THE TOXICITY OF FIVE ORGANIC PHOSPHORUS INSECTICIDES TO CHICKENS

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PREFACE

Thousands of organic phosphorus insecticides have received wide attention in the last few years, and the interest shown in their effectiveness for insect control and their effects upon animals led to the research reported in this paper.

The writer is indebted to many individuals for their help in various ways and wishes to thank the following: the advisory committee that reviewed the work, for the advice and criticisms that helped in the preparation of this paper; Dr. D. E. Howell, for the special tasks he performed; Fay A. Warnhoff, the writer's wife, for her unfailing encouragement and for her typing of this thesis; Dr. G. E. Bair, for his assistance in checking this paper for composition and grammar; Florence Hermenze, for her aid in the laboratory work; Drs. G. W. Anderson and W. J. Goodwin, for their help with autopsies; Mr. B. J. Todd, for his assistance in the statistical analysis of the results of this study; Dr. J. H. Cochran, for his help in setting up the research project; American Cyanamid Company, Chemagro Corporation, and Florida Agricultural Supply Company, for their cooperation; and Mr. Charles Arrington, pastor of the Clemson Baptist Church, for his invaluable service in multilithing this paper.

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INTRODUCTION

It is imperative that entomologists and workers in related fields become familiar with the use of organic phosphorus compounds in the control of insects. These materials, because of their toxic nature, were at one time restricted to use in plant protection. However, with the development in the last few years of newer and less hazardous compounds, new emphasis is being placed upon their use to protect animals from both destructive and annoying pests.

The field of poultry production appears to be a fertile area for use of the organic phosphorus compounds. The control of house flies, lice, and mites is always of importance to the poultryman. Since the materials mentioned show promise for the control of these pests, it seemed reasonable to study in some detail their effect upon chickens. To do this the writer conducted tests to determine the effects of five organic phosphorus insecticides on chickens.

In cooperation with the Florida Agricultural Supply Company, tests using bait materials of oyster shell flakes treated with malathion, diazinon, and Dipterex (L13/59) were carried on. The main object of these tests was to determine the acceptability of the material to chickens, the toxicity of the material if accepted in the ration, and the effects the material might have on chickens.

A second series of tests using parathion, malathion, Dipterex, chlorthion, and diazinon was conducted to determine respective lethal dosages, symptoms of poisoning, and cholinesterase inhibition. Autopsies and tissue residue analyses were made at the end of the test period.

It was believed that tests of the nature enumerated would be of great value in adding to the information on organic phosphorus materials. It was also the writer's belief that work of this nature would be of practical value to poultrymen and livestock men beset by an imposing assortment of pests.

REVIEW OF LITERATURE

Even though literature dealing specifically with the effects of the organic phosphorus insecticides on chickens is very meager, there is an abundance of literature dealing with topics related to the problems considered in this paper. This review of literature covers work published on cholinesterase and cholinesterase inhibition, analyses of residues in animal tissue, development and use of various organic phosphorus insecticides, chemotherapeutic use of insecticides, and the toxicity and symptoms of poisoning of organic phosphorus materials.

One aspect under consideration in this thesis is a study of the inhibition of cholinesterase by the organic phosphorus insecticides. As early as 1906 Hunt and Taveau isolated choline esters from certain animal tissues and showed the potency of acetylcholine in biological action (Hazelton 1955). Hazelton stated:

> A modern concept of neurohormonal transmission is that acetylcholine is the chemical mediator for all autonomic ganglia, the endings of the parasympathetic and somatic nervous systems, and perhaps the central nervous system. With the exception of the central nervous system these nerves, whose impulses are mediated by acetylcholine, are termed "cholinergic", and the term is frequently used to describe the effect that is produced by their function or stimulation.

Von Rumker (1955) compared the action at the neuromuscular junction with an electric motor. In this comparison the acetylcholine would be comparable to the electrical current which is transformed into mechanical energy by the motor. As a result of acetylcholine stimulation, the effector cell induces muscular action in a manner similar to the activation of the motor by electrical current. According to Augustinsson (Sumner 1950) the impulse transmission is both chemical and electrical in nature, but the chemical action is primary. Dale (1914) suggested that it was obvious that since acetylcholine, so biologically potent, was present in tissue following nerve function or stimulation, there must be some detoxifying mechanism to prevent its accumulation in the effector cells. Stedman and his co-workers (1933) applied the name "choline-esterase" to the enzyme responsible for splitting acetylcholine into choline and acetic acid. Von Rumker (1955) likened this cholinesterase material to the rheostat that controls the motor. Augustinsson (Sumner 1950) stated that it seemed undesirable to obscure the text of his study by too many detailed references to the more than 1300 papers presented on the subject of acetylcholine and cholinesterase. Since 1950 many more papers have been written on the subject. This recent attention was brought about by the increased interest in the so-called cholinesterase inhibitors, organic phosphates, for insect control.

Stedman and his co-workers (1933) presented a method of determination of cholinesterase activity in blood sera from several animal species. The method employed by this group was a direct titration method, considered to be the pioneer method. Ammon (Hazelton 1955) is given credit for evolving a reliable method of determination based on manometric techniques. Since 1933 new methods and variations of the ones mentioned have been employed. Perhaps the method most widely used today is that of Michel (1949). This technique was based on an electrometric method of determining red blood cell and plasma cholinesterase activity. Modifications of this technique were described by Brown and Bush (1950) and Wolfsie and Winter (1952). Micro sampling techniques were developed by Marchand and Hermenze (Hamblin 1953). These techniques included directions for collection, storage, and shipment of micro blood samples.

Several methods of analysis of residue in animal tissues have been perfected. Two methods are reviewed here because of their application to this thesis. Averell and Norris (1948) presented a method of residue analysis for parathion involving a colorimetric technique for estimating small amounts of the material. Chemagro Corporation, Pittsburg, Pennsylvania, employs this method for analysis of chlorthion residue (Chemagro 1955). The American Cyanamid Company, Stamford, Connecticut, employs a colorimetric method originated by Norris, Vail, and Averell (1954).

The insecticides included for use in this study have been given wide attention. Hazelton (1955) asserted that probably no other group of insecticides had so much known about them before they were put to practical use as did the organic phosphorus compounds. Intensive research on the part of industrial, independent, governmental, and academic laboratories made this knowledge possible.

Schrader, whose work in this field began as early as 1934, is considered the pioneer in the development of the organic phosphorus insecticides (DuBois 1952). In 1947 Schrader's studies were published, and since then thousands of these compounds have been evaluated for insecticidal properties. Many of these compounds are in commercial use (Metcalf 1955). Parathion, one of the materials tested by the writer, is the most widely used and is said by Metcalf (1955) to be manufactured and used at the rate of millions of pounds annually. Because of the much publicized hazards of the earlier group of organic phosphorus compounds, much interest has been shown in newer materials of considerably less hazardous nature.

Four of the less hazardous materials considered in this paper are malathion, chlorthion, Dipterex, and diazinon. Great interest in these four materials was evinced as a result of the resistance of certain insect species to the chlorinated hydrocarbons. Marsh and Eden (1955) in tests against two strains of the house fly, used six insecticides—three chlorinated hydrocarbons and three phosphorus compounds. They found that there was a distinct difference in effectiveness between the phosphorus compounds and the chlorinated hydrocarbons. Diazinon, American Cyanamid 4124, and chlorthion all showed extreme toxicity in these tests. Molpus and Hutchins (1955) tested several insecticides for use in control of fly larvae in chicken droppings and found that diazinon gave excellent results, but malathion and Dipterex gave very little promise. Studies have been made of these materials applied as sprays and mixed with feed. Diazinon as a spray had definite adverse effects on poultry of various age groups. This finding seemed to concur with reports concerning diazinon and its toxicity to turkeys, ducks, and geese (Alexander 1955). In tests by Molpus and Hutchins, malathion when applied as a spray on poultry produced no visible effects. Neither malathion nor diazinon showed any deleterious effects on the birds fed these materials in their rations. However, the researchers stated that the amount of feed eaten was below normal; and they assumed this was because the food was not palatable.

In 1954 Gahan, Wilson, and McDuffie (1954) reported extensive tests to evaluate the effectiveness of dry sugar baits for the control of house flies. These tests were conducted during 1953 at the Orlando, Florida, laboratory of the Entomology Research Branch of the United States Department of Agriculture. The flies used in the evaluation had been determined to be resistant to DDT and other chlorinated hydrocarbon insecticides. Baits containing only 0.1% of malathion, diazinon, or Bayer L13/59 (Dipterex) gave 99% kill of flies in sixteen hours while higher concentrations gave faster kill. In open dairy barns and poultry houses, applications of 100 grams of the bait per 2500 to 5000 square feet of floor space resulted in reductions in fly population of 90% or higher within four hours. Some important aspects of this type of treatment were: the material could be spread so sparsely that animals would be unlikely to eat much of it, the bait was very inexpensive, it was easily prepared, and it was easily applied.

Several authors have reported success in using these same organic phosphorus insecticides to control other important insect and arthropod pests. Smith and Richards (1955) reported excellent control by the phosphorus compounds malathion and diazinon in tests of several materials against livestock and poultry lice. The phosphorus insecticides lack the residual effectiveness of the chlorinated hydrocarbons, but in the same tests it was found that the organic phosphorus compounds were effective at much lower concentrations than those of the chlorinated hydrocarbons.

Harding (1955) found that 4.0% malathion dust used both as a litter treatment and as a direct application to individual birds gave effective control of the northern fowl mite. Vincent, Lindgren, and

Krohne (1954) also reported success using malathion both as a litter treatment and as a direct application to the bird in tests against the northern fowl mite. The investigators reported no off-flavor in eggs from hens fed a ration containing 50 parts per million, and no adverse effect on egg production or upon hatchability. Moore and Schwart (1954) stated that malathion gave excellent control of both the chicken red mite and chicken lice at 1.00% and 3.00% respectively.

Recently there has been renewed interest in another field of investigation--that of the chemotherapeutic use of insecticides for control of blood-sucking parasites. Bishopp and his co-workers (1926) reported no success in the use of chemicals then available as internal medication for the control of various ectoparasites. Parman (1928) reported similar results in tests on poultry. Bruce (1940) administered rotenone and zinc oxide to cattle as internal medication for control of hornfly larvae in droppings and reported success. Rotenone at the rate of 0.4 gram per 100 pounds of body weight and zinc oxide at the rate of 1.5 gram per 100 pounds of body weight killed 100% of the larvae. No harmful effects were noted on the cattle.

More recent investigation by McGregor, Radeleff, and Bushland (1954) indicated a revival in research attempting to find an internal treatment for ectoparasites. These researchers attempted to find a material toxic to ectoparasites, non-toxic to the host, and one which would not leave questionable residue in the tissues or products of the treated animals. After a period of testing, the investigators still considered the goal unattained; but with some organic phosphorus compounds showing promise, they regarded the matter with renewed hope of success. Diazinon, Dipterex, and chlorthion were administered to cattle infested with cattle grubs. Diazinon given orally at 10 and 25 milligrams per kilogram of body weight gave almost complete kill of grubs present at time of treatment. The lower dosage was completely effective against grubs for one week but less effective the second week, and no new grubs were found after treatment at the higher dosage. Dipterex gave excellent control of the grubs present at time of treatment; however, within four weeks after treatment with 100 milligrams per kilogram of body weight no kill was noticed. Chlorthion at 500 milligrams per kilogram of body weight killed only about 66% of the grubs present at time of treatment.

Bruce, Howard, Sauveur, and Hazelton (1954), testing diazinon on mice and cats, found that technical diazinon (95%) gave an LD50 reading of 82 milligrams per kilogram of body weight on mice, and an LD50 reading of 100-150 milligrams per kilogram of body weight on cats. The symptoms of poisoning were a mild depression and lack of appetite.

Dubois, Doull, and Coon (1948) recorded that parathion administered to cats gave a generalized parasympathetic and central nervous system stimulation, and death from primary respiratory paralysis occurred within one hour. Sublethal doses of five milligrams per kilogram of body weight produced tremors, muscular twitching, and fluid in the respiratory tract. Autopsy showed an intensely contracted intestinal tract and urinary bladder as well as fluid in the respiratory tract.

Hazelton and Godfrey (1948) reported muscarinic and/or nicotinic effects on dogs treated with parathion. Holland, Hazelton, and Hanzal (1952), not naming any particular animal in the discussion, stated that malathion was relatively nontoxic to warm blooded animals. These investigators reported that the 99+% form was appreciably less toxic than material less pure.

Hagan and Woodard (1948) using parathion in corn oil solution, found an LD50 reading of 25 milligrams per kilogram of body weight for guinea pigs. These authors reported that death from respiratory failure occurred within twenty-four hours.

Radeleff and fellow workers (1955), using several organic phosphorus insecticides on livestock, recorded the following results on several materials:

Parathion produced symptoms of poisoning in calves within one hour after administration of 0.5 milligram per kilogram of body weight. Malathion produced cholinesterase inhibition as do other organic phosphorus materials. A minimum toxic dose to calves, when administered orally, was between 10 and 20 milligrams per kilogram of body weight. Diazinon was determined to have a minimum toxic dosage to baby calves of 1.0 milligram per kilogram of body weight. Definite drowsiness appeared in the calves after treatment. It was stated, "Diazinon appeared to give a better chance of recovery than parathion when administered at approximately the same degree". The symptom of drowsiness appearing in diazinon tests was not noted in tests using the other related organic phosphorus materials. Chlorthion was perhaps the least toxic of the group. A minimum toxic dosage to baby calves ranged from 50 to 100 milligrams per kilogram of body weight. Dipterex showed a minimum toxic dosage of 5 to 10 milligrams per kilogram of body weight to baby calves, and 50 to 100 milligrams per kilogram on cattle. The authors stated, "All phosphorus compounds used produced symptoms of poisoning indistinguishable to us. The symptoms are those that are associated with the destruction of or interference with the enzyme that destroys esters of choline".

In order of appearance the symptoms recorded by Radeleff *et al.* (1955) were: excessive salivation, respiratory difficulty (breathing with mouth open and with great effort), fasciculation of all skeletal muscles, loud rales from lungs, and convulsions (noted after high dosages).

Autopsy reports showed lesions in heart, lungs, and gastro-intestinal tract though the exact location was not consistent. Frothy exudates were often present in bronchi and trachea. Animals treated over a long period showed typical symptoms of pneumonia.

In a summary of oral toxicity tests from the Livestock Laboratory of the United States Department of Agriculture, Kerrville, Texas (1955), the following data were available on hens one year old:

Parathion----1 milligram per kilogram toxic in 5 doses, or
2 milligrams per kilogram toxic in 4 doses.

Malathion----1 dose of 200 milligrams per kilogram toxic;
2 doses of 100 milligrams per kilogram toxic;
2 doses of 50 milligrams per kilogram and
3 doses of 20 milligrams per kilogram not toxic.

Diazinon-----3 doses of 1 milligram per kilogram toxic, or
2 doses of 2 milligrams per kilogram toxic.

Chlorthion---1 dose of 500 milligrams per kilogram toxic.

Dipterex-----1 dose of 50 milligrams per kilogram toxic;
2 doses of 20 milligrams per kilogram toxic; or
4 doses of 10 milligrams per kilogram toxic.

The Hazelton Laboratories were commissioned by Geigy Chemical Company, New York, N. Y., to determine the LD50 of diazinon on domestic fowl (Alexander 1955). These laboratories found the approximate LD50 on chickens to be 41 milligrams per kilogram of body weight; on turkeys, 7 milligrams per kilogram; on geese, 15 milligrams per kilogram; and on ducks, 20 milligrams per kilogram.

Deichman (1954) reported that Dipterex, when administered as a 10.0% aqueous compound, was lethal to female chicks at the rate of 0.08 gram per kilogram. Brelsford (1955), writing on the toxicity of parathion and malathion to chickens, stated that technical parathion showed an acute oral LD50 to New Hampshire roosters of 8.7 milligrams per kilogram of body weight when administered as a 0.1% solution in corn oil. Autopsies conducted on the birds showed no significant pathology. The acute oral LD50 to New Hampshire roosters of malathion technical (95%), as a 1.5% solution in corn oil, was approximately 850 milligrams per kilogram. A dosage of 1000 milligrams per kilogram killed six of seven young roosters within eighteen hours.

Brelsford (1955) also reported that in feeding studies using malathion and parathion, day old chicks were obtained and fed on starting mash containing one part per million of parathion and ten parts per million of malathion. After two weeks the birds were divided into groups and placed on diets of ten, fifty, and one hundred parts per million of parathion. Other birds were placed on diets containing one hundred, one thousand, and five thousand parts per million of malathion. The tests covered a period of twelve weeks—from the time the chicks were obtained until completion of the tests. Growth retardation was slight for birds on ten parts per million of parathion and one hundred and one thousand parts per million of malathion. Parathion at fifty parts per million and malathion at five thousand parts per million caused marked growth retardation. One hundred parts per million of parathion was lethal to the majority of the birds within two weeks. Plasma cholinesterase inhibition was significant at fifty and one hundred parts per million of parathion levels. After eight weeks, malathion at one thousand and five thousand parts per million inhibited plasma cholinesterase.

Metcalf (1955) included at the end of his chapter on organic phosphorus insecticides a very comprehensive bibliography of articles dealing with all aspects of the subject. Hazelton (1955) gives a more or less complete review of the knowledge of the toxicity of cholinesterase inhibiting insecticides. In his review there is included a bibliography of some length. Augustinsson (Summer 1950) reviewed the pertinent information on acetylcholine esterase and cholinesterase and included a bibliography.

DISCUSSION OF CHEMICALS USED

Since the publication in 1947 of Gerhard Schrader's work in the development of the organic phosphorus insecticides, thousands of phosphorus compounds of many types have been evaluated for insecticidal properties (Metcalf 1955). Of this vast number of compounds, only five were considered in this study; and a brief characterization of each follows:

Parathion--O,O-diethyl O-p-nitrophenyl thionophosphate. Parathion in technical form (95% purity) is a dark brown liquid with a slight garlic odor. It is slightly soluble in water (about 20-25 p.p.m.) and is miscible in esters, ketones, ether, benzene, toluene, chloroform, carbon tetrachloride, and animal and vegetable oils. It hydrolyzes rapidly in alkaline solutions (Metcalf 1955).

Because of the extremely small amounts to be used in tests, parathion was mixed with peanut oil, with which it is miscible, at the rate of one part parathion to nine parts of peanut oil to facilitate weighing of the insecticide.

Malathion--O,O-dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate. The technical material is a dark brown viscous liquid with a rather disagreeable garlic odor. It is slightly soluble in water (145 p.p.m.), is miscible with most organic solvents, and rapidly hydrolyzes in alkaline media (Metcalf 1955).

Since malathion was used in relatively large amounts in these tests with chickens, it was administered as a technical material without further dilution.

Chlorthion--O,O-dimethyl O-3-chloro-4-nitrophenyl thionophosphate. Chlorthion in technical grade is a yellowish brown viscous liquid with an ester-like odor. It is slightly soluble in water but readily soluble in most organic solvents such as benzene, toluene, alcohols, ethers, and in animal and vegetable oils. It hydrolyzes quickly in an alkaline media (Metcalf 1955). In the tests completed, chlorthion was used as technical grade material without dilution.

Dipterex--O,O-Dimethyl 1-hydroxy-2-trichloromethyl phosphonate. The technical grade of Dipterex is a white crystalline solid with a slightly sweetish odor. Dipterex is soluble in water to about 16% and in aromatic hydrocarbons, alcohol, and acetone (Metcalf 1955). It was administered as the technical crystalline material to chickens in this series of tests.

Diazinon--O,O-diethyl O,2-isopropyl-4-methylpyrimidyl-(6) thionophosphate. Diazinon as a technical material is a pale brown liquid, soluble to about 0.004% in water. The odor of diazinon is of a rather pleasant aromatic nature. It is readily miscible in alcohol, xylene, acetone, and petroleum solvents (Metcalf 1955).

Diazinon, because of its reported toxicity to poultry, was used in very minute quantities. To be accurate in measurement of these small samples, the writer mixed technical material in peanut oil, with which it was miscible, at the rate of one part diazinon to nine parts peanut oil.

Parathion and malathion, technical grade, were furnished by the American Cyanamid Company while chlorthion and Dipterex, technical grade, were supplied by the Chemagro Corporation. Diazinon was furnished in technical grade by the Geigy Chemical Corporation.

MATERIALS AND METHODS

All chickens used in tests conducted to determine the toxicity of certain organic phosphorus insecticides were purchased from the Clemson Poultry Department. The birds were picked from the flock after having fallen off in egg production. They were apparently normal, but each was examined and weighed before being included in the experiment. Only chickens meeting a weight standard of one kilogram or more were retained, and all were Leghorn hens one to one and one-half years old.

The test hens were held in single row laying cages or in Walker small animal cages. The writer found that the laying cage type was more satisfactory in several ways. The Walker type cages, each holding two birds in separate compartments divided by a solid metal partition, were completely closed on top and back; and during hot weather air circulation was somewhat restricted because of this feature. Cleaning was simplified in the laying cages because the droppings could fall more easily through the mesh into the dropping trays which were suspended at some distance under the cages. The laying cage type was very much lighter in weight and more easily handled--a battery of six layer type cages was lighter than just one of the Walker cages. There was one disadvantage to the laying cages, however, in their having to be suspended from the ceiling in some manner, though this feature did not present a problem to the writer as rafters from which to suspend the cages were available.

Feed and water troughs which would accommodate an entire battery of cages were available from the cage manufacturers, but it was the belief of the investigator that the results from this study should be based as nearly as possible on the reaction of the individual bird and that any possibility of contamination from one bird to another should be eliminated. Therefore individual feed and water cans were devised from 46 ounce fruit juice cans readily available from the college kitchen. The cans were cut so that by turning the flap of metal downward into the can there was a narrow opening in the side large enough for the birds to feed easily, but the combination of narrow opening and flap seemed to prevent much food and bait waste. Hooks made from wire were soldered onto the cans and served as means of attachment. The bait material to be discussed in this section was also placed in this type of can and could be changed easily. Figure 1 shows the layer type cages with feed and water containers attached.

The Florida Agricultural Supply Company, Jacksonville, Florida, manufacturers of "Fly Flakes", supplied formulations of 1.0% Dipterex, 1.0% diazinon, and 1.0% malathion on very small flakes of oyster shell. The usual "Fly Flake" material is oyster shell flakes treated so that they hold 1.0% malathion, but for these studies the manufacturer also treated flakes to hold 2.0% malathion as the company was interested in determining the effects of a 2.0% product. Information concerning the materials used in preparation of the flake products was not available.

In tests where oyster shell bait material was used, fifty grams of the material were weighed and introduced into the individual feeding cans suspended at an opening on the back of the cage. Each day the old material remaining in the can was removed and weighed so as to get an

Figure 1. Layer Type Cages with Feed and Water Containers.

accurate record of the amount consumed during that period. New bait in fifty gram lots was placed in the cans daily. The hens used as checks received untreated oyster shell supplied by the Clemson Poultry Department in the same manner as those receiving treated material.

The tests using oyster shell baits were carried on over a period of six weeks. Before the experiment was begun, weights of the birds were recorded so that any weight change could be noted at the end of the testing period. A daily individual record was kept of eggs laid, and any abnormal actions of the chickens were noted.

The liquid insecticides used in the forced feeding tests were administered in #00, #0, and #1 gelatin capsules. Some difficulty was encountered in weighing the liquids as the capsules would not stand upright while being filled. After trying several methods, the writer found that a cork, bored partly through from the small end and with the small cork plug removed, made a very efficient holder for the capsules while they were being filled on the pan of the balances. Figure 2 shows the apparatus used in the balance pan to hold each capsule upright for filling.

To introduce the liquid insecticides into the capsules, a medicine dropper with the outlet reduced in size or a one milliliter tuberculin type hypodermic syringe was used. Some of the liquid materials could not be introduced in small enough droplets for accurate weighing. It was found that very small amounts of the excess material which dropped into the capsule could be removed by drawing the excess back up into the dropper or syringe.

Another difficulty occurred—that of the materials leaking from the capsules which were not in an upright position after being filled.

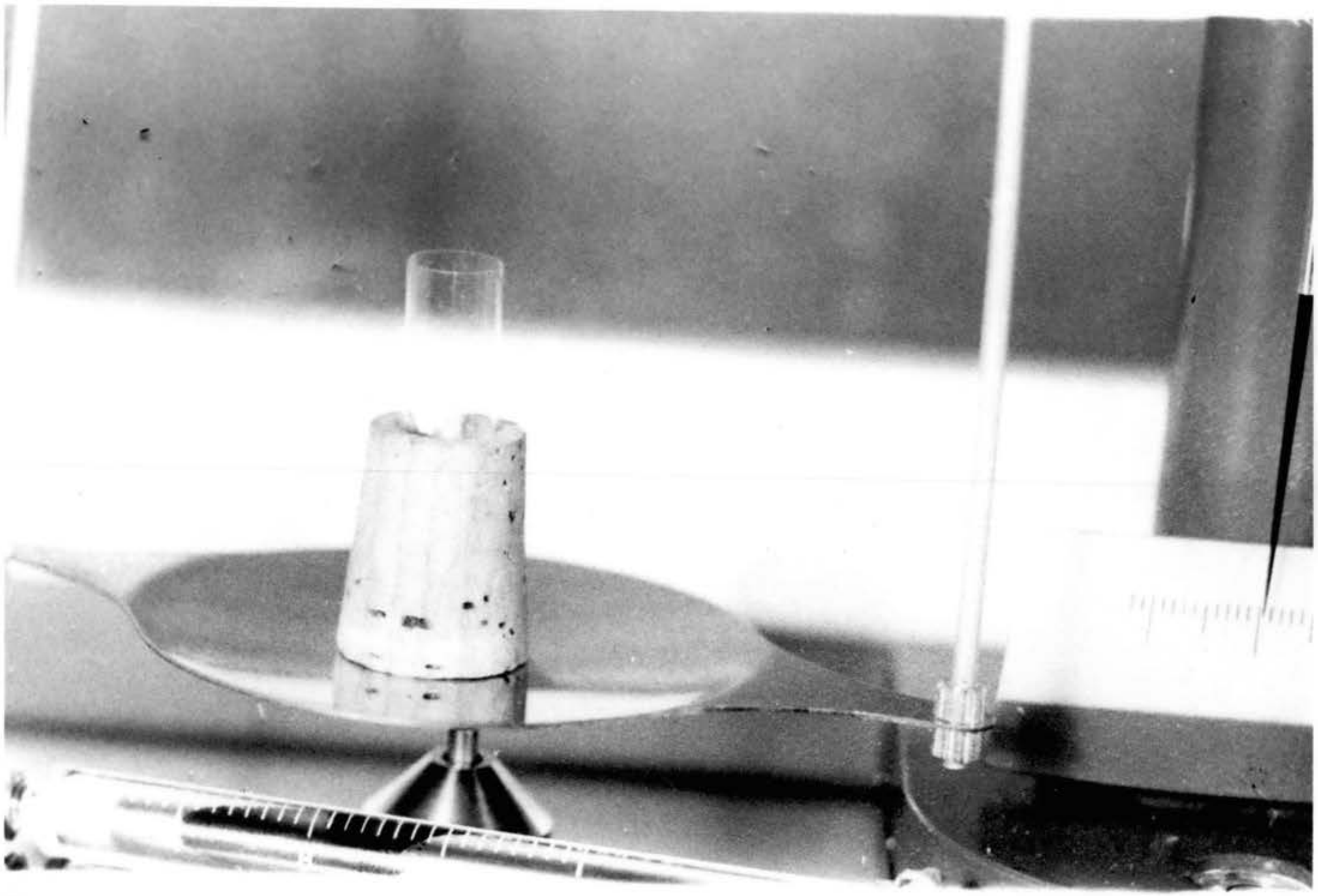

Figure 2. Apparatus Used to Hold Capsule Upright.

Wetting welded one portion of the capsule to the other, but this method was rather inefficient in trying to prevent loss of the insecticides. Using a box top with holes the size of the capsules punched in it relieved the trouble with leaking and made transportation of the capsules from place to place considerably easier. Figure 3 shows the box top used for transporting filled capsules in an upright position.

The liquid materials were administered to the chickens in milligram amounts based on the weight in kilograms of the birds. Birds were picked at random but in equal numbers from the closed Walker cages and the open laying cages. Most of the birds were tagged by the Poultry Department with leg bands which served as identification in the tests. Untagged birds were identified by cage number.

The materials used in the forced feeding tests were administered by holding the chicken with the mouth open and forcing a capsule some distance down the throat. If the capsule was not placed far enough down the throat, the hen could expel the material with little difficulty.

Malathion was given in doses of 100, 200, and 400 milligrams per kilogram of body weight, and four birds were used for each level of concentration.

Parathion was given at the rates of 2.5, 5, and 10 milligrams per kilogram of body weight, using four birds for each level of concentration.

Dipterex was administered at 25, 50, and 100 milligrams per kilogram of body weight. Of the ten birds tested at the rate of 100 milligrams per kilogram, four were given the material at one time while the other six received it at a later date. Four birds received 50 milligrams per kilogram of body weight, and four others received 25 milligrams per kilogram.

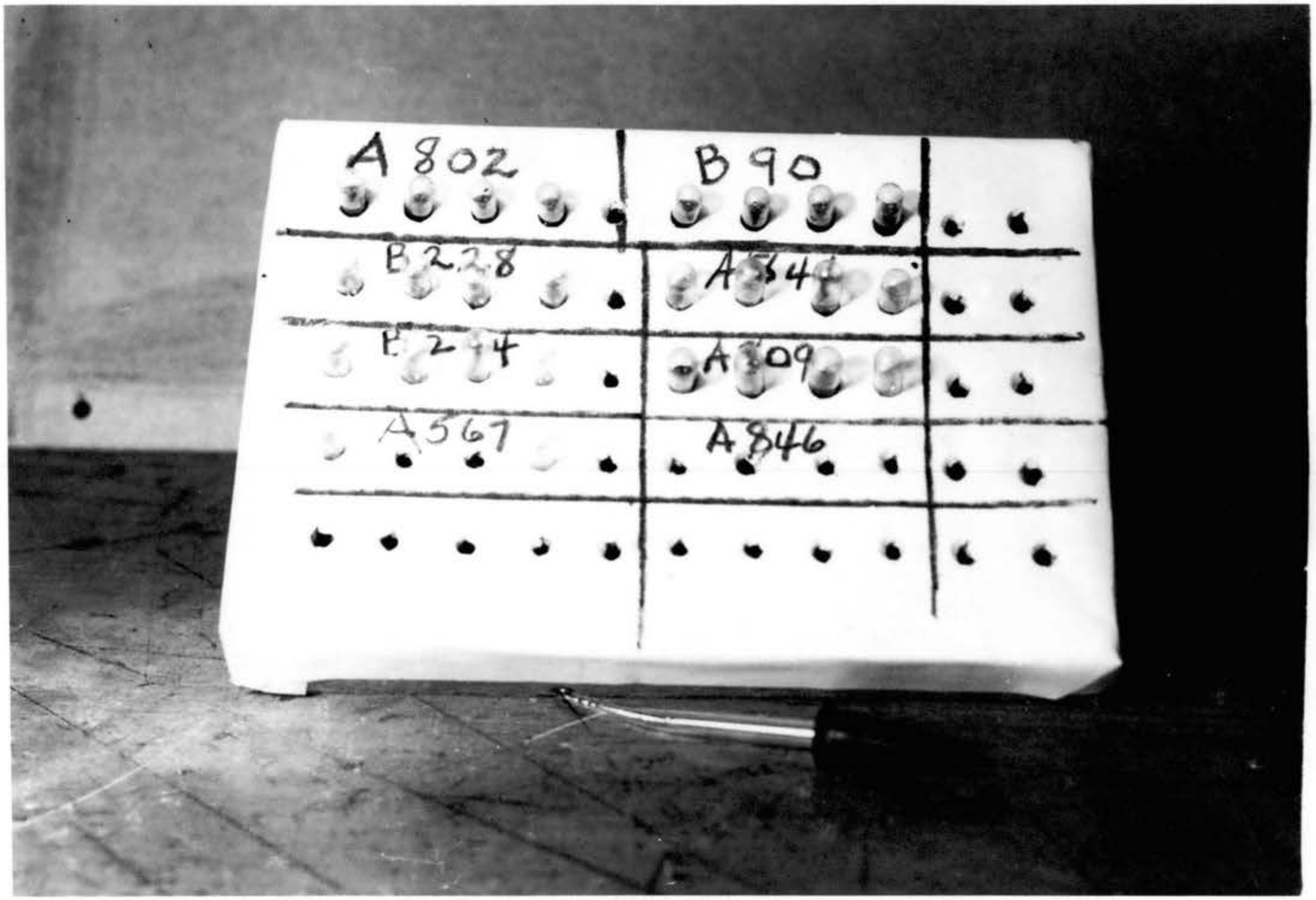

Figure 3. Box Top Used to Hold Filled Capsules.

Diazinon at 20 milligrams per kilogram of body weight was forced upon ten birds. The material was given to four birds at the rate of 5 milligrams per kilogram and to four other birds at the rate of 10 milligrams per kilogram.

The levels of treatment used in the administration of chlorthion were 100, 200, 400, 800, and 1000 milligrams per kilogram of body weight. Four birds were treated at the rate of 100 milligrams per kilogram, four at the rate of 200 milligrams per kilogram, and four at the rate of 400 milligrams per kilogram. Six birds were given 800 milligrams per kilogram, and six birds were given 1000 milligrams per kilogram.

The levels of materials to be used were arbitrarily determined by reviewing such toxicity data as could be gathered from previous tests of this nature. Data supplied by the Hazelton Laboratories (Alexander 1955), the United States Department of Agriculture Livestock Laboratory (U.S.D.A. 1955), Brelsford (1955), and Diechman (1954) were used as criteria upon which the levels were established. The writer attempted to establish dosage levels both below and above those reported in the data to compensate for differences in technique, experimental conditions, and certain expected biological variations. There were at least ten untreated birds kept during all the writer's tests to serve as checks.

Blood samples for cholinesterase determinations were taken before the tests began and at intervals throughout the period of the investigation. The blood samples were taken in small quantities so that a capillary tube was of sufficient size to accommodate the material. These tubes had been treated previously with heparin to prevent clotting.

Michel's method (Hamblin 1953) for determining cholinesterase activity in red blood cells and plasma was used in these analyses.

At the end of the test period all birds were sacrificed, and gross autopsies were performed with the aid of Dr. G. W. Anderson, a veterinarian with the Zoology and Entomology Department of the Clemson Agricultural College who has worked extensively in poultry pathology, and Dr. W. J. Goodwin, Associate Entomologist, South Carolina Experiment Station, Clemson, South Carolina.

DISCUSSION OF TESTS CONDUCTED

One series of tests conducted was with the use of oyster shell treated so as to contain 1% malathion, 2% malathion, 1% diazinon, and 1% Dipterex. Untreated oyster shell was used as a check material.

The test material in the amount of 50 grams was placed in specially prepared individual feed containers attached to the back of the layer type cages. The treated and untreated shell was removed daily and weighed, thereby determining the amount consumed. This figure was reasonably accurate as there was very little evidence of waste. The test was carried on for a period of six weeks, and at all times the chickens were fed a balanced laying ration. Weights of the birds were recorded before the tests began and at the close of the six weeks' period. Table 1 shows amount of material consumed during the test period, number of eggs laid, and weight change. Table 2 shows a resume of the findings at autopsy.

Table 3 shows the analysis of variance of feeding oyster shell baits to chickens. Based on the analysis of variance technique there was a highly significant difference in the average rate of consumption between the five feeding treatments. An inspection of the average rate of consumption in conjunction with the difference required for significance revealed that only the check group differed significantly from the treated groups of birds. A difference of about 68 grams was needed for significance, and only the check group differed by that amount. The

Table 3 shows the analysis of variance of feeding oyster shell baits to chickens. Based on the analysis of variance technique, there was a highly significant difference in the average rate of consumption between the five feeding treatments. An inspection of the average rate of consumption in conjunction with the difference required for significance revealed that only the check group differed significantly from the treated groups of birds. A difference of about 68 grams was needed for significance, and only the check group differed by that amount. The large amount of variation indicated that a considerable increase in replication would be necessary in order to detect a significant difference between the treated oyster shell bait groups. Because of the limited number of observations of the variables involved in the tests, no attempt was made to correlate the shell consumption with the other variables.

Tables 4 through 7 show the cholinesterase activity of the birds receiving oyster shell bait. Considering the average delta pH per hour of the four groups of birds before treatment as .185 and the delta pH per hour of four untreated birds as .175, it can be observed from the tables that there was no significant variance in the average of the four groups of treated birds.

In the series of tests using forced feeding methods, technical chlorthion, diazinon, parathion, Dipterex, and malathion were administered in gelatinous capsules. Each chicken treated was given only one level of the insecticide, and very close observations were made of the effects of the poisons on each chicken. The following is a discussion of the external symptoms of poisoning for each material at the various levels of administration:

Chlorthion at the rate of 1000 milligrams per kilogram of body weight killed three of six birds in one dose. These chickens displayed, as symptoms of poisoning, drowsiness and frothing from the mouth. The three birds that survived the treatment displayed the symptoms mentioned but were normal within 24 hours. Chickens treated with chlorthion at the rates of 800, 400, and 200 milligrams per kilogram of body weight displayed none of the symptoms described, except for one bird that showed a drowsiness and some frothing after the sixth dose.

Diazinon, when administered at the rate of 20 milligrams per kilogram of body weight, killed six of ten birds within two hours. The symptoms of poisoning were the same as for chlorthion poisoning--drowsiness and frothing from the mouth. In addition, just before death the birds fluttered wildly. In the birds that survived, there was a marked recovery within 24 hours. At lower rates of ten and five milligrams of diazinon per kilogram of body weight, the symptoms of drowsiness and frothing were present. At the five milligram per kilogram rate, two of the four birds treated died after one dose. There was no apparent explanation for this since the other two birds treated at this level survived 20 doses, and none of the hens receiving ten milligrams per kilogram died.

Parathion at ten milligrams per kilogram of body weight was lethal to four of four birds in one dose. At five milligrams per kilogram, two of four chickens were killed with one dose; one bird survived two doses; and another survived until the tenth dose. At the 2.5 milligrams per kilogram level, two birds were killed with one dose, and the other two birds survived 20 doses. The symptoms of poisoning were severe tremors, frothing from mouth, inability to stand or control body, loud squawking, and wild fluttering just before death.

Dipterex was lethal to six of ten birds when administered at 100 milligrams per kilogram of body weight. At 50 milligrams per kilogram two of four birds were killed with one dose, and at 25 milligrams per kilogram the four birds treated survived 20 doses. The symptoms of poisoning were frothing from mouth and eyes, coughing, inability to remain upright or control body action, and wild fluttering just before death.

Malathion at 400 milligrams per kilogram of body weight killed four of four birds treated in one dose. At the rate of 200 milligrams per kilogram one bird survived until the eleventh dose, and the other three chickens survived 20 doses. Four of four hens received 20 doses at the 100 milligram per kilogram level and survived without apparent ill effects. The symptoms of poisoning noted were drowsiness, loss of body control, and wild fluttering just before death.

All untreated birds used as checks survived all the test periods.

Tables 8 through 15 show the results of plasma cholinesterase analysis of birds in forced feeding tests. Each of these tables shows a distinct decrease in cholinesterase activity after forced feeding treatment with five organic phosphorus insecticides. In nearly all cases there was a rapid recovery of cholinesterase activity within four days and complete recovery within 25 days after cessation of treatment.

The readings for all cholinesterase determinations in this paper are plasma analyses since in seventeen tests of the red cells of chickens for cholinesterase activity by Michel's method of analysis the delta pH per hour ranged from 0.00 to 0.03. With each series of plasma tests, a blank determination with the delta pH per hour ranging from 0.09 to 0.13 was used for the non-enzymatic correction factor.

Table 16 shows the results of tissue analysis of malathion treated birds by the American Cyanamid Company, Stamford, Connecticut. The table shows that there were appreciable amounts of malathion in the tissues of these chickens. It can be noted from Tables 15 and 16 that the results of cholinesterase analysis and tissue analysis of bird #B90 were compatible, i. e., the tissue of this bird showed in a composite analysis 94 p.p.m. of malathion and a delta pH reading of .045 after seven doses of malathion at 200 milligrams per kilogram of body weight.

Tissues of chickens treated with chlorthion and Dipterex were sent to the Chemagro Corporation, Pittsburgh, Pennsylvania, for analysis, but due to a reorganization within the company this material was not processed.

DISCUSSION OF TESTS CONDUCTED

One series of tests conducted was with the use of oyster shell treated so as to contain 1% malathion, 2% malathion, 1% diazinon, and 1% Dipterex. Untreated oyster shell was used as a check material.

The test material in the amount of 50 grams was placed in specially prepared individual feed containers attached to the back of the layer type cages. The treated and untreated shell was removed daily and weighed, thereby measuring the amount consumed. This figure was reasonably accurate as there was very little evidence of waste. The test was carried on for a period of six weeks, and at all times the chickens were fed a balanced laying ration. Weights of the birds were recorded before the tests began and at the close of the six weeks' period.

Table 1 shows amount of material consumed during the test period, number of eggs laid, and weight change. There was a decided preference for the untreated oyster shell over the shell that had been treated with the insecticides. No clear indication concerning weight change could be attributed to any material in this series of tests. There was some evidence that egg production increased; however, further tests using large numbers of chickens would be necessary to verify this finding.

Table 2 shows a resume of the findings at autopsy. None of the physical abnormalities found could be shown clearly to be the direct result of the birds having received treated oyster shell.

Table 1. The Results of Oyster Shell Bait Feeding Tests.

Bird Number	Treatment	Total Amount Consumed (grams)	Weight Change (oz.)	No. Eggs Laid
B69	1% diazinon	37.01	- 3½	7
Z472	1% diazinon	4.02	- 2	1
Z403	1% diazinon	31.89	- 3	27
Z340	1% diazinon	19.30	+ 5	17
A719	1% malathion	148.07	- 12	24
Z383	1% malathion	60.49	+ 7½	1
A418	1% malathion	39.52	0	17
A442	1% malathion	4.09	+ 4	1
A622	2% malathion	124.87	+ ½	23
B125	2% malathion	69.73	+ 1	14
B243	2% malathion	13.69	- 25	8
Z399	2% malathion	48.36	+ 5½	5

Table 1. (continued) The Results of Oyster Shell Bait Feeding Tests.

Bird Number	Treatment	Total Amount Consumed (grams)	Weight Change (oz.)	No. Eggs Laid
Z368	1% Dipterex	51.32	+ 8	13
A477	1% Dipterex	83.44	- 1	19
Z420	1% Dipterex	43.96	+ 9	11
A824	1% Dipterex	41.05	+ 8	9
Z393	* Check	102.21	+ 11	3
B7	Check	235.52	+ 3	16
A709	Check	131.61	+ $\frac{1}{2}$	3
B423	Check	193.01	+ 1	5

* Chickens used as checks received untreated oyster shell.

Table 2. Results of Autopsies on Birds Used in Oyster Shell Bait Feeding Tests.

Bird Number	Treatment	Results
B69	1% diazinon	Normal
Z472	1% diazinon	Normal
Z403	1% diazinon	Messy tail feathers; odor of insecticide
Z340	1% diazinon	Normal
A719	1% malathion	Normal
Z383	1% malathion	Liver slightly yellow
A418	1% malathion	Normal
A442	1% malathion	Normal
A622	2% malathion	Liver slightly yellow; odor of insecticide
B125	2% malathion	Yellow liver; odor of insecticide
B243	2% malathion	Breast muscle wasting away; spleen enlarged three times normal; odor of insecticide
Z399	2% malathion	Liver slightly yellow; odor of insecticide

Table 2. (continued) Results of Autopsies on Birds Used in Oyster Shell Bait Feeding Tests.

Bird Number	Treatment	Results
Z368	1% Dipterex	Liver slightly yellow
A477	1% Dipterex	Liver very yellow
Z420	1% Dipterex	Liver yellow
A824	1% Dipterex	Liver yellow
Z393	Check	Normal
B7	Check	Normal
A709	Check	Liver slightly yellow
B423	Check	Normal

Table 3. Analysis of Variance of Feeding Oyster Shell Baits to Chickens.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio (F)
Treatment	46,269.1111	4	11,567.2777	5.71 *
Error	30,365.4580	15	2,024.3639	
Total	76,634.5691	19		

* Significant at the .01 level.

67.81 = Difference between average required for significance.

Table 4. Results of Plasma Cholinesterase Analysis Before and After Feeding Hens 1% Diazinon on Oyster Shell.

Number of Chicken	Total Intake (grams)	Before	After
		△ pH/hr.	
Z472	4.02	.230	.210
Z340	19.30	.233	.228
Z403	31.98	.190	.156
B69	37.01	.123	.090
Average	23.05	.194	.171

Table 5. Results of Plasma Cholinesterase Analysis Before and After Feeding Hens 1% Malathion on Oyster Shell.

Number of Chicken	Total Intake (grams)	Before	After
		Δ pH/hr.	
A418	39.52	.200	.207
Z383	60.49	.200	.200
A442	4.09	.220	.200
A719	148.07	.140	.135
Average	63.04	.190	.186

Table 6. Results of Plasma Cholinesterase Analysis Before and After Feeding Hens 2% Malathion on Oyster Shell.

Number of Chicken	Total Intake (grams)	Before	After
		Δ pH/hr.	
B243	13.69	.160	.140
Z399	48.36	.255	.247
B125	69.73	.135	.110
A622	124.87	.150	.168
Average	64.16	.175	.166

Table 7. Results of Plasma Cholinesterase Analysis Before and After Feeding Hens 1% Dipterex on Oyster Shell.

Number of Chicken	Total Intake (grams)	Before	After
		Δ pH/hr.	
Z368	51.32	.195	.190
Z420	43.96	.185	.175
A824	41.05	.177	.180
A477	83.44	.180	.140
Average	54.94	.184	.171

Table 8. Results of Plasma Cholinesterase Analysis of Birds Fed Chlorthion at the Rate of 200 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses 2	Number of Doses 5	Number of Doses 10	After Being Removed from Test*
	△ pH/hr.			
Z316	.150	.135	.100	
Z418	.145	.140	.105	
Z575	.120	.100		
A751	.165	.135	.110	
Average	.145	.127	.101	

* None of the birds survived the twenty doses set as a stopping point for the test. Four check birds showed an average cholinesterase value of .175.

Table 9. Results of Plasma Cholinesterase Analysis of Birds Fed Chlorthion at the Rate of 400 Milligrams Per Kilogram of Body Weight.

Number of Chickens	Number of Doses: 2	Number of Doses: 5	After Being Removed from Test
	Δ pH/hr.		Δ pH/hr.
Z304	.130	.110	*
Z379	.100		**
B1384	.150	.140	*** .230
A297	.120	.110	****
Average	.125	.120	.230

* Bird died after 8th dose.

** Bird died after 5th dose.

*** This figure was derived 15 days after end of test.

**** Bird died after 9th dose.

Table 10. Results of Plasma Cholinesterase Analysis of Birds Fed Diazinon at the Rate of 10 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses		Before Treatment
	2	5	
	Δ pH/hr.		Δ pH/hr.
A95	.170	.115	.215
A406 *	.110	____	.175
Z535 **	.115	____	.190
B244 ***	.105	____	.165
Average	.125	.115	.186

* Bird did not survive second dose.

** Bird did not survive second dose.

*** Bird did not survive second dose.

Table 11. Results of Plasma Cholinesterase Analysis of Birds Fed Diazinon at the Rate of 20 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses		Before Treatment
	2	5	
	Δ pH/hr.		Δ pH/hr.
B1353	.150	.100	.175
#2	.135	.110	.230
Z448 *	.135	___	.195
B258 **	.130	___	.155
#5 ***	.100	___	.120
Average	.130	.105	.193

* This bird did not survive second dose.

** This bird did not survive second dose.

*** This bird did not survive second dose.

Table 12. Results of Plasma Cholinesterase Analysis of Birds Fed Parathion at the Rate of 2.5 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses 7	Number of Doses 11	After Being Removed from Test
	Δ pH/hr.		Δ pH/hr.
B181	.105	.070	.165 (4 days) *
Z347	.110	.110	.196 " " **
Average	.108	.090	.180

* 25 days after completion of test the cholinesterase value was .230.

** 25 days after completion of test the cholinesterase value was .230.

Table 13. Results of Plasma Cholinesterase Analysis of Birds Fed Dipterex at the Rate of 100 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses 2 (Δ pH/hr.)	Number of Doses 5 * (Δ pH/hr.)	Before Treatment (Δ pH/hr.)
B1371	.135	—	.210
#16	.110	—	.205
B1051	.130	—	.170
B24	.105	—	.195
B417	.135	—	.260
Average	.123	—	.208

* None of the birds survived until the fifth dose.

Table 14. Results of Plasma Cholinesterase Analysis of Birds Fed Malathion at the Rate of 100 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses: 7	Number of Doses: 13	After Being Removed from Test
	Δ pH/hr.		Δ pH/hr.
B244	___*	.136	.205 (4 days) **
A809	.110	.110	.180 " " ***
A846	.130	.100	.170 " "
A567	.090	.110	.130 " " ****
Average	.110	.114	.171

* Blood clotted.

** 27 days after end of treatment the cholinesterase value was .200.

*** 25 " " " " " " " " " .190.

**** 25 " " " " " " " " " .183.

Table 15. Results of Plasma Cholinesterase Analysis of Birds Fed Malathion at the Rate of 200 Milligrams Per Kilogram of Body Weight.

Number of Chicken	Number of Doses: 7	Number of Doses: 13	After Being Removed from Test
	Δ pH/hr.		Δ pH/hr.
B90	.045	___ *	___
B228	.075	.050	.135 (4 days) **
A802	.150	.100	.140 " " ***
A844	.125	.100	___ ****
Average	.098	.083	.137

* This bird died after eleventh dose.

** 25 days after end of test the cholinesterase level was .130.

*** 25 " " " " " " " " " .260.

**** This bird died after eighteenth dose.

Table 16. Results of Analysis of Chicken Tissue.

			Concentration of Malathion, PPM							
			Meat		Skin		Fat		Composite	
Bird No.	Dosage	Dressed Wt.	Wt. Gms.	PPM Malathion	Wt. Gms.	PPM Malathion	Wt. Gms.	PPM Malathion	Wt. Gms.	PPM Malathion
A822	400 mg/kg 1 dose	1130 g.	–	–	–	–	–	–	528	3.5
Z397	400 mg/kg 1 dose	1704 g.	514	6	175	31	150	18	839	55
A750	400 mg/kg 1 dose	1496 g.	320	1.6	97	7.5	–	–	–	–
B90	200 mg/kg 11 doses	1202 g.	–	–	–	–	–	–	545	94

SUMMARY

From the tests conducted using oyster shell flakes treated to hold 1% malathion, 2% malathion, 1% diazinon, and 1% Dipterex, it was learned that the treated flakes were considerably less palatable to the chickens than were untreated oyster shell flakes. Whereas the chickens having access to untreated shell consumed an average of 165.58 grams over a six weeks' period, those receiving 1% malathion flakes consumed 63.04 grams, those receiving 2% malathion flakes consumed 64.16 grams, those receiving 1% diazinon consumed 23.05 grams, and those receiving 1% Dipterex flakes consumed 54.94 grams. This indicated that although oyster shell is an important component in the diet of a chicken, when the shell has been treated the chickens refused to eat it in quantity. An analysis of variance showed that there was a significant difference between the amounts of treated and untreated material consumed. This finding would further indicate that oyster shell bait treated with 1% to 2% malathion or 1% Dipterex might be used safely around poultry plants for insect control.

There was no significant reduction in plasma cholinesterase activity of the chickens that consumed a considerable amount of the oyster shell bait material over a six weeks' period.

The autopsy findings for those birds fed on the bait material gave no pathological evidence of organic phosphorus poisoning. This was due perhaps to the fact that relatively small amounts were consumed at any given time. There were no deaths caused by insecticides used.

Organic phosphorus insecticides when administered in gelatin capsules produced more cholinesterase inhibition than was observed in the tests previously described. All materials were observed to have reduced the plasma cholinesterase level of chickens when administered in sublethal doses, but the chickens seemed to be able to reverse this inhibition rather rapidly when treatment was stopped. This conclusion is derived from data in Tables 8 through 15. These tables show that within four days after treatment was ended there had been a sharp recovery in plasma cholinesterase activity. Within twenty-five days the normal average was reached.

The symptoms of poisoning were generally the same for all the tested insecticides. Diazinon gave the same appearance of drowsiness in chickens as was observed by Radeleff and his fellow workers (1955) in calves. Chlorthion and malathion often produced these same symptoms.

Autopsies nearly always revealed fluid in the lungs of the birds used in the oral feeding studies. This would appear to confirm the findings of DuBois (1948) in work on cats, and of Hagan (1948) on guinea pigs.

When administered at the level of 1000 milligrams per kilogram, chlorthion caused a much contracted and irritated gastro-intestinal tract. The droppings from chickens treated with chlorthion were bloody, a condition which had not existed prior to treatment. Bailey found these same conditions existed when dogs were treated with chlorthion.[1] This condition in the chickens might be attributed to some intestinal

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[1]C. C. Bailey. Unpublished data, South Carolina Experiment Station, Clemson, S. C., 1956.

parasite except that it was observed only in treated birds and not in the checks.

Dipterex at the rate of 100 milligrams per kilogram of body weight was toxic in one dose to six of ten birds; at 50 milligrams per kilogram it was toxic in from one to three doses; and at 25 milligrams per kilogram it was found non-toxic in 20 doses.

Chlorthion was found non-toxic at rates up through 800 milligrams per kilogram of body weight. One dose of chlorthion at 1000 milligrams per kilogram killed three of six birds within twenty-four hours.

Diazinon was found toxic to six of ten hens when administered at the rate of 20 milligrams per kilogram of body weight. Below this amount there was considerable variation in the results.

Parathion was toxic to all hens when administered at ten milligrams per kilogram of body weight. In one group of four birds treated at the rate of five milligrams per kilogram, one bird survived ten doses while the others were killed by one or two doses. One dose of 2.5 milligrams per kilogram was lethal to two of four birds, but two birds survived twenty doses.

One dose of malathion was lethal to four of four birds at the rate of 400 milligrams per kilogram of body weight. At rates of 200 and 100 milligrams per kilogram, only one bird did not survive the full 20 doses. This single bird survived ten doses without showing any visible symptoms of poisoning.

From the results obtained in these studies, it would seem that parathion and diazinon, because of their extreme toxicity, should be excluded from use near chickens; but malathion, Dipterex, and chlorthion are less toxic in nature and might be used safely.

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