at the termination of the experiment, all groups had gained weight. Generally, male birds gained more weight than did the female birds (Table III).

Bayer 9001 T showed 100 per cent efficacy in all groups that had helminths and that were challenged (Table VIII). The worm burden in all infected groups was low.

Trial 2. The objective was to determine the efficacy of Bayer 9001 T against A. galli adults and pre-adults. Secondly, in order to better evaluate the efficacy of Bayer 9001 T, hydrocortisone was used as an immunosupressor (Johnson, 1963) for increasing the number of worms per bird.

Per the experimental design, given in Table IV, 7 birds in each group were given hydrocortisone; the remaining birds were untreated controls. Weights were taken at the beginning of the experiment, after final treatment and at the end of the

experiment. Loss of weight generally occurred among the controls and the treated groups during treatment with Bayer 9001 T (Table IV). At termination of the experiment, all groups with the exception of 14 and 13 gained weight.

Birds receiving no hormone generally showed little or no weight gains during treatment with the organo-phosphate (Table V). However, at the end of the experimental period, most of the birds, excepting 2177, had gained weight. Hormone-treated birds (Table VI) had gained weight at the conclusion of the experiment excepting the controls (Groups 13-14). This inconsistency cannot be explained.

Comparing total weight gained during treatment in Trials 1 and 2 (Table VII), the hormone-treated birds generally showed loss in weight, whereas the birds not receiving hormone in Trial 1 gained weight. At the conclusion of the experiment the weight gains of Trial 1 were much greater than those of Trial 2. Apparently the hormone treatment interferred with weight gain.

Hydrocortisone increased the worm burden in birds (Table IX). Bayer 9001 T administered in a single dose showed a 100 per cent efficacy against A. galli. However, its efficacy declined when an effective single dosage level was administered in multiple dosages over a period of days (Table VIII). Efficacy of Bayer 9001 T went as low as 25 per cent when an efficacious dose was given over a 5-day period.

Trial 3. This trial was a replication of Trial 2. The treated birds were older in Trial 2 than in Trial 1; however, birds were treated as closely as possible to the procedure in Trial 1. Hydrocortisone was used to increase the worm burden.

The experimental design given in Table XI shows 8 groups of 7 birds each, 4 of which received hydrocortisone and 3 of which did not receive the hormone. Since the hydrocortisone-treated birds showed smaller weight gain than the untreated birds before treatment with Bayer 9001 T, the dosage of the organo-phosphate was reduced for the hydrocortisone-treated birds (Table XI).

Table X shows that efficacy was 98 per cent at the highest dose. As in Trial 2, efficacy declined when the dosage was divided and treatment extended over a period of days. Table XI shows that treated and untreated birds all had positive weight gains at the end of the experiment. Table XII is an effort to show the total mg. per kg. dosage of the experiment. Table IX shows the worm burden in Trial 3 was much higher. This was probably due to the age at which the birds were exposed.

TABLE II

AVERAGE WEIGHT GAINS (%) OF BIRDS TREATED WITH BAYER 9001 T, EXPERIMENT I, TRIAL I

Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain during Treatment (%)	Weight Gain from Initial Treatment - Death (%)
1	8	+	688	1	12.00	31.92
2	8	+	688	1	5.84	33.11
3	8	+	688	1	9.34	46.46
4	8	+	344	2	12.19	40.55
5	8	+	344	2	27.05	53.43
6	8	+	344	2	13.51	36.84
7	8	+	138	5	41.91	74.34
8	8	+	138	5	39.28	66.66
9	8	+	138	5	42.62	69.53
10	8	-	0	0	7.62	45.94
11	8	-	0	0	-11.52	25.19
12	8	-	0	0	20.34	42.03
13	8	+	0	0	18.89	34.22
14	8	+	0	0	14.48	40.95
15	8	+	0	0	19.87	33.37
16	8	-	688	1	2.29	43.28
17	8	-	344	2	20.17	41.32
18	8	-	138	5	29.98	61.83

(+) = infected
(-) = non-infected
(0) = non-treated

TABLE III

WEIGHT GAINS OF MALE, FEMALE AND ISOLATED BIRDS TREATED WITH BAYER 9001 T AS COMPARED TO CONTROLS, EXPERIMENT I, TRIAL I

Group	Parasitism	Dosage (mg. per treatment)	Per Cent Weight Gains During Treatment			Isolates			Per Cent Weight Gain from Initial Treatment-Death					
			Male	Female	Sex Unknown	Male	Female	Sex Unknown	Male	Female	Sex Unknown	Male	Female	Sex Unknown
1-3	+	688	9.37	8.61	-12.34	-12.34	4.41	-8.45	44.59	41.47		24.37	42.15	18.30
4-6	+	344	15.77	14.96	29.02	11.27	15.15		42.51	44.89	56.99	34.77	30.50	
10-11	+	0	22.38	1.41	-17.74	22.50	13.34		45.31	33.48	22.88	59.16	37.43	
13-14	-	-	12.82	22.08		20.00	16.41		42.11	40.96		43.82	39.56	

TABLE IV

AVERAGE WEIGHT GAINS (%) OF BIRDS TREATED WITH BAYER 9001 T, EXPERIMENT I, TRIAL 2

Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain during Treatment (%)	Weight Gain from Initial Treatment - Death (%)
1	8	+	688	1	- 6.57	1.71
2	8	+	688	1	- 2.41	0.95
3	8	+	688	1	- 5.46	2.19
4	8	+	344	2	- 4.00	4.80
5	8	+	344	2	- 0.37	2.81
6	8	+	344	2	- 7.75	0.64
7	8	+	138	5	11.71	14.50
8	8	+	138	5	10.34	16.67
9	8	+	138	5	15.67	23.99
10	8	-	688	1	-14.67	0.44
11	8	-	344	2	- 1.03	6.21
12	8	-	138	5	20.81	27.86
13	8	+	0	0	- 5.62	- 1.80
14	8	+	0	0	- 6.09	- 0.26
15	8	+	0	0	3.60	8.26
16	8	-	0	0	- 5.90	2.47
17	8	-	0	0	- 1.93	4.00
18	8	-	0	0	7.01	18.23

(+) = infected
(-) = non-infected
(0) = non-treated

TABLE V

WEIGHT GAINS OF BIRDS NOT RECEIVING HYDROCORTISONE, EXPERIMENT I, TRIAL 2

Group	Bird Number	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain during Treatment (%)	Weight Gain from Initial Treatment - Death (%)
1	2204	+	688	1	5.82	4.89
2	2158	+	688	1	died before	
3	2177	+	688	1	- 9.40 treatment	- 2.75
4	2172	+	344	2	- 2.09	13.10
5	2237	+	344	2	- 4.40	2.78
6	2150	+	344	2	- 3.38	4.27
7	2173	+	138	5	9.53	12.74
8	2162	+	138	5	4.51	9.65
9	2187	+	138	5	3.98	4.78
10	2110	-	688	1	-13.08	1.57
11	2197	-	344	2	- 6.19	3.53
12	2151	-	138	5	28.67	30.80
13	2188	+	0	0	3.33	3.66
14	2154	+	0	0	- 3.65	4.31
15	2196	+	0	0	0.60	4.06
16	2114	-	0	0	- 4.32	2.10
17	2192	-	0	0	- 5.17	6.46
18	2175	-	0	0	11.98	18.45

(+) = infected
(-) = non-infected
(0) = non-treated

TABLE VI

AVERAGE WEIGHT GAINS OF BIRDS RECEIVING HYDROCORTISONE, EXPERIMENT I, TRIAL 2

Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain from Initial Treatment - Death (%)
1	7	+	688	1	1.87
2	7	+	688	1	0.95
3	7	+	688	1	2.90
4	7	+	344	2	3.61
5	7	+	344	2	2.82
6	7	+	344	2	1.31
7	7	+	138	5	14.90
8	7	+	138	5	17.63
9	7	+	138	5	26.73
10	7	-	688	1	0.56
11	7	-	344	2	6.59
12	7	-	138	5	27.44
13	7	+	0	0	- 2.58
14	7	+	0	0	- 0.91
15	7	+	0	0	10.36
16	7	-	0	0	2.55
17	7	-	0	0	3.51
18	7	-	0	0	18.19

TABLE VII

COMPARISON OF AVERAGE WEIGHT GAINS OF BIRDS RECEIVING BAYER 9001 T
EXPERIMENT I, TRIAL I AND EXPERIMENT I, TRIAL 2

Group	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain during Treatment (%)		Weight Gain from Initial Treatment-Death (%)	
				Trial I	Trial 2	Trial I	Trial 2
1	+	688	1	12.00	- 6.57	31.92	1.71
2	+	688	1	5.84	- 2.41	33.11	0.95
3	+	688	1	9.34	- 5.46	46.46	2.19
10	-	688	1		-14.67		0.44
16	-	688	1	2.29		43.28	
13	+	0	0	18.89	- 5.62	34.22	- 1.80
16	-	0	0		- 5.90		
10	-	0	0	7.62		45.94	
4	+	344	2	12.19	- 4.08	40.55	4.80
5	+	344	2	27.05	- 1.37	53.43	2.81
6	+	344	2	13.51	- 7.75	36.84	0.64
17	-	344	2	20.17		41.32	
11	-	344	2		- 1.03		6.21
14	+	0	0	14.48	- 6.09	40.95	- 0.26
11	-	0	0	-11.52		25.19	
17	-	0	0		- 1.93		4.00
7	+	138	5	41.19	11.71	74.34	14.50
8	+	138	5	39.28	10.34	66.66	16.67
9	+	138	5	42.62	15.67	69.53	23.99
18	-	138	5	29.98		61.83	
12	-	138	5		20.81		27.86
15	+	0	0	19.87	3.60	33.37	0.26
12	-	0	0	20.34		42.03	
18	-	0	0		7.01		18.23

TABLE VIII

PERCENT EFFICACY OF BAYER 9001 T AGAINST
ASCARIDIA GALLI, EXPERIMENT I, TRIALS 1 AND 2

Group	Dosage (mg. per treatment)	Treatment (consecutive days)	Efficacy (%) Trial 1	Trial 2
1	688	1	100	100
2	688	1	*no challenge	100
3	688	1	100	100
4	344	2	100	100
5	344	2	100	100
6	344	2	100	83
7	138	5	100	80
8	138	5	*no challenge	100
9	138	5	100	25

*No worms were present.

TABLE IX

COMPARISON OF WORM BURDENS OF BIRDS RECEIVING BAYER 9001 T, TRIALS 1, 2, AND 3, EXPERIMENT I

Trial	Group	Total Number Worms	Average Worms per Group
1	1	1	2.42
	2	0	
	3	4	
	4	2	
	5	2	
	6	6	
	7	3	
	8	0	
	9	1	
	13	1	
	14	6	
	15	3	
2	1	9	8.33
	2	3	
	3	16	
	4	5	
	5	4	
	6	6	
	7	5	
	8	8	
	9	4	
	13	17	
	14	14	
	15	9	
3	1	88	89.50
	2	93	
	3	87	
	8	90	

TABLE X

ANTEMORTEM AND POSTMORTEM FINDINGS OF BIRDS RECEIVING BAYER 9001 T, EXPERIMENT I, TRIAL 3

Group	Isolate Band Number	Antemortem Post Treatment Days 1	2	3	4	5	6	7	Worms Recovered Postmortem (number)	Efficacy of Entire Group (%)
		Number Worms Eliminated								
1		56	7	4	0	0			1	98
	3268	7	7	0	0	0			0	
	3274	6	0	0	0	0			0	
2		41	40	1	0	0			4	95
	3241	4	1	1	0	0			0	
	3237	1	0	0	0	0			0	
3		15	28	1	0	6	1	0	29	64
	3235	2	1	0	0	0	0	0	0	
	3206	3	1	0	0	0	0	0	0	
8 (control)		1	0	2	0	0	1	11		
	3201	1	1	0	0	0	0	0		
	3015								20	
	2301								4	
	3208								21	
	3243								12	
	3249								19	

TABLE XI

AVERAGE WEIGHT GAINS (%) OF BIRDS TREATED WITH BAYER 9001 T, EXPERIMENT I, TRIAL 3

Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain during Treatment (%) + Hormone	Weight Gain during Treatment (%) - Hormone
1	4	+	313	1	55.82	
	3	+	688	1		26.63
2	4	+	156	2	59.31	
	3	+	344	2		25.72
3	4	+	62	5	53.90	
	3	+	138	5		40.86
4	4	-	313	1	53.38	
	3	-	688	1		22.48
5	4	-	156	2	57.01	
	3	-	344	2		12.94
6	4	-	62	5	43.31	
	3	-	138	5		28.57
7	4	-	0	0	33.50	
	3	-	0	0		15.04
8	4	+	0	0	33.06	
	3	+	0	0		8.93

TABLE XII

SUMMARY OF DOSAGES OF BAYER 9001 T
GIVEN IN EXPERIMENT I, TRIAL I

Group	Dosage (mg. per treatment)	Treatment (consecutive days)	Average Dose in mg. per kg.	Total Average Dose Level (mg. per kg.)
1	688	1	1261.4	1259.3
2	688	1	1360.2	
3	688	1	1255.6	
13	688	1	1160.2	
4	344	2	730.0	662.2
5	344	2	619.4	
6	344	2	617.9	
14	344	2	627.8	
7	138	5	254.0	239.7
8	138	5	251.5	
9	138	5	227.8	
15	138	5	225.5	

Experiment II

Trial I. The objective of this trial was to indicate the efficacy of Bayer 9001 T against A. galli larvae while they were still in the tissue phase of their development. Hormone (hydrocortisone) was used as a means to increase the worm burden.

Fourteen-day-old White Rocks were arranged into 8 groups (Table XIII) containing 7 birds of which 4 received hormone and 3 did not.

Treatment was begun 10 days post-exposure. Ackert (1931) reported that the tissue phase lasts from 10-17 days after exposure with embryonated eggs. However, because of many deaths among experimental birds, subsequent treatment was discontinued. All birds receiving hormone (Group 1) died within 3 hours of treatment. Those not receiving hormone were listless and showed inappetence. One bird from each of groups 2, 4 and 5 died within 24 hours. The history of rapid death is strong and convincing evidence that a synergism between the hormone and anthelmintic was the cause of death in these birds. Thus, it was decided, 10 days must lapse between treatment with hydrocortisone and anthelmintic compounds.

In subsequent experimental work milligram per kilogram dosages were used. It was necessary to compare the dosage levels based on food consumption on a milligram per kilogram basis (Table XIV). As can be seen, birds receiving hormone received much higher dosages on a milligram per kilogram basis than those not receiving hormone, e.g. 2139 mg. per kg. versus

TABLE XIII

WEIGHT GAINS OF BIRDS RECEIVING BAYER 9001 T
EXPERIMENT II, TRIAL I

Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain from Initial Treatment to Death (%)	
					+ Hormone	- Hormone
1	7	+	313	1	*	15.16
2	7	+	156	2	15.81	17.42
3	7	+	62	5	14.34	20.00
4	7	-	313	1	21.62	19.67
5	7	-	156	2	15.31	19.14
6	7	-	62	5	19.58	25.57
7	7	-	0	0	17.90	25.57
8	7	+	0	0	18.87	15.31

*All birds died.

TABLE XIV

SUMMARY OF DOSAGES OF BAYER 9001 T BASED ON FOOD CONSUMPTION AS COMPARED ON A MILLIGRAM PER KILOGRAM BASIS EXPERIMENT II, TRIAL I

Group	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Average Mg. per Kg. Dose per Day	
				+ Hormone	- Hormone
1	+	313	1	2139	1441
4	-	313	1	2410	1402
2	+	156	2	1116	694
5	-	156	2	1218	752
3	+	62	5	398	270
6	-	62	5	460	340

TABLE XV

EFFICACY OF BAYER 9001 T AGAINST *A. GALLI* LARVAE
EXPERIMENT II, TRIAL I

Group	Bird Number	Hormone Treatment	Dosage (mg. per kg. per day)	Number Worms Found at Necropsy	Average Worms Per Group	
					+ Hormone	- Hormone
1	3252	-	1565	1	4.00	
	3310	-	1337	7		
2	3303	+	1122	23	21.33	4.67
	3258	+	1114	22		
	3307	+	1114	19		
	3320	-	757	2		
	3277	-	650	8		
	3323	-	675	4		
3	3257	+	298	13	15.75	5.00
	3313	+	492	23		
	3279	+	413	9		
	3304	+	392	18		
	3255	-	295	11		
	3283	-	269	1		
	3225	-	248	3		
4	3284	+	control	14	12.75	2.50
	3298	+		16		
	3276	+		5		
	3254	+		16		
	3299	-		4		
	3317	-		1		

1441 mg. per kg. This compound given in a single dose had little effect on A. galli larvae in the intervillar migration phase (Table XV). By the conclusion of the experimental period all surviving birds had shown a weight gain (Table XIII).

Trial 2. The objective of this trial was to test the efficacy of Bayer 9001 T against A. galli larvae. Hydrocortisone was not used as an immunosuppressor.

The safe level of the compound was found to be 1200 mg. per kg. in Experiment I, Trial 3 (Table XVI). In this trial the average weight of all birds at the time of treatment was 105 g. The dose administered was 1200 mg. per kg. The treatment was begun 14 days post-exposure. The compound was found to be slightly effective against the larval stage of this roundworm (Table XVII).

Experiment III

Trial 1. This test was done to establish lethal dosage of Bayer 9001 T. Three dosage levels were selected and were given to 53-day-old White Rocks (Table XVIII). Bird 3290 was given Bayer 9001 T at a dosage rate of 10,000 mg. per kg. There was immediate vomiting and dyspnea. Regurgitation of part of the compound was noted. Four hours later dyspnsa, violent shaking, extreme diarrhea, foaming at the mouth, and dialation of the pupils was noted. Death occurred 6 hours after treatment. Hemorrhage of the cecae, bleaching of the crop and intestine, and inhibition of the clotting mechanism were observed.

TABLE XVI

SUMMARY OF THE DOSAGE OF BAYER 9001 T
EXPERIMENT I, TRIAL 3

Group	Dosage (mg. per treatment)	Treatment (consecutive days)	Parasitism	Hormone * Treatment	Average Mg. per Kg. Dose per Day	
					+ Hormone	- Hormone
1	313	1	+	+	1200	1281
	688	1	+	-		
4	313	1	-	+	1156	1198
	688	1	-	-		
2	156	2	+	+	597	686
	344	2	+	-		
5	156	2	-	+	584	551
	344	2	-	-		
3	62	5	+	+	216	242
	138	5	+	-		
6	62	5	-	+	224	234
	138	5	-	-		

* Hormone = Hydrocortisone

TABLE XVII

EFFICACY OF BAYER 9001 T, EXPERIMENT II, TRIAL 2

Group	Bird Number	Dosage (mg. per kg. per day)	Number Worms Found at Necropsy	Average Worms per Bird	Average Dosage per Group (mg. per kg.)
1	2743	1032	1	1.17	1150
	2739	1145	0		
	2746	926	0		
	2732	1166	6		
	2741	1235	0		
	2744	1400	0		
2	2734	700	1	1.33	585
	2736	562	0		
	2747	656	1		
	2745	500	2		
	2731	572	1		
	2726	525	3		
3	2728	183	-	0.02	227
	2729	245	0		
	2737	271	0		
	2750	250	0		
	2748	208	1		
	2735	208	0		
4	2751	control	-	4.20	
	2733	control	3		
	2730	control	1		
	2738	control	6		
	2742	control	6		
	2749	control	5		

Bird 1673 was administered 5,000 mg. per kg. No particular symptoms were noted until 6 hours after treatment. At that time dialation of the pupil, diarrhea, and ataxia were noted. Six and one-half hours after treatment there was severe ataxia, listlessness, violent shaking and dyspnea. Death occurred seven hours after treatment. Necropsy examination was conducted by Poultry Pathology, Department of Pathology, Parasitology, and Public Health and revealed hydropericardium and fibrinous pericarditis, particularly of the auricles. There was hemorrhage or congestion in the fat of the heart and gizzard. A defect in the clotting mechanism was noted. A slight amount of feed remained and the gizzard was stained yellow. The bile was pale and watery. The spleen was small and pale. Terminal arterial hemorrhages were noted. The liver was mottled and congested with grayish areas which extended deeply into the substance. The kidney was enlarged and pale. The thyroid was congested. There was a slight excess of cerebrospinal fluid. Slight congestion of the mucous membrane of the trachea was noted. The pancreas was smaller than normal. The anterior thigh muscles were erratically hyperemic. In the lateral thoracic area gelatinous material was noted. The intestine was fragile, the mucosa thickened; some feed was present. Serosal vessels were injected. The compound had been regurgitated into the mouth area and nasal passages; the nasal passages were congested. The crop was still full of compound.

Bird 1545 showed no pathological effects from the compound. It was euthanitized approximately two weeks later. Necropsy

TABLE XVIII

DOSAGE LEVELS OF BAYER 9001 T GIVEN
TO BIRDS IN EXPERIMENT III

Trial	Bird Number	Number of Birds	Dosage (mg. per kg.)	Weight (grams)	Amount Given (mg.)
1	1545	1	1000	1108	1108
	1673	1	5000	786	3903
	3290	1	10000	820	8200
2	1675	1	1000	1068	1068
	2974	1	2000	1036	2072
	1674	1	3000	1035	3105
	1553	1	4000	988	3952
	1548	1	5000	812	4060

revealed osteodystrophy, pale heart muscles, moderate edema of the lung, slightly thickened intestine, and an enlarged, pale, mottled kidney.

Trial 2. The objective of Trial 2 was to establish the lethal dosage range between 1000-5000 mg. per kg. Five dosage levels were selected, and 55-day-old White Rocks were given the dosages listed in Table XVIII. Rectal temperatures were taken at various intervals over a 48 hour period. At the beginning of the experiment the average temperature was 107°F. Temperatures declined one to two degrees within two hours. The temperature returned to normal within 24 hours.

The following symptoms were seen antemortem. Bird 2974 showed bloody stool 8 hours after treatment. Bird 1553 had diarrhea, ataxia, listlessness 8 hours after treatment, and was inappetent. Similar symptoms were noted in Bird 1548.

Necropsy on Bird 1675 showed that the heart was pale and there was an area of fibrotic pericarditis as well as slight hydropericardium. The kidney was enlarged and pale. Osteodystrophy was observed.

Postmortem findings showed osteodystrophy in all birds. Bird 1675 had a slight hydropericardium. The heart was pale, and an area of fibrotic pericardiatis was noted. Bird 2974 showed slight lung edema in the ventral half, and the myocardium was pale and mottled. Subepicardial ecchymosis which may have been agonal was noted. Birds 1553 and 1674 had enlarged parathyroids and lung edema. Subepicardial ecchymosis was noted. Slight hyperemia of the intestine with scattered ecchymotic hemorrhage was seen in Bird 1553.

Experiment IV

Trial 1. The objective of this experiment was to determine the efficacy of Bayer 9001 T against H. gallinarum adults and pre-adults. The dosage was determined by food consumption as in Experiment I (Table XIX). Cecal droppings were collected for seven days. During this interval no pathological symptoms were noted. During the experimental period no H. gallinarum were found in the feces. It was concluded that the compound was not effective against this parasite. Positive weight gains were shown for all groups at the end of the experimental period (Table XIX).

Trial 2. The objective of this trial was to determine if the levels double the average maximum dosage given in Experiment IV, Trial 1, were toxic. Ten (five male, five female) White Leghorns were given 2584 mg. per kg. which is double the average maximum dosage of Experiment IV, Trial 1 (Table XX). The following observations were made during the antemortem period. There was a steady drop in rectal temperature beginning in approximately five hours after treatment. Rectal temperatures were regained by the third day after treatment. Ataxia, inappetence and bloody stool were noted in addition to a drop in temperature. Even after normal temperature was regained it was noted that ataxia was present. Anemic infarct of the heart and subcapsular hemorrhage of the kidney were general postmortem findings.

Experiment V

Trial I. The objective of this experiment was to determine the efficacy of Bayer 9002 against A. galli adults and juveniles.

Fifty-eight-day-old White Leghorns were divided into groups (Table XXI) and were treated with Bayer 9002. The birds were euthanitized five days after final treatment and the intestine was examined for nematodes. Efficacy was higher when the compound was administered over a period of three days (Table XXII); however, it was as high as 92 per cent at a dosage level of 25 mg. per kg. for one day.

Due to an ambiguous statement on the Bayer data sheet, it was thought that 50 mg. per kg. should be given on three separate days. It was found that this level was lethal. Three of five birds died in Group I and four of five died in the control Group 3. This varifies a statement in the data sheet that the LD 50 is 35 mg. per kg. of body weight.

Those birds treated with 25 mg. per kg. body weight showed ataxia, inappetence, and bloody discharge with severe diarrhea. The birds given 16 mg. per kg. of body weight for three days showed slight ataxia, inappetence, bloody stool and diarrhea. These symptoms disappeared two days after the final treatment with this compound. It was noted that worms were being passed in the feces within one hour after treatment.

The birds which received the compound showed very poor weight gains when compared to the untreated controls at the end of the experimental period (Table XXI).

Trial 2. The objective of this experiment was to test the efficacy of Bayer 2353 against A. galli adults and pre-adults. Bayer 2353 was given to 58-day-old White Leghorns (Table XXI). During the experimental period there were no evidences of deleterious effects. The fecal droppings appeared normal in all groups. Efficacy was very poor in all groups (Table XXIII). When the compound was given over a period of four days, efficacy was higher than when the compound was given in a single dose. The compound had little or no effect on weight gains (Table XXI).

TABLE XIX

DATA CONCERNING WEIGHT GAINS OF BIRDS TREATED WITH BAYER 9001 T AND EFFICACY OF THE COMPOUND, EXPERIMENT IV, TRIAL I

Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain from Initial Treatment - Death (%)	Average Worm Burden Found at Necropsy	Average Worm Burden for Dosage Level
1	5	+	688	1	33.80	7.00	7.80
2	5	+	688	1	31.51	7.25	
3	5	+	688	1	23.09	9.25	6.53
4	5	+	344	2	34.38	5.00	
5	5	+	344	2	35.47	6.40	
6	5	+	344	2	38.38	8.25	
7	5	+	138	5	35.53	9.40	9.73
8	5	+	138	5	37.68	7.80	
9	5	+	138	5	24.63	12.00	
10	5	+	0	0	37.77	6.80	9.00
11	5	+	0	0	33.33	7.20	
12	5	+	0	0	41.28	13.00	
13	5	-	688	1	49.78		
14	5	-	344	2	37.85		
15	5	-	138	5	42.89		
16	5	-	0	0	39.78		
17	5	-	0	0	32.25		
18	5	-	0	0	38.70		

TABLE XX

DOSAGE LEVEL OF BAYER 9001 T GIVEN IN EXPERIMENT IV, TRIAL 2

Band Number	Sex*	Weight of Bird (grams)	Treatment (mg. per treatment)
2752	F	600	1550
2706	F	610	1576
2715	M	586	1514
2713	M	576	1488
2708	F	588	1519
2798	F	520	1343
2709	F	522	1349
2716	M	586	1514
2717	M	658	1700
2799	M	540	1395

*F = Female
M = Male

TABLE XXI

WEIGHT GAINS OF BIRDS TREATED WITH BAYER 9002 AND BAYER 2353 AGAINST *A. GALLI*, EXPERIMENT V, TRIALS 1 AND 2

Trial	Group	Number Birds	Parasitism	Dosage (mg. per treatment)	Treatment (consecutive days)	Weight Gain Initial Treatment to Death (%)
1	1	5	+	50	1	0.45*
	2	5	+	25	1	6.90
	3	5	-	50	1	- 3.44#
	4	5	-	25	1	1.82
	5	5	+	0	0	21.08
	6	5	-	0	0	15.92
	7	5	+	16	3	- 9.37
2	1	5	+	50	1	14.47
				100	1	
				100	1	
				150	1	
	2	5	-	400	1	16.56
	3	5	+	400	1	24.03
	4	5	+	0	0	16.25
	5	5	-	0	0	12.11

*Two birds only.
#One bird only.

TABLE XXII

EFFICACY OF BAYER 9002 AGAINST *A. GALLI*,
EXPERIMENT V, TRIAL I

Group	Antemortem Post Treatment Days						Numbers of Worms Recovered Postmortem	Dosage (mg. per treatment)	Efficacy of Entire Group (%)
	1	2	3	4	5	6			
	Number Worms Eliminated								
2	32	1	3	0	0	0	3	25	92
7	47	5	0	3	1	0	1	16	98
5	1	0	0	0	0	0		0	control
							247 8		
							252 9		
							292 12		
							274 0		
							253 5		

TABLE XXIII

EFFICACY OF BAYER 2353 AGAINST A. GALLI,
EXPERIMENT V, TRIAL 2

Group	Antemortem Post Treatment Days: Number Worms Eliminated 1	2	3	4	5	6	Numbers of Worms Recovered Postmortem	Efficacy of Entire Group (%)
1	0	0	3	0	5	10	43	34
3	2	0	0	0	0	0	59	3
4	0	0	0	0	0	0		control
							20	
							7	
							8	
							43	
							4	

Experiment VI

Trial I. The objective of this trial was to test the efficacy of Bayer 9002 against H. gallinarum. The compound was given at two dose levels (Table XXIV). The feces were collected daily and were examined for the nematode. At the end of a five day period no nematodes had been found in the feces. Table XXV compares the worm burdens found at necropsy. When comparing treated groups with controls, it would appear that the compound was highly effective. The reason no worms were found in the feces might be explained on the basis of deterioration of the worms in the gut or feces.

Within the first day after treatment, Bird 1827 which received 25 mg. per kg. and Bird 1893 which received 16 mg. per kg. died. Necropsy showed a small amount of gelatinous material around the crop of Bird 1893. Both birds, 1893 and 1827, had a slight amount of yellowish fluid in the abdominal cavity. The hearts of both were mottled and vessels were injected. Livers were enlarged, friable, dark and mottled. The spleen of Bird 1827 was normal size but was very pale, and there were petechial hemorrhages. The spleen of Bird 1893 was one-half the normal size and very pale. The serosal vessels of the intestine were markedly injected and there were hemorrhages in the mucosa; some seem to extend completely throughout. There was excessive, rather thick mucus in the intestines. The proventriculus of both was dilated $2\frac{1}{2}$ or 3 times normal size and there were hemorrhages in the glandular portions as well as beneath the serosa. There was an increase

in the pericardial fluid and it appeared straw colored. There were hemorrhages in the pectoral muscle of Bird 1827; this change was not seen in the other bird. Some straw colored fluid was noted beneath the skin of the breast and thigh area. The kidneys of both were enlarged and mottled.

Antemortem, the experimenter noted ataxia, severe diarrhea and bloody stool in Groups 1, 2, 3, and 4. This did not clear up until several days after final treatment. At the end of the experimental period one bird from each group was necropsied. No gross pathological findings were observed.

Bird 1837 (Group 2) died 24 hours after final treatment with Bayer 9002. Upon necropsy it was found that the spleen appeared one-half the normal size. The subcutaneous fat was very gelatinous and mucoid in nature. The liver was discolored and mottled. Adrenals were enlarged and hypertrophic. Kidneys appeared normal. The heart was atonic in appearance and there was myocardial degeneration. There were two petechial hemorrhages of the pancreas. The intestine showed inflammation and petechial hemorrhages and there was hyperemia and excess mucus. The proventriculus showed two hemorrhages.

Bayer 9002 seems to depress weight gains to some extent (Table XXIV).

Trial 2. The objective of this trial was to test the efficacy of Bayer 2353 against H. gallinarum. There were no adverse symptoms noted throughout the experimental period. Efficacy of Bayer 2353 was better when it was administered in a single dose (Table XXV). As in Experiment VI, Trial 1, no H. gallinarum were found in the feces during the experimental period. Bayer 2353 had little effect on weight gains (Table XXIV).

TABLE XXIV

WEIGHT GAINS OF BIRDS TREATED WITH BAYER 2353 AND BAYER 9002
EXPERIMENT VI, TRIALS 1 AND 2

Trial	Group	Number Birds	Parasitism	Dosage (mg. per kg. per treatment)	Treatment (consecutive days)	Weight Gain Initial Treatment to Death (%)
1	1	4	+	25	1	11.21
	2	4	+	16	3	9.68
	3	4	-	25	1	17.91
	4	4	-	16	3	22.40
	5	4	+	0	0	28.16
	6	4	-	0	0	22.27
2	1	4	+	400	1	24.90
	2	4	-	400	1	29.79
	3	4	+	100	4	24.31
	4	4	-	100	4	27.07
	5	4	+	0	0	30.16
	6	4	-	0	0	25.27

TABLE XXV

EFFICACY OF BAYER 2353 AND BAYER 9002 AGAINST *H. GALLINARUM*, EXPERIMENT VI, TRIALS 1 AND 2

Trial	Group	Dosage (mg. per kg.)	Treatment (consecutive days)	Average Number Worms per Bird Found at Necropsy
1	1	25	1	6.00
	2	16	3	4.33
	5	control		40.72
2	1	400	1	7.50
	2	100	4	54.25
	5	control		40.72

DISCUSSION

The difference in effectiveness of given anthelmintics against various Orders, Families, Genera and even species of parasitic forms may be due in part to the location of the parasite within the host. For example, Ascaridia galli is found in the lumen of the small intestine where it is subjected to substances which are introduced into the alimentary canal; therefore, an orally administered anthelmintic such as Bayer 9001 T easily comes in contact with the parasite. Conversely, H. gallinarum is located in the fundus of the cecum, a blind pocket which receives relatively small quanities of intestinal material. In many cases the compound may never reach the parasite because the peristaltic stream may push the drug through the system too quickly to be effective. Another example where the location is very important in the effectiveness of an antiparasitic drug is the larvae of Ascaridia galli. The newly hatched larvae of A. galli, according to Ackert (1931), live in the lumen of the posterior portion of the duodenum and intevillar spaces until the 10th day after hatching. At that time the worms move deeply between the villi and penetrate the intestinal mucosa, destroying Lieberkühn's gland; the larvae remain there until the 17th day. Because of this close association of the parasite to the host and the natural protection from material in the lumen of the duodenum, the larvae are not subjected to the full effect of any non-systemic anthelmintic substance.

Once a drug reaches the site of the parasite it must either immobilize or kill the parasite so that it may be eliminated by the peristaltic action of the gut. Two major factors control this antiparasitic activity; the power of penetration of a drug into the parasite and the influence of environment on the rate of penetration (Trim, 1944). The power of penetration is determined by something inherent within the parasite such as the cuticle. Little work has been done on the mode of action of anthelmintics in respect to their action on parasitic forms; however, Trim (1949) studied the rate of penetration of certain phenolic compounds, alkaloid nicotine, and a chlorinated hydrocarbon *in vitro* into *Ascaris suum*. His experimental results suggested that the penetration of many substances into *Ascaris* depends upon their being partitioned between the medium and a thin outer lipoid layer of the cuticle. For example, penetration of nicotine was greatly enhanced by the use of a surface active agent. Knapp *et. al.* (1960), in studies done *in vitro* with ligated and non-ligated worms, found CS_2^{35} was taken up by *A. galli* as a vapor via the cuticle.

Research has revealed that drugs which are active against the adult *A. galli* are generally ineffective against larval forms. In experiments done with Bayer 9001 T this was demonstrated as being true. Besides location of the larval parasite, the cuticle may also be important in protecting the larva from various drugs. Studies by Bird and Rogers (1956) concerning the chemical composition of adult and larval cuticle of various nematodes show that the third stage larval

cuticle of Ascaris lumbricoides was much simpler in structure than that of the adult. Disulfide bonds and quinone tanning which seemed to be important in adult Ascaris were not found in the larval cuticle. It would be interesting to see if this difference or a similar difference was true in the fourth stage larva and if the difference in cuticle composition might also be true of A. galli. In addition, studies on the influence of anthelmintic effectiveness in relation to these differences would be of interest.

Bayer 2353 showed poor efficacy against both A. galli and H. gallinarum. This compound is generally recommended as a taenicide. In addition to the above factors such as location of the parasite within the host, another factor may be important in the failure of this compound to react against nematodes; the nematode differs morphologically, physiologically and chemically from the cestode. Waitz et. al. (1964) found the cuticle of four species of cestodes, Hymenolepis diminuta, Hydatigera taeniaeformis, Hymenolepis nana, and Dipylidium caninum, contained moderate levels of lipid. The cuticle was essentially non-reactive for glycogen in all four species. In studies by Bird (1956) the cuticle of various nematodes including Ascaris showed very little carbohydrate and lipid. Rothman (1959) has found that the cuticle of Hymenolepis diminuta consists of microtriches and there are numerous mitochondria seen within its matrix. He suggests that the cestode cuticle be called a tegument connotating that it is a living substance rather than non-living. As of yet electron microscope studies of the nematode cuticle do not reveal this

type of tegument. Bird (1957) gives evidence that the cuticle of Ascaris is secreted collagen and that the external cortical layer consists of tanned protein, the outermost part of which is covered by a thin lipid layer. Further, the cestode has no alimentary canal and the food is absorbed through the outer surface of the body (Rothschild, 1952). The nematode, on the other hand, ingests food by means of the sucking action of the pharyngeal or esophageal muscles. The cuticle is permeable to water, some ions and certain hydrophobic material but not glucose, so cuticular feeding does not occur (Fairbairn, 1957).

Some environmental factors such as hydrogen ion concentration can be influential in the antiparasitic activity of a drug. Trim (1949) found that the hydrogen ion concentration in a medium was influential in the rate of penetration of nicotine into Ascaris. Therefore it is not safe to assume that compounds tested in vitro will be effective in vivo for the chemical composition or effectiveness can be changed within the digestive tract of the host animal.

In spite of the fact that a compound is able to reach and penetrate a parasite resulting in a high efficacy, it is not necessarily a marketable compound. Such might be the case of Bayer 9002. This compound, which is used for the treatment of Capillaria, was relatively effective against both A. galli and H. gallinarum. However, toxicity to the host was extreme at low dosage levels of the compound and its effects upon the host were harsh. Diarrhea, salivation, cyanosis, uncontrollable muscle twitches, and loss of reflexes were observed in chickens treated in this study. The effect of organic phosphorous

poisons such as Bayer 9001 T and 9002 on the host animal have been studied to some extent. These types of compounds affect the host by acting as irreversible inhibitors of cholinesterase, thus allowing the acetycholine to accumulate. The cholinesterase content of various tissues is not equally affected within the same poisoned animal, i.e. the level of all tissues including the brain can be lowered to about 30 per cent of pre-poisoning levels without seriously affecting normal function. However when critical levels of cholinesterase depletion is reached, grave symptoms occur. Poisoned animals show various degrees of heart block and cardiac arrest usually occurs. In man, signs of organic phosphate poisoning include weakness, nausea, cramps, diarrhea, nervousness, and discomfort in the chest. Sweating, miosis, lacrimation, salivation, pulmonary edema, cyanosis, papilledema, uncontrollable muscle twitches, convulsion, coma, loss of reflex and loss of sphincter control were seen in advanced cases. If the poisoned animal is able to reform his critical quota of cholinesterase, recovery can be complete (clinical memoranda).

A problem which confronts the investigator attempting to evaluate efficacy of candidate anthelmintics against experimentally induced A. galli infections is the inability to predict worm burdens. A few birds have large numbers of worms while the majority will have few or none. This distribution of worms is not related to the methods of exposure but rather is related to something inherent in the birds. Following some work done in this laboratory (Johnson, 1963) hydrocortisone was administered to birds to increase and get consistent worm burdens.

It has been reported that corticosteroids increase the susceptibility of animals to parasitism. Dougherty (1953), Thomas (1953) and Robinson and Smith (1953) propose that this may be due to inhibition of inflammation and suppression of antibody formation. Other investigators such as Michael and Whorton (1951), Mogabgab and Thomas (1952), Schwartzman and Aronson (1953), and Coker (1955) found increased susceptibility to parasitism was due to corticosteroid inhibition of the inflammatory response. Fischel (1953), Bjønreboe, et. al. (1951) and Briggs (1959) found corticosteroid suppression of antibody production resulted in increased susceptibility to parasitism. Johnson (1963) suggests that it is possible that both inhibition of inflammation and antibody suppression take place in the fowl as in the mammal. He found injections of hydrocortisone during the first 20 days post-exposure increased the worm burdens of A. galli. In the present investigation hydrocortisone increased the numbers of worms per bird, and it was noted that there was a marked difference in weight gain between the hydrocortisone treated and untreated birds. Johnson (1963) suggests that the failure to gain weight is due to an alteration in the carbohydrate and protein metabolism.

Although the present investigation showed that A. galli developed more readily in birds receiving hydrocortisone than in those not receiving hormone, it should be noted that further research must be done before the procedure can be used in studying anthelmintic properties of organic phosphates.

SUMMARY

Efficacy of Bayer 9001 T, 9002, and 2353 was tested against experimentally induced infections of Ascaridia galli and Heterakis gallinarum. Studies concerning the toxicity of Bayer 9001 T to the chicken were performed and all unusual symptoms were noted. The results of these investigations were as follows:

1. In tests using 344 chickens, efficacy of Bayer 9001 T was 100 per cent effective against A. galli, but efficacy decreased as low as 25 per cent when the dosage was fractionated over several days.
2. A synergism between Bayer 9001 T and hydrocortisone was shown to exist if the two were given concurrently. It was suggested that 10 days should transpire between the last hormone injection and the first treatment with an anthelmintic compound.
3. At levels of 2000 mg. per kg. Bayer 9001 T was found to be toxic to the host animal. Symptoms of poisoning included salivation, cyanosis, uncontrollable muscle twitches, and loss of reflexes.
4. In tests employing 86 birds at a level of approximately 1200 mg. per kg. Bayer 9001 T was slightly effective against fourth stage larvae of A. galli. The average worms per bird treated with Bayer 9001 T at necropsy was 0.84 compared to the non-treated controls which was 4.2.

5. When given at a dose level of 1259 mg. per kg. Bayer 9001 T was not effective against H. gallinarum. At 2584 mg. per kg. the compound was too toxic to be of value as an anthelmintic drug.
6. Bayer 9002 was 92 per cent effective against A. galli at a dosage level of 25 mg. per kg. per one day; however, at a dosage level of 16 mg. per kg. per three days, Bayer 9002 was found to be 98 per cent effective.
7. Bayer 9002 was found to be severely toxic at the dosage levels tested and weight was affected. A dosage level of 50 mg. per kg. was found to be lethal.
8. In experiments using 25 birds, Bayer 2353 was ineffective against A. galli when given in dosage levels of 400 mg. per kg.
9. Bayer 9002 was effective against H. gallinarum in experiments employing 26 birds at levels of 16 mg. per kg. per three days and 25 mg. per kg. per one day. The average worms per treated bird found at necropsy was 5.1 compared to 40.7 in the control group.
10. Bayer 2353 showed poor efficacy for H. gallinarum in at levels of 400 mg. per kg. in experiments using 26 birds.

Under the conditions of this study the efficacy of Bayer 9002, 2353 and 9001 T against A. galli and H. gallinarum was disappointing. Where efficacy was high, toxicity was generally present. At the dosage levels and route of administration utilized in the present trials, Bayer 9001 T will have limited use against A. galli adults and pre-adults. Bayer 9002 should

be tested at lower dosage levels to determine efficacy since the levels and the conditions in which it was tested in this study were toxic to the host. Bayer 2353 appears to have little or no efficacy against A. galli or H. gallinarum under the conditions of this study.

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THE EFFICACY OF THREE CANDIDATE ANTHELMINTICS AGAINST ADULTS AND PRE-ADULTS OF *HETERAKIS GALLINARUM* AND *ASCARIDIA GALLI* OF CHICKENS

by

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ABSTRACT

Tests utilizing 624 chickens (White Rocks and White Leghorns) were conducted to investigate the anthelmintic activity of three compounds--two organo-phosphates (Bayer 9001 T, formula restricted information and Bayer 9002, N-hydroxynaphthalimide diethyl phosphate) and one which was not an organo-phosphate (Bayer 2353, 2', 5-dichloro-4' - nitro-salicylanilide) against pre-adults and adults of Ascaridia galli and Heterakis gallinarum.

The compounds were administered orally in milligram per kilogram dosages or dosages based on food consumption. Bayer 9001 T was given on basis of food consumption at rates of 688 mg. for one day, 344 mg. for 2 consecutive days, and 138 mg. for 5 consecutive days. Bayer 9002 was given at dosage levels of 25 mg. per kg. (1 day) and 16 mg. per kg. (3 consecutive days). Bayer 2353 was given at a rate of 400 mg. per kg. as a single dose or fractionated doses over 4 consecutive days.

The toxicity of various compounds to the host was observed. Bayer 9001 T was found to be toxic at doses over 2000 mg. per kg. Toxicity was noted at rates of 16 mg. per kg. and 25 mg. per kg. in birds treated with Bayer 9002. Bayer 2353 produced no toxic effects in the host at levels of 400 mg. per kg.

Hydrocortisone was administered subcutaneously in 3 experiments to increase the worm burden. Rates of 0.625 mg. were given every 3 days for a total of 3 to 5 injections.

Bayer 9001 T was found to be 100 per cent effective against A. galli adults when given in one dose, but when the doses were fractionated over several days, efficacy decreased to as low as 25 per cent. This compound was found to have no efficacy for H. gallinarum and questionable efficacy against the larval stages of A. galli.

Bayer 9002 was 92 per cent effective against A. galli at 25 mg. per kg. but 98 per cent effective at 16 mg. per kg. given for 3 consecutive days. It was somewhat effective against H. gallinarum. (The average worms per bird found at necropsy was 5.1 in the treated birds as compared to 40.7 in the control group.)

Under the condition of this study, Bayer 2353 was found to be ineffective against both A. galli and H. gallinarum.