

The Effectiveness of Insecticides Administered Orally to the Fowl as a Deterrent to the Breeding of Flies in Droppings^{1,2}

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Filth-inhabiting flies are capable of transmitting at least 30 human diseases. Poultry droppings are of special significance as one of the major sources of fly breeding, particularly when birds are maintained in wire cages. At least 24 species of flies belonging to 14 families breed in chicken manure in Hawaii (Tanada, *et al*, 1950). *Musca domestica* (L.) is the predominant species, yet there are nine other economically important species breeding in large numbers. The treatment of poultry manure to control fly larvae is generally effective for a comparatively short time (Hoffman and Monroe, 1956, 1957; Wilson and Gahan, 1957). The major factors involved in the failure of such larvicidal sprays were discussed by Sampson (1956) and are mainly associated with the lack of penetration of the insecticide deep into the manure mass.

The use of animals as vehicles for the distribution of insecticides in the manures would offset some of the difficulties inherent in the conventional methods of application, especially that of penetration. Over seven years of intensive investigation in our laboratory showed that ingestion by poultry of certain organophosphorous insecticides will prevent fly breeding in the droppings. However, the inclusion of toxic substances in the diet of poultry is particularly hazardous from the public health standpoint. Relatively little information is available on this subject. In recent years the publication of Rachel Carson's *Silent Spring* has stimulated discussion and concern regarding the effect of the use of pesticides on wild life as well as on humans.

It is essential, therefore, to clarify the actual extent of the hazard. With this in mind, the specific aims of this research program in our laboratory are:

- (1) to determine the relative toxicity of droppings from hens on an insecticide-treated diet to the larvae of several species of flies;
- (2) to determine the acute, subacute, and chronic toxicity of insecticides to poultry;

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(3) to determine quantitatively the deposition of these insecticides in the tissues and eggs after ingestion by poultry; and

(4) to determine the metabolic fate of certain organophosphorus insecticides after ingestion by poultry.

The bioassay procedure has been developed and described (Sherman, *et al.*, 1962, etc.) to determine the relative toxicity to fly larvae of droppings from chickens on an insecticide-treated diet. Our work has shown that there is a differential susceptibility in fly species to manure from poultry treated with insecticides. Four species of flies representing four important families or subfamilies breeding in poultry manure in Hawaii are presently being utilized in this study: *Musca domestica* (L.), Muscidae, subfamily Muscinae; *Fannia pusio* (Wiedemann), Muscidae, subfamily Anthomyiinae; *Chrysomya megacephala* (F.), Calliphoridae; and *Parasarcophaga argyrostoma* (Robineau-Desvoidy), Sarcophagidae. Additional species of medically important Diptera also will be cultured and utilized. This study is being conducted concurrently with the same animals used in the subacute toxicity studies, the chronic toxicity studies, and the residue studies.

Over the years, standardized techniques have been developed in our laboratory to determine the acute, subacute, and chronic toxicity of insecticides to poultry. These techniques have proven both successful and reliable (Sherman and Ross, 1961b, etc.). Acute toxicity studies consist of giving single oral dosages, usually encapsulated in gelatin to one-week old chicks, the dose collated with the weight of the animal. Symptoms of intoxication are recorded and the median-lethal dosages and their confidence limits are calculated by probit analysis. Subacute toxicity studies are usually combined with the larvicidal bioassay in which insecticide-treated feed or water is administered over a one- or two-week period to the animals. Chronic toxicity studies consist of including insecticides in the diet of laying hens over a period of one year or longer. The insecticides chosen are those which have shown no toxicity to the chicks in the subacute toxicity studies and have caused the droppings from these chicks to be highly larvicidal. Studies are also conducted on the inhibitory effect of these insecticides on the cholinesterase activity of chicken blood plasma.

General procedures are available to detect residues of well-established insecticides in animal tissues spectrophotometrically or by use of gas chromatography. Many of the insecticides of interest to us have not had a definitive microanalytical method developed. Therefore, with some of the residue problems inherent in this project we have had to develop microanalytical methods. The development of electron capture gas chromatography has done much to solve this problem, especially with halogen-containing insecticides.

Collection of tissues for analysis is generally made as follows: the insecticides to be tested are mixed in a standard laying mash (or in the water supply) and administered to laying hens for a prolonged period (6 to 12 months). At the end of each experiment, while they are still on the treated diet, some hens are killed and tissues (liver, abdominal fat, and leg muscle) are quick frozen and stored until analyzed. In another group of hens, tissue samples are taken one, two and three weeks after they are taken off the insecticide-treated diet. Random

sampling of eggs is made during the treatment period and after removal of the hens from treatment. All animals are autopsied at time of death or at the end of the experiment for detection of pathological conditions, especially for tumorous growths and incidence of other aspects of avian leucosis complex.

During the next few years emphasis will be placed on studying the metabolism of organophosphorus insecticides to the fowl. A number of insecticides have been discovered which, when fed to chicks or hens, caused greater than 90 percent mortality to fly larvae in the manure. Isotopes of these insecticides, labeled with P^{32} , will be orally administered to mature hens. At various intervals after treatment, standard radioisotope tracer techniques will be used to determine the distribution pattern in various tissues and organs. Similarly, radioactivity will be determined in urine and feces. Because the fowl normally voids both feces and urine through a common opening, the cloaca, surgical methods will be necessary to effect a separation. We are experimenting with colostomy and exteriorization of the ureters to separate the urine from the feces.

This paper will review some of the entomological aspects of this research program. The toxicity of these insecticides to the fowl is discussed in detail elsewhere (Ross and Sherman, 1960; Sherman and Rosenberg, 1953, 1954; Sherman and Ross 1959, 1960,a, 1960,b, 1961,b; and Sherman, *et al*, 1963).

In our initial experiment (Sherman and Ross, 1959), the acute toxicity of Diazinon (0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dipterex (0,0-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate), Dow ET-14 (0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate), Dow ET-15 (0-methyl 0-(2,4,5-trichlorophenyl) phosphoramidothioate), malathion, and phenothiazine to one-week-old chicks and the toxic effect of the droppings from these treated chicks on one-day-old larvae of the house fly, *Musca domestica* (L.) were investigated. The degree of fecal toxicity to house-fly larvae was directly related to the dosage of insecticide ingested by the chick and inversely related to the period of time after ingestion that the feces were voided. Feces collected daily from chicks fed non-lethal levels of insecticides caused greater than 90 percent mortality in house fly larvae for varying periods of time, as follows: malathion, 1 day; Dipterex, 2 days; Dow ET-14, 3 days; and Dow ET-15, 6 days. Feces from Diazinon- and phenothiazine-treated chicks caused relatively low mortality in the larvae.

The toxicity to house fly larvae of feces from chickens on a continuous dietary regimen of laying ration containing Co-Ral (0-(3-chloro-4-methylumbelliferone) 0, 0-diethyl phosphorothioate), Diazinon, Dipterex, malathion, phenothiazine, ronnel and Dow ET-15 was next investigated (Sherman and Ross, 1960,a). The insecticide levels in the feed that caused greater than 90 percent mortality in larvae placed in the manure were: Co-Ral, 89 p.p.m.; Diazinon, 154 p.p.m.; Dipterex, 89-132 p.p.m.; ronnel, 176-220 p.p.m.; and Dow ET-15, 89 p.p.m. Feces from chickens on malathion- and phenothiazine-treated rations caused relatively low mortality in the larvae at concentrations as great as 1,102 p.p.m. and 11,023 p.p.m., respectively.

The effect of continuous administration via the water supply to chicks of Dipterex on the toxicity of the manure to house fly larvae was also investigated

(Sherman and Ross, 1960,b). The hydrogen ion concentration of the water used in the Dipterex solutions influenced the toxicity of the manure. Water solutions at pH 8.0 resulted in a rapid decrease in toxicity to larvae. However, Dipterex at 30 p.p.m. in distilled or acidified water at pH levels below 7.0 caused high levels of larval mortality in the droppings. At this concentration no adverse effect on weight gain, water consumption or feed conversion was noted in the chicks. It appears that the chick is able to metabolize Dipterex and excrete a highly larvicidal product but when conversion to DDVP (0,2, 2-dichlorovinyl 0,0-dimethyl phosphate) occurs under alkaline conditions, the metabolic pathway is different and results in products having low larvicidal activity.

The toxicity to house fly larvae of droppings from chicks administered the following 23 insecticides was determined (Sherman and Ross, 1961,a): American Cyanamid 12008 (0,0-diethyl S-isopropylthiomethyl phosphorodithioate), American Cyanamid 18706 (0,0-dimethyl S-(N-ethylcarbamoylmethyl) phosphorodithioate), Bayer 22408 (0,0-diethyl 0-naphthaloximidophosphorothionate), Bayer 25141 (0,0-diethyl 0-p-(methylsulfinyl) phenyl phosphorothioate), Bayer 29493 (0,0-dimethyl 0-(4-(methylthio)-m-tolyl) phosphorothioate), Co-Ral (0-(3-chloro-4-methylumbelliferone) 0,0-diethyl phosphorothioate), DDVP, Delnav (2,3-p-dioxanedithiol S.S-bis (0,0-dimethyl phosphorodithioate), dicapthon, dimethoate, Dowco 105 (0-methyl 0-(4-*tert*-butyl-2-chlorophenyl) ethylphosphoramidothioate), Dowco 109 (0-(4-*tert*-butyl-2-chlorophenyl) 0-methyl methylphosphoramidothioate), heptachlor, Isolan (dimethyl 5-(1-isopropyl-3-methylpyrazolyl) carbamate), Kepone (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta(cd) pentalen-2-one), Perthane (1,1-dichloro-2,2-bis(p-ethylphenyl)ethane), Phosdrin (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), phosphamidon, Pyrolan (dimethyl 5-(3-methyl-1-phenylpyrazolyl) carbamate), Ruelene (0-4-*tert*-butyl-2-chlorophenyl 0-methyl methylphosphoramidate), Sevin (1-naphthyl N-methylcarbamate), Strobane (a mixture of chlorinated terpenes with about 66 percent Cl (chlorinated α -pinene isomers) and *Bacillus thuringiensis* Berliner. Feces collected daily from chicks given single oral dosages of insecticides caused greater than 90 percent mortality in house fly larvae for varying periods of time as follows: Bayer 22408, 6 days; dicapthon, 5 days; dimethoate, 4 days; Dowco 105, 2 days; *Bacillus thuringiensis*, 1 day; Co-Ral, 1 day; Dowco 109, 1 day; and Perthane, 1 day. Single oral dosages of American Cyanamid 12008, American Cyanamid 18706, Bayer 25141, Bayer 29493, DDVP, Delnav, heptachlor, Isolan, Kepone, Phosdrin, phosphamidon, Pyrolan, Ruelene, Sevin, and Strobane caused little or no mortality in house fly larvae placed, within 24 hours, in droppings from treated chicks. The insecticide levels in the feed that caused greater than 80 percent mortality in larvae placed in the manure were: American Cyanamid 12008, 110 p.p.m.; American Cyanamid 18706, 220 p.p.m.; Bayer 22408, 22 p.p.m.; Bayer 25141, 80 p.p.m.; Bayer 29493, 220 p.p.m.; dicapthon, 440 p.p.m.; dimethoate, 44 p.p.m.; and phosphamidon, 440 p.p.m. Somewhat less toxic to the larvae were: DDVP, Delnav, Kepone, and Pyrolan. Of little or no toxicity to the larvae at the highest concentration level tested were: Dowco 105, Dowco 109, Isolan, Perthane, Ruelene, Sevin, and *Bacillus thuringiensis*. Dimethoate and phospho-

midon administered to chicks in the drinking water at 22 and 220 p.p.m., respectively, resulted in greater than 90 percent mortality in larvae placed in the droppings. Chicks given solutions of 220 p.p.m. Isolan produced droppings which were non-toxic to house fly larvae.

In another experiment with these same compounds the comparative susceptibility of larvae of *Musca domestica*, *Fannia pusio*, *Chrysomya megacephala*, and *Parasarcophaga argyrostoma* to droppings from poultry given feed containing these insecticides was determined (Sherman, *et al*, 1962). The two muscid species were in most instances more tolerant of the insecticide-containing manure than either the sarcophagid or calliphorid. Several organic phosphate insecticides (American Cyanamid 12008, Bayer 22408, and dimethoate) were highly toxic to the larvae of all four species at levels in the feed ranging between 40 and 80 p.p.m. The carbamate insecticides included in the feed had no apparent effect on larval mortality while the two chlorinated hydrocarbon insecticides, Kepone and Perthane, exhibited but slight toxicity. The bacillus appeared to have limited usefulness for general fly control when administered in the feed to chickens.

The effects of administering technical and emulsifiable dimethoate (0,0-dimethyl S-methylcarbamoylmethyl phosphorodithioate) at a concentration of 30 p.p.m. in the drinking water of laying hens were studied over a 59-week period (Sherman, *et al*, 1963). The estimated mean daily intake of the technical and emulsifiable dimethoate was 0.010 gm. and 0.0092 gm., respectively. These treatments resulted in excellent larval control of *Musca domestica*, *Fannia pusio*, *Chrysomya megacephala*, and *Parasarcophaga argyrostoma* in the droppings from these hens. No hen mortality attributable to the treatment occurred. Although decreased feed consumption occurred during the first two weeks of the experiment, by the fifth week normal feed consumption and egg production levels were reached. There were no detrimental effects on the economic qualities of the eggs nor were there any off-flavors, -odors, -texture, or -color attributable to the treatment. Blood-plasma cholinesterase activity was inhibited by the dimethoate treatment to a maximum of approximately 40 percent within two weeks. Recovery of enzyme activity was complete one week after the birds were removed from the dimethoate treatment.

It was necessary to develop a method for the analysis of dimethoate in the tissues and eggs of these hens. Attempts to determine dimethoate residues by enzymatic methods were unsuccessful. A successful procedure was finally developed (Sherman and Chang, in press). Animals were killed while on treatment, and 7, 14, and 35 days after removal from treatment. Eggs were also sampled from birds on treatment and after removal from treatment. Fifty-gram samples of liver, egg white, and yolk were extracted with solvent from a sodium sulfate column while muscle and fat were directly extracted by solvent in a blender. The insecticide was extracted from the stripping solvent into 47-49 percent HBr. Upon boiling the acid solution, the thiono sulfur is converted to H₂S and collected in zinc acetate solution. The zinc sulfide formed is reacted with p-aminodimethylaniline reagent to produce methylene blue which is determined spectrophotometrically at 670 mu. Approximately 200 samples of tissues and

eggs were analyzed. In all instances apparent dimethoate found in either tissues or eggs was less than 0.10 p.p.m. The highest apparent residue detected was found in liver; however, since a normal constituent of liver apparently reacts to form methylene blue, this amount can be considered insignificant.

These studies have both practical and fundamental implications. This research holds promise of aiding in the development of a unique, inexpensive, convenient, and effective method of fly control with universal application. Conventional methods of fly control are expensive, time-consuming, and often ineffective. As a result, this aspect of farm sanitation is frequently neglected or even omitted by poultrymen. The inclusion of larvicidal chemicals in the feed or water would make fly control an integral part of an essential operation.

In addition, these studies will add to our basic knowledge of the toxicity of insecticidally active chemicals to birds and will help to clarify the metabolic pathways of these materials. The distribution of insecticides and their metabolites in the various tissues and excretory products in relation to time will help clarify the mechanism of detoxification.

Poultry products constitute an important segment of the American diet. Therefore, the intentional or accidental contamination of poultry feedstuffs with substances known to be toxic is potentially hazardous to man, and the necessity for determining insecticide residues is obvious. The residue analyses will determine those materials which would be safe to use in feeds for fly control. In addition, the knowledge of the relative persistency of these residues in tissues would be useful in case of accidental contamination. It would be possible to estimate the period of time after such contamination during which the poultry should not be marketed.

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