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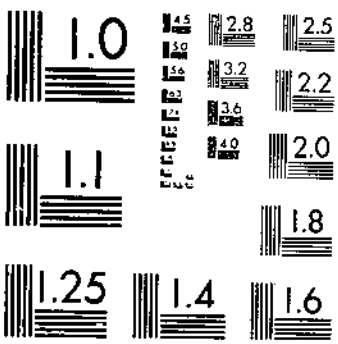
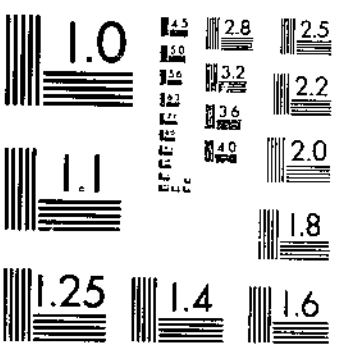


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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BIOASSAY OF SOIL Containing Residues of Chlorinated Hydrocarbon Insecticides

WITH SPECIAL REFERENCE TO
CONTROL OF
JAPANESE BEETLE GRUBS

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Bioassay of Soil Containing Residues of Chlorinated Hydrocarbon Insecticides, With Special Reference to Control of Japanese Beetle Grubs

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Studies were conducted at Moorestown, N.J., from 1956 through 1960 to develop a practical bioassay method for determining effective quantities of the chlorinated hydrocarbon insecticides, alone or in mixtures, which remain in the soil at intervals after treatment, for control of grubs of the Japanese beetle (*Popillia japonica* Newman).

The chlorinated hydrocarbon insecticides—DDT, toxaphene, chlordane, dieldrin, heptachlor, and aldrin—are authorized by the Plant Pest Control Division (*128*) [1] for use in eliminating these grubs in soil about the roots of plants in commercial nurseries subject to the restrictions of the quarantine on this pest. These restrictions are intended to prevent the spread of the beetle in agricultural commodities to uninfested localities. Treatments with the same materials are also recommended for control of the grubs in lawns, golf courses, parks, and other turf areas (*56*).

It is the practice of the Plant Pest Control Division to analyze the soil of certified plots periodically and to require the application of additional insecticide as needed to maintain adequate toxicity. The additions are held to a minimum to avoid the accumulation of excessive insecticide residues. Inordinate amounts of these chlorinated hydrocarbon insecticides in the soil may be toxic to some plants.

The analysis is complex if two or more of the chlorinated hydrocarbon insecticides have been applied to a soil in nursery plots, or if other insecticides have been applied to the foliage of plants. Much of the residue of insecticides applied to the foliage eventually gets into the soil. The problem is complicated further by the conversion of some of the chlorinated hydrocarbons in the soil to other compounds. Aldrin is converted to dieldrin and heptachlor to its epoxide (*27, 49, 65, 91, 93, 94, 95*); some of the dieldrin may be transformed to aldrin (*27*), and there may be some modification of the other chlorinated hydrocarbons.

The chemical determination of a chlorinated hydrocarbon insecticide in the soil includes sampling the soil, removing the insecticide res-

[1] Italic numbers in parentheses refer to Literature Cited, p. 38.

idue with a solvent, and analyzing the recovered material. Although specific colorimetric and spectrophotometric methods are available for determining most of these insecticides, Carter (*34*) pointed out that these methods are tedious and time consuming, and that homologous compounds, decomposition products, and plant extractives sometimes interfere with the analysis. Chisholm and Koblitsky (*36*) found that many soils contain extractable substances that cause interference. Usually with specific methods no information is obtained on the breakdown and conversion products that might be toxic to insects. The analytical procedure generally used is the determination of the organic chlorine in the extract and the calculation of the equivalent amount of insecticide with an appropriate conversion factor, as described by Agazzi et al. (*1*) and Koblitsky and Chisholm (*35*).

This nonspecific chemical procedure is a relatively quick screening method to establish the presence of chlorinated organic compounds in a soil. Only the organic chlorine is measured. Since the inorganic chlorides are not soluble in the organic solvents used for extraction, they cause a minimum of interference. The method does not distinguish between organic chlorine in an insecticide and that in a nontoxic compound nor between available and nonavailable insecticide in a soil. It does not identify the insecticide. It is inadequate when two or more chlorinated hydrocarbon insecticides differing greatly in chlorine content are present, when there has been decomposition or conversion of the insecticides, or when fertilizers containing organic chlorine have been applied to a soil. The organic chlorine in mixtures of compounds can be determined, but there is no valid factor for calculating the equivalent amounts of the individual insecticides.

Chemical analysis does not necessarily establish the presence of toxic substances in a soil; toxicity is revealed by its effect on a living organism. In the absence of suitable chemical methods, consideration was given to the development of a biological procedure for the evaluation of complex insecticide residues in soil. This situation was not unusual. Davis (*42*) pointed out in 1951 that the lack of satisfactory chemical methods for some of the more potent organic insecticides had stimulated wide interest in the application of bioassays to the residue problems.

Bioassay is a method for determining the amount of a toxic substance present by measuring its effect on a living organism. Bioassay or biological procedures are of interest because of the wide range of compounds to which they can be applied and in some instances their high sensitivity. Bioassays sometimes indicate the presence of toxic residues that have not been detected by chemical analysis (*34*). Various biological techniques have been used for many years in assays of insecticide formulations and residues. These assays evaluate toxicity but give little other information about the toxicant.

However, Laug (*88*) differentiated the gamma isomer from the other isomers of benzene hexachloride by their relative toxicities to the house fly (*Musca domestica* Linnaeus). Fleming et al. (*57*) distinguished residues of DDT from those of chlordane in the soil by the slower response of *Macrocentrus ancylivorus* Rohwer to DDT and noted differences in the symptoms of intoxication produced by these insecticides. Davidow and Sabatino (*41*) differentiated chlordane

and related cyclodienes from other chlorinated hydrocarbons by differences in the response of goldfish, *Carassius auratus* Linnaeus. Harwood and Arceknl (*73*) used four unrelated test animals—the pomace fly *Drosophila melanogaster* Meigen, the rusty grain beetle (*Cryptolestes ferrugineus* (Stephens)), the mushroom mite (*Tyrophagus putrescentiae* (Schrank)), and the brine shrimp *Artemia salina* (Linnaeus)—and obtained mortality patterns that distinguished DDT, aldrin, dieldrin, heptachlor, parathion, Sevin, and allethrin.

The basic principles of quantitative determination used in analytical chemistry apply to bioassay. Gunther and Blinn (*69*), Healy (*74*), and Van Middelem (*129*) reviewed these principles. Hoskins (*76*) discussed the use of bioassay in entomological research.

Bioassays of soil containing the residue of only one insecticide have been made by extracting the residue in the same manner as for chemical analysis and exposing a test organism to the extract, by exposing a test organism directly to the soil, or by introducing a test organism into the soil. The response of the test organism to the extract or to the soil was compared with its response to a standard with known amounts of the insecticide under standardized conditions, and by interpolation of the response data the amount of toxicant present was calculated. Bioassays of soil extracts have been made by Banks and Reed (*5*), Berger and Randolph (*13*), Fleming et al. (*57*), Gannon and Bigger (*65*), Kiigemagi et al. (*81*), Lange and Carlson (*87*), Lichtenstein et al. (*91*), and Terriere and Ingalsbe (*127*). Direct bioassays of an insecticide residue in soil have been made by Edwards et al. (*50*), Fleming et al. (*62*), Fleming and Maines (*60, 61*), Kloke (*83*), Lichtenstein and Polivka (*93*), Lichtenstein and Schulz (*94, 95*), Lichtenstein et al. (*91*), Wylie (*135*), and Young and Rawlins (*136*).

A bioassay of a soil extract is an evaluation of the total potential toxicity in the soil, because the solvent extracts both the free and the adsorbed insecticide. On the other hand, the direct bioassay measures only the active toxicity, the adsorbed part of the residue apparently is not available as an insecticide. The direct exposure methods have certain advantages over the extraction methods in that they are relatively simple and the potential danger of poor extraction or the elimination of the insecticide in the cleanup procedures is not encountered. Lichtenstein (*90*) discussed the advantages and disadvantages of direct bioassay of soil.

The active toxicity, not the total potential toxicity, in a soil is the criterion as to whether sufficient insecticide is present to kill Japanese beetle grubs. From the viewpoint of regulatory agencies, it is more important to have information on the active toxicity than to know the identity and the amount of various insecticide residues in a soil. Fleming et al. (*57*) developed a method for measuring the total toxicity of mixtures of DDT and chlordane, but as far as is known no consideration has been given to evaluating the active toxicity of complex mixtures of the chlorinated hydrocarbon insecticides. Under these circumstances an investigation was undertaken to develop a practical biological method for assaying the active toxicity of these mixtures in soil so as to determine whether the toxicity was sufficient to prevent the development of newly hatched Japanese beetle grubs.

BIOASSAY OF SOIL WITH JAPANESE BEETLE GRUB AS TEST INSECT

The first-instar Japanese beetle grub is the logical organism to use in evaluating the active toxicity in soil treated with the chlorinated hydrocarbon insecticides to control this stage of the insect, but its usefulness is limited. Grubs at this stage are usually available for only a few weeks during the summer; they have not been propagated successfully at other seasons. They must be handled with extreme care because of their susceptibility to mechanical injury and to adverse environmental factors, such as desiccation, excess moisture, or an increase in the carbon dioxide tension in the soil. The third-instar grub, which is present for 9 months of the year and is more readily handled and less affected by its environment, appeared to be a practical substitute for the newly hatched grub.

Third-instar grubs have been used for many years in biological screening tests of arsenicals, chlorinated hydrocarbons, and other compounds as soil insecticides. The grubs have also been used in direct bioassays of insecticide residues in soil (*57, 60, 61, 62*). These bioassays were rather simple in that only one insecticide was in a soil and the exposure could be adjusted according to the toxicity. When a soil contains residues of several chlorinated hydrocarbons that differ greatly in toxicity and in the velocity of insecticidal action, the biological evaluation is more complex. The problem was to develop an adequate standard for evaluating the toxicity of such mixtures and to correlate the mortality of third-instar grubs with that of newly hatched grubs.

The initial step in the development of a composite toxicity standard was to determine the exposure required at 80° F. to kill 50 percent of the third-instar grubs with a dosage of each of the chlorinated hydrocarbon insecticides that would prevent the development of newly hatched grubs. It had been determined in experiments over several years that newly hatched grubs could not survive in Sassafras sandy loam containing 5 pounds of DDT, 2.5 pounds of toxaphene, 0.4 pound of chlordane or endrin, 0.3 pound of dieldrin, or 0.07 pound of heptachlor or aldrin per 3-inch acre. Usually these dosages are about double the amounts actually needed to kill most of these grubs. When these insecticides were mixed at these rates with the sandy loam, the desired level of mortality with third-instar grubs was attained in 2 weeks with endrin, dieldrin, heptachlor, and aldrin, but almost 3 weeks were required for DDT, toxaphene, and chlordane. In view of these results, it was decided to use an exposure of 2 weeks and to increase accordingly the dosages of the less toxic insecticides.

The tests were continued to develop data for standard concentration-mortality curves, with four or five dosages for each insecticide and not less than 500 grubs for each dosage. When the mortalities were plotted against the dosages, typical asymmetrical sigmoid curves were obtained with all the chlorinated hydrocarbon insecticides. It is not easy, however, to draw freehand an adequate asymmetrical curve through a series of scattered points. Such a curve cannot be calculated as readily as a straight line. Gaddum (*64*) and O'Kane et al. (*113*) noted the possibility of a logarithmic and probability transformation of a sigmoid curve to a straight line, and Bliss (*14, 15*) devel-

oped this transformation. Further consideration of this log-probit transformation has been given by Bliss (*16*, *17*, *18*, *19*, *20*, *21*), Bliss and Marks (*23*), Bliss and Cattell (*24*), Finney (*52*, *53*), Morrison (*105*), Wadley (*130*, *131*), Wadley and Sullivan (*132*), and others. This conversion, which facilitates the determination of the dosage-mortality relationship, is now widely accepted by entomologists.

The mortalities were converted to probits and the concentrations to logarithms and the log-probit regression was calculated for each insecticide by the method of least squares. The fitted lines were then transformed back to the original units and plotted as concentration-mortality curves. The average pounds per 3-inch acre of each of the chlorinated hydrocarbons needed in Sassafras sandy loam for 10- to 90-percent mortality of third-instar grubs are given in table 1. As shown in table 6 (p. 24), a significant change in the mortality at the 50-percent level is produced by increasing or decreasing the number of pounds of each insecticide per 3-inch acre as follows: Aldrin or heptachlor 0.02, dieldrin 0.14, endrin 0.12, chlordane 0.43, toxaphene 1.27, and DDT 2.21.

TABLE 1.—*Amounts per 3-inch acre of chlorinated hydrocarbon insecticides required in Sassafras sandy loam for 10- to 90-percent mortality of third-instar Japanese beetle grubs with a 2-week exposure at 80° F.*

Mortality (percent)	DDT	Toxaphene	Chlordane	Endrin	Dieldrin	Heptachlor	Aldrin
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
10	1.8	2.4	0.11	0.08	0.02	0.03	0.02
20	3.0	3.4	.24	.13	.04	.04	.03
30	4.4	4.3	.41	.20	.08	.06	.04
40	6.0	5.3	.66	.28	.15	.08	.06
50	8.0	6.5	1.02	.40	.26	.10	.08
60	10.7	8.0	1.58	.54	.45	.12	.11
70	14.6	10.0	2.52	.76	.81	.16	.15
80	21.0	12.7	4.34	1.14	1.64	.23	.22
90	34.8	18.0	9.23	1.98	4.35	.37	.38

The dosages of aldrin, heptachlor, dieldrin, and endrin required to kill 50 percent of the third-instar grubs under these conditions are about the same as those needed to prevent the development of newly hatched grubs. The dosages for chlordane, toxaphene, and DDT are definitely higher than required for the elimination of first instars. It was apparent that the third-instar grubs are more resistant to chlordane, toxaphene, and DDT than would be expected from the reactions of the small grubs.

The standard deviation in the mortality of groups of grubs exposed to a soil is usually within 10 percent. When the mortality is more than 60 percent, the toxicity in a soil is adequate to prevent the development of newly hatched grubs, but when it is less than 40 percent, some insecticide should be applied to fortify the treatment. When the mortality is between 40 percent and 60 percent, additional tests may be required to establish definitely the level of the toxicity. For many

routine analyses it may be necessary to determine only if the toxicity in a soil is above or below that needed to kill 50 percent of the third-instar grubs. By comparing the mortality in a soil being tested with the mortality rates in table 1 and interpolating, the toxicity may be expressed as an equivalent amount of DDT, toxaphene, chlordane, endrin, dieldrin, heptachlor, or aldrin.

Although third-instar grubs have been used for several years in evaluating the toxicity of insecticide residues in soil, the procedure was considered to be only provisional until a more practical one could be developed. The grubs have not been propagated satisfactorily in the laboratory. Field-collected grubs vary from place to place and from season to season in their resistance to insecticides, so that adjustments have to be made in the exposure period. Although third-instar grubs are available from September until June, they are satisfactory as test insects only during the fall and winter; in the spring their reaction to insecticides is confounded by pupation.

The evaluation of toxicity in the spring is more important than at other periods of the year, because it shows, just prior to the hatching of the new annual brood, whether there is sufficient insecticide residue to kill the first-instar grubs. The procedure is slow and laborious; at least a month elapses before the initial and the confirmatory tests are completed.

In view of these limitations with the grubs, an attempt was made to find a suitably susceptible organism for use as a test animal that could be in continuous laboratory production and that would both accelerate and facilitate assay procedure.

SEARCH FOR SUBSTITUTE TEST ORGANISM

A search was made of the literature to determine what organisms had been used by other investigators in laboratory assays of the toxicity of insecticides and insecticide residues. Busvine and Barnes (*32*) critically reviewed the techniques that had been devised for laboratory tests of insecticides and acaricides, and Dewey (*46*) and Nagasawa (*108*) reviewed bioassay techniques for the determination of pesticide residues. These reviews were of great assistance.

They showed that many organisms have been used for the detection and quantitative determination of toxic materials. These include bacteria, fungi, yeasts, daphnids, shrimps, several species of fish, collembola, symphilids, the larvae of several species of mosquitoes, a parasite of the oriental fruit moth, several species of insects that attack stored products, the house fly, the pomace fly, termites, wireworms, and several species of white grubs. Many of these organisms are easily handled under laboratory conditions.

The pomace fly *Drosophila melanogaster* was selected as apparently the most suitable substitute test organism. Although this insect is probably best known as a well-adapted organism for studies in theoretical biology, it has been used by several investigators to evaluate insecticides and insecticide residues.

Drosophila was used to study the toxicity of various insecticides (*9*, *12*, *23*, *28*, *29*, *30*, *31*, *39*, *47*, *48*, *80*, *84*, *97*, *98*, *99*, *100*, *101*, *103*, *104*, *105*, *106*, *118*, *120*, *124*), to identify insecticides and acaricides in comparative bioassays (*73*), to demonstrate that the toxicity of DDT

sprays varies inversely with the size of the droplets (*3*), to study the translocation of BHC in plants (*51*), and to determine the effect of captan, dichlone, and thiram on the toxicity of dieldrin (*4*). It was also used to determine residues of the chlorinated hydrocarbon insecticides in or on fruits and vegetables [2] [3] (*49, 68, 71, 114, 117, 125*) and residues of parathion on cauliflower (*78*), of dieldrin in applesauce (*54*), of aldrin, dieldrin, and heptachlor on alfalfa (*86, 92*), of SD–4402 on tobacco (*70*), and of aldrin, dieldrin, heptachlor, BHC, DDT, chlordane, and methoxychlor in soil (*50, 91, 93, 94, 95, 135, 136*).

For the direct bioassay of residues of the chlorinated hydrocarbon insecticides in soil, *Drosophila* has the following advantages: (1) It is susceptible to these insecticides, and slight changes in the concentration are reflected by modifications in the mortality; (2) it reacts to small insecticide residues in the soil; (3) an assay can usually be completed in about 24 hours at any time of the year; (4) it can be reared with reasonable facility throughout the year; (5) continuous propagation does not appear to modify its susceptibility to insecticides; (6) it can be handled in large numbers without difficulty; and (7) its natural mortality during a bioassay is negligible.

PROPAGATION OF DROSOPHILA

Stock

Since a stabilized population was desired for test insects, a culture of the pomace fly *Drosophila melanogaster* was obtained in 1953 from the laboratory stock of the Department of Entomology of Rutgers University. This strain has been propagated continuously at Moorestown, N.J., since that time.

Wild *Drosophila* may be found in abundance on various kinds of overripe fruits and vegetables. The adult flies and the larvae feed on the fermenting juices, yeasts, and other micro-organisms. Wild flies can be collected readily in the field during the summer, but such populations are heterogeneous and heterozygous and are likely to be variable in their reactions to insecticides.

Bartlett (*10*) found great differences in the susceptibility of laboratory and field-collected strains to insecticides. Merrell and Underhill (*102*) observed the most marked changes in resistance to insecticides with flies that had not been inbred. Laboratory strains of flies tend to become somewhat inbred and to lose their generic variability, so that they are relatively stable in their reaction to insecticides (*82, 102*).

Culture Medium

Many different media have been used in propagating *Drosophila*, including fermenting fruit, canned pumpkin, banana-agar, and several

[2] DEWEY, J. E. HIGHLIGHTS OF THE BIOASSAY PROGRAM FOR RESIDUE DETERMINATION. Paper presented at N.Y. State Insecticide and Fungicide Conf. 14. 1952. [Unpublished.]

[3] DEWEY, J. E. A BIOASSAY METHOD USING DROSOPHILA AND SOME PRELIMINARY RESULTS FROM NEW YORK CROP ADSORPTION STUDIES. Paper presented at 48th Ann. Conv. Natl. Canners Assoc., Feb. 1955. [Unpublished.]

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media in which a sugar and either cornmeal, oatmeal, agar, or potatoes were constituents. After various factors had been studied and preliminary tests had been made, a medium prepared according to the following formula was adopted:

Agar	22.8 grams
Corn sirup	300.0 ml.
Yellow cornmeal	190.0 grams
Sodium propionate, 20-percent solution	45.0 ml.
Water	1.5 liters

This medium satisfied the following requirements, which are necessary for a large-scale production of the flies for bioassay: (1) It must be a complete diet for rearing the flies to optimum size and uniform vigor; (2) it must be an adequate diet for the larvae, the brood flies, and the emerging flies, producing high yields per unit of medium with little variation in the ratio of the sexes; (3) it must be easily prepared from readily available materials; (4) it must contain sufficient moisture so that a culture can be carried for several weeks without drying out; (5) it must be of a consistency favorable for the developing larvae, but firm enough so that the container can be handled without loosening the medium; (6) it must be of such a composition that adult flies do not stick to it; and (7) it must be relatively resistant to harmful molds and bacteria.

To prepare this medium, bring 1 liter of water to a boil in a stainless-steel cooking pot and dissolve the agar in the boiling water. Add the corn sirup and again bring the mixture to a boil. Mix the cornmeal with 500 ml. of water in a separate container, add it to the boiling mixture, and continue cooking until the medium is fairly thick. Just before the cooking is completed, add the sodium propionate and stir it in thoroughly. Pour the hot medium into 500-ml. Erlenmeyer flasks and sterilize at a pressure of 20 pounds for 15 minutes.

Remove the flasks from the sterilizer, cap each with a square of sterilized linen made of tracing cloth, from which the sizing has been removed, and allow to cool at room temperature. After the flasks have stood for 24 hours, dissipate any condensate on their sides by directing a stream of air from an electric fan over them. However, there should be sufficient moisture on the surface of the medium to show a slight movement when the flask is tilted. If the moisture is insufficient, add about 1 ml. of sterile distilled water.

The quantities in this formula will be sufficient for 200 ml. in each of 10 flasks. The average depth of the medium in a flask is 1⅜ inches.

Rearing Procedure

One-half teaspoonful of dry baker's yeast is distributed evenly over the surface of the medium in each flask immediately before introducing the flies. Approximately 100 brood flies, up to 5 days of age and about equally divided as to sex, are put into each flask. The ratio of the sexes is determined periodically with at least 1,000 flies. The phototropic response of the flies is utilized in transferring them from a plastic randomizing cylinder to the rearing flasks. The 200 ml. of medium per flask is adequate to support the progeny of 100

flies without overcrowding. Kerr (*80*) noted that overcrowding delays development and tends to reduce the size of the adult flies.

The brood flies in the rearing flasks are incubated in a well-lighted room, where the temperature is maintained at 74° F. and the relative humidity at 40 percent. It is important to hold approximately this temperature to avoid a possible modification in the ratio of the sexes or a change in the resistance of the progeny to insecticides. Williams (*134*) found that males were more numerous than females at temperatures below 70° and that the females predominated at temperatures above 80°, but within the range of 70° to 80° the ratio of the sexes was about 1:1. Munson et al. (*107*) found that flies reared at 64° were more resistant to DDT than those reared at 86°.

The air in the flasks with the medium has a high humidity, probably close to saturation. Demerec (*43*) pointed out that oviposition is induced in a humid atmosphere; no eggs are deposited when the air is dry. However, excess moisture is undesirable, because flies maturing from larvae that develop in the condensate on the side of a flask are small and undesirable as test insects. When sufficient condensate develops on the side of a flask to permit larvae to crawl from the medium, the flask is placed in front of an electric fan until the excess moisture is dissipated.

The brood flies are removed from the rearing flasks and destroyed on the 11th day, and the flasks are transferred to the production unit.

Before the 500-ml. Erlenmeyer flask was adopted as the rearing container, various types and sizes of containers were tested, including 1-gallon widemouthed candy jars, canning jars, milk bottles, and various flasks. The production was better when the same amount of medium and the same number of brood flies were distributed in several small containers rather than in one large one. The use of several small containers is of advantage in that when an undesirable condition develops in one of them, the contents of that container can be discarded without interfering seriously with the production. The Erlenmeyer flask has the advantage over a jar in that its shape holds the medium firmly on the bottom.

Drosophila cannot be reared continuously without the possibility of contaminating the culture by undesirable organisms. The molds have not been troublesome, because their growth is inhibited by the sodium propionate. Early in 1958, before sterilization of the medium and the rearing flasks became routine procedure, reddish and brownish spots developed in the substratum of the medium in some of the flasks. It was evident that the medium had been contaminated by one or more anaerobic organisms. As deterioration of the medium progressed, mortality among the brood flies and the developing larvae increased. This situation was remedied by sterilizing the medium and the flasks.

An equally serious situation developed when the rearing flasks were invaded by predaceous mites, probably *Histiostoma* sp. By the time they were discovered, the infestation had become general. It was necessary to eliminate the infested cultures and destroy the mites. The mites on the glassware and other equipment were destroyed by heat and other sanitary measures, and the room was fumigated with formaldehyde. The propagation was continued with uncontaminated

flies. Since then stricter sanitary measures have been followed to prevent further invasions of mites.

Production Unit

The production flasks were maintained in the same environment as the rearing flasks. The average production of a flask during a 2-week period is about 350 flies daily. Two units of 14 flasks each were operated for the production of about 10,000 flies each day. Each week 14 flasks from which flies were beginning to emerge were added to the production unit and the 14 oldest flasks were discarded. When a flask was added, an additional one-fourth teaspoonful of baker's yeast was distributed uniformly over the medium.

All emerging flies were removed from the production flasks each day. The transfer of the flies from the flasks to a plastic randomizing cylinder was facilitated by utilizing the natural tendency of flies to move upward and toward a bright light. When the production flask was darkened and its mouth inserted into the open end of the cylinder, placed in a horizontal position, the flies moved rapidly toward a bright light at the opposite end of the cylinder. This movement was facilitated further by having a gentle flow of air from the flask through the cylinder.

Gerolt (*66*) and Harwood and Areekul (*73*) used a trap to collect the flies as they emerged. Their production units were kept in a darkened room, and as the flies emerged they were attracted to light and so moved continuously into a trap. However, this method did not assure that all flies in the trap had emerged during a 24-hour period. With enlarged ovaries as a criterion of age, Harwood and Areekul found that up to 2 percent of the flies in the trap and in the production unit were more than 24 hours old.

REACTIONS OF DROSOPHILA TO CHLORINATED HYDROCARBON INSECTICIDES IN SOIL

Drosophila is very sensitive to residues of DDT, toxaphene, chlordane, dieldrin, endrin, heptachlor, and aldrin in soil. The speed of reaction to these residues varies with the inherent toxicity and concentration of the insecticide, the nature of the soil, the temperature, and other factors, but the symptoms of intoxication are much the same with all of them.

Five stages of intoxication are recognized. In the first stage the insecticide has no visible effect on the flies. They appear to be normal. They rest quietly, walk leisurely, and fly occasionally. They are not easily disturbed. The second stage is a period of excitement. The flies do little resting and appear to be irritated. They walk and fly rapidly and respond readily to any disturbance, such as a shadow or a tap on the petri dish. In the third stage the flies do more resting and begin to walk unsteadily. As the legs become paralyzed, the flies tend to stand in a fixed position with an occasional violent movement of the wings. This movement of the wings pushes the flies aimlessly and rapidly over the surface of the soil. The fourth stage is exhaustion. The flies lie most of the time on their

sides or backs with only occasional movement of the legs; they are unable to move from their positions. The final stage is death. The point of death is difficult to determine by observation. The absence of any movement in a relaxed fly was considered to be indicative of the cessation of all vital functions.

Since the chlorinated hydrocarbons produce progressive irreversible characteristic symptoms with *Drosophila*, any stage in the intoxication beginning with the onset of paralysis may be used as the criterion of toxic action. The affected individuals are most easily counted when the intoxication has progressed to the advanced stage of paralysis, so that the flies are incapable of changing their position or they are dead. Permanent immobilization is a stable end point. It was selected as the criterion of toxic action in this investigation.

PRELIMINARY TESTS WITH DROSOPHILA

In order to standardize the testing procedure before undertaking bioassays with *Drosophila* as the test insect, there were several factors that had to be resolved. These included the sex and age of the test insects, separation of flies into groups for testing, number of flies per test, feeding them during the testing period, and the container for tests.

Sex

Lord (*97*, *98*), McLeod (*99*), Knutson (*84*), Kerr (*80*), and Gerolt (*66*) reported that the males were more susceptible than the females to nicotine, arsenicals, and the chlorinated hydrocarbon insecticides. Tests with residues of DDT, chlordane, and dieldrin in soil confirmed that the male flies were easier to kill than the female flies. In these tests 10 to 20 percent more males than females succumbed to the insecticides.

The variability in the reaction of the flies to insecticide residues in soil can be reduced by using only one sex in the bioassays. This has been recognized by several investigators, including Bliss (*17*), Bochnig (*26*), Crow (*30*), Edwards et al. (*50*), Fisher and Smallman (*54*), Fleming et al. (*57*), Gerolt (*66*), Lord (*97*), Merrell and Underhill (*102*), and others. The males would be preferred because of their greater response to changes in the concentration of the insecticides. However, it is a slow, tedious task to sort a large number of flies according to their sex. The time required for such an operation makes it impractical, except for small special tests.

When males are not separated from females, it is essential that the ratio of the sexes be maintained throughout each series of tests. Bliss (*17*), Fisher and Smallman (*54*), Edwards et al. (*50*), and others have considered such balanced populations of flies to be satisfactory. In view of the importance of the sex ratio, determinations have been made periodically of the relative numbers of males and females in successive populations. Since 1957, when these determinations were undertaken, there has been no significant deviation from the 1:1 ratio of the sexes.

Age

There is little agreement among investigators with reference to the optimum age of flies that are to be used as test insects. The ages used by various workers are as follows: 5–28 hours (Young and Rawlins *136*), 1 day (Sun and Pankaskie *125*, Earle et al. *49*), 2 days (Bartlett *9*, Fisher and Smallman *54*, Wylie *135*), 3 days (Kerr *79*, Arnold and Apple *4*, Edwards et al. *50*), and 5 days (Kerr *80*).

Only a few investigators have studied the effect of age on the susceptibility of the flies to insecticides. Kerr (*80*) found that young flies succumbed more readily to DDT, but Broadbent and Bliss (*29*) found that old flies were easier to kill with hydrocyanic acid than young flies. The reports on the effect of age on the susceptibility of flies to nicotine sulfate are conflicting. Morrison (*105*) found that susceptibility tended to decrease with age, but McLeod (*99*) found that it increased.

In the studies conducted at Moorestown groups of flies ranging in age from 1 to 8 days were exposed to soil containing residues of chlordane and dieldrin. There seemed to be no consistent relationship between the age of the flies and their speed of reaction to the insecticide residues. On some days the older flies reacted quicker, but on other days the younger flies appeared to be more susceptible. The differences in mortality of the various age groups were small. It was not possible to establish that any age group was consistently more susceptible or less variable in its reaction to the insecticides.

It is not desirable to use flies of mixed ages in bioassay, because an unnecessary variable would be introduced. Since the additional labor and equipment did not seem justified in aging flies, it was decided to use, as test insects, flies that had emerged during the preceding 24-hour period.

Separation of Flies Into Groups

There seems to be little agreement among investigators as to how a population of *Drosophila* can best be separated into groups for testing. Stultz (*124*) and Wylie (*135*) aspirated the flies from the collecting unit to their testing containers.

Morrison (*105*), McLeod (*99*), Edwards et al. (*50*), and others placed a bright light on the far side of the test unit and counted the flies as they moved from the darkened collecting unit through an aperture that permitted the passage of only one fly at a time.

Proverbs and Morrison (*120*) used a mixture of ether and ethyl alcohol to keep the flies under anesthesia while they were being separated into groups. Demerec and Kaufmann (*44*), Crow (*39*), and Merrell and Underhill (*102*) used ether to anesthetize the flies. Caldwell (*33*), Tattersfield et al. (*126*), Sun and Pankaskie (*125*), Arnold and Apple (*4*), and Earle et al. (*49*) used carbon dioxide for this purpose.

Proverbs and Morrison (*120*) found no difference in the susceptibility of anesthetized and nonanesthetized flies to deposits of DDT. On the other hand, Kerr (*80*) found it difficult to interpret his results, largely because of the unpredictable toxic effect of carbon dioxide on the flies. L'Heritier and Sigot (*89*) and de Scoeux (*45*)

reported that some strains of flies were more sensitive to carbon dioxide than others. Tattersfield et al. (*126*) discovered that a relatively resistant strain can develop a sensitivity to carbon dioxide when flies exposed to sublethal dosages of the gas are used for propagation. Beard (*11*) found that the pattern of susceptibility of insects exposed to sublethal dosages of toxicants was essentially the same as that of untreated insects. Bliss and Beard (*22*) reported that there were dynamic and static variations in the response of the large milkweed bug (*Oncopeltus fasciatus* (Dallas)) to sublethal dosages of carbon dioxide and nitrous oxide. Some of the individual resistance to toxicants can be phenotypic rather than genotypic. Kerr (*80*) and Edwards et al. (*50*) questioned the use of an anesthetic to facilitate sorting the flies and attributed erratic results to the effects of anesthesia.

There was a possibility that the flies could be chilled to inactivity for a sufficient time to separate them into groups. When they were placed in the freezing compartment of an electric refrigerator at approximately 5° F., they remained active for almost 10 minutes. No flies were killed by chilling for 15 minutes at this temperature, but at 20 minutes 75 percent were killed and at 30 minutes all of them. When the flies that had been chilled for 15 minutes were transferred to room temperature, they began to recover in 1½ minutes and all were active within 5 minutes. From this preliminary test, chilling appeared to be a hazardous and impractical procedure to use to facilitate sorting the flies.

Aspirating the active flies from a randomizing cylinder to petri dishes was slow and tedious. There was a tendency to collect the less active flies from the sides of the container. Such a selection does not constitute random sampling, and it would be likely to enhance the variability in the response of the groups of flies to insecticides. Aspirating the active flies did not seem to be a satisfactory method for large-scale tests.

A study was made of separating the flies into groups by means of the phototropic response and also after anesthesia with carbon dioxide to facilitate the separation. In the first method the flies were counted as they moved from the randomizing cylinder into the petri dishes through an aperture that permitted the passage of only one fly at a time.

In the second method sufficient carbon dioxide was introduced into the randomizing cylinder to lightly anesthetize the flies. The flies dropped onto a fine mesh screen, where they were kept under light anesthesia by introducing carbon dioxide below this screen as needed. The anesthetized flies were selected at random from this screen and counted as they were aspirated into shell vials for transfer to petri dishes. The anesthetized flies became active within a few seconds after being transferred to the dishes. The tests were repeated on 5 successive days. Each day a determination was made of the percentage of males in the population from which the groups were drawn and in the groups. These data are summarized in table 2.

The sexes were within ±6 percent from the 1:1 ratio in the five tests. The number of males in the groups separated by phototropic response varied by as much as 11 percent and the number in the groups separated after anesthesia varied within 5 percent from the

TABLE 2.—*Sex ratios of population of flies* (Drosophila) *and of groups separated from population by phototropic response and after anesthesia with carbon dioxide*

Test	Population		Groups of flies separated—			
			By phototropic response		After anesthesia	
	Flies	Males	Flies	Males	Flies	Males
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	2, 166	49	303	58	297	54
2	1, 799	55	305	63	298	55
3	4, 627	45	297	50	296	44
4	2, 789	52	289	63	300	49
5	2, 744	53	332	44	293	58
Total or average	14, 125	51	1, 526	56	1, 484	52

populations from which both groups were drawn. Most of the flies separated by their reaction to light were dominantly males.

Randomization in the separation of the flies into groups is necessary to avoid unrepresentative results in bioassays. It is important that the ratio of the sexes in the groups be approximately the same as that in the population from which they were drawn. An analysis of the variance showed that the separation of the anesthetized flies into groups satisfied the requirement for randomization. On the other hand, separating the flies according to the rapidity of their response to light was a nonrandom selection of the more active individuals. Morrison (*105*) tried to compensate for the lack of randomization among flies separated in this manner by randomizing the groups of flies among his test units.

The study was continued to compare the reaction of anesthetized and nonanesthetized flies to dieldrin in Sassafras sandy loam. Groups of 100 anesthetized and nonanesthetized flies were exposed simultaneously for approximately 24 hours on 5 successive days to soil containing 0.2, 0.3, and 0.4 pounds of dieldrin per 3-inch acre. The mortality obtained in these tests is summarized in table 3.

The anesthetized flies were significantly more susceptible to dieldrin than were the nonanesthetized flies. From the concentration-mortality curves, which were practically parallel, the median lethal concentration of dieldrin was 0.27 pound per acre for the anesthetized flies and 0.35 pound per acre for the nonanesthetized flies. The increased susceptibility is of advantage in bioassay, but the consistency of the results in replicated tests is a more important factor.

The reactions of the anesthetized flies in the replicated tests were very consistent. The standard deviation of the mortality from the averages with the different dosages of dieldrin was 5 percent. On the other hand, the nonanesthetized flies selected by their phototropic response varied considerably in their reactions to the insecticide. This higher variability in the mortality was expected because of the non-

random selection of these flies. Bliss (*17*), in discussing heterogeneity in bioassays, pointed out that no method of sampling populations of flies that depended on the activity of the insect would give successive samples of equivalent susceptibility.

TABLE 3.—*Mortality of anesthetized and nonanesthetized flies* (Drosophila) *exposed for 24 hours to Sassafras sandy loam containing various amounts of dieldrin*

Test	Mortality with indicated amounts of dieldrin per 3-inch acre		
	0.4 pound	0.3 pound	0.2 pound
ANESTHETIZED FLIES			
	Percent	*Percent*	*Percent*
1	96	57	22
2	85	62	23
3	87	59	24
4	94	66	30
5	90	54	11
Average	90	60	22
NONANESTHETIZED FLIES			
1	94	53	15
2	49	30	8
3	67	24	5
4	83	50	15
5	42	20	5
Average	67	35	10

After considering the different methods for sorting flies into groups for bioassays, it was concluded that the use of carbon dioxide to anesthetize the flies lightly during this operation was the most satisfactory and practical method.

Number of Flies per Test

There is no fixed standard as to the number of flies to use in a bioassay of an insecticide residue. The number used by different investigators varied from 75 to 800. There was no agreement on the number of flies per test unit nor on the number of times the test unit was replicated.

With stomach poison insecticides, the dosage obtained by a fly depends on its activity and the extent of feeding, as well as on the amount of insecticide present. These conditions tend to make the results with this type of insecticide more variable than with fumigants, and larger numbers of insects are required to establish the concentration-mortality relationship. In general, the experimental error is

629838—62——3

reduced more by increasing the number of replications than by increasing the number of flies per test unit.

In preliminary tests a unit of 100 flies seemed to be about the minimum needed to obtain consistent results in the reaction of the flies to a residue in soil. For convenience in observation, this unit was subdivided into four groups of 25 flies each and exposed in four petri dishes containing the same slurry of soil. It was necessary to determine how many times the test unit should be replicated to evaluate adequately the toxicity in a soil.

Data had been obtained in tests with chlordane, dieldrin, and endrin, in which the average mortality was approximately 50 percent. The standard deviation in the 106 units in those tests was 8.7 percent. The standard error of the average mortality with various replications of these units can be calculated by dividing this statistic by $\sqrt{N}$, where N is the number of replications. Thus, to reduce the error by one-half, the number of replications must be multiplied by 4.

It was estimated that adequate bioassays could be made when the standard error of the mortality was within 3 percent. This level of variation appeared to be obtainable with 10 replications. With this number of replications the variation at the 50-percent level of mortality would be between 47 and 53 percent in two-thirds of the tests. Therefore, it was decided to use 10 units of 100 flies each to evaluate the toxicity of an insecticide residue in soil.

Feeding Flies During Testing Period

The adult *Drosophila* must be fed during the testing period to prevent starvation from becoming a limiting factor. Stultz (*124*), Morrison (*105*), Lord (*97*), McLeod (*99*), and Bartlett (*9*) introduced a cotton plug or paper saturated with a 3- to 5-percent aqueous honey solution as food. Later Morrison (*106*) substituted molasses for the honey. Kerr (*79, 80*) used water containing 5 percent of molasses and 5 percent of baker's yeast, and Glasser et al. (*68*) made a solution containing 5 percent of "bee candy" and 2.5 percent of brewer's yeast.

In the direct bioassay of fruit and vegetables, Pankaskie and Sun (*117*), Sun and Pankaskie (*125*), Dewey,[4] and Glasser et al. (*68*) exposed flies to the macerated tissues without additional food. In studies of the toxicity of insecticides, Fisher and Smallman (*54*) and Arnold and Apple (4) blended the materials with macerated pumpkin. Pankaskie and Sun (*117*) suggested blending equal parts of soil and canned pumpkin. Young and Rawlins (*136*) mixed soil with applesauce. Wylie (*135*) used a 25-percent aqueous solution of corn sirup, and Edwards et al. (*50*) used applejuice in preparing slurries of soil.

When water was used to prepare slurries of soil containing no insecticide residues, about one-fourth of the flies confined with these slurries were dead within 24 hours and all of them within 72 hours. However, when the slurries were prepared with applejuice, a 10-percent honey solution, or a 10-percent corn sirup, practically no mortality occurred within 120 hours. The addition of one of these sugars to the soil did not modify the response of the flies to residues

[4] See footnote 3, p. 7.

of chlordane, DDT, or dieldrin in the soil. Since corn sirup was cheaper and apparently a more standardized material, it was selected for feeding the flies during the testing period.

Further consideration was given to the concentration of the corn sirup solution. Flies were exposed to slurries of soil containing no insecticide, which were prepared with corn sirup solutions ranging in concentration from 1 to 20 percent by volume. When the concentration exceeded 10 percent, the slurries dried slowly and the surface remained sticky, a potential hazard to the flies. A 1-percent sirup was sufficient to sustain the flies for at least 48 hours and a 5-percent sirup for 120 hours. It was decided to use a 5-percent sirup in the preparation of the slurries. Since this solution was adopted, several hundred tests have been conducted with practically no natural mortality during an exposure period of 24 or 48 hours.

According to Waksman (*133*), corn sirup—a commercial glucose—is used very readily as a source of energy by the soil fungi, actinomyces, and most of the heterotrophic soil bacteria. The decomposition with the accompanying evolution of carbon dioxide is very rapid; 1 percent of corn sirup in a soil may be decomposed in 48 hours. Such a condition in a closed petri dish could be very detrimental to the flies and could induce heterogeneous reactions to insecticides. The decomposition of the corn sirup in the soil was prevented for at least 72 hours at 80° F. by adding 6 grams of sodium propionate to each liter of the 5-percent corn sirup.

Container for Tests

Investigators have used various containers to confine *Drosophila* with insecticide residues. Some used vials (*39*, *84*, *103*, *105*, *106*, *120*, *135*), others used test tubes[8] (*54*, *68*, *115*, *136*), 4-ounce bottles (*4*, *49*), small glass jars (*50*, *125*), and petri dishes (*3*, *9*, *47*, *100*, *102*, *121*). The petri dish has also been used to advantage in studying the reaction of house flies to residual-type sprays (*38*, *96*, *109*).

Various containers were tested for their suitability in the direct bioassay of insecticide residues in soil. Consideration was given to the facility with which the soil and the flies could be introduced into the container, the ease with which their reactions could be observed, and the cleaning of the containers. The 9-cm. petri dish was found to be the most suitable. The flies confined in a petri dish are close to the surface of the soil, and thus the possibility of their contact with it is increased. It appeared, in general, that the deeper the air space above the soil the fewer the contacts the flies made with the soil.

TOXICITY STANDARDS WITH DROSOPHILA FOR CHLORINATED HYDROCARBON INSECTICIDES IN SOIL

Each of the chlorinated hydrocarbon insecticides was mixed with Sassafras sandy loam in a progressive series of dosages. The median dosage of each series was the amount of each insecticide needed to

[8] See footnote 3, p. 7.

kill all newly hatched Japanese beetle grubs. The treated soil was made into slurries, and *Drosophila* was exposed at a temperature of 80° F. (See p. 21.) The median dosages of endrin, dieldrin, heptachlor, and aldrin killed 50 percent of the flies in about 24 hours; the median dosage of chlordane required about 36 hours and that of DDT and of toxaphene more than 48 hours.

To be practical, a bioassay should last approximately 24 or 48 hours in order that the period might be prolonged or shortened by 2 or 3 hours to compensate for variations in the resistance of different batches of flies without having to extend the observations beyond the normal working hours. Therefore, dosages of chlordane, endrin, dieldrin, heptachlor, and aldrin were selected that killed 50 percent of the flies in approximately 24 hours and dosages of DDT and toxaphene that killed half of them in approximately 48 hours.

The tests were continued with four or five dosages of each insecticide, including the new median dosage, until 1,000 or more flies had been exposed to each dosage of an insecticide. The mortalities were converted into probits and the dosages to logarithms and the log-probit regressions were calculated by the method of least squares. The fitted lines were then transformed back to the original units and plotted as concentration-mortality curves. The average amounts of each of the chlorinated hydrocarbon insecticides needed for 10- to 90-percent mortality of the flies and for 100-percent mortality of the first-instar Japanese beetle grubs are given in table 4.

The dosages of endrin, dieldrin, heptachlor, and aldrin for a 50-percent mortality of *Drosophila* were practically the same as those required to prevent development of newly hatched grubs, but dosages of DDT, toxaphene, and chlordane were about double the amounts needed for the grubs.

The mortality of *Drosophila* obtained with an exposure of approximately 24 hours to a sample of soil is attributed to aldrin, chlordane, dieldrin, endrin, or heptachlor, alone or in combination. The toxicity may be expressed as equivalent pounds per 3-inch acre of one of these insecticides. When there is no reaction of the flies in 24 hours, but a mortality is obtained in 48 hours, the toxicity is attributed to DDT or toxaphene. In view of the difference in the exposure required for the more toxic and the less toxic insecticides, the toxicity attributed to aldrin, chlordane, dieldrin, endrin, or heptachlor can be evaluated in the presence of the usual amounts of DDT or toxaphene. Normally, less than 25 pounds per 3-inch acre of DDT or toxaphene are in the soil. However, without some adjustment for the difference in the exposure periods, the toxicity of mixtures of DDT or toxaphene with the more potent insecticides cannot be evaluated.

The evaluation of the toxicity of soil with residues of DDT mixed with the more potent chlorinated hydrocarbons is important, because several of these insecticides have been applied to soil treated previously with DDT. Soils treated with toxaphene have not been retreated in this manner.

About 15 percent of the flies exposed to Sassafras sandy loam containing 30 pounds of DDT per 3-inch acre were killed with an exposure of 24 hours. This was about the minimum amount of DDT that would produce a consistent reaction with this exposure.

By adding 30 pounds of DDT per 3-inch acre to a sample of a DDT-treated soil to fortify the residue, the toxicity was so increased that residues of DDT could be evaluated within a 24-hour exposure period. A new standard for evaluating the toxicity of soil fortified by the addition of DDT was developed and is presented in table 5.

TABLE 4.—*Amounts per 3-inch acre of chlorinated hydrocarbon insecticides required in Sassafras sandy loam for 10- to 90-percent mortality of flies* (Drosophila) *and 100-percent mortality of first-instar Japanese beetle grubs with 48- and 24-hour exposures at 80° F.*

Mortality (percent)	48-hour exposure		24-hour exposure				
	DDT	Toxaphene	Chlordane	Endrin	Dieldrin	Heptachlor	Aldrin
	FLIES						
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
10	2.7	1.9	0.41	0.16	0.14	0.014	0.034
15	3.6	2.3	.49	.19	.16	.028	.038
20	4.5	2.7	.57	.22	.18	.034	.042
25	5.4	3.0	.65	.26	.20	.040	.046
30	6.4	3.3	.73	.29	.22	.045	.050
35	7.5	3.7	.81	.32	.23	.051	.054
40	8.7	4.1	.89	.36	.25	.058	.058
45	10.0	4.5	.98	.40	.26	.065	.062
50	11.6	4.9	1.07	.44	.28	.073	.066
55	13.3	5.4	1.18	.49	.30	.082	.070
60	15.4	5.9	1.30	.54	.32	.092	.075
65	17.9	6.5	1.42	.60	.34	.104	.081
70	21.0	7.2	1.58	.67	.37	.118	.087
75	24.9	8.0	1.76	.76	.40	.136	.094
80	30.1	9.0	2.00	.86	.43	.158	.103
85	37.5	10.4	2.30	1.01	.48	.189	.114
90		12.4	2.76	1.23	.54	.237	.130
	FIRST-INSTAR GRUBS						
100	5.0	2.5	0.40	0.40	0.30	0.07	0.07

TABLE 5.—*Amounts per 3-inch acre of DDT required in Sassafras sandy loam for 10- to 90-percent mortality of flies* (Drosophila) *with a 24-hour exposure at 80° F.*

Mortality (percent)	DDT	Mortality (percent)	DDT
	Pounds		*Pounds*
10	29.2	55	52.5
15	32.0	60	55.0
20	35.0	65	58.5
25	37.5	70	62.0
30	40.0	75	66.0
35	42.5	80	71.0
40	45.0	85	77.0
45	47.5	90	85.0
50	50.0		

The mortality in a treated soil fortified by the addition of DDT was compared with the mortality rates in table 5, and by interpolation the amount of DDT corresponding to this mortality was determined. The 30 pounds of added DDT was then subtracted from this value to give the accelerated DDT equivalent of the residue in the soil. The mortality of the flies corresponding to this DDT equivalent is the measure of the toxicity in the soil.

This procedure with *Drosophila* was tested in comparison with a bioassay with grubs, where the total toxicity was determined directly, to evaluate the toxicity of soil in a commercial nursery to which both DDT and chlordane had been applied. The results of these determinations are summarized in table 12 (p. 35). With both biological procedures, the mortality of the test insects exceeded 50 percent in seven plots, was below 50 percent in five plots, and in disagreement in two plots, where lead arsenate was also suspected of being in the soil. This finding tended to establish the validity of this procedure with *Drosophila* for determining the toxicity of binary mixtures of DDT and the more potent chlorinated hydrocarbon insecticides.

When DDT was added to soil to accelerate the reaction of *Drosophila* to a residue and an adjustment was made for this addition, the DDT equivalent of the residue as determined from table 5 was much higher than the actual residue in the soil. This situation did not occur when DDT residues were in excess of 30 pounds per acre and no additional insecticide was added to the sample. When the mortality corresponding to the accelerated DDT equivalent of the residue was compared with that in table 4 and the data were interpolated, a good estimate of the original residue was obtained.

In bioassays of binary mixtures of DDT and chlordane in soil in which both grubs and *Drosophila* were used as test insects, the toxicity was expressed as equivalent pounds of chlordane per acre. In the bioassays with grubs, the chlordane equivalent of the toxicity of a residue was determined directly from table 1 (p. 5). With *Drosophila*, the mortality corresponding to the accelerated DDT equivalent of the residue, as determined from table 5, was compared with the mortality rates in table 4, and by interpolation the equivalent amount of chlordane was calculated. Since substantially the same results were obtained with both biological procedures, as shown in table 12, it was demonstrated that in bioassays of binary mixtures of DDT and a more potent chlorinated hydrocarbon insecticide with *Drosophila*, the toxicity can be expressed as an equivalent amount of any of the insecticides given in table 4.

PROCEDURE FOR BIOASSAY WITH DROSOPHILA

Soil Sample

Adequate sampling is the first important step in either a chemical or a biological analysis. Even though extreme care is exercised in spreading formulations of the chlorinated hydrocarbon insecticides over turf or cultivated land, homogeneity in the distribution may be approached but it is rarely attained. Heterogeneity in the dispersion

of the insecticides is a factor that must be considered in sampling soil. For this reason, it is necessary to take a sufficient number of subsamples at random over a treated area in order that the composite is representative.

Most of the residues of the chlorinated hydrocarbon insecticides applied as topdressings to turf remain within the upper 1-inch layer of soil. All these insecticides were recovered within the upper 3 inches (*35*, *36*). In cultivated land practically all the residues were found within the layer of tillage (*2*, *35*, *36*, *37*). The toxicity in the upper 3 inches of soil is most essential in the control of Japanese beetle grubs.

In sampling the soil of land treated with insecticides, it has been the practice at Moorestown to take 50 plugs, each 2 inches in diameter and 3 inches deep, at random over an area not larger than 20,000 square feet, with a sampling tool similar to that described by Johnson and Caskey (*77*). Thus approximately 1.1 square feet of plot area constitutes a composite sample of slightly more than one-fourth cubic foot in volume. The close agreement in the analyses of duplicate samples from plots has demonstrated repeatedly that this method of sampling is usually adequate.

Preparation of Sample

The composite sample of soil is exposed in shallow trays until nearly air-dry. It is then passed through a 6-mesh sieve to remove stones and miscellaneous debris. The stones are discarded. The small roots and grass are shredded and combined with the soil. The sample is mixed thoroughly to assure homogeneity and placed in a can with a tight cover.

A 160-ml. aliquant part of a composite sample of dry soil is needed for four petri dishes. However, it is easier to determine the apparent specific gravity of a soil and to weigh the amount of soil required than to measure it by volume. The approximate apparent specific gravity of a soil is determined by putting it in a receptacle of known volume and obtaining its weight. The apparent specific gravity of the mineral soils may range from 1.10 to 1.20 for the clays to 1.65 to 1.75 for the sands. Humous soils may have an apparent specific gravity as low as 1.00, and muck often reaches a low value of 0.40.

When a soil is known to contain residues of DDT and the more potent chlorinated hydrocarbons or these mixtures are suspected of being present, DDT in the form of a dilute dust is mixed with the soil at the rate of 1.25 grams per cubic foot, equivalent to 30 pounds per acre, to accelerate the response of the flies to the DDT. No insecticide is added to a soil containing only one of the chlorinated hydrocarbons, mixtures of the more potent insecticides, or mixtures of DDT and toxaphene.

A 160-ml. soil sample is mixed with 7.5 grams of plaster of paris in a stainless-steel beaker, and enough of a 5-percent aqueous solution of corn sirup, containing 6 grams of sodium propionate per liter, is added to make a free-flowing slurry. The slurry is blended thoroughly with an electric mixer. The plaster of paris is added so that the slurries of all soils will present a moderately hard smooth surface to the flies. The corn sirup provides food for the flies during the

testing period. The sodium propionate retards the decomposition of the sugar in the soil.

The slurry is distributed at random in the lids of four petri dishes. The containers are jarred slightly to distribute the mass uniformly over the surface and to expel any air bubbles. An embossed number for identification is pressed lightly into the surface of the slurry in each dish. When the plaster of paris has set and there is no free water on the surface, the soil is ready for exposure to the flies.

Preparation of Test Standards

The manufacturers of the chlorinated hydrocarbon insecticides have provided analyzed samples of the materials in the form of dilute dusts for use in preparing standards of insecticidal action. These materials are renewed annually. When a new sample of a material is obtained, comparative tests are conducted with it and the previous sample to establish their relative toxicities before it is used in bioassay.

Test standards are used to govern the exposure in each series of tests. In preparing these standards, each of the following amounts of insecticides (milligrams) is mixed thoroughly with 1 cubic foot of Sassafras sandy loam, which has been passed through a 6-mesh screen: DDT 483, toxaphene 204, chlordane 45, endrin 18, dieldrin 12, heptachlor 3, and aldrin 2.7. These rates are equivalent to the pounds per 3-inch acre of the insecticides at the 50-percent mortality level given in table 4 (p. 19). When DDT at the level of 50 pounds per acre is needed as a test standard for soil fortified with DDT, 2.08 grams of the insecticide is mixed with 1 cubic foot of the soil. The soil and the insecticide in the form of a dilute dust are blended intimately in a small concrete mixer. Then the soil is placed in a tight container and held for at least 5 days to establish an equilibrium between the soil and the insecticide. A slurry of a standard is prepared in the usual manner for each series of tests. The test standards are replaced monthly.

Testing for Toxicity

A test series normally consists of 10 to 14 samples of soil and one of the standards discussed in the previous paragraph; 44 to 60 petri dishes are used. Occasionally a check with four dishes of untreated soil is added to the series. An untreated check is not included in each series because from past experience practically no natural mortality occurred during the testing period.

The flies anesthetized with carbon dioxide are separated into groups of 25 each and distributed among the petri dishes. Sufficient carbon dioxide is introduced into the randomizing cylinder to lightly anesthesize the flies. The flies drop on a fine mesh screen, where they are kept under light anesthesia by the introduction of carbon dioxide below the screen as needed. The anesthetized flies are selected at random from this screen and are counted as they are aspirated into shell vials for transfer to the petri dishes. Immediately after the flies are placed on the hardened slurry of soil, the bottom of the petri dish, serving as the cover, is pressed into the soil to confine the flies and conserve moisture.

The test series is incubated at 80° F. in a well-lighted room until the mortality with the standard soil is 50 percent. An exposure of approximately 24 hours is required for soil containing chlordane, endrin, dieldrin, heptachlor, and aldrin and mixtures of DDT with these insecticides, and an exposure of approximately 48 hours is needed for soil containing DDT or toxaphene. After a test is completed, the slurries are discarded. The tests with a sample of soil are repeated on successive days with freshly prepared slurries until at least 1,000 flies have been exposed to the soil.

Evaluation of Toxicity

Since the exposure is governed by the test standard—Sassafras sandy loam containing a sufficient amount of one of the chlorinated hydrocarbon insecticides to kill 50 percent of the flies—the toxicity in a soil under investigation is actually expressed in terms of the standard. With this procedure soils with a high adsorptive capacity for the insecticides show a lower active toxicity than those with a low fixing power, even when the same total amount of insecticide is present.

The mortality of the flies is determined directly for all the chlorinated hydrocarbons, except mixtures of DDT and the more potent insecticides, when an adjustment is made for the toxicity of the added DDT (see p. 19).

The standard deviation in the mortality of groups of flies exposed to an insecticide residue in soil is usually less than 10 percent. When the mortality is more than 60 percent, the toxicity in a soil is adequate to prevent the development of newly hatched Japanese beetle grubs, but when it is less than 40 percent, additional insecticide should be added to fortify the treatment. When the mortality is between 40 percent and 60 percent, additional tests may be necessary to establish more definitely the level of the toxicity. For many routine analyses it may be necessary only to determine whether the toxicity in a soil is sufficient to kill 50 percent of the *Drosophila*.

The toxicity in a soil may be expressed as equivalent pounds per acre of any one of the chlorinated hydrocarbon insecticides given in table 4 (p. 19) by comparing the mortality of the flies with the mortality rates in the table and interpolating the data. The equivalent amount of insecticide may also be calculated by converting the mortality to its corresponding probit, substituting this value for "E" in the log-probit regression, and solving for "X," the logarithm of the concentration of the insecticide. The antilogarithm, or the concentration of the insecticide in pounds per acre, can be obtained readily from a table of logarithms. The log-probit regressions for the chlorinated hydrocarbons with *Drosophila* are as follows:

Aldrin	$X=(E-10.1398)/4.3514$
Chlordane	$X=(E-4.9081)/3.1123$
DDT	$X=(E-2.8427)/2.0291$
Dieldrin	$X=(E-7.4902)/4.5104$
Endrin	$X=(E-6.0246)/2.8776$
Heptachlor	$X=(E-7.8521)/2.5090$
Toxaphene	$X=(E-2.8172)/3.1688$

Sensitivity of Direct Bioassay of Soil

It is well known that the determination of insecticide residues is affected by various factors associated with a procedure. Therefore, the sensitivity of a chemical or biological analytical method should be indicated in order that the significance of the determinations may be evaluated.

Sensitivity usually refers to the minimum amount of a substance that can be detected consistently by an analytical method. Accurate evaluations of a residue cannot be made by bioassay when the mortality of the test insect is approaching zero. Therefore, the sensitivity of a biological procedure cannot be established by attempting to determine the minimum amount of an insecticide that will cause some mortality. Since the most accurate biological determinations are made at the 50-percent level of mortality, the sensitivity of a bioassay may be considered to be the amount of an insecticide needed to cause a significant change in the mortality at that level.

When parallel tests with a soil containing a chlorinated hydrocarbon and with the standard soil containing a dosage of the insecticide for 50-percent mortality are repeated 10 times, a deviation of 9.4 percent in the mortality of Japanese beetle grubs in the test soil from that of the standard is usually significant, and a deviation of 12.9 percent is highly significant. A deviation of 8.2 percent in the mortality of *Drosophila* is usually significant and one of 11.2 percent highly significant. From the concentration-mortality data given in tables 1 and 4, the amounts of each of the insecticides needed to produce such changes in the mortality of the grubs and of the flies at the 50-percent level of mortality were calculated and expressed as pounds of insecticide per 3-inch acre of soil. The sensitivity of these bioassays in Sassafras sandy loam is summarized in table 6.

TABLE 6.—*Sensitivity in pounds per 3-inch acre of bioassays with Japanese beetle grubs and flies* (Drosophila) *in determining residues of chlorinated hydrocarbon insecticides in Sassafras sandy loam*

Insecticide	With grubs		With flies	
	Odds 1:19	Odds 1:99	Odds 1:19	Odds 1:99
	Pounds	*Pounds*	*Pounds*	*Pounds*
Aldrin	0.02	0.03	0.007	0.01
Heptachlor	.02	.03	.01	.02
Dieldrin	.14	.24	.03	.04
Endrin	.12	.18	.07	.10
Chlordane	.43	.68	.17	.23
Toxaphene	1.27	1.84	.74	1.05
DDT	2.21	3.29	2.75	3.88

There were great differences in the amounts of these insecticides needed in Sassafras sandy loam to cause a significant change in the mortality of the grubs or the flies. With the exception of DDT,

where the sensitivity of both methods was relatively low and of about the same order, the bioassays with the flies were more sensitive than those with the grubs. In most tests the flies reacted to about one-half of the dosages needed to produce significant changes in the mortality of the grubs. With the flies, the most accurate determinations can be made of residues of aldrin, where only 0.007 pound per acre is needed to produce a significant change in the mortality, and the least accurate of DDT residues, where 2.75 pounds are required for such a modification in the mortality.

The sensitivity of a bioassay is also modified by the nature of the soil to which the chlorinated hydrocarbon insecticides are applied. It was estimated that to produce a significant change in the mortality of grubs or flies with a sand, only about one-half the amount of insecticide needed in Sassafras sandy loam would be required. On the other hand, to cause such a change in the mortality with a muck, the amounts of insecticide would have to be increased fivefold to tenfold.

Population Variations

When a test was repeated several times on the same day with groups of flies selected at random from the same population, there was usually a difference of only a few minutes in the exposure required to reach a certain level of mortality with the different groups, but when it was repeated on different days with successive populations of flies, the exposures of the groups might vary as much as 4 hours. Although much has been done to standardize the rearing procedure and the testing method, it has not been possible to eliminate this variation in the resistance of successive populations. However, it is generally considered to be better experimental design when tests are replicated on different days with successive populations rather than with a single population.

Sokal (*123*) in studying the susceptibility of *Drosophila* to DDT found similar fluctuations occurred throughout 76 generations of flies reared under apparently identical conditions. He attributed these fluctuations to the microenvironmental changes in the containers used for propagation. Newman et al. (*110*) found a similar variation with laboratory-reared southern house mosquitoes (*Culex pipiens quinquefasciatus* Say) and Gersdorff et al. (*67*) with the house fly.

Since the resistance of *Drosophila* to insecticides over a series of generations is likely to fluctuate, bioassay has to be on a relative basis with reference to the exposure. The principle of parallel testing of insecticides can be employed to advantage in correcting to a large extent for these fluctuations. Each day parallel tests are conducted with the soils under investigation and with a soil receiving a standard insecticidal treatment that will kill one-half of the flies in approximately 24 hours. The exposure is prolonged until 50 percent of the flies are killed by the standard treatment. Then the mortality with the soils under investigation is determined. With this modification in the procedure, reproducible results have been obtained consistently with successive populations of flies.

COMPARISON OF BIOASSAYS WITH JAPANESE BEETLE GRUBS AND WITH DROSOPHILA AS TEST INSECTS

A few samples of soil were collected from field plots that had been treated with formulations of aldrin, dieldrin, and heptachlor to compare the results obtained in bioassays with third-instar Japanese beetle grubs and with adult *Drosophila*. These determinations are summarized in table 7.

TABLE 7.—*Comparison of bioassays of field plots treated with aldrin, dieldrin, and heptachlor, using Japanese beetle grubs and flies* (Drosophila) *as test insects*

Insecticide	Amount per 3-inch acre determined—	
	With grubs	With flies
	Pounds	*Pounds*
Aldrin	0. 09	0. 03
	. 25	. 15
	1. 39	1. 20
	2. 65	2. 60
Dieldrin	. 44	. 38
	. 28	. 23
	. 30	. 23
	. 30	. 37
Heptachlor	. 33	. 23
	. 40	. 48

In these tests substantially the same results were obtained with both test insects. The average deviation between the two methods of assay was equivalent to 0.10 pound of insecticide per 3-inch acre. About the same appraisal of toxicity in a soil was made with the grubs burrowing through and ingesting soil as with the flies coming into contact with only the surface soil. Although these tests were not extensive, they tend to establish that either of these organisms can be used in evaluating the toxicity in a soil.

BIOASSAY VERSUS CHEMICAL ANALYSIS FOR DETERMINING RESIDUES OF CHLORINATED HYDROCARBON INSECTICIDES IN SOIL

Samples of soil were collected for bioassay and for chemical analysis in Connecticut, Massachusetts, New Jersey, North Carolina, Ohio, and Pennsylvania from experimental turf plots to which the chlorinated hydrocarbon insecticides had been applied to control Japanese beetle grubs. The sampling covered the weathering of heptachlor during a period of 3 years, aldrin and dieldrin during 5 years, toxaphene during 7 years, and chlordane and DDT during 9 years. Endrin was not included in these tests.

The toxicity in each sample was determined by bioassay with third-instar Japanese beetle grubs as test insects. The equivalent amount of insecticide residue was calculated by comparing the mortality in the sample and the mortality when known amounts of the insecticides were used and interpolating. The amount of organic chlorine in each sample was determined by the Pesticide Chemicals Research Branch. The method of Koblitsky and Chisholm (*85*) was used, and the equivalent amount of insecticide residue was calculated by multiplying the amount of organic chlorine by an appropriate factor. The data were arranged in groups and the average residue for each group was determined. The summary of the biological and the chemical determinations of the insecticide residues is given in table 8.

The average discrepancy between the biological and chemical determinations of the chlorinated hydrocarbon insecticide residues was as follows:

Insecticide	*Pounds per 3-inch acre*
Aldrin	0.60
Chlordane	.89
DDT	2.14
Dieldrin	.67
Heptachlor	.89
Toxaphene	4.14

The average discrepancy with chlordane and DDT was less than 10 percent of the initial applications of these insecticides, but with the other materials it ranged from 16.6 percent with toxaphene to 44.5 percent with heptachlor. The relationship between the biological and chemical determinations remained fairly constant throughout the sampling periods with chlordane and DDT, but as the other materials weathered, there was a trend for larger amounts to be recovered by chemical analysis than by bioassay.

Other investigators have also observed discrepancies between biological and chemical determinations of residues of the chlorinated hydrocarbon insecticides in soil. Terriere and Ingalsbe (*127*) usually recovered greater amounts of these insecticides by the organic chlorine method (*85*) than by biossay with larvae of *Culex quinquefasciatus* Say. Kiigemagi et al. (*81*) found good agreement initially between the organic chlorine method (*1*) and bioassay with culicine larvae in the analysis of soils containing aldrin, dieldrin, and heptachlor, but after the soils had weathered for several months, larger amounts of these insecticides were obtained by chemical analysis than by bioassay.

Discrepancies have also been noted in the results obtained by specific chemical methods and bioassays of insecticide residues in soil. Young and Rawlins (*136*) reported significantly higher amounts of heptachlor in mineral soils by colorimetric analysis (*115, 119*) than by direct bioassay with *Drosophila;* in muck the results obtained by both methods were not significantly different. Lichtenstein and Polivka (*93*) found more chlordane and less aldrin and heptachlor in soil by colorimetric analysis (*111, 112, 116, 119*) than by bioassay with this fly. Lichtenstein and Schulz (*94*), Edwards et al. (*50*), and Lichtenstein et al. (*91*) also found less aldrin in soil by colorimetric analysis (*40, 111, 112*) than by bioassay with *Drosophila.* Lichtenstein et al. (*91*) recovered more lindane by chemical analysis (*122*) than by bioassay with this fly.

TABLE 8.—*Amounts per 3-inch acre of chlorinated hydrocarbon insecticide residues in soil during weathering as determined by bioassay and chemical analysis*

Insecticide and years residue weathered	Plots	Residues determined by—		Difference	Insecticide and years residue weathered	Plots	Residues determined by—		Difference
		Bioassay	Chemical analysis				Bioassay	Chemical analysis	
Aldrin:	*Number*	*Pounds*	*Pounds*	*Pounds*	Dieldrin:	*Number*	*Pounds*	*Pounds*	*Pounds*
0.5	6	1.77	1.07	−0.70	0	5	3.04	3.66	+0.62
2	8	.43	.64	+.21	1	4	2.10	2.60	+.50
3	5	.26	.92	+.66	2	4	1.78	2.38	+.60
5	11	.10	.90	+.80	3	7	1.13	1.61	+.48
Chlordane:					4	7	.83	1.81	+.98
0.5	9	5.8	3.3	−2.5	5	4	.55	1.35	+.80
1	12	3.3	3.9	+.6	Heptachlor:				
2	13	1.5	2.0	+.5	0	2	2.15	1.85	−.30
5	11	.7	.5	−.2	1	4	.35	1.80	+1.45
9	17	.3	1.3	+1.0	2	4	.20	.55	+.35
DDT:					3	4	.10	1.25	+1.15
0	8	24.5	21.1	−3.4	Toxaphene:				
0	3	51.0	51.1	+.1	0	3	30.5	33.1	+2.6
4	7	17.8	14.0	−3.8	1	3	16.6	18.2	+1.6
4	3	36.5	31.8	−4.7	3	1	13.1	10.5	−2.6
6	12	11.6	10.3	−1.3	5	3	7.9	8.2	+.3
7	16	7.5	9.0	+1.5	6	7	3.9	9.8	+5.9
9	3	2.5	3.8	+1.3	7	4	.7	8.1	+7.4

Since the evidence indicated that some of the chlorinated hydrocarbons are converted to other compounds in the soil, bioassay appears to be the more reliable and realistic method for assaying residues of these materials.

EFFECT OF LEAD ARSENATE IN SOIL ON BIOASSAYS OF CHLORINATED HYDROCARBON INSECTICIDES

Before DDT and the other chlorinated hydrocarbons were authorized for the treatment of plots in commercial nurseries to satisfy the requirements of the Japanese beetle quarantine, lead arsenate at the rate of 1,000 pounds per 3-inch acre was authorized for this purpose. In some nurseries the chlorinated hydrocarbons have been applied to soil containing arsenical residues. Experiments were undertaken to determine the effect of the arsenical on bioassays of residues of the chlorinated hydrocarbons.

When third-instar Japanese beetle grubs were introduced into Sassafras sandy loam containing lead arsenate and held there for 2 weeks at 80° F.—the conditions under which the chlorinated hydrocarbons were assayed—the mortality of the grubs increased progressively with the increment in the amount of the arsenical. The amounts of lead arsenate and the mortality of grubs were as follows:

Lead arsenate (pounds per acre)	*Mortality (percent)*
200	8
400	27
600	43
800	57
1,000	65

Since the grubs react to the arsenical and to the chlorinated hydrocarbons under the same conditions, the grubs can be used to assay the toxicity of mixtures of these insecticides.

On the other hand, lead arsenate affected *Drosophila* much slower than did the chlorinated hydrocarbons. When the flies were exposed to slurries of soil containing lead arsenate at rates up to 2,000 pounds per acre, no mortality occurred within 24 hours at 80° F. The mortality was 2, 45, and 98 percent in 48, 72, and 96 hours, respectively. There appeared to be no relationship between the amount of the arsenical in the soil and the speed of insecticidal action.

Wylie (*135*) also observed that the mortality of the flies exposed to soil containing lead arsenate was not primarily a result of the dosage. With 0.28 pound of dieldrin and 1,000 pounds of lead arsenate per acre in the soil and an exposure of 24 hours, the presence of the arsenical did not modify the reaction of the flies to the dieldrin. Therefore, with *Drosophila* as the test insect, the toxicity of residues of the chlorinated hydrocarbons can be determined in the presence of lead arsenate, but the combined toxicity of these insecticides cannot be measured.

INACTIVATION OF CHLORINATED HYDROCARBON INSECTICIDES IN SOIL

Soil is not an inert medium. It is a complex dynamic mixture of mineral and organic matter that is the habitat of many forms of animal and plant life. It furnishes the foothold and, in part, the sustenance of higher plants. The flora and fauna and the chemical and physical properties of soil vary greatly from place to place.

Many investigators have observed that the introduction of chlorinated hydrocarbons in the soil affected plants differently in various parts of the country. These insecticides were more toxic to plants in sand than in the heavier mineral soils or muck. Edwards et al. (*50*), Fleming (*55*), Fleming and Maines (*58*, *59*, *60*, *61*), Forbes and King (*63*), and others have found a high negative correlation between the amount of organic matter in soil and the toxicity of the chlorinated hydrocarbons to insects. The studies of Barlow and Hadaway (*6*, *7*, *8*) have indicated that when aldrin, DDT, dieldrin, and lindane are applied to a soil, a part of the insecticide vaporizes and is adsorbed by the particles of soil; the adsorbed material is inactive as an insecticide.

A study was undertaken to determine more definitely to what extent the chlorinated hydrocarbons are inactivated insecticidally in different soils. Through the cooperation of the Soil Conservation Service, representative agricultural soils were obtained from various parts of New Jersey for these exploratory tests.

Drosophila was exposed for 24 hours at 80° F. to soils containing progressively larger amounts of aldrin, chlordane, dieldrin, and heptachlor and for 48 hours to soils with DDT and toxaphene until the amount of each insecticide needed for 50-percent mortality of the flies exposed to each soil was determined. Only about three-fourths of the planned tests could be completed, but they showed some very significant trends. The results are summarized in table 9.

TABLE 9.—*Amounts per 3-inch acre of chlorinated hydrocarbon insecticides needed for 50-percent mortality of flies* (Drosophila) *in various soils*

Soil	DDT	Toxaphene	Chlordane	Dieldrin	Heptachlor	Aldrin
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Monmouth loam	3.7	1.7	0.35	0.10	0.03	0.03
Lakewood sand	4.2	2.5	.41	.13	.06	.06
Downer sandy loam	5.8		.50	.19		
Bucks silt loam	10.9		1.34	.33		
Sassafras sandy loam	11.6	4.9	1.07	.28	.07	.07
Annandale silt loam	17.9		1.29	.37		
Collington sandy loam	18.6	14.5	2.36	.76		.19
Croton silt loam	21.1		1.94	.44		
Weeksville sandy loam	38.5	12.5	4.61	1.17		.21
Cokesbury silt loam	43.8		5.91			
Muck	88.4	29.7	5.00	1.20	.50	.36

It was assumed that all the insecticide required for 50-percent mortality of the flies exposed to Monmouth loam, the least adsorptive soil, was insecticidally active. The increase in the amount of insecticide for 50-percent mortality with another soil was considered to be the amount inactivated by the other soil. For example, 3.7 pounds of DDT were required in Monmouth loam and 11.6 pounds in Sassafras sandy loam, indicating that 7.9 pounds per acre or 68 percent of the insecticide added to the Sassafras soil was not functioning. In like manner it was determined that the Sassafras soil inactivated 65 percent of the toxaphene, 67 percent of the chlordane, 64 percent of the dieldrin, and 57 percent of the heptachlor and aldrin added to it. Muck inactivated 96 percent of the DDT, 94 percent of the toxaphene, 93 percent of the chlordane, 92 percent of the dieldrin, 94 percent of the heptachlor, and 92 percent of the aldrin.

In view of the great differences in the amounts of the chlorinated hydrocarbon insecticides needed to obtain the same level of toxicity in the various soils, more consideration should be given to the nature of the soil to which these insecticides are applied for the control of the grubs.

PRETREATMENT ASSAY TO DETERMINE AMOUNT OF CHLORINATED HYDROCARBON INSECTICIDES NEEDED TO KILL JAPANESE BEETLE GRUBS IN SOIL

The Plant Pest Control Division authorizes the application of 25 pounds of DDT or toxaphene, 10 pounds of chlordane, and 3 pounds of dieldrin, heptachlor, or aldrin per 3-inch acre to eliminate newly hatched Japanese beetle grubs in the soil of nursery plots. These rates are 5 to 40 times as much as are needed to kill all these grubs in Sassafras sandy loam in order to allow for some unevenness in the distribution and for the effect of different soils on the insecticidal action and to provide for the longevity of the treatment. The dosages of these insecticides needed in Sassafras sandy loam are given in table 10. Although a single application of one of these insecticides is usually effective for 4 or 5 years, there is need for a practical method to determine before treatment how much insecticide is actually required to assure the destruction of the grubs in the soil of a plot.

Tests were undertaken with 10 agricultural soils, with *Drosophila* as the test insect, to determine how much of these insecticides was needed in these soils to kill all newly hatched grubs. As a standard in each series of tests, one of the insecticides was mixed with Sassafras sandy loam at a rate that would kill 50 percent of the flies. Parallel tests were conducted with the standard and with the other soils. The amount of insecticide in each soil was increased progressively until the toxicity was equivalent to that in the standard.

With information on the amounts of insecticide needed for (*a*) 50-percent mortality of the flies in the standard soil, (*b*) 50-percent mortality of the flies in the soil under investigation, and (*c*) 100-percent mortality of newly hatched grubs in the standard soil, an

estimate can be made of (X) the pounds of insecticide per 3-inch acre for 100-percent mortality of the grubs in the test soil by means of the equation $X=(b\times c)/a$. The amounts of the chlorinated hydrocarbons needed for 100-percent mortality of the newly hatched grubs were calculated for the different soils and are given in table 10.

TABLE 10.—*Estimated amounts per 3-inch acre of chlorinated hydrocarbon insecticides to eliminate newly hatched Japanese beetle grubs in various soils, using flies* (Drosophila) *as the test insect*

Soil	DDT	Toxaphene	Chlordane	Dieldrin	Heptachlor	Aldrin
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Sassafras sandy loam [1]	5.0	2.5	0.40	0.30	0.07	0.07
Monmouth loam	1.6	.9	.13	.11	----	.03
Lakewood sand	1.8	1.3	.15	.14	.06	.06
Downer sandy loam	2.5	----	.19	.20	----	----
Bucks silt loam	4.7	----	.50	.35	----	----
Annandale silt loam	7.7	----	.48	.40	----	----
Collington sandy loam	8.0	----	.88	.82	----	.19
Croton silt loam	9.1	7.4	.72	.47	----	----
Weeksville sandy loam	16.6	6.4	1.72	1.26	----	.21
Cokesbury silt loam	18.9	----	2.21	----	----	----
Muck	38.2	15.2	1.87	1.29	.50	.36

[1] Amount needed to eliminate grubs determined directly for this standard soil. All other values estimated from reactions of *Drosophila*.

These calculated values are subject to an experimental error owing to variations in the reactions of the insects. Since the dosages for 100-percent mortality of the grubs in the standard soil contributed little to this error, because the dosage selected for each insecticide exceeded the actual minimum required, there is little possibility of any variation in the mortality. The dosages for 50-percent mortality of the flies in the standard soil also contributed little to this error, because they were used to govern the exposure of the flies to other soils; a series of tests was terminated when the mortality with the standard reached 50 percent. In the other soils it was estimated that the dosages for 50-percent mortality of the flies were determined with an error of about 9 percent for DDT, 6 percent for toxaphene and chlordane, and 4 percent for dieldrin, heptachlor, and aldrin. It is believed, therefore, that the values given for DDT in table 10 have an experimental error of not more than 20 percent, those for toxaphene and chlordane of not more than 15 percent, and those for dieldrin, heptachlor, and aldrin of not more than 10 percent.

It appeared that not more than 25 percent of the authorized amount of chlordane, dieldrin, heptachlor, or aldrin was needed to eliminate the grubs in most of these test soils; therefore, at least 75 percent of the initial application was left to provide for the longevity of the treatments. However, 60 percent of the authorized amount of toxaphene was needed in the muck and the authorized amount of DDT appeared to be inadequate in that soil. It seemed that about one-third of the authorized amount of toxaphene would be adequate for all these mineral soils, but with DDT the dosages

for some mineral soils approached the authorized amount. It would seem that chlordane, dieldrin, heptachlor, and aldrin are adapted for use under a wider range of conditions in commercial nurseries than either DDT or toxaphene.

This pretreatment assay of soil should be of use to the Plant Pest Control Division in determining the suitability of a soil for treatment with one of the chlorinated hydrocarbon insecticides and in determining how much the residue in a soil could be decreased before retreatment was required to maintain the plot in a certified status.

TOXICITY OF MIXTURES OF CHLORINATED HYDROCARBON INSECTICIDES IN SOIL

A study was made of the toxicity of binary mixtures of the chlorinated hydrocarbon insecticides in Sassafras loam. Each insecticide was thoroughly mixed with the soil at a rate that would kill 50 percent of the *Drosophila* exposed to the soil for about 24 hours at 80° F. The rates were as follows:

Insecticide	*Pounds per 8-inch acre*
DDT	50
Toxaphene	15
Chlordane	1.07
Endrin	.44
Dieldrin	.28
Heptachlor	.07
Aldrin	.07

Each batch of soil was held for 5 days in a closed container to allow the insecticide and the soil to come to an equilibrium. Then 1:1 mixtures were prepared by blending equal weights of the batches. Each binary mixture contained each insecticide at a concentration of half that originally applied to the soil. *Drosophila* was exposed simultaneously to slurries of each binary mixture and the components of the mixture until the mortality with the mixture was approximately 50 percent. Then the mortality with the components was determined.

Following the procedure proposed by Horsfall (*75*), a graph was prepared for each mixture. The mortality with component *A* was on the left and that with component *B* on the right. A straight line connecting the points for *A* and *B* represented the mortality expected with mixtures of these components. The mortality with each binary mixture was compared with that expected for the mixture, and the difference was determined. These deviations in the mortality with the various mixtures are given in table 11.

A mortality significantly above the expected would show synergism and one significantly below would show antagonism between the components of a mixture. There was evidence of synergism with 11 of the 21 mixtures but no indication of antagonism. There appeared to be a synergistic action when DDT was mixed with aldrin, chlordane, dieldrin, endrin, heptachlor, or toxaphene; with mixtures of heptachlor and aldrin, toxaphene, or perhaps endrin; and with mixtures of toxaphene and aldrin or endrin.

TABLE 11.—*Deviation in mortality from that expected with flies* (Drosophila) *exposed to Sassafras sandy loam containing binary mixtures of the chlorinated hydrocarbon insecticides* [1]

Component *A*	Component *B*						
	Aldrin	Chlordane	DDT	Dieldrin	Endrin	Heptachlor	Toxaphene
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aldrin	-------	+0.2	+23.0	−5.4	+4.1	+16.6	+16.7
Chlordane	+0.2	-------	+23.2	+2.5	+1.5	+3.8	+5.8
DDT	+23.0	+23.2	-------	+15.2	+23.9	+24.4	+17.0
Dieldrin	−5.4	+2.5	+15.2	-------	−3.0	−3.3	+1.3
Endrin	+4.1	+1.5	+23.9	−3.0	-------	+8.9	+30.0
Heptachlor	+16.6	+3.8	+24.4	−3.3	+8.9	-------	+17.3
Toxaphene	+16.7	+5.8	+17.0	+1.3	+30.0	+17.3	-------

[1] A deviation of 8.2 percent is significant and one of 11.2 percent highly significant.

The toxicity of the other mixtures did not differ significantly from that expected. This finding indicates that with these mixtures the response of the flies to each component was not affected by the presence of the other component. Since there appeared to be no antagonism between any of these chlorinated hydrocarbon insecticides, a soil containing a residue of one of them may be retreated with one of the others without loss of toxicity due to a reaction between the insecticides, and sometimes the toxicity may be enhanced.

BIOASSAY OF TOXICITY IN FIELD PLOTS WITH RESIDUES OF DDT AND CHLORDANE

In the fall of 1959 samples of soil were taken from 14 plots in a commercial nursery to evaluate the toxicity of the soil to which during the past 10 years both DDT and chlordane had been applied to satisfy the requirements of the Japanese beetle quarantine. The DDT had been applied initially in 1950 and 1951. One or more supplementary applications of DDT had been made to some of the plots before the chlordane was applied during 1954–59. During the period since the initial insecticide treatment, all the plots have been plowed, harrowed, and planted in green manure crops for soil improvement. At the time of sampling, some of the plots were in a certified status with reference to the quarantine, others were uncertified.

The evaluations of the toxicity of the residues of DDT and chlordane in each sample were made with 200 third-instar Japanese beetle grubs and with 1,000 flies (*Drosophila*). The results of these determinations, expressed as equivalent pounds of chlordane per 3-inch acre, are summarized in table 12.

TABLE 12.—*Bioassay of toxicity of residues of DDT and chlordane in soil with Japanese beetle grubs and flies* (Drosophila)

[Toxicity as equivalent amounts of chlordane per 3-inch acre]

Sample No.	Total active toxicity			Toxicity attributed to chlordane
	With grubs	With flies	Average	
	Pounds	*Pounds*	*Pounds*	*Pounds*
1 [1]	1. 97	1. 91	1. 94	1. 94
2 [1]	1. 47	1. 91	1. 69	1. 86
3	. 50	. 20	. 35	. 25
4 [1]	1. 64	2. 70	2. 17	1. 98
5 [2]	4. 76	. 27		. 31
6	. 04	. 15	. 10	. 24
7 [1]	21. 60	20. 00	20. 80	16. 36
8 [1]	1. 59	1. 91	1. 75	1. 64
9 [1]	1. 51	1. 73	1. 62	. 44
10	. 39	. 86	. 63	. 38
11 [2]	3. 07	. 62		. 24
12 [1]	2. 92	3. 82	3. 37	3. 36
13	. 37	. 31	. 34	. 31
14	. 32	. 20	. 26	. 32

[1] More than 50-percent mortality of grubs and flies.
[2] More than 50-percent mortality of grubs and less than 50-percent mortality of flies.

The mortality of both test insects exceeded 50 percent with seven samples, was below 50 percent with five samples, and was in disagreement with two samples. The high mortality of the grubs and the low mortality of the flies with those two samples suggest that some insecticide in addition to the chlorinated hydrocarbons may have been in the soil of those plots. Since the grubs react to lead arsenate during the long period required for assay, and the flies are not affected by it during a 24-hour exposure, and since lead arsenate had been applied to many plots at the nursery prior to 1950, it is suspected that the discrepancy in the reaction of the grubs and the flies to those samples could be attributed to the presence of lead arsenate in the soil. The results of these bioassays show that the toxicity in the soil was adequate to kill newly hatched grubs in nine of the plots and inadequate in five of them.

Since the toxicity of chlordane can be evaluated in the presence of the normal amounts of DDT when *Drosophila* is used as the test insect, the toxicity of the mixtures that could be attributed to chlordane was determined. These determinations, expressed as equivalent pounds of chlordane per 3-inch acre, are also given in table 12. Most of the toxicity was caused by the more recently applied chlordane; the weathered DDT seemed to contribute little except in two of the plots.

SUMMARY

Residues of the chlorinated hydrocarbon insecticides applied to soil to control grubs of the Japanese beetle (*Popillia japonica*

Newman) may persist for several years. It is the practice of the Plant Pest Control Division of the Agricultural Research Service to analyze periodically the soil of treated plots in commercial nurseries subject to the restrictions of the quarantine on this pest and to require the application of additional insecticide as needed to maintain adequate toxicity. Applications are held to the minimum required, since inordinate amounts of these insecticides may be toxic to plants.

The analysis of soil becomes more complex when two or more of these insecticides, differing in insecticidal potency, have been applied to a soil, and when some of them in the soil are converted to other compounds. In the absence of suitable chemical methods for analyzing these complex residues, consideration was given to the development of a biological procedure to determine whether the toxicity was sufficient to kill newly hatched grubs.

A provisional method, using third-instar Japanese beetle grubs as test insects, was developed for assaying the toxicity of soil containing residues of aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, and toxaphene. This procedure was not practical, because it was slow and laborious and could be used only in the fall and winter.

After various organisms were considered as test animals, including fish, daphnids, shrimp, and many species of insects, the pomace fly *Drosophila melanogaster* Meigen appeared to be the most suitable substitute test organism.

A bioassay procedure was developed with *Drosophila* as a test insect for the direct evaluation of the toxicity in soil containing residues of these chlorinated hydrocarbon insecticides, alone or in combination. When 50 percent of the flies were killed with an exposure of 24 hours, the toxicity of a soil was adequate to prevent the development of newly hatched grubs. The toxicity may be expressed as equivalent pounds per acre of any one of these insecticides.

Substantially the same results were obtained in bioassays of field plots containing residues of aldrin, dieldrin, and heptachlor when both third-instar Japanese beetle grubs and *Drosophila* were used as test insects. About the same appraisal of toxicity was made with the grubs burrowing through and ingesting soil as with the flies coming into contact with only the surface soil.

Bioassays and chemical analyses were made of residues of the chlorinated hydrocarbon insecticides in experimental turf plots after these residues had weathered for various periods. Comparable results were obtained in the biological and chemical determinations of chlordane and DDT. There was initially fair agreement between the methods in the determinations of aldrin, dieldrin, heptachlor, and toxaphene, but as these insecticides weathered, larger amounts were obtained by chemical analysis than by bioassay. Since some of these insecticides are converted to other compounds in the soil, bioassay appears to be the more reliable and realistic method of assay.

In a study of the inactivation of the chlorinated hydrocarbon insecticides in various soils, it was assumed that practically all the insecticide applied to the least adsorptive soil would be insecticidally active and that the increase in dosage needed to obtain a comparable mortality of *Drosophila* with another soil was a measure of the

adsorptive and inactivating capacity of that soil. The amounts of these insecticides that were inactivated varied with the different soils; muck, the most adsorbent, inactivated about 90 percent. In view of the great differences in the amounts of the chlorinated hydrocarbon insecticides needed to obtain the same level of toxicity in various soils, more consideration should be given to the nature of the soil to which these insecticides are applied for control of the grubs.

There is need for a practical method to determine before treatment how much of a chlorinated hydrocarbon insecticide is actually required to assure the destruction of newly hatched grubs in a plot. With *Drosophila* as the test insect, a method was developed for estimating how much of each of these insecticides was required. In tests with 10 agricultural soils, it appeared that not more than one-fourth of the authorized amount of aldrin, chlordane, dieldrin, or heptachlor was needed to eliminate the grubs in these soils, and therefore, three-fourths of the initial application was left to provide for the longevity of the treatments. With toxaphene, about one-third of the authorized amount was needed for the mineral soils and about two-thirds for muck. With DDT, the required dosages for some of the mineral soils approached the authorized amount, and the dosage for muck exceeded it.

In a study of the toxicity of binary mixtures of the chlorinated hydrocarbon insecticides in Sassafras sandy loam, there appeared to be a synergistic action with mixtures of DDT and aldrin, chlordane, dieldrin, endrin, heptachlor, or toxaphene; with mixtures of heptachlor and aldrin, toxaphene, or perhaps endrin; and with mixtures of toxaphene and aldrin or endrin. The toxicity of the other mixtures did not differ significantly from that expected. This finding indicates that with these mixtures the response of *Drosophila* to each component was not affected by the presence of the other component. There was no evidence of antagonism between any of these insecticides.

Bioassays were made of plots in a commercial nursery, where from 1949 to 1959 both DDT and chlordane had been applied to satisfy the requirements of the Japanese beetle quarantine. About the same evaluations of the total toxicity in the soil were obtained with third-instar grubs as with *Drosophila* as test insects, except in two plots where a high toxicity was obtained with grubs and a low toxicity with flies. It is suspected that this discrepancy was due to the presence of lead arsenate in the soil of these plots. Most of the toxicity in the soil could be attributed to the more recently applied chlordane.

The development of a biological procedure for assaying residues of the chlorinated hydrocarbon insecticides, alone or in combination in soil, makes available a practical method for evaluating the toxicity and determining whether there is sufficient insecticide present to prevent the development of newly hatched Japanese beetle grubs. Since the toxicity is expressed in terms of a standard soil and only the active toxicity is measured, insecticides in soil with a high adsorptive capacity show a lower active toxicity than in soils with a low fixing power, even when the same total amount of insecticide is present in all of them.

LITERATURE CITED

(1) AGAZZI, E. J., PETERS, E. D., and BROOKS, F. R.
1953. COMBUSTION TECHNIQUES FOR THE DETERMINATION OF RESIDUES OF HIGHLY CHLORINATED PESTICIDES BY TOTAL CHLORINE ANALYSIS. Analyt. Chem. 25: 237–240.

(2) ALLEN, N., WALKER, R. L., FIFE, L. C., and others.
1954. PERSISTENCE OF BHC, DDT, AND TOXAPHENE IN SOIL AND THE TOLERANCES OF CERTAIN CROPS TO THEIR RESIDUES. U.S. Dept. Agr. Tech. Bul. 1090, 19 pp.

(3) ANDERSON, R. F.
1948. RELATION OF DROPLET SIZE TO TOXICITY IN RESIDUAL-TYPE DDT-OIL SPRAYS. Jour. Econ. Ent. 41: 974–976.

(4) ARNOLD, E. W., and APPLE, J. W.
1957. THE COMPATIBILITY OF INSECTICIDES AND FUNGICIDES USED FOR THE TREATMENT OF CORN SEED. Jour. Econ. Ent. 50: 43–45.

(5) BANKS, W. A., and REED, J. K.
1956. BIOASSAY OF DIELDRIN IN SOIL USING LARVAE OF AEDES AEGYPTI (L.). (Abstract) Assoc. South. Agr. Workers Proc. 53: 136.

(6) BARLOW, F., and HADAWAY, A. B.
1955. STUDIES ON AQUEOUS SUSPENSIONS OF INSECTICIDES. PART V. SORPTION OF INSECTICIDES BY SOILS. Bul. Ent. Res. 46: 547–559.

(7) ———
1958. STUDIES ON AQUEOUS SUSPENSIONS OF INSECTICIDES. PART VI. FURTHER NOTES ON THE SORPTION OF INSECTICIDES BY SOILS. Bul. Ent. Res. 49: 315–331.

(8) ———
1958. STUDIES ON AQUEOUS SUSPENSION OF INSECTICIDES. PART VII. THE INFLUENCE OF RELATIVE HUMIDITY UPON THE SORPTION OF INSECTICIDES BY SOILS. Bul. Ent. Res. 49: 333–354.

(9) BARTLETT, B. R.
1951. A NEW METHOD FOR REARING DROSOPHILA AND A TECHNIQUE FOR TESTING INSECTICIDES WITH THIS INSECT. Jour. Econ. Ent. 44: 621.

(10) ———
1952. A STUDY OF INSECTICIDAL RESISTANCE IN STRAINS OF DROSOPHILA MELANOGASTER MEIG. Canad. Ent. 84: 189–205.

(11) BEARD, A. L.
1952. EFFECT OF SUB-LETHAL DOSAGES OF TOXICANTS ON THE SUSCEPTIBILITY OF INSECTS TO INSECTICIDES. Jour. Econ. Ent. 45: 561–567.

(12) BERG, P. W.
1949. LABORATORY TESTS ON THE TOXICITY OF TECHNICAL DDT, DDT ISOMERS AND ANALOGUES, AND DDD. Ind. Acad. Sci. Proc. 59: 157–163.

(13) BERGER, R. S., and RANDOLPH, N. M.
1958. THE DETERMINATION OF SOME INSECTICIDAL RESIDUES IN SOILS BY BIOASSAY. Jour. Econ. Ent. 51: 186–187.

(14) BLISS, C. I.
1935. THE CALCULATION OF THE DOSAGE-MORTALITY CURVE. Ann. Appl. Biol. 22: 134–167.

(15) ———
1935. THE COMPARISON ON DOSAGE-MORTALITY DATA. Ann. Appl. Biol. 22: 307–333.

(16) ———
1938. THE DETERMINATION OF THE DOSAGE-MORTALITY CURVE FROM SMALL NUMBERS. Quart. Jour. Pharm. and Pharmacol. 11: 192–216.

(17) ———
1939. FLY SPRAY TESTING. Soap 12: 103, 105, 107, 109, 111.

(18) ———
1939. THE TOXICITY OF POISONS APPLIED JOINTLY. Ann. Appl. Biol. 26: 585–615.

(19) ———
1945. CONFIDENCE LIMITS FOR BIOLOGICAL ASSAYS. Biometrics Bul. 1: 57–65.

(20) ———
1956. CONFIDENCE LIMITS FOR MEASURING THE PRECISION OF BIOASSAYS. Biometrics Bul. 12: 491–526.

(21) BLISS, C. I.
1957. SOME PRINCIPLES OF BIOASSAY. Amer. Sci. 45: 449–466.
(22) ——— and BEARD, R. L.
1953. STATIC AND DYNAMIC VARIATION IN THE RESPONSE OF AN INSECT TO SUBLETHAL DOSES OF TWO GASES. Conn. Agr. Expt. Sta. Bul. 577, 24 pp.
(23) ——— and BROADBENT, B. M.
1935. A COMPARISON OF CRITERIA OF SUSCEPTIBILITY IN THE RESPONSE OF DROSOPHILA TO HYDROCYANIC ACID GAS. I. STUPEFACTION TIME AND MORTALITY. Jour. Econ. Ent. 28: 989–1001.
(24) ——— and CATTELL, McK.
1943. BIOLOGICAL ASSAY. Ann. Rev. Physiol. 5: 479–539.
(25) ——— and MARKS, H. P.
1939. THE BIOLOGICAL ASSAY OF INSULIN. II. THE ESTIMATION OF DRUG POTENCY FROM A GRADED RESPONSE. Quart. Jour. Pharm. and Pharmacol. 12: 182–205.
(26) BOCHNIG, V.
1954. GENETISCHE UNTERSUCHUNGEN ZUR DDT RESISTENZ AN DROSOPHILA MELANOGASTER. Ztschr. f. Induktive Abstam. u. Vererbungslehre 86: 185–209.
(27) BOLLEN, W. B., ROBERTS, J. E., and MORRISON, H. E.
1958. SOIL PROPERTIES AND FACTORS INFLUENCING ALDRIN-DIELDRIN RECOVERY AND TRANSFORMATION. Jour. Econ. Ent. 51: 214–219.
(28) BOYCE, A. M.
1928. STUDIES ON THE RESISTANCE OF CERTAIN INSECTS TO HYDROCYANIC ACID. Jour. Econ. Ent. 21: 715–720.
(29) BROADBENT, B. M., and BLISS, C. I.
1936. COMPARATIVE CRITERIA OF SUSCEPTIBILITY IN RESPONSE OF DROSOPHILA TO HYDROCYANIC ACID GAS. II. RECOVERY TIME. Jour. Econ. Ent. 29: 143–155.
(30) BROWNING, H. C., FRASER, F. C., SHAPIRO, S. K., and others.
1948. THE BIOLOGICAL ACTIVITY OF DDT AND RELATED COMPOUNDS. Canad. Jour. Res. Sect. D, Zool. Sci. 26: 282–300.
(31) ——— SHAPIRO, S. K., and DUBRULE, M.
1948. THE INSECTICIDAL ACTIVITY OF DDT AND RELATED COMPOUNDS AGAINST DIFFERENT INSECT SPECIES. Canad. Jour. Res. Sect. D, Zool. Sci. 26: 301–306.
(32) BUSVINE, J. R., and BARNES, S.
1957. A CRITICAL REVIEW OF THE TECHNIQUES FOR TESTING INSECTICIDES. 208 pp. Commonwealth Institute of Entomology, London.
(33) CALDWELL, A. H.
1949. MASS REARING OF DROSOPHILA. Jour. Econ. Ent. 42: 707.
(34) CARTER, R. H.
1955. STATUS OF ANALYTICAL METHODS WITH RESPECT TO THE DETERMINATION OF MINIMAL QUANTITIES OF INSECTICIDES. Jour. Econ. Ent. 48: 424–425.
(35) CHISHOLM, R. D., and KOBLITSKY, L.
1952. TESTING SOILS FOR INSECTICIDE RESIDUES. Agr. Chem. 7: 47–49, 119.
(36) ——— and KOBLITSKY, L.
1959. ACCUMULATION AND DISSIPATION OF PESTICIDE RESIDUES IN SOIL. No. Amer. Wildlife Conf. Trans. 24: 118–123.
(37) ——— KOBLITSKY, L., FAHEY, J. E., and WESTLAKE, W. E.
1950. DDT RESIDUES IN SOIL. Jour. Econ. Ent. 43: 941–942.
(38) ——— NELSON, R. H., and FLECK, E. E.
1949. THE TOXICITY OF DDT DEPOSITS AS INFLUENCED BY SUNLIGHT. Jour. Econ. Ent. 42: 154–155.
(39) CROW, J. F.
1954. ANALYSIS OF A DDT-RESISTANT STRAIN OF DROSOPHILA. Jour. Econ. Ent. 47: 393–398.
(40) DANISH, A. A., and LIDOV, R. E.
1950. COLORIMETRIC METHOD FOR ESTIMATING SMALL AMOUNTS OF ALDRIN. Analyt. Chem. 22: 702–706.
(41) DAVIDOW, B., and SABATINO, F. J.
1954. BIOLOGICAL SCREENING TEST FOR CHLORINATED INSECTICIDES. Assoc. Off. Agr. Chem. Jour. 37: 902–905.

(42) DAVIS, A. C.
1951. THE BIOASSAY FOR ANALYSIS OF TOXIC SPRAY RESIDUES. Farm Res. 17 (3): 3.
(43) DEMEREC, M.
1950. BIOLOGY OF DROSOPHILA. 632 pp. New York.
(44) ——— and KAUFMANN, B. P.
1950. DROSOPHILA GUIDE. Ed. 5, 44 pp. Carnegie Institution, Washington, D.C.
(45) DE SCOEUX, F. H.
1952. HEREDITARY CARBON DIOXIDE SENSITIVITY IN DROSOPHILA. ATTEMPTS AT PURIFICATION OF THE GENOID. Rev. Canad. de Biol. 11: 1–3.
(46) DEWEY, J. E.
1958. UTILITY OF BIOASSAY IN THE DETERMINATION OF PESTICIDE RESIDUES. Jour. Agr. and Food Chem. 6: 274–281.
(47) DORMAL, S., DELVAUS, E. L., DILLS, L. E., and FREAR, D. E. H.
1950. SYNTHESIS OF CHLORINATED AROMATIC COMPOUNDS, CHEMICAL STRUCTURE, AND INSECTICIDAL EFFICIENCY. Jour. Econ. Ent. 43: 915–918.
(48) DORST, H. E.
1959. LABORATORY TESTS WITH INSECTICIDES AGAINST DROSOPHILA MELANOGASTER. Jour. Econ. Ent. 52: 172.
(49) EARLE, N. W., PANKASKIE, J. E., and SUN, Y. P.
1959. MICROBIOASSAY OF INSECTICIDE RESIDUES IN PLANT TISSUES WITHOUT EXTRACTION, WITH SPECIAL REFERENCE TO ALDRIN AND DIELDRIN. Assoc. Off. Agr. Chem. Jour. 42: 586–592.
(50) EDWARDS, C. A., BECK, S. D., and LICHTENSTEIN, E. P.
1957. BIOASSAY OF ALDRIN AND LINDANE IN SOIL. Jour. Econ. Ent. 50: 622–626.
(51) EHRENHARDT, H.
1954. ÜBER DIE WIRKUNG DES HEXACHLORCYCLOHEXANS ALS SYSTEMISCHES INSEKTIZID. Anz. f. Schädlingsk. 27 (pt. 1): 1–5.
(52) FINNEY, D. J.
1952. THE ANALYSIS OF TOXICITY TESTS ON MIXTURES OF POISONS. Ann. Appl. Biol. 29: 82–94.
(53) ———
1952. STATISTICAL METHOD IN BIOLOGICAL ASSAY. 661 pp. New York.
(54) FISHER, R. W., and SMALLMAN, B. N.
1954. STUDIES ON A DIRECT FEEDING METHOD FOR USE IN BIOASSAY OF INSECTICIDE RESIDUES. Canad. Ent. 86: 562–569.
(55) FLEMING, W. E.
1950. PERSISTENCE OF EFFECT OF DDT ON JAPANESE BEETLE LARVAE IN NEW JERSEY SOILS. Jour. Econ. Ent. 43: 87–89.
(56) ———
1960. THE JAPANESE BEETLE: HOW TO CONTROL IT. U.S. Dept. Agr. Farmers' Bul. 2151, 16 pp.
(57) ———COLES, L. W., and MAINES, W. W.
1951. BIOLOGICAL ASSAY OF RESIDUES OF DDT AND CHLORDANE IN SOIL USING MACROCENTRUS ANCYLIVORUS AS A TEST INSECT. Jour. Econ. Ent. 44: 310–315.
(58) ——— and MAINES, W. W.
1947. THE EFFECTIVENESS AND DURATION OF TREATMENTS WITH TECHNICAL DDT IN DIFFERENT SOILS AGAINST LARVAE OF THE JAPANESE BEETLE. U.S. Bur. Ent. and Plant Quar. E-716, 20 pp. [Processed.]
(59) ——— and MAINES, W. W.
1951. EXPERIMENTS WITH TOXAPHENE AGAINST THE JAPANESE BEETLE. U.S. Bur. Ent. and Plant Quar. E-821, 9 pp. [Processed.]
(60) ——— and MAINES, W. W.
1953. PERSISTENCE OF DDT IN SOILS OF THE AREA INFESTED BY THE JAPANESE BEETLE. Jour. Econ. Ent. 46: 445–449.
(61) ——— and MAINES, W. W.
1954. PERSISTENCE OF CHLORDANE IN SOILS OF THE AREA INFESTED BY THE JAPANESE BEETLE. Jour. Econ. Ent. 47: 165–169.
(62) ——— MAINES, W. W., and COLES, L. W.
1951. PERSISTENCE OF CHLORINATED HYDROCARBON INSECTICIDES IN TURF TREATED TO CONTROL THE JAPANESE BEETLE. U.S. Bur. Ent. and Plant Quar. E-829, 6 pp. [Processed.]

(63) FORBES, A. R., and KING, K. M.
1957. CONTROL OF ROOT MAGGOTS IN RUTABAGAS, ESPECIALLY IN MUCK SOILS. Jour. Econ. Ent. 50: 89–91.

(64) GADDUM, J. H.
1933. REPORTS ON BIOLOGICAL STANDARDS. III. METHODS OF BIOLOGICAL ASSAY DEPENDING UPON A QUANTAL RESPONSE. [Gt. Brit.] Med. Res. Council, Spec. Rpt. 183, 46 pp.

(65) GANNON, N., and BIGGER, J. H.
1958. THE CONVERSION OF ALDRIN AND HEPTACHLOR TO THEIR EPOXIDES IN SOIL. Jour. Econ. Ent. 51: 1–2.

(66) GEROLT, P.
1957. METHOD FOR BREEDING, HANDLING AND SEXING ADULTS OF DROSOPHILA MELANOGASTER MG. AS A TEST INSECT FOR BIOASSAY. Bul. Ent. Res. 48: 311–315.

(67) GERSDORFF, W. A., NELSON, R. H., and MITLIN, N.
1950. CHLORINATED HYDROCARBONS. Soap and Sanit. Chem. 26 (4): 137–139.

(68) GLASSER, R. F., BLENK, R. G., DEWEY, J. E., and others.
1958. OCCURRENCE OF A TOXIC NON-ALDRIN RESIDUE IN CARROTS GROWN IN ALDRIN-TREATED SOIL. Jour. Econ. Ent. 51: 337–341.

(69) GUNTHER, F. A., and BLINN, R. C.
1953. PESTICIDE RESIDUES; BASIC PRINCIPLES FOR QUANTITATIVE DETERMINATION. Jour. Agr. and Food Chem. 1: 325–330.

(70) GUTHRIE, F. E., RABB, R. L., and BOWERY, T. G.
1959. EVALUATION OF CANDIDATE INSECTICIDES AND INSECT PATHOGENS FOR TOBACCO HORNWORM CONTROL, 1956–1958. Jour. Econ. Ent. 52: 798–804.

(71) GYRISCO, G. G., EVANS, W. G., BURRAGE, R. H., and BRIANT, A. M.
1955. FURTHER STUDIES ON THE EFFECTS OF SOIL TREATMENTS WITH INSECTICIDES ON RESIDUES AND FRUIT QUALITY OF STRAWBERRIES. Jour. Econ. Ent. 48: 700–706.

(72) HARWOOD, R. F., and AREEKUL, S.
1957. A REARING TRAP FOR PRODUCING POMACE FLIES FOR BIOASSAY OF INSECTICIDES. Jour. Econ. Ent. 50: 512–513.

(73) ——— and AREEKUL, S.
1960. IDENTIFICATION OF INSECTICIDES AND ACARICIDES BY COMPARATIVE BIOASSAY. Science 131: 1369–1370.

(74) HEALY, M. J. R.
1956. PRINCIPLES OF BIOLOGICAL ASSAY. *In* Paech, K., and Tracey, M. V., Modern Methods of Plant Analysis, v. I, pp. 305–324. Berlin.

(75) HORSFALL, J. G.
1945. FUNGICIDES AND THEIR ACTION. 239 pp. Chronica Botanica, Waltham, Mass.

(76) HOSKINS, W. M.
1957. BIOASSAY IN ENTOMOLOGICAL RESEARCH. Ztschr. f. Pflanzenkrank. u. Pflanzenschutz 64: 498–505.

(77) JOHNSON, V. A., and CASKEY, W. C.
1952. SOIL SAMPLER. U.S. Bur. Ent. and Plant Quar. ET–298, 4 pp. [Processed.]

(78) KASTING, R., and HARCOURT, D. G.
1952. PARATHION RESIDUES OF CAULIFLOWER HEADS AFTER SPRAYING. Sci. Agr. 32: 299–303.

(79) KERR, R. W.
1954. REARING DROSOPHILA MELANOGASTER MG. FOR INSECTICIDE INVESTIGATIONS. Bul. Ent. Res. 45: 313–316.

(80) ———
1954. VARIATION WITH AGE IN THE SUSCEPTIBILITY TO DDT AND THE RESPIRATION RATE OF MALE AND FEMALE DROSOPHILA MELANOGASTER. Bul. Ent. Res. 45: 323–328.

(81) KITGEMAGI, U., MORRISON, H. E., ROBERTS, J. E., and BOLLEN, W. B.
1958. BIOLOGICAL AND CHEMICAL STUDIES ON THE DECLINE OF SOIL INSECTICIDES. Jour. Econ. Ent. 51: 198–204.

(82) KING, J. C.
1954. THE GENETICS OF RESISTANCE TO DDT IN DROSOPHILA MELANOGASTER. Jour. Econ. Ent. 47: 387 393.

(83) KLOKE, A.
1953. DIE ANWENDUNG VON PFLANZENSCHUTZMITTELN UND DAS BIOLOGISCHE GLEICHGEWICHT IM BODEN. Ztschr. f. Pflanzenernähr. Dungung u. Bodenk. 63: 150–154.

(84) KNUTSON, H.
1953. SUSCEPTIBILITY OF A NORMAL AND AN INBRED STRAIN OF DROSOPHILA MELANOGASTER TO DIELDRIN, TOXAPHENE, DDT AND METHOXYCHLOR. Jour. Econ. Ent. 46: 137–141.

(85) KOBLITSKY, L., and CHISHOLM, R. D.
1949. DETERMINATION OF DDT IN SOILS. Assoc. Off. Agr. Chem. Jour. 32: 781–786.

(86) KOEHLER, C. S., MUKA, A. A., GYRISCO, G. G., and EDMONDS, C. M.
1958. STUDIES ON THE CONTROL OF THE ALFALFA SNOUT BEETLE LARVA. Jour. Econ. Ent. 51: 682–685.

(87) LANGE, W. H., and CARLSON, E. C.
1955. ZONAL DISPERSION OF CHEMICALS IN SOIL FOLLOWING SEVERAL TILLAGE METHODS. Jour. Econ. Ent. 48: 61–67.

(88) LAUG, E. P.
1948. TISSUE DISTRIBUTION OF A TOXICANT FOLLOWING ORAL INGESTION OF THE GAMMA ISOMER OF BENZENE HEXACHLORIDE BY RATS. Jour. Pharmacol. and Expt. Ther. 93: 277–281.

(89) L'HERITIER, P., and SIGOT, A.
1948. CONTRIBUTION À L'ÉTUDE DU PHÉNOMÈNE DE LA SENSIBILITÉ AU CO_2 CHEZ LA DROSOPHILE. INFLUENCE DU CHAUFFAGE AUX DIFFÉRENTS STADES DU DÉVELOPPEMENT SUR LA MANIFESTATION DE LA SENSIBILITÉ CHEZ L'IMAGO. Ann. Biol. [Paris] 52: 88–89.

(90) LICHTENSTEIN, E. P.
1958. THE ADVANTAGES AND DISADVANTAGES ASSOCIATED WITH BIOASSAY OF SOILS AND CROPS USING A DIRECT EXPOSURE METHOD WITH DROSOPHILA. Ent. Soc. Amer., No. Cent. States Br., Proc. 13: 44.

(91) ——— DEPEW, L. J., ESHBAUGH, E. L., and SLEESMAN, J. P.
1960. PERSISTENCE OF DDT, ALDRIN, AND LINDANE IN SOME MIDWESTERN SOILS. Jour. Econ. Ent. 53: 136–142.

(92) ——— and MEDLER, J. T.
1958. PERSISTENCE OF ALDRIN AND HEPTACHLOR RESIDUES ON ALFALFA. Jour. Econ. Ent. 51: 222–226.

(93) ——— and POLIVKA, J. B.
1959. PERSISTENCE OF SOME CHLORINATED HYDROCARBON INSECTICIDES IN TURF SOILS. Jour. Econ. Ent. 52: 289–293.

(94) ——— and SCHULTZ, K. R.
1959. BREAKDOWN OF LINDANE AND ALDRIN IN SOILS. Jour. Econ. Ent. 52: 118–124.

(95) ——— and SCHULZ, K. R.
1960. EPOXIDATION OF ALDRIN AND HEPTACHLOR IN SOILS AS INFLUENCED BY AUTOCLAVING, MOISTURE, AND SOIL TYPE. Jour. Econ. Ent. 53: 192–197.

(96) LINDSAY, D. R., and HAINES, T. W.
1951. A METHOD OF TESTING THE RESISTANCE OF HOUSE FLIES TO RESIDUAL-TYPE INSECTICIDES. Jour. Econ. Ent. 44: 104–106.

(97) LORD, F. T.
1942. THE RELATIVE SUSCEPTIBILITY OF THE SEXES OF DROSOPHILA MELANOGASTER MEIG. TO NICOTINE ALKALOID USED IN CONTACT INSECTICIDES. Ontario Ent. Soc. Ann. Rpt. 72 (1941): 32–34.

(98) ———
1944. THE USE OF DROSOPHILA MELANOGASTER MEIG. FOR COMPARING THE TOXICITY OF STOMACH POISON DUSTS. Sci. Agr. 24: 320–326.

(99) MCLEOD, W. S.
1944. FURTHER REFINEMENT OF A TECHNIQUE FOR TESTING CONTACT INSECTICIDES. Canad. Jour. Res. Sect. D, Zool. Sci. 22: 87–104.

(100) MAXWELL, K. E., and SWAIN, A. F.
1949. TETRAETHYL PYROPHOSPHATE DUSTS. Jour. Econ. Ent. 42: 848–849.

(101) MAYER, E. L., MCGOVRAN, E. R., TALLEY, F. B., and WILLAMAN, J. J.
1950. TESTS FOR SYNERGISM BETWEEN NICOTINE AND PHTHALONITRILE AND BETWEEN NICOTINE AND 2,3,4,5,6-PENTACHLOROANISOLE. Jour. Econ. Ent. 43: 533–537.

(102) MERRELL, D. J., and UNDERHILL, J. C.
1956. SELECTION FOR DDT RESISTANCE IN INBRED, LABORATORY AND WILD STOCKS OF DROSOPHILA MELANOGASTER. Jour. Econ. Ent. 49: 300–306.
(103) METCALF, R. L.
1948. SOME INSECTICIDAL PROPERTIES OF FLUORINE ANALOGUES OF DDT. Jour. Econ. Ent. 41: 416–421.
(104) ——— and LINDGREN, D. L.
1948. INSECTICIDAL PROPERTIES OF ELEMENTAL FLUORINE. Jour. Econ. Ent. 41: 522.
(105) MORRISON, F. O.
1943. THE STANDARDIZING OF A LABORATORY METHOD FOR COMPARING THE TOXICITY OF CONTACT INSECTICIDES. Canad. Jour. Res. Sect. D, Zool. Sci. 21: 35–75.
(106) ———
1946. COMPARING THE TOXICITY OF SYNTHETIC ORGANIC COMPOUNDS. Ontario Ent. Soc. Ann. Rpt. 76 (1945): 18–20.
(107) MUNSON, S. C., PADILLA, G. M., and WEISSMANN, M. L.
1954. INSECT LIPIDS AND INSECTICIDAL ACTION. Jour. Econ. Ent. 47: 578–587.
(108) NAGASAWA, S.
1959. BIOLOGICAL ASSAY OF INSECTICIDE RESIDUES. *In* Steinhaus, E. A., and Smith, R. F., eds., Annual Review of Entomology, v. 4, pp. 319–342. Palo Alto, Calif.
(109) NELSON, R. H.
1949. A LABORATORY METHOD FOR EVALUATING DDT RESIDUES. Jour. Econ. Ent. 42: 151.
(110) NEWMAN, J. F., AZIZ, M. A., and KOSHI, T.
1949. STUDIES IN CONTACT TOXICITY. PT. 1. RESISTANCE OF SUCCESSIVE GENERATIONS OF CULEX FATIGANS WIED. TO INSECTICIDES. Indian Acad. Sci. Proc. 30B: 61–68.
(111) O'DONNELL, A. E., JOHNSON, H. W., JR., and WEISS, T. F.
1955. CHEMICAL DETERMINATION OF DIELDRIN IN CROP MATERIALS. Jour. Agr. and Food Chem. 3: 757–762.
(112) ——— NEAL, M. M., WEISS, F. T., and others.
1954. CHEMICAL DETERMINATION OF ALDRIN IN CROP MATERIALS. Jour. Agr. and Food Chem. 2: 573–580.
(113) O'KANE, W. C., WESTGATE, W. A., and GLOVER, L. C.
1934. METHODS OF EXPRESSING TOXICITY. N.H. Agr. Expt. Sta. Tech. Bul. 58, 13 pp.
(114) OLSON, R. E.
1954. THE USE OF THE FRUIT FLY DROSOPHILA MELANOGASTER IN DETECTING MINUTE QUANTITIES OF BENZENE HEXACHLORIDE IN PLANT TISSUES. Ph. D. thesis, Cornell Univ., Ithaca, N.Y., Univ. Microfilms, Ann Arbor Mich. Pub. 9767.
(115) OPPENOORTH, F. J., and DRESDEN, D.
1953. SELECTION OF A BHC-RESISTANT STRAIN OF DROSOPHILA MELANOGASTER MG. Bul. Ent. Res. 44: 395–400.
(116) ORDAS, E. P., SMITH, V. C., and MEYER, C. F.
1956. SPECTROPHOTOMETRIC DETERMINATION OF HEPTACHLOR AND TECHNICAL CHLORDANE ON FOOD AND FORAGE CROPS. Jour. Agr. and Food Chem. 4: 444–451.
(117) PANKASKIE, J. E., and SUN, Y. P.
1954. BIOASSAY OF CERTAIN INSECTICIDAL RESIDUES BY EXPOSING DROSOPHILA MELANOGASTER MEIG. TO MACERATED FOOD. Cong. Internatl. de Phytopharm. (1952) [Compt. Rend.] 3 (2): 52–55.
(118) PATTERSON, N. A.
1935. INDICES OF TOXICITY OF VARIOUS POISONS TO DROSOPHILA AMPELOPHILA LOEW. Ontario Ent. Soc. Ann. Rpt. 65 (1934): 78–80.
(119) POLEN, P. B., and SILVERMAN, P.
1952. DETECTION AND DETERMINATION OF 1,4,5,6,7,8,8-HEPTACHLORO-3A,4,7,7A-TETRAHYDRO-4,7-METHANOINDENE. Analyt. Chem. 24: 733–35.
(120) PROVERBS, M. D., and MORRISON, F. O.
1947. THE RELATIVE INSECTICIDAL ACTIVITIES OF DDT AND RELATED ORGANIC MOLECULES. Canad. Jour. Res. Sect. D, Zool. Sci. 25: 12–44.

(121) RIEMSCHNEIDER, R., and ROHRMANN, B.
1950. ÜBER DIE ZUCHT DDT-RESISTENTER DROSOPHILA MELANOGASTER M. Anz. f. Schädlingsk. 23: 148–149.

(122) SCHECHTER, M. S., and HORNSTEIN, I.
1952. COLORIMETRIC DETERMINATION OF BENZENE HEXACHLORIDE. Analyt. Chem. 25: 544.

(123) SOKAL, R. R.
1958. ENVIRONMENTALLY CAUSED FLUCTUATIONS IN QUANTITATIVE CHARACTERS OF DROSOPHILA. 10th Internatl. Cong. Ent. (Montreal 1956) Proc. 2: 843–854.

(124) STULTZ, H. T.
1939. METHODS AND MATERIALS OF A NEW TECHNIQUE FOR USING POMACE FLIES IN BIOLOGICAL TESTS WITH CONTACT INSECTICIDES. Ontario Ent. Soc. Ann. Rpt. 70 (1939): 72–80.

(125) SUN, Y. P., and PANKASKIE, J. E.
1954. DROSOPHILA, A SENSITIVE INSECT, FOR MICROBIOASSAY OF INSECTICIDE RESIDUES. Jour. Econ. Ent. 47: 180–181.

(126) TATTERSFIELD, F., KERRIDGE, J. B., and TAYLOR, J.
1953. THE EFFECT OF REPEATED SPRAYING OF INSECTS IN INCREASING THEIR RESISTANCE TO INSECTICIDES. I. DEVELOPMENT OF RESISTANCE TO DDT BY DROSOPHILA. II. EFFECT OF CO_2 SENSITIVITY ON TOXICITY OF DDT TO DROSOPHILA. Ann. Appl. Biol. 40: 498–536.

(127) TERRIERE, L. C., and INGALSBE, D. W.
1953. TRANSLOCATION AND RESIDUAL ACTION OF SOIL INSECTICIDES. Jour. Econ. Ent. 46: 751–753.

(128) U.S. DEPARTMENT OF AGRICULTURE, PLANT PEST CONTROL DIVISION.
1958. MANUAL OF APPROVED TREATMENT PROCEDURES TO BE USED UNDER THE JAPANESE BEETLE QUARANTINE. 30 pp. Washington, D.C. [Processed.]

(129) VAN MIDDELEM, C. H.
1958. HOW TO GET VALID PESTICIDE RESIDUE DATA. Jour. Agr. and Food Chem. 6: 581–582.

(130) WADLEY, F. M.
1943. STATISTICAL ASPECTS OF LABORATORY TESTS OF INSECTICIDES. *In* Campbell, F. L., and Moulton, F. R., eds., Laboratory Procedures in Studies of the Chemical Control of Insects. American Association for the Advancement of Science Publication 20, pp. 177–188. Washington, D.C.

(131) ———
1955. ADAPTATION OF DOSAGE-MORTALITY CURVES TO MACHINE CALCULATION. Jour. Econ. Ent. 48: 335–336.

(132) ——— and SULLIVAN, W. M.
1943. A STUDY OF THE DOSAGE-MORTALITY CURVE. Jour. Econ. Ent. 36: 367–371.

(133) WAKSMAN, S. A.
1927. PRINCIPLES OF MICROBIOLOGY. 897 pp. Baltimore, Md.

(134) WILLIAMS, D. D.
1951. TEMPERATURE EFFECTS ON THE PREVALENCE OF MALE AND FEMALE DROSOPHILA MELANOGASTER MEIGEN. N.Y. Ent. Soc. Jour. 59: 213–216.

(135) WYLIE, W. D.
1956. DETERMINATION OF INSECTICIDAL RESIDUES IN SOIL USING DROSOPHILA. Jour. Econ. Ent. 49: 638–640.

(136) YOUNG, W. R., and RAWLINS, W. A.
1958. THE PERSISTENCE OF HEPTACHLOR IN SOILS. Jour. Econ. Ent. 51: 11–18.

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