BIOLOGICAL ASSAY OF INSECTICIDES IN
PROCESSED VEGETABLES

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the
Graduate School of The Ohio State
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By

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I. Introduction

Tolerances and exemptions from tolerances for pesticide chemicals in or on raw agricultural commodities have been established by the Federal Food and Drug Administration (5U). Tolerances for pesticide residues in or on fresh fruits and vegetables have been established. The findings of fact and regulations are based on the Food and Drug Administration hearings (13, 14) held in 1950, and published data and information collected relative to many important compounds up until 1950. The findings of fact and regulations do not take into account technical and research advances made since 1950.

Tolerances have been established by the recent order for such important organic insecticides as the following:

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>1,2,3,4,10,10-hexachloro-1,4,9a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene</td>
</tr>
<tr>
<td>Chlordane</td>
<td>1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene</td>
</tr>
<tr>
<td>DDD</td>
<td>1,1-dichloro-2,2-bis(p-chlorophenyl) ethane</td>
</tr>
<tr>
<td>DDT</td>
<td>1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,9a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene</td>
</tr>
<tr>
<td>EPN</td>
<td>O-ethyl O-paranitrophenyl benzene thiophosphonate</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene</td>
</tr>
<tr>
<td>Insecticide</td>
<td>Chemical Name</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Lindane</td>
<td>The gamma isomer of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane)</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane</td>
</tr>
<tr>
<td>Parathion</td>
<td>0,0-diethyl-O-paranitrophenyl thiophosphate</td>
</tr>
<tr>
<td>TEPP</td>
<td>Tetraethyl pyrophosphate</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>Chlorinated camphene</td>
</tr>
</tbody>
</table>

The Federal Food, Drug and Cosmetic Act of 1938 (Section 106) empowers the Secretary of the Department of Health, Education and Welfare (formerly the Federal Security Administrator) to promulgate regulations limiting the quantity of poisonous or deleterious substances in food.

The Miller bill or Public Law 518 becomes Section 408 of the Food, Drug and Cosmetic Act of 1938. Section 408 outlines the responsibilities of all concerned with production and processing. The regulations will become effective on July 22, 1955, one year after the signing of the bill by the President, unless there is an extension of the above date.

Public opinion concerning the importance of spray residues is at a very high peak. The hazard to health has increased greatly since the advent of many compounds which are much more toxic to animals than DDT. Much work has been done from the physiological and toxicological approach. Chronic toxicity, the toxicity due to the assimilation of small amounts of toxicant over a long period of time, i.e., the residues in or on foodstuffs, has been the concern
of the toxicologist and of great interest to the public.

Many more useful organic insecticides have been manufactured and approved for use on agricultural commodities since 1950. These toxicants must be continually tested in the field and laboratory before practical tolerances may be evoked. Many conscientious groups associated with either the safeguarding of public health, the protection of the world's food supply, or the manufacturing or control of the chemicals themselves are conducting these field and laboratory experiments.

There are many facets to the problem of insecticidal residues on or in foodstuffs. Since the tolerance for an insecticide is established for many of the fruits and vegetables, a great burden of responsibility will be placed on the food processing industry. According to Hartsell and Storrs (29), the processors will have to know what materials were used on the crop, what concentrations remain at harvest, and whether these toxic compounds will persist throughout washing and the many processing operations. This is indeed a large assignment when one considers the many new compounds on the market and the many others in the developmental stage. There is a great need for simple, practical analytical methods for the quantitative detection of these insecticides, and for information on the fate of the different insecticides at each stage of processing.

At the present time there are two general types of analysis for the quantitative detection of insecticidal residues, chemical analysis and biological assay.

Several chemical methods have been developed for the
determinations of small amounts of certain insecticidal residues. In general these chemical methods involve colorimetric procedures. A good rapid colorimetric analysis is very desirable. Many workers are now striving to develop new colorimetric methods and perfect established methods.

Chemical procedure includes such spectrophotometric methods as the Schechter-Haller Method (46) for DDT, its metabolites and analogs, the Averell-Norris Method (3) for organic phosphorous insecticides, the detection of dieldrin residues by a new method published by Gunther, Kolbezon, and Blinn (26), and the Danish and Lidou (17) method for the determination of aldrin.

The total organic chlorine determination as developed by Koblitsky and Chisholm (35) and the potentiometric chloride method of Helmkaop et. al. (30) are often used for the quantitative determination of chlorinated organic insecticides.

Metcalf and March (40, 41) have used paper chromatographic procedures for the quantitative estimation of parathion and related phosphate esters and for the systemic insecticides, systox (O,O-diethyl-O-ethylmercapto-ethyl thiophosphate) and schradan (octamethyl pyrophosphoramide).

The best known biochemical or enzymatic method of analysis is that developed by Giang and Hall (25) for organic phosphorous insecticides. The procedure is based on the inhibitive effect of the organic phosphate toxicant on the activity of the enzyme, cholinesterase.

Biological assay is extremely important for pesticidal compounds for which there is no good available chemical method. In some
instances, bioassay may be the only method of analysis. It is also very important as a supplementary or comparative method for those compounds for which simple, rapid, accurate chemical procedures of analyses are available.

In biological assay, a living organism is exposed to known and unknown concentrations of the pesticidal compound. Some criterion based on a change in the organism is recorded. In the case of pesticides, some characteristic symptom or mortality or morbidity is measured. The results from the unknown concentration are compared to those of the known concentrations.

Biological assay techniques are also employed in studies of nutrition and pharmacology.

In toxicant residue studies, where small quantities of chemical are involved, sensitive animals are used. For this reason, insects have been utilized as test animals in biological assay. Both mature and immature insect forms are used.

The entomological testing procedures in biological assay most generally utilize one of the following three insects as test animals; the house fly, the mosquito larva, or the fruit fly (also designated as pomace or vinegar fly).

The fruit fly, Drosophila melanogaster Meig., was chosen as the test insect because of its sensitivity and because it is relatively easy to rear. Of the three common forms of insects used in biological assay of insecticides the mosquito larva is the most sensitive and also the most difficult to rear. The house fly is also considered as more difficult to rear than the fruit fly and it is in most cases less
susceptible (immature male house flies are more sensitive than fruit flies in the detection of aldrin, dieldrin, lindane and DDT in milk in a feeding method, Sun, 1953). The rearing of mosquito larvae and house flies takes considerably more time than the rearing of fruit flies.

In the chemical methods of residue analysis, a solvent extraction of the plant or animal tissues (usually macerated) is utilized. In biological assay, there are two alternatives, placing the test insect in direct contact with the evaporated tissue extract or placing the test insect in direct contact with the macerated tissue. In this study, the biological assay technique involves exposing the test insect to the macerated tissue.

Malathion, demeton, perthane, Strobane, dieldrin, and endrin were evaluated for desirable assay properties by preparing dosage-effect curves employing the Modified Sun Method. D. melanogaster was exposed to different concentrations of insecticide blended into pumpkin.

Dieldrin and endrin residue determinations were made on canned and frozen lima beans and frozen snap beans.

A preliminary investigation was carried out for dieldrin and endrin using the Culture Tube Method with pumpkin spread over wood stakes and stainless steel strips.
II. Literature Review

1. Examples of Living Forms Other Than Insects Used in Biological Assay

Certain living forms, both from the plant and animal kingdoms, other than insects have been employed as test subjects.

Anderson (1) in 1944, published the toxicity thresholds of various substances found in industrial wastes when added to Lake Erie water. The determinations were made with the water flea, Daphnia magna Straus (a micro crustacean). Wollerman and Putnam (64) found that Daphnia pulex de Geer, can be used as a test animal to screen systemic toxicants. Systox translocation was tested in cut branches of black locust placed in 50 per cent demeton solution and in branches from trees receiving soil applications.

Kocher et. al. (36) in 1953 undertook to improve the precision of known methods of bioassay, using Daphnia pulex, particularly for quantities of insecticides of less than 1 mg. per kg. of plant material. Examples were given for the determination of 0.7 ppm diazinon (0,0-diethyl-0-[2-isopropyl-4-methylpyrimidyl (6)] thiophosphate) on cauliflower, 1 ppm isolan (3-methyl-1-isopropyl pyrazolyl (5) dimethyl carbamate), and 0.1 to 1 ppm parathion on cherries. Parathion could be determined in the presence of DDT.

Wilson and Choudhri (60) reported in 1946 that the quantity of DDT mixed with soil or spread on the surface of an agar medium in test tubes was much in excess of that which will ever be applied for the control of insects yet there was no evidence that this material
was injurious to microorganisms. It also was reported by Wilson and Choudhuri (61) that an application of benzene hexachloride considerably in excess of the amount recommended to control such organisms as wireworms had no pronounced deleterious effect on certain heterotrophic microorganisms of the soil, but was toxic to other soil organisms and to various species of algae.

Pagan and Hageman (44) in 1950 used fish as test animals in a biological assay of DDT residues on vegetables. Samples were prepared in the laboratory and acetone extracts were tested on the fish. The results showed good recoveries for all concentrations of DDT in tomatoes and beans (the median lethal dosage approximated 60 ppm). Erratic results and high mortality checks were obtained, when diluted milk samples contaminated with DDT were tested.

Angelotti et al. (2) attempted to utilize microbiological techniques as a method of detecting spray residues. Bacteria, yeasts and protozoa were used as test organisms.

Parathion, DDT, and lindane (gamma isomer of benzene hexachloride) were tested in various ways and were ineffective against the following test microorganisms: Sarcina leutea, Micrococcus pyogenes var. epidermidis, Micrococcus pyogenes var. albus, Escherichia coli, Proteus vulgarus, Pseudomonas aeruginosa, Serratia marcescens, Serratia indica, Bacillus mycoides, Erwinia carotovora, Azotobacter chroococcum, Azotobacter vinelandii, Saccharomyces cerevisiae and Torulopsis utilis.

The following free swimming protozoa were used as test organisms: Paramecium multimicromeleatum, Paramecium paranema, Blepharisma,
Spirostomum, Englena deses and Tetrahymena. Only Paramecium multimicromelaetum showed any response to parathion in concentrations as low as 1 ppm. DDT and lindane had no apparent effect on any of these organisms.

Bacteria-free Tetrahymena were grown in sterile media and mixed with the evaporated ether extracts of solutions of insecticides in a slurry of lima beans, peaches and tomato juice before and after canning. After an incubation period of 37° C., the growth was measured on an Evelyn colorimeter. Also, Angelotti et. al. demonstrated that lindane and parathion in the canned lima beans, peaches and tomato juice caused no inhibition of growth.

2. House Flies As Test Insects In Biological Assay

Laug (37, 38) made a pioneer study of the distribution of DDT and lindane in animal tissues by means of bioassay. Laug (37) in 1946 developed a method depending on the toxic response of the house fly to determine DDT (100 flies were exposed to DDT residues for a 20 hour period) in animal tissues and excreta. The biological assay method was compared with the Schechter colorimetric method (46). Quantities of DDT of the order of 2.5 ppm were determined.

In 1948, Laug (38) again using the house fly as a sensitive test insect developed a biological assay method for lindane based upon fly mortality to the deposit of a given tissue extract as compared with a set of standards made from known amounts of the insecticide. About 20 mg. corn oil were added to each flask to prevent losses of lindane in the standards during and subsequent to the evaporation of the ether solution.

House flies were used by Wingo and Crisler (63) to bioassay
milk from cows that received oral doses of DDT in a study of the effect of DDT on dairy cattle (total gains) and milk.

Carter et al. (12) in 1949 stated that with the synthetic cream which was made with skim milk and butter, DDT concentrations of 50 ppm or less could not be definitely detected on the basis of house fly mortalities.

Wiesmann (57) described a method of biological assay, for investigating cases of poisoning in honey bees due to DDT, benzene hexachloride and parathion, exposing *Musca domestica* L to evaporated acetone extract residues in petri dishes. The insecticide concerned can be identified from the symptoms shown by the flies. Mosquitoes were used for the bioassay of DDT. The benzene hexachloride in mixtures of benzene hexachloride and DDT were determined by exposing flies to the fumigant action of the residue or by additional tests with insects such as *Carausius morosus* Brunn, that are susceptible to benzene hexachloride but not to DDT.

Furman and Bankowski (23) in 1949 demonstrated by biological assay technique using the house fly as the test insect, that benzene hexachloride was absorbed by poultry tissues following treatment of roosts.

Hoskins (31) used the small vial method for biological assay of pesticidal residues of lindane with house flies as test insects in a study of the deposit and residue of recent insecticides resulting from various control practices in California.

Furman and Hoskins (24) in 1948 developed a biological assay method for the detection of benzene hexachloride in terms of the lindane equivalent in milk from dairy cows following spraying with
aqueous suspensions of benzene hexachloride. Twenty five to 30 house flies, 4 or 5 days old were introduced and exposed for 30 minutes to evaporated acetone extracts after which they were transferred to observation cages. Twenty four hour mortality was determined.

Instead of exposing house flies continuously in treated vessels, Furman and Hoskins (24) and Hoskins and Messenger (32) exposed the flies for 30 minutes in small shell vials. In Lang's pioneer study (37) exposures lasted 20 hours and thereby required that the test insects be fed during this period.

Hoskins, Witt and Erwin (33) improved the small vial method for bioassay of insecticidal residues of lindane with house flies as test insects by adding a minute quantity of light spray oil which was non-toxic by itself and had the functions of spreading the toxicant in solution over the inner surface of the vials and enabling a large and consistent fraction of the total deposit to reach the test insects. The uniformity of contact and high pick up contributed to consistency of mortality.

Dahm and Pankaski (16) presented a method similar to that used by Lang (37, 38), which consisted essentially of exposing adult house flies to a residue of aldrin for a period of 48 hours. A standard assay curve was established by plotting the per cent mortalities as probits against the milligrams of the insecticides per kilogram of house flies. Residues of the toxicant were concurrently assayed with uniformly sampled lots of flies from the same rearing. Values of the insecticide in the unknown residues were obtained from the standard curve for that day.

Stansbury and Dahm (49) employed a biological assay method
using the house fly, *M. domestica*, to determine the effect of commercial alfalfa dehydration upon aldrin, chlordane, parathion, and toxaphene residues. House flies 3 to 4 days old were exposed to evaporated benzene extracts in petri dishes. One ml. of corn oil solution (20 mg. of corn oil per ml. of benzene) was added to each petri dish. Each dish was fitted with a cylindrical screen wire cage. Fifty carbon dioxide anesthesized house flies (Williams, 1946) were put into a cage. A cardboard disc plunger was then lowered to confine the flies in a small volume just above the residue. After one hour the cages were removed from the dishes and placed in an 80°F. incubator. After 48 hours mortality counts were made. The per cent reduction of the residue due to dehydration was recorded by Stansbury and Dahm (1951).

Sun (53) evaluated the insecticidal residue by continuously exposing adult house flies to a film of deposit.

Sun suggested that when using the extraction procedure, that the amount of total residues should be first determined, since, an extract may contain varying amounts of dissolved substances which affect the toxicity of an insecticide in the extract in various ways.

Sun presented the following 4 methods of biological assay:

Method 1 - Banking method for analyzing the presence or absence of insecticidal residue.

Method 2 - LD$_{50}$ method for calculating traces of insecticidal residue.

Method 3 - Interpolation method for evaluating small amounts of insecticidal residue.
Method 4 - Interpolation method for determining large amounts of insecticidal residue.

Dahm (15), in a study of the effects of weathering and commercial dehydration upon residues of aldrin, chlordane, and toxaphene applied to alfalfa, employed Sun's bioassay technique (53) to test for aldrin. Dahm used 100 house flies on each of six replicates and the mortality counts were made after 18 to 42 hours exposure, depending on the time necessary to attain mortalities in the 30 to 80 per cent range.

Sun and Sun (52) in 1953 reported a method of analyzing micro-quantities of dieldrin in cow's milk by feeding the house fly, *M. domestica*, with treated milk. The feeding method can be used for the biological assay of other toxicants in milk obtained from other animals or in liquid food which can be fed to house flies or other insects.

3. Mosquito Larvae As Test Insects In Biological Assay

Nolan and Wilcoxon (43) using *Aedes aegypti* L. larvae, developed a biological assay method capable of detecting and estimating small amounts of parathion in or on plant tissue for correlation with the colorimetric procedure developed by Averell and Norris (3) in 1948.

The method of Nolan and Wilcoxon is suitable for concentrations of parathion as low as 0.02 ppm.

Starnes (50) compared two methods of assaying plant tissue (beans, beets, corn, and potatoes) biologically with the aid of the fourth instar mosquito larvae, *A. aegypti*, and both were found to be sufficiently sensitive to yield a quantitative indication of the presence of a toxicant in the presence of plant tissue.

Hartzell and Storrs (29) used a modified Nolan and Wilcoxon (43)
method for parathion to biologically assay processed foods for the known amounts of spray residues of ten insecticides.

Three day old yellow fever mosquito larvae, *A. aegypti*, were used in these tests. The two products assayed were snap beans and a mixture of two parts apricots to one part apples put up as baby food.

Hartzel (27) tested apricots, prunes and peaches from sprayed trees. Two day old larvae of *A. aegypti* were used to test the toxicity due to spray residues. The spray schedules for the different fruit trees were reported.

Bushland (10) described a method of detecting insecticide contamination of meat and milk by biological assay with fourth instar larvae of the mosquito, *A. aegypti*. Tests were made with butterfat samples containing DDT, DDD, methoxychlor, lindane, chlordane, and toxaphene. Some constituent of the emulsified butterfat, according to Bushland, rendered all of these insecticides nontoxic to larvae. Sulfuric acid treatment of emulsified butterfat did not remove all the substances that interfered with DDT and DDD toxicity, but it did eliminate the ingredient that reduced the toxicity of lindane. Toxaphene and chlordane were not sufficiently toxic to be detected by the method that was effective for lindane.

Burchfield, Hilchey, and Storrs (8) developed a rapid procedure based on the tendency of mosquito larvae to move away from a strong light source (the negative phototaxic response). A number of physiological responses of larvae were investigated.

The larvae of *A. aegypti* were confined behind a porous barrier in a shallow glass trough containing a dilute aqueous suspension of toxicant. Viable larvae rapidly migrate to the far end when a
500-watt light source parallel to the longitudinal axis of the trough was turned on and the barrier removed. A second barrier was dropped into place after an exposure of one minute and the larvae that were trapped behind it were regarded as moribund.

The method was used successfully to determine paralytic action of serial dilution tests, and to measure the time required to inactivate 50 per cent of the population. The technique can be used in the secondary screening of new insecticides and was semi-specific for liposoluble neural poisons and narcotics.

Early in 1954, Burchfield and Storrs (9) using the photomigration technique determined the time required for immobilization of 50 per cent of a population of larvae of *A. aegypti* in concentration series for lindane, methoxychlor, Dilan (53.3 per cent "Bulan" - 2-nitro-1,1-bis (p-chlorophenyl) butane, 26.7 per cent "Prolan" - 2-nitro-1,1-bis (p-chlorophenyl) propane, and 20 per cent related compounds), parathion, DDT, DDD, dieldrin, heptachlor, chlordane, Strobane, (terpene polychlorinates), aldrin and toxaphene. Bioassay results can be obtained on all of these toxicants in an hour or less at concentrations of 0.1 to 1.0 ppm.

Later in 1954, Hartzell, Storrs and Burchfield (28) analyzed vegetables grown under field conditions by bioassay and chemical methods. Samples of cabbage, carrot, corn, onion, potato, turnip, and yams were tested for chlordane and heptachlor. The bioassay method (photomigration technique) gave lower results on the heptachlor samples because of the masking effect of plant lipids present in the extracts. The two methods agreed closely for the samples containing chlordane.
Fruit Flies As Test Insects In Biological Assay

Bartlett (5) assembled from field and laboratory sources 16 strains of *D. melanogaster* representative of diversified climate and food habits. These strains were isolated as family lines and tested for extent of susceptibility to DDT, a contact poison; and to hydrocyanic acid gas, a respiratory poison. Suitable test methods for adequate evaluation of small differences in susceptibility between the 16 strains were developed.

Differences in insecticidal resistance between strains was demonstrated to occur in treatment with DDT, tartar emetic, and HCN. Resistance was shown to be inherited and not associated with nutrition. Resistance for anesthesia and certain physical factors were also tested.

Cauliflower curds are often attacked by insects just prior to harvest. Although the manufacturers recommended that parathion should not be applied within 30 days of harvest or after the heads are formed, such applications are sometimes made. Kasting and Harcourt (34) in 1952 analyzed cauliflower heads for parathion by chemical means and with a quantitative biological test in which *D. melanogaster* were exposed to benzene extracts. No residue was detected chemically; biologically a residue of less than 0.06 ppm was reported.

Sun and Pankaskie (51) were the first workers to publish a method of biological assay using the fruit fly, *D. melanogaster*, as the test insect. Only one laboratory strain was used in their research, because no appreciable difference in susceptibility was found for one wild strain and two laboratory strains. The fruit
flies were reared on canned pumpkin as described by Bartlett (4). Only one day old flies were used for testing.

Sun and Pankaskie have described two general methods for biological assay using the fruit fly. One method involved the direct exposure of fruit flies to macerated tissues, the method upon which the current study is based; the second method involved the exposure of pomace flies to dry residues.

Results obtained in the determination of dieldrin in milk by using house flies led to the development of a method of analyzing plant tissues for insecticidal residues by exposing adult fruit flies to macerated plant tissues. "To evaluate the insecticidal content of a sample, mortalities resulting from exposure to treated plant tissues are compared with mortalities resulting from exposure to standards containing known amounts of the same insecticide" (Sun and Pankaskie, 1954).

The same methods described for house flies by Sun and Sun (53) can be used when fruit flies are used for the biological assay of dry residues deposited from the evaporation of animal or plant tissue extracts. The extracts are deposited on the bottoms of 1/2-ounce wide-mouth jars. Canned pumpkin serves as the food for the pomace flies. Each jar is covered with a piece of tissue paper.

The extraction method has been successfully used for determining the amount of aldrin or dieldrin in brain, liver, kidney, muscle and fatty tissues obtained from rats used in toxicological studies. According to Sun, 0.05 microgram of aldrin or dieldrin can be detected by this method.
Fisher and Smallman (19) reported on the investigation of the influences of the cultural and testing techniques on the sensitivity and reliability of the toxicological response of the fruit fly, D. melanogaster.

Fisher and Smallman conducted two types of experiments, (1) the effects of variables such as age, sex, and numbers of fruit flies were assessed by the mortality resulting from exposure to a range of concentrations of aldrin in canned pumpkin and (2) the residue of toxicant in a "sample" of treated plant material was estimated by comparison with a standard made up with the same plant material.

Fisher and Smallman presented a sample biological assay of dieldrin in applesauce to demonstrate the sensitivity of the method applied to plant material. Two samples of canned applesauce were assayed from apple trees sprayed with dieldrin and from non-sprayed apple trees using the same method that was used on the added aldrin residues in pumpkin. Three lots of 30 unanesthetized flies were used for each dosage. The assay revealed a residue of $0.36 \pm 0.03$ ppm in one sample and $0.18 \pm 0.02$ ppm in the other, though the large numbers of flies undoubtedly reduced the sensitivity of the test. These results are in the correct order, as determined from information on the dosages applied to the trees.
III. Methods

A. Rearing Procedure For Drosophila melanogaster Meig.

The assay organism for this study, *D. melanogaster*, was obtained from the Division of Genetics, Department of Zoology and Entomology, The Ohio State University. The Division of Genetics originally obtained the culture from the Laboratories of Jack Schultz, Philadelphia, Pa.

This particular strain of *Drosophila* had been cultured in the laboratory for 88 generations when first obtained from the Division of Genetics. The cultures have been maintained up through about 132 generations at the conclusion of the testing.

The culture was a wild, inbred strain, maintained by making brother-sister matings, and designated as "forked reversed forked". Sun (51) in his extensive testing was unable to perceive any significant difference in susceptibility between wild and laboratory strains when tested against several insecticides.

*D. melanogaster*, the test organism, was reared on a corn meal-molasses-agar media, which is used by geneticists and those biologists interested in entomological testing. The culturing method used was similar to that given by Demerec and Kaufmann (18). The following recipe makes approximately 20 bottles of media:

- Corn meal: 95 g.
- Agar: 15 g.
- Brewer's yeast: 5 g.
- Tegosept P (1 g./10 ml. alcohol): 10 ml.
- Corn syrup: 75 ml.
Molasses 75 ml.
Water 850 ml.

The solids — corn meal, agar, and Brewer's yeast — were weighed out on a triple beam balance and poured into a pot. The liquids — Tegosept P solution, corn syrup, and molasses were measured out in graduate cylinders and poured into the cooking container holding the solids. These constituents were mixed together thoroughly. The measured amount of water was added to the mixture and the entire mass was stirred thoroughly. Heat was applied, with a gas burner with constant stirring, until the boiling point was reached. The brown colored media was allowed to simmer for a short period or until the presence of the large gelatinous agar particles were no longer discernible. The hot media was poured into a funnel with a large neck, to which was attached a piece of rubber hose in which a short length of pyrex glass tubing had been inserted. A Fischer clamp, was used on the rubber hose to check the flow of the hot media until it was time to fill the culture containers.

Square based half pint Duraglass milk bottles were used as the culture containers. Between 20 and 25 of these bottles may be prepared from the above recipe. Media was allowed to flow into the milk bottle to a height of approximately one half to three fourths of an inch. The bottles were stoppered with cotton and autoclaved for 20 minutes at 15 pounds pressure. After the culture bottles had cooled at room temperature they were stored in a refrigerator (See Photograph 1).
An active culture, a newly established culture and a culture bottle (from left to right).
Demerec and Kaufmann (18) suggest that Moldex (sodium propionate) be used as the fungicide in the preparation of the corn meal-agar-molasses media. Because of the recommendation and the successful use of Tegosept P (purified propyl parahydroxybenzoate) by the geneticists, this fungicide was used in the media. By careful handling, the use of the fungicide Tegosept P, and storing the uncultured bottles in a refrigerator, the occurrence of mold was held down to a minimum. It was not necessary to use a flaming technique.

A special coarse flake type agar (20 per cent moisture basis) was used in the preparation of the media. The agar used was manufactured by the American Agar and C. Company, San Diego, California.

At least 10 males and 10 females and not more than 25 of each sex (from emergence to 24 hours old) from an active established culture were used to start a new culture. The individual fly size varies noticeably, if large numbers were used to initiate a new culture.

In making a transfer, carbon dioxide gas was used to anesthetize the fruit flies. The gas was blown into an empty half pint bottle. The flies were shaken from a culture bottle into the bottle containing the carbon dioxide. The anesthetized flies were then placed on a 4 x 5 card and by means of a small brush were swept into a small aluminum foil cup. The cup was then inserted into a fresh culture bottle. Just before this transfer takes place, a half sheet of Kleenex tissue saturated with a Baker's yeast suspension was dropped onto the surface of the culture media. The cultures were incubated at 25°C.

The small aluminum foil cups were made by pressing aluminum foil squares (1 to 1 1/2 inch size) around a mold which has a height of one fourth inch and a base about three eighths of an inch square. The
mold was made from a small piece of 2x4 lumber (See Photograph 2). The aluminum foil was cut from rolls of the type commonly used for household purposes.

The Baker's yeast suspension was prepared by shaking one package of dried viable yeast with three fourths of a pint of water. The suspension was refrigerated.
Photograph 2.

Aluminum cup mold, cup, and small square of aluminum.
At the incubation temperature of 25° C., it took approximately 10 days for the life cycle to be completed. Subsequent stages in the life cycle include larva, pupa, and adult (imago).

"The larvae are such intensely active and voracious feeders that the culture medium in which they are crawling becomes heavily channelled and furrowed" (Demerec and Kaufmann, 1945). The channeling and furrowing of the larvae is the simplest criterion by which the worker is able to decide at a glance, a few days after the beginning of egg laying, whether the expected generation is developing successfully.

The flies are relatively light in color upon emergence, but they darken during the first few hours. It is therefore possible to distinguish the more recently emerged pomace flies from the older flies present in the same culture bottle.

After making the transfer and holding the culture bottle in a 25° C. incubator for 10 days for the life cycle to be completed, an additional 10 days were employed to supply pomace flies for testing and to make transfers. After the culture was 20 days old it was discarded. In this way it was possible to keep track of the generations. Also a culture seems to have its best production of flies from about the twelfth to the eighteenth day. By the time the culture was 20 days old few flies were emerging.

Male and female pomace flies can be distinguished from each other by several apparent characteristics. In recognizing the sex of the adult fly, the tip of the abdomen is elongated in the female and somewhat more rounded in the male. The abdomen becomes distended with maturing eggs, as the female fly ages. Pomace flies of this sex
are recognizable at a glance. In many cultures, including the wild-type, the pattern of darker markings on the abdominal segments is sufficiently distinct in the two sexes to permit their separation on this basis without recourse to the microscope or hand lens.

B. Spray Schedules For Lima Beans and Snap Beans

Lima bean samples and snap bean samples were obtained from Dr. J. P. Sleesman, Leader of State Project 198, "Biology and Control of Vegetable Crop Insects," The Ohio Agricultural Experiment Station, Wooster, Ohio.

The test plots were arranged according to random distribution with five replications. Each plot consisted of a single row, 20 feet long.

A tractor mounted Bean 7 gallon pump, 300 pounds p.s.i., with cone type nozzles was used for the spraying operation. The rate of application was 160 gallons per acre.

The Fordhook 242 variety of lima beans and the Wade variety of snap beans were employed in this study. The spray schedule information for the years 1953 and 1954 is as follows:
### Schedule 1

<table>
<thead>
<tr>
<th></th>
<th>Dithane Z-78</th>
<th>Dieldrin</th>
<th>Endrin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation</strong></td>
<td>65 per cent</td>
<td>50 per cent</td>
<td>18.5 per cent</td>
</tr>
<tr>
<td><strong>1953</strong></td>
<td>wettable powder</td>
<td>wettable powder</td>
<td>emulsifiable concentrate</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>2 lbs./100 gals.</td>
<td>1 pt./100 gals.</td>
<td>1 pt./100 gals.</td>
</tr>
<tr>
<td><strong>1953</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dates of Application</strong></td>
<td>June 24</td>
<td>July 6, 16 and 27</td>
<td>August 5, 17 and 26</td>
</tr>
<tr>
<td><strong>1953</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Formulation</strong></td>
<td>65 per cent</td>
<td>50 per cent</td>
<td>19.5 per cent</td>
</tr>
<tr>
<td><strong>1954</strong></td>
<td>wettable powder</td>
<td>wettable powder</td>
<td>emulsifiable concentrate</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>2 lbs./100 gals.</td>
<td>1 pt./100 gals.</td>
<td>1.5 pts./100 gals.</td>
</tr>
<tr>
<td><strong>1954</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dates of Application</strong></td>
<td>June 28</td>
<td>July 7, 16 and 26</td>
<td>August 6, 19 and 31</td>
</tr>
<tr>
<td><strong>1954</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C. Processing of the Lima Beans and Snap Beans

1. Canned Lima Beans

The lima beans were harvested by hand on September 9, 1953. They were placed in cold storage (32-34°F) for two days. The lima beans were handled and canned using standard pilot plant procedures.

The lima beans were shelled by hand and were washed with an overhead spray. They were next steam blanched for 3 minutes and then were filled into No. 2 C-enameded cans and exhausted in live steam to 160°F. The filled cans were given a steam flow closure. The cans were coded and the cans and contents processed for 20 minutes at 250°F. The cans were cooled to 90°F in an overflowing water bath. After cooling, they were cased and stored at room temperature until the time of testing.

2. Frozen Lima Beans

The lima beans were harvested by hand on September 3, 1953. They were shelled, washed and blanched as for canning.

After the 3 minute steam blanch, the lima beans were washed and cooled for 1.5 minutes in an overhead water spray, after which they were drained and packaged in a Kordite carton with polyethylene liner. The coded packages were placed on a plate freezer (-20°F) for at least 12 hours and then stored in a freezer held at 0°F until the time of testing.

3. Frozen Snap Beans

The snap beans were harvested by hand on August 25, 1954. The beans were sorted by hand. An overhead spray of water was used to wash the beans. After snapping by hand, the beans were blanched for
minutes with live steam. Immediately after blanching they were washed and cooled for 90 seconds in a water spray.

The snap beans were packaged, coded, frozen and stored as were the lima beans.

When blanched 4 minutes, the beans gave a negative peroxidase test.

D. Preparation of Insecticidal Solutions

All the insecticides were dissolved in reagent grade acetone. The stock solutions were diluted with reagent grade acetone, (specific gravity 0.788 at 25° C.). In all the concentration calculations, 0.8 was used as the approximate specific gravity. The concentrations are expressed in parts per million on a weight-weight basis.

For the preparation of each stock insecticidal solution either one or two hundred milligrams of insecticide were weighed on an analytical balance and dissolved in 100 ml. of acetone. The resultant stock solution concentration was 1250 ppm or 2500 ppm. The stock solutions were stored for not more than 14 days in a dark cabinet.

In the preparation of the stock solutions and in the serial dilution procedure, clear 125 ml. ground glass stoppered bottles, volumetric flasks and volumetric pipettes were used. In the serial dilution, the smallest volume used was 10 ml. For example, if it was necessary to cut the concentration from 100 ppm to 50 ppm, 10 ml. of the 100 ppm solution was added to 10 ml. of acetone, resulting in 20 ml. of a 50 ppm solution.

On each day that an experiment was conducted, acetone-insecticide solutions were prepared from the stock solution which would give the
The final desired insecticide concentrations when 5 ml. aliquots of these solutions were thoroughly blended into 200 gram samples of plant tissue.

The insecticides that were used were all of high known purity. In all the concentration calculations the insecticides were considered as being 100 per cent chemically pure. The insecticides and their respective purities are as follows:

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Per Cent Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dieldrin</td>
<td>99.6</td>
</tr>
<tr>
<td>Endrin</td>
<td>99.6</td>
</tr>
<tr>
<td>Malathion*</td>
<td>95</td>
</tr>
<tr>
<td>Demeton</td>
<td>100</td>
</tr>
<tr>
<td>Perthane**</td>
<td>99 plus</td>
</tr>
<tr>
<td>Strobane</td>
<td>100</td>
</tr>
</tbody>
</table>

*Malathion — S-1,2-bis (ethoxycarbonyl) ethyl-0,0-dimethyl dithiophosphate

**Perthane — 1,1-bis (p-ethylphenyl)-2,2-dichloroethane
E. Maceration and Blending Procedure

An Osterizer (Model No. 10; 18,000 to 20,000 rpm.; John Oster Manufacturing Company, Racine, Wisconsin), hooked to a variable transformer, was used to blend the 5 ml. of acetone-insecticide solution with the 200 grams of test material (plant tissue) (See Photograph 3).

Two hundred grams of test material were weighed into a pint Ball jar on a triple beam balance. Five ml. of acetone-insecticide solution, or just 5 ml. of acetone in the case of the check, were pipetted into the jar. The Osterizer lid was screwed onto the jar and the jar was inverted and placed on the Osterizer. The motor was allowed to run for 4 or 5 minutes. The mixing speed was regulated by means of the variable transformer.

For canned lima beans, the Osterizer macerated the whole beans and blended the acetone-insecticide solution with the test material in one operation. Because of their maturity, the frozen lima beans and the frozen snap beans were ground (ground frozen for greater shearing action) in an overhand kitchen grinder. One hundred eighty grams of ground beans and 20 ml. of tap water plus the 5 ml. of acetone-insecticide solution were blended together. Sun (51) stated that for certain materials, a small amount of water should be added during the maceration process. "To avoid high natural mortality among the fruit flies, the slurry should have a high water content, but it should not be so high as to permit the fruit flies to drown. The same amount of water should be added to all samples of an assay series to equalize the dilution factor."

The acetone-tissue ratio in this study was not great enough to
Variable transformer and Osterizer.
cause significant mortality in the untreated checks. Sun (45, 51) states that the concentration of the acetone-insecticide solution added to the macerated, untreated tissue for making standards should be such that not more than 0.5 ml. of acetone solution is added to 25 grams of sample.

F. Direct Exposure Methods For Macerated Plant Tissue

1. Modified Sun Method

After the mixing operation, level tablespoonfuls of slurried test material were measured out and placed in 4 ounce jars. A level tablespoonful of pumpkin and other pureed plant tissue weighed approximately 20 grams. Six jars or replicates were set up at each insecticide level (See Photograph 4).

In all the preliminary screening work with pumpkin and all the test runs with lima beans and snap beans, a check and four concentrations constituting a standard series were run. In all experiments, an attempt was made to set up concentrations for the standard series which would result in mortalities ranging from 30 to 85 per cent.

After the mortality data were recorded, the average mortality for each dosage level was calculated. The average values were plotted on logarithmic-probability paper. Per cent mortality was expressed in probit units along the ordinate and the insecticide concentration was expressed in parts per million, in this case, on a logarithmic scale along the abscissa. Each time a sample was assayed, a standard curve was prepared.

For each assay, a small concentration of dieldrin or endrin was added and blended into the macerated sample. By using this addition
Photograph 4.

Test containers for the Modified Sun Method.
procedure, it is possible to detect residues which by themselves are not large enough to cause mortality.

If an unknown macerated sample, upon exposure to fruit flies, does not contain enough toxicant to give a moderate kill, then the method of adding a small amount of insecticide to the sample is employed. By knowledge of previous investigation, one concentration is selected which by itself gives a mortality which approximates the median lethal dosage. A concentration of toxicant producing 40 to 60 per cent mortality is considered as a desirable range for more accurate comparison. The unknown residue plus the increment gives a greater mortality than that produced by the added increment, alone. The difference, between the sample plus the increment of toxicant and the selected level in the standard series (the same level as the amount added), is that mortality which is due to the unknown effective residue.

The flies were stripped from the active cultures every twenty four hours. On the day a test was to be run, the flies were removed from the culture bottles and held in empty, cotton stoppered bottles for 6 hours at 25°C. The age of the insects used in the tests varied from 6 to 30 hours.

After the 6 hour holding period, the fruit flies were anesthetized in the holding bottle with carbon dioxide. The flies were then spread on the surface of the modified Williams' apparatus (62). The flies were given a continuous carbon dioxide anesthesia on the Buchner funnel-cellucotton apparatus (See Photograph 5) until they were drawn up by an aspirator gun (See Photograph 6). Fifty fruit flies, from 6 to 30 hours old, were counted, by means of the aspirator gun, and placed
Buchner funnel-cellucotton apparatus for continuous carbon dioxide anesthesia.
Photograph 6.

Aspirator gun.
in an aluminum foil cup (See Photograph 2). The cup was immediately placed on the surface of the pumpkin or test material. A piece of cheesecloth about 3 or 4 inches square was placed over the top of the 4-ounce jar. The cheesecloth was held in place with a rubber band. After all the pomace flies have been counted and checked for recovery, the test jars were placed in a 25° C. incubator (See Photograph 7).

No specific length of exposure time was used. It was desirable to carry out the exposure until the mortalities fell between approximately 30 and 85 per cent. Sun (45, 51) used exposures varying from 17 to 48 hours. Mortality data were taken at regular intervals until the desired mortalities were obtained.

2. Culture Tube Method

The test materials were prepared for the Culture Tube Method as they were for the Modified Sun Method previously described.

The test materials were measured out with teaspoons and spread over pieces of untreated wood stake (7/8"x6") (used as markers in vegetable gardening plots) or pieces of stainless steel sheet (7/8"x 6") (No. 22 gauge) (See Photograph 8) with stainless steel spatulas. The contents of one level teaspoon weighed approximately 3.2 grams.

The fruit flies were handled in exactly the same manner as given above. The culture tubes (pyrex culture tubes, 32x200 mm.) were used to receive the flies from the aspirator gun instead of the usual small vial. Twenty flies were placed in each culture tube. The tube was immediately stoppered with cotton.

After the 6 to 30 hour old flies revived from the carbon dioxide anesthesia, they were shaken to the bottom of the tube, the
Photograph 7.

Incubator, 25° C.
Photograph 8.

(From left to right): wood stake, cotton stoppered test tube with wood stake, test tube and cotton, cotton stoppered test tube with stainless steel strip, and stainless steel strip.
cotton stopper was removed, the test material either on the wood stake or the strip of stainless steel sheet was inserted, and the cotton stopper was replaced.

Six replicates were used for the check, each of the four standard series concentrations, and the unknown sample in each assay using the Culture Tube Method.

The range of exposure times and the incubation temperature were the same as in the Modified Sun Method.

G. De-contamination Procedure For Glassware and Other Equipment

Because contamination is possibly the greatest single hazard in biological assay, special handling procedures are necessary for all equipment involved in rearing, preparation and dilution of insecticidal solutions, and experimentation.

All glassware and metal utensils were first rinsed with tap water. Next, the equipment was placed in a detergent-water bath where it was scrubbed by hand with an assortment of brushes, rinsed in the detergent-water bath, rinsed in warm tap water three times and placed in wire baskets to drain.

The washed equipment was then heated at 250° C. for at least 24 hours.

Sun (53) suggests that all glassware be soaked in hot water, washed thoroughly with cleanser, rinsed with water, dried and heated overnight at 90° C. or higher in order to eliminate the last trace of toxicant.

Hartzell and Storrs (29) described a rather elaborate method of cleaning glassware for mosquito larvae tests. "The glassware was first
rinsed with acetone, then placed in hot trisodium phosphate solution to soak overnight. The glassware was then successively rinsed with hot running tap water, chromate-sulfuric acid cleaning solution, and finally thoroughly rinsed in hot running tap water" (Hartzell and Storrs, 1950).

H. Statistical Treatment

The dosage-mortality curves were plotted using the simplified method of Litchfield and Wilcoxon (39) for evaluating dose-effect experiments.

By employing Tukey's (56) method of statistical analysis for several groups of measurements, the effective residues are expressed as ranges of concentration (parts per million).

In determining the potency of an insecticide the dosage-response curve is used in reverse and $X$ (dosage) is estimated from $Y$ (mortality). The computation of variability for such reverse estimates is different from the computation that would be made if the independent variate ($X$) was used to estimate the dependent variate ($Y$) (Bliss, 1952). This difference in computation of variability has not been taken into account here in the interests of the quick method.

By using Tukey's method of statistical analysis (56) for several groups of measurements, it can be stated that the residue value lies between two concentrations of insecticide. This range of concentration, which has been used to express the effective residue of dieldrin or endrin, is based primarily on (1) the per cent mortality difference between the sample plus the added increment of toxicant and the corresponding value of the standard series and (2) the WSD value. The
WSD value or "Wholly Significant Difference" expresses the range of variation between replicates for all the concentration levels of the standard series and the sample plus added insecticide increment.
IV. Presentation of Results

A. Exploratory Tests For Six Insecticides Using Pumpkin As A Base

Six organic insecticidal compounds - dieldrin, endrin, malathion, demeton, perthane and Strobane - were evaluated by the Modified Sun Method. *D. melanogaster*, the assay organism, was exposed at 25° C. to canned pumpkin with varying concentrations of toxicants. Six replicates, using 50 fruit flies, varying from 6 to 30 hours in age from a mixed population, per replicate, were prepared for each insecticide level.

Canned pumpkin was used in the evaluation studies because it is a desirable food for the fruit flies and it has been used as a rearing medium (Bartlett, 1952) in several studies.

This evaluation procedure was followed to find four concentrations that would give a range of mortalities between 30 and 85 per cent, which when plotted on logarithmic-probability paper would approximate a straight line. The per cent mortality was recorded at various time intervals until the four concentrations in the standard series gave mortalities in the desired range. This preliminary evaluation was not meant to be a comparative toxicity study, thus exposure time was a variable.

By using this testing method it was possible to eliminate 4 of the 6 toxicants from further consideration. Only the chlorinated naphthalenes, dieldrin and endrin, were found suitable for biological assay by the given method of placing the test insect in direct contact with the macerated tissue.

The data obtained from the preliminary evaluations were plotted on logarithmic-probability paper and are presented in Graphs 1 to 6.
1. Malathion

The fruit fly mortalities produced by malathion (an organic thio-
phosphate which is 50 to 100 times less toxic - acute toxicity - to
certain mammals than parathion) were variable. Although the variation
between replicates was not extreme when compared to other compounds,
the day to day variation in mortalities obtained for the standard
series was great. It proved impossible in 6 experiments to obtain h
concentrations which would give a desirable spread of values over the
sought after 30 to 85 per cent mortality range. The data in Table 1
were obtained in the very first of the 6 experiments with malathion.

It is not desirable to use this method for malathion, because of
the difficulty with reproducibility in preparing a standard series.
Malathion and most organic phosphates as a class are thought to be
unstable and particularly when in a water system (hydrolysis).
Table 1

DOSAGE-MORTALITY DATA FOR MALATHION IN PUMPKIN

(Modified Sun Method)

29 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Malathion ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.6</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>12.3</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>68.8</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>90.3</td>
<td>10</td>
</tr>
</tbody>
</table>
Graph 1

DOSAGE - MORTALITY CURVE FOR MALTHION IN PUMPKIN

(Modified Sun Method)

29 Hrs. Exposure at 25°C.
2. Demeton

The mortalities effected by demeton (an organic thiophosphate com-
 pound with marked systemic properties) varied in the tests with pump-
 kin. It was similar to malathion in variability. Certain concentra-
 tions of demeton resulted in different mortalities when tested over
 the same exposure period on different days. Demeton had greater var-
 iation between replicates than did malathion. Both hydrolysis and the
 fact that demeton is a systemic insecticide may explain why seemingly
 high concentrations of such a toxic substance, 8, 9, 10 and 12.5 ppm,
 were required to bring about the recorded mortality range (See Table
 2).

The curve on Graph 2 was drawn by sight and not according to the
 Litchfield and Wilcoxon Method (39).
Table 2
DOSAGE-MORTALITY DATA FOR DEMETON IN PUMPKIN
(Modified Sun Method)
22 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Demeton ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>12.6</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>50.3</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>59.6</td>
<td>20</td>
</tr>
<tr>
<td>12.5</td>
<td>88.3</td>
<td>28</td>
</tr>
</tbody>
</table>
Graph 2

DOSAGE - MORTALITY CURVE FOR DEMETON IN PUMPKIN
(Modified Sun Method)

22 Hrs. Exposure at 25° C.
3. **Perthane**

High concentrations of perthane, a DDT analog, were necessary to prepare a standard series. Concentrations of 100, 200, 300 and 400 ppm brought about 12.3, 46.6, 74.6 and 90.0 mean per cent mortalities, respectively, for a 15 hour exposure period (See Table 3). Although the concentrations could have been cut down considerably with longer exposure times, it would not have been enough to warrant the assay of small quantities of perthane by the Modified Sun Method of biological assay.
Table 3
DOSAGE-MORTALITY DATA FOR PERTHANE IN PUMPKIN
(Modified Sun Method)
15 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Perthane ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>12.3</td>
<td>12</td>
</tr>
<tr>
<td>200</td>
<td>46.6</td>
<td>16</td>
</tr>
<tr>
<td>300</td>
<td>74.6</td>
<td>24</td>
</tr>
<tr>
<td>400</td>
<td>90.0</td>
<td>14</td>
</tr>
</tbody>
</table>
Graph 3

DOSAGE - MORTALITY CURVE FOR PERTHANE IN PUMPKIN

(Modified Sun Method)

15 Hrs. Exposure at 25° C.
h. Strobane

From the preliminary evaluation, it does not seem feasible to assay for small quantities of Strobane by the procedure of placing the assay organism in direct contact with the macerated tissue. From the data in Table h, it can be seen that Strobane has a low toxicity for fruit flies. After 48 hours, relatively large quantities of Strobane blended into pumpkin at concentrations of 20, 25, 30 and 40 ppm resulted in fruit fly mortalities of 13.0, 34.3, 68.0 and 90.0 percent, respectively.
Table 4

DOSAGE-MORTALITY DATA FOR STROBANE IN PUMPKIN
(Modified Sun Method)

48 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Strobane ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.0</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>13.0</td>
<td>26</td>
</tr>
<tr>
<td>25</td>
<td>34.3</td>
<td>18</td>
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<tr>
<td>30</td>
<td>68.0</td>
<td>26</td>
</tr>
<tr>
<td>40</td>
<td>90.0</td>
<td>4</td>
</tr>
</tbody>
</table>
Graph 4

DOSEAGE - MORTALITY CURVE FOR STROBANE IN PUMPKIN

(Modified Sun Method)

48 Hrs. Exposure at 25° C.
5. Dieldrin

Much assay work has been done biologically with dieldrin. Dieldrin has the longest residual properties of any of the popular organic insecticides. It is used on many economic crops and always at a relatively low concentration, because of its residual properties.

In the preliminary work with pumpkin, it was possible with dieldrin to closely reproduce the standard series mortality values in successive experiments, more readily than it was for malathion, demeton, perthane and Strobane. Typical dosage-mortality data for the fruit fly exposed to dieldrin in pumpkin can be seen in Table 5. The average per cent mortalities for concentrations of 0.75, 1, 1.25 and 1.75 ppm were 26.0, 40.3, 61.6 and 77.3, respectively. The differences in per cent between the highest and lowest replicates for concentrations of 0.75, 1, 1.25 and 1.75 ppm were 18, 16, 18 and 16, respectively.

The dosage levels for dieldrin, a stomach and contact poison for most insects, are relatively low for obtaining the 30 to 85 per cent range of mortalities as compared to the other compounds already mentioned. The mortality values for the mortality-dosage curve are recorded in 18 to 20 hours, which is also very desirable.
Table 5
DOSAGE-MORTALITY DATA FOR DIELDRIN IN PUMPKIN
(Modified Sun Method)
19 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>0.75</td>
<td>26.0</td>
<td>18</td>
</tr>
<tr>
<td>1</td>
<td>40.3</td>
<td>16</td>
</tr>
<tr>
<td>1.25</td>
<td>61.6</td>
<td>18</td>
</tr>
<tr>
<td>1.75</td>
<td>77.3</td>
<td>16</td>
</tr>
</tbody>
</table>
Graph 5

**DOSAGE - MORTALITY CURVE FOR DIELDRIN IN PUMPKIN**

(Modified Sun Method)

19 Hrs. Exposure at 25° C.

- Per Cent Mortality - Probit Units
- Concentration - ppm

X - Standard Series

Log Scale
6. Endrin

Endrin brought about results similar to dieldrin when tested against the fruit fly in canned pumpkin. Endrin is a stereoisomer of dieldrin and therefore similar in physical, chemical and biological properties. Endrin as yet has not been as widely used as dieldrin or aldrin, another of the chlorinated naphthalenes.

The average per cent mortalities for endrin at dosages of 0.75, 1, 1.5 and 2 ppm were 38.3, 57.3, 75.0 and 91.0 per cent, respectively. The differences in per cent between the highest and lowest replicates for the various concentrations were relatively constant. For 0.75, 1, 1.5 and 2 ppm the differences in per cent between the highest and lowest replicates were 18, 18, 14 and 16, respectively (See Table 6).

Endrin, as for dieldrin, required a relatively short exposure time (18 hours) to effect mortalities for the various insecticide levels over the mortality range of 30 to 85 per cent.
Table 6

DOSAGE-MORTALITY DATA FOR ENDRIN IN PUMPKIN

(Modified Sun Method)

18 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6</td>
<td>4</td>
</tr>
<tr>
<td>0.75</td>
<td>38.3</td>
<td>18</td>
</tr>
<tr>
<td>1</td>
<td>57.3</td>
<td>18</td>
</tr>
<tr>
<td>1.5</td>
<td>75.0</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>91.0</td>
<td>16</td>
</tr>
</tbody>
</table>
Graph 6

DOSAGE - MORTALITY CURVE FOR ENDRIN IN PUMPKIN

(Modified Sun Method)

18 Hrs. Exposure at 25°C.
B. **Biological Assays Employing Modified Sun Method**

Actual assays were run for dieldrin and endrin on canned lima beans, frozen lima beans and frozen snap beans.

The fruit fly mortality was counted at different intervals of time until the mortalities fell into the proper range (30 to 85 per cent). Sun (51) suggests that the time of observing mortality may vary from 17 to 24 hours (or more) from one assay to another. This suggestion was well grounded, because after 30 hours of incubation at 25°F, the beans soured and gave off odors indicating the beginning of putrefaction.

When fruit flies become 72 hours old, natural mortality is then a factor for consideration. The exposure period for flies which are between 6 and 30 hours of age should not be for longer than 48 hours, to avoid the effect of natural mortality.

Two assays were made for dieldrin and two for endrin in canned and frozen lima beans and in frozen snap beans. The two assays for each toxicant in each processed product, were determined within a two week period and were successive in almost every case.

All the canned lima bean samples had been held in storage at room temperature for over one year.

1. **Dieldrin Treated Canned Lima Beans**

The standard series for dieldrin and canned lima beans was the same as that worked out in the evaluation studies using pumpkin (0.75, 1, 1.25 and 1.75 ppm). The WSD values for the two assays are ± 11.4 and ± 10.6, which are only slightly different. The differences between the average per cent mortality for the sample plus 1 ppm and the average per cent mortality for the 1 ppm level of the standard series
were 4.0 (See Table 7) and 4.4 (See Table 8).

The high and the low values for the sample plus 1 ppm as given in Tables 7 and 8 were 66.4 and 43.6 per cent and 51.6 and 38.8 per cent, respectively. Using these values, the range of the sample plus 1 ppm may be determined from the standard curve. For dieldrin in canned lima beans these values are 0.92 and 1.30 ppm for the first assay (See Table 7) and are 0.89 and 1.07 ppm for the second assay (See Table 8). By subtracting 1 ppm from each of these values, the range that the residue value lies between may be determined. One subtracted from 0.89 and 0.92 leaves negative numbers, but since it is impossible to have a negative residue, zero is taken as the low concentration. One subtracted from 1.30 and 1.07 ppm equals 0.30 and 0.07 ppm, respectively. These data show that the residue values of dieldrin lie between 0 and 0.30 ppm in the first assay and 0 and 0.07 ppm in the second assay (See Tables 7 and 8).

The two assays for dieldrin in canned lima beans were run approximately 6 months apart. This may in part explain the marked difference in the results. Also the two points on the curve, 1 ppm and the sample plus 1 ppm both lie below the dosage-mortality curve for the second assay (See Graph 8). All curves are plotted according to the simplified method of Litchfield and Wilcoxon (39). In using this type of curve it is possible for the experimental sample (i.e. sample + added increment) to fall below the curve.
Table 7
DOSAGE-MORTALITY DATA FOR DIELDRIN IN CANNED LIMA BEANS
(Modified Sun Method)
25 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>0.75</td>
<td>31.6</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>47.0</td>
<td>24</td>
</tr>
<tr>
<td>Sample</td>
<td>51.0</td>
<td>24</td>
</tr>
<tr>
<td>‡ 1 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>60.0</td>
<td>22</td>
</tr>
<tr>
<td>1.75</td>
<td>87.3</td>
<td>10</td>
</tr>
</tbody>
</table>

WSD = ‡ 11.4

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(51.0 - 47.0) \pm 11.4 = 4.0 \pm 11.4 = -7.4 \text{ to } 15.4 \text{ per cent.}\]

The sample plus 1 ppm may be as low as 43.6 per cent and as high as 66.4 per cent, these values correspond to 0.92 and 1.30 ppm respectively. Therefore, the residue value lies between 0 and 0.30 ppm.
Graph 7

DOSEAGE - MORTALITY CURVE FOR DIELDRIN IN CANNED LIMA BEANS

(Modified Sun Method)

25 Hrs. Exposure at 25°C.
Table 8

DOSAGE-MORTALITY DATA FOR DIELDRIN IN CANNED LIMA BEANS

(Modified Sun Method)

23 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6</td>
<td>4</td>
</tr>
<tr>
<td>0.75</td>
<td>31.6</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>40.6</td>
<td>26</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>45.0</td>
<td>20</td>
</tr>
<tr>
<td>1.25</td>
<td>59.0</td>
<td>10</td>
</tr>
<tr>
<td>1.75</td>
<td>84.0</td>
<td>14</td>
</tr>
</tbody>
</table>

WSD = $\frac{1}{4} 10.6$.

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

$((45.0 - 40.6) \div 10.6 = 4.4 \div 10.6 = -6.2$ to 15 per cent.

The sample plus 1 ppm may be as low as 38.8 per cent and as high as 51.6 per cent, these values correspond to 0.89 and 1.07 ppm, respectively. Therefore, the residue value lies between 0 and 0.07 ppm.
Graph 8

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN CANNED LIMA BEANS

23 Hrs. Exposure at 25°C.

Per Cent Mortality - Probit Units

Concentration - ppm

Log Scale

x - Standard Series
○ - Sample + 1 ppm
2. Endrin Treated Canned Lima Beans

The concentrations of endrin used in pumpkin to obtain a standard curve were not potent enough to produce similar mortalities in macerated canned lima beans. The concentrations of 0.75, 1, 1.5 and 2 ppm had to be increased by 1 ppm in all treatments to produce mortalities ranging from 30 to 85 per cent. The actual concentrations used were 1.75, 2, 2.5 and 3 ppm. Slurried canned lima beans mask the effects of endrin more than canned pumpkin. Canned lima beans did not have this effect on dieldrin, as the concentration levels of dieldrin were the same in the preparation of the dosage-mortality curves for dieldrin in pumpkin and canned lima beans (0.75, 1, 1.25 and 1.75 ppm).

In the first assay for endrin in canned lima beans, the mean percent mortality value for the sample plus 2 ppm was less than the value for the 2 ppm endrin level in the standard series (See Table 9 and Graph 9). This is the only time in all the determinations that such a result was recorded.

The residue value is reported as zero ppm for the first assay (See Table 9). For the second assay, the residue value is reported as lying between 0 and 0.20 ppm (See Table 10). This difference in results is due to the greater range in mortalities between the replicates of each concentration in the second assay. The WSD values vary by $\mu$, being ±9.1 and ±13.1, respectively. The results indicate that there is no residue or a very small residue of endrin in the year old samples of canned lima beans.
Table 9

DOSAGE-MORTALITY DATA FOR ENDRIN IN CANNED LIMA BEANS

(Modified Sun Method)

18 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.75</td>
<td>36.0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>47.0</td>
<td>16</td>
</tr>
<tr>
<td>Sample</td>
<td>40.0</td>
<td>24</td>
</tr>
<tr>
<td>+ 2 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>70.3</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>83.0</td>
<td>6</td>
</tr>
</tbody>
</table>

WSD = ± 9.1

The difference between the sample plus 2 ppm and 2 ppm in the standard series is stated to be

\[(40.0 - 47.0) ± 9.1 = -7.0 ± 9.1 = -16.1 \text{ to } 2.1 \text{ per cent.}\]

The sample plus 2 ppm may be as low as 23.9 per cent and as high as 42.1 per cent, these values correspond to 2.5 and 1.88 ppm respectively. Considering dilution, the residue value is zero.
Graph 9

DOSAGE - MORTALITY CURVE FOR ENDRIN IN CANNED LIMA BEANS

(Modified Sun Method)

18 Hrs. Exposure at 25°C.
Table 10

DOSAGE-MORTALITY DATA FOR ENDRIN IN CANNED LIMA BEANS

(Modified Sun Method)

18 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.75</td>
<td>27.3</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>39.6</td>
<td>24</td>
</tr>
<tr>
<td>Sample + 2 ppm</td>
<td>41.0</td>
<td>18</td>
</tr>
<tr>
<td>2.5</td>
<td>64.0</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>86.3</td>
<td>10</td>
</tr>
</tbody>
</table>

WSD = ± 13.1

The difference between the sample plus 2 ppm and 2 ppm in the standard series is stated to be

\[(41.0 - 39.6) \pm 13.1 = 1.4 \pm 13.1 = -11.7 \text{ to } 14.5 \text{ per cent}\]

The sample plus 2 ppm may be as low as 29.3 per cent and as high as 55.5 per cent, these values correspond to 1.80 and 2.25 ppm, respectively. Therefore, the residue value lies between 0 and 0.25 ppm.
Graph 10

DOSAGE - MORTALITY CURVE FOR ENDRIN IN CANNED LIMA BEANS

(Modified Sun Method)

18 Hrs. Exposure at 25° C.

Per Cent Mortality - Profilt Units

Log Scale

Concentration - ppm

x - Standard Series
o - Sample + 2 ppm
3. **Dieldrin Treated Frozen Lima Beans**

Frozen lima beans and snap beans were ground in the frozen state in an overhand kitchen grinder and were then allowed to thaw. To facilitate blending the toxicant (dieldrin or endrin) and the 180 g. of the thawed ground frozen lima beans, a 20 ml. volume of water was added. To take into account the dilution the two concentrations between which the residue lies (found statistically) have been multiplied by a factor of 10/9.

There was a large difference between the highest and lowest replicates for each concentration level in the two assays for dieldrin in frozen lima beans. The differences for 0.75, 1, sample plus 1, 1.25 and 1.75 ppm are 22, 46, 26, 42 and 36 for the first assay (See Table 11) and 16, 26, 30, 32 and 30 for the second assay (See Table 12), respectively. The WSD values are relatively high, ±22.9 and ±17.9, which connotes that there is a large difference between the highest and lowest replicates for the given levels of dieldrin concentrations.

The dieldrin residue values lie between 0 and 0.83 ppm in the first assay and 0 and 0.64 ppm in the second assay, respectively (See Tables 11 and 12).
Table 11

DOSAGE-MORTALITY DATA FOR DIELDRIN IN FROZEN LIMA BEANS

(Modified Sun Method)

18 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>4</td>
</tr>
<tr>
<td>0.75</td>
<td>18.3</td>
<td>22</td>
</tr>
<tr>
<td>1</td>
<td>42.3</td>
<td>46</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>48.6</td>
<td>26</td>
</tr>
<tr>
<td>1.25</td>
<td>59.1</td>
<td>42</td>
</tr>
<tr>
<td>1.75</td>
<td>76.6</td>
<td>36</td>
</tr>
</tbody>
</table>

WSD = ± 22.9

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(48.6 - 42.3) \pm 22.9 = 6.3 \pm 22.9 = -16.6 \pm 29.2 \text{ per cent}\]

The sample plus 1 ppm may be as low as 32.0 per cent and as high as 77.8 per cent, these values correspond to 0.89 and >1.75 ppm respectively. Considering dilution, the residue value lies between 0 and >0.83 ppm.
Graph 11

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN FROZEN LIMA BEANS

(Modified Sun Method)

18 Hrs. Exposure at 25° C.

x - Standard Series
○ - Sample ± 1 ppm

Per Cent Mortality - Probit Units

Concentration - ppm
Log Scale
Table 12

DOSE-MORTALITY DATA FOR DIELDREN IN FROZEN LIMA BEANS

(Modified Sun Method)

21 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>0.75</td>
<td>36.6</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>56.0</td>
<td>26</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>57.0</td>
<td>30</td>
</tr>
<tr>
<td>1.25</td>
<td>63.6</td>
<td>32</td>
</tr>
<tr>
<td>1.75</td>
<td>76.3</td>
<td>30</td>
</tr>
</tbody>
</table>

WSD = ± 17.9

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(57.0 - 56.0) \times 17.9 = 1.0 \times 17.9 = -16.9 \text{ to } 18.9 \text{ per cent}\]

The sample plus 1 ppm may be as low as 40.1 per cent and as high as 73.9 per cent, these values correspond to 0.71 and 1.58 ppm respectively. Considering dilution, the residue value lies between 0 and 0.64 ppm.
Graph 12

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN FROZEN LIMA BEANS

(Modified Sun Method)

21 Hrs. Exposure at 25° C.
1. **Endrin Treated Frozen Lima Beans**

The endrin residue in frozen lima beans taking dilution into account, lies between 0.22 and >1.11 ppm in the first assay (See Table 13) and between 1.11 and >1.11 ppm in the second assay (See Table 14). The respective WSD values were ±12.5 and ±16.6. In each case, a large variation between the highest and lowest replicates for the 2.5 ppm concentration level in the standard series was recorded.

By observing the plot of the dosage-mortality curves and the sample plus 2 ppm (See Graphs 13 and 14), in both assays it can be seen that the sample plus 2 ppm is approximately 0.6 ppm greater than the 2 ppm concentration level of the standard series.

Frozen lima beans had a masking effect on endrin toxicity. The same levels of endrin that were used to produce the standard curve in the canned lima bean assays were used for frozen lima beans. The dosages used to produce a curve in the evaluation study with pumpkin had to be increased by 1 ppm in all instances. To produce fruit fly mortality for the construction of the dosage-mortality curve, 1.75, 2, 2.5 and 3 ppm concentrations were employed.
Table 13
DOSAGE-MORTALITY DATA FOR ENDRIN IN FROZEN LIMA BEANS
(Modified Sun Method)
25.5 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>2</td>
</tr>
<tr>
<td>1.75</td>
<td>27.0</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>36.0</td>
<td>14</td>
</tr>
<tr>
<td>Sample + 2 ppm</td>
<td>66.6</td>
<td>18</td>
</tr>
<tr>
<td>2.5</td>
<td>57.3</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>85.0</td>
<td>10</td>
</tr>
</tbody>
</table>

WSD = ± 12.5

The difference between the sample plus 2 ppm and 2 ppm in the standard series is stated to be

\[(66.6 - 36.0) \pm 12.5 = 30.6 \pm 12.5 = -13.1 \text{ to } 43.1 \text{ per cent}\]

The sample plus 2 ppm may be as low as 48.5 per cent and as high as 109.7 per cent, these values correspond to 2.20 and >3.00 ppm respectively. Considering dilution, the residue value lies between 0.22 and >1.11 ppm.
Graph 13
DOSAGE - MORTALITY CURVE FOR ENDRIN IN FROZEN LIMA BEANS
(Modified Sun Method)

25.5 Hrs. Exposure at 25° C.

Graph showing a dosage-mortality curve for endrin in frozen lima beans. The x-axis represents concentration in ppm on a log scale, while the y-axis represents percent mortality in probit units. The graph includes two sets of data points:
- 'x' - Standard Series
- 'o' - Sample + 2 ppm

The data points are plotted to illustrate the relationship between concentration and mortality.
Table 14

DOSE-MORTALITY DATA FOR ENDRIN IN FROZEN LIMA BEANS

(Modified Sun Method)

23 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.3</td>
<td>4</td>
</tr>
<tr>
<td>1.75</td>
<td>46.3</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>48.3</td>
<td>12</td>
</tr>
<tr>
<td>Sample + 2 ppm</td>
<td>75.6</td>
<td>10</td>
</tr>
<tr>
<td>2.5</td>
<td>72.3</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>86.3</td>
<td>16</td>
</tr>
</tbody>
</table>

WSD = ± 16.6

The difference between the sample plus 2 ppm and 2 ppm in the standard series is stated to be

$$(75.6 - 48.3) ± 16.6 = 27.3 ± 16.6 = 10.7 \text{ to } 43.9 \text{ per cent}$$

The sample plus 2 ppm may be as low as 86.3 per cent and as high as 119.5 per cent, these values correspond to 3.00 and $>3.00$ ppm respectively. Considering dilution, the residue value lies between 1.11 and $>1.11$ ppm.
Graph 14

DOSEAGE - MORTALITY CURVE FOR ENDZRIN IN FROZEN LIMA BEANS

(Modified Sun Method)

23 Hrs. Exposure at 25°C.

Per Cent Mortality - Probit Units

Concentration - ppm

Log Scale

x - Standard Series
o - Sample + 2 ppm
5. Dieldrin Treated Frozen Snap Beans

The residue value for dieldrin treated frozen snap beans lies between 0.11 and 0.64 ppm in the first assay and 0.10 and >1.11 ppm in the second assay (See Tables 15 and 16). Snap beans were ground, thawed and diluted in the same manner as the frozen lima beans, so that the above concentrations are values which have been corrected for dilution (multiplied by a factor of 10/9).

The WSD value was ±10.4 for the first assay of dieldrin in frozen snap beans and ±17.9, a relatively high value, for the second assay of dieldrin in frozen snap beans. It can be seen in Table 16 that there was a large difference in the per cent mortalities between the highest and lowest replicates for both the sample plus 1 ppm and the 1.5 ppm level of the standard series. Each of these two ranges had a value of 38.

The mortality due to the effective dieldrin residue in each assay was approximately the same. These values of 9.7 and 12.4, varied only by approximately 3 per cent.

The dieldrin dosages used in the preparation of the standard curve, varied from that employed in the dosage-mortality curves with pumpkin in the preliminary evaluation. The levels of dieldrin used with pumpkin were 0.75, 1, 1.25 and 1.75 ppm, and those utilized in the preparation of the standard series in frozen snap beans were 0.75, 1, 1.5 and 2 ppm. For the higher ranges of mortality more dieldrin was required to produce the desired kill.
Table 15

DOSAGE-MORTALITY DATA FOR DIELDRIN IN FROZEN SNAP BEANS

(Modified Sun Method)

20 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>0.75</td>
<td>34.0</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>47.6</td>
<td>24</td>
</tr>
<tr>
<td>Sample</td>
<td>57.3</td>
<td>16</td>
</tr>
<tr>
<td>+ 1 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>72.3</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>88.3</td>
<td>14</td>
</tr>
</tbody>
</table>

WSD = ± 10.4

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(57.3 - 47.6) \pm 10.4 = 9.7 \pm 10.4 = -0.7 \text{ to } 20.1 \text{ per cent}\]

The sample plus 1 ppm may be as low as 56.6 per cent and as high as 77.4 per cent, these values correspond to 1.10 and 1.58 ppm respectively. Considering dilution, the residue value lies between 0.11 and 0.64 ppm.
Graph 15

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN FROZEN SNAP BEANS

(Modified Sun Method)

20 Hrs. Exposure at 25° C.

Per Cent Mortality - Probit Units

x - Standard Series
o - Sample + 1 ppm

Concentration - ppm

Log Scale
Table 16

DOSAGE-MORTALITY DATA FOR DIELDRIN IN FROZEN SNAP BEANS

(Modified Sun Method)

21 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and LowestReplicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>0.75</td>
<td>28.6</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>46.6</td>
<td>22</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>59.0</td>
<td>38</td>
</tr>
<tr>
<td>1.5</td>
<td>75.0</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>88.3</td>
<td>16</td>
</tr>
</tbody>
</table>

WSD = ± 17.9

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(59.0 - 46.6) \pm 17.9 = 12.4 \pm 17.9 = -5.5 \text{ to } 30.3 \text{ per cent}\]

The sample plus 1 ppm may be as low as 53.5 per cent and as high as 89.3 per cent, these values correspond to 1.09 and >2.00 ppm. Considering dilution, the residue value lies between 0.10 and >1.11 ppm.
Graph 16

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN FROZEN SNAP BEANS

(Modified Sun Method)

21 Hrs. Exposure at \( 25^{\circ} \text{C} \).

Per Cent Mortality - Probit Units

\[ x - \text{Standard Series} \]
\[ o - \text{Sample + 1 ppm} \]

Concentration - ppm

Log Scale
6. **Endrin Treated Frozen Snap Beans**

The difference between the mortalities of the sample plus 1 ppm and 1 ppm in the standard series was 10.7 in the first assay and 1.6 in the second assay for endrin in frozen snap beans (See Tables 17 and 18). This large difference is responsible for the variation in results. The per cent mortalities plotted against the 4 dosage-levels, 0.75, 1, 1.5 and 2 ppm, on logarithmic-probability paper, fell almost on a straight line for each assay (See Graphs 17 and 18). The WSD values are low, being ±8.0 and ±6.1 for the two assays, respectively. These values indicate the small variation between the high and the low replicates for each standard series concentration and sample plus the added toxicant. These values were 8, 14, 14, 14 and 10 for the first assay and 8, 12, 14, 10 and 2 for the second assay of endrin in frozen snap beans.
Table 17
DOSAGE-MORTALITY DATA FOR ENDRIN IN FROZEN SNAP BEANS
(Modified Sun Method)
23 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>22.0</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>40.3</td>
<td>14</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>51.0</td>
<td>14</td>
</tr>
<tr>
<td>1.5</td>
<td>66.6</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>86.0</td>
<td>10</td>
</tr>
</tbody>
</table>

WSD = $\pm 8.0$

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(51.0 - 40.3) \pm 8.0 = 10.7 \pm 8.0 = 2.7 \text{ to } 18.7 \text{ per cent}\]

The sample plus 1 ppm may be as low as 53.7 per cent and as high as 69.7 per cent, these values correspond to 1.18 and 1.49 ppm. Considering dilution, the residue value lies between 0.20 and 0.54 ppm.
Graph 17

DOSAGE - MORTALITY CURVE FOR ENDREN IN FROZEN SNAP BEANS

(Modified Sun Method)

23 Hrs. Exposure at 25°C.
Table 18

DOSAGE-MORTALITY DATA FOR ENDRIN IN FROZEN SNAP BEANS

(Modified Sun Method)

24 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>19.3</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>40.0</td>
<td>12</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>41.6</td>
<td>14</td>
</tr>
<tr>
<td>1.5</td>
<td>71.0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>93.0</td>
<td>2</td>
</tr>
</tbody>
</table>

WSD = ± 6.1

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(41.6 - 40.0) \pm 6.1 = 1.6 \pm 6.1 = -4.5 \text{ to } 7.7 \text{ per cent}\]

The sample plus 1 ppm may be as low as 37.1 per cent and as high as 49.3 per cent, these values correspond to 0.97 and 1.10 ppm. Considering dilution, the residue value lies between 0 and 0.11 ppm.
Graph 18

DOSAGE - MORTALITY CURVE FOR ENDRIN IN FROZEN SNAP BEANS

(Modified Sun Method)

24 Hrs. Exposure at 25°C.

Per Cent Mortality - Probit Units

0.75 1 1.50 2
Concentration - ppm
Log Scale

- Standard Series
- Sample + 1 ppm
C. Preliminary Investigation Using The Culture Tube Method

In the Culture Tube Method, canned pumpkin was blended with the insecticides, dieldrin and endrin, in the same manner as in the Modified Sun Method. A smaller amount of pumpkin was used for each replicate in the evaluation studies.

In the Culture Tube Method, 20 fruit flies were exposed to the test material as compared to 50 in the Modified Sun Method.

1. The Wood Stake Procedure

In the first experiments with the Culture Tube Method, the pumpkin was spread out on wood stakes. Many experiments were carried out, using different concentrations and exposure times. In all cases with pumpkin the results were extremely variable.

With increasing concentration of dieldrin or endrin, from one dosage level to another, there was often a decrease in mortality rather than the expected increase. In Table 19, 0.75 ppm of dieldrin effected a mean per cent mortality of 58.0, while 1 ppm produced an average per cent mortality of 39.1. The mean fruit fly mortality was approximately 58 per cent for both the 0.75 and 1.75 ppm dieldrin levels. The effect of 1.75 ppm dieldrin was less than that of 1.5 ppm. In Table 20, the mean per cent mortality for 2 ppm dieldrin was 24.1 per cent, which is less than one half that recorded for 1 ppm. More examples of unpredicted response can be cited from Tables 19, 20, 21 and 22 for both dieldrin and endrin in pumpkin.

The extreme per cent mortality variation between the highest and lowest replicate for the individual concentration levels of dieldrin and endrin can also be seen in Tables 19, 20, 21 and 22. The
differences between the highest and lowest replicates for 0.75, 1, 1.5, 1.75 and 2 ppm dieldrin are 35, 45, 30, 85 and 55 in the first test (See Table 19) and 30, 35, 20, 75 and 30 for the second test (See Table 21). For endrin concentrations of 0.75, 1, 1.5 and 2 ppm in the first test (See Table 21), the differences between the highest and lowest replicates were 20, 70, 80 and 95; though in the second test (See Table 22), for 1.5, 2, 2.5 and 3 ppm, the differences were 20, 70, 15 and 55.

In the second test with endrin in canned pumpkin, 12 additional tubes with 50 flies instead of the usual 20 were exposed to concentration levels of 2 and 2.5 ppm. The average per cent mortality was 21.3 and 38.0 per cent for six replicates at 2 and 2.5 ppm, respectively. At 2 ppm the mortality for 20 flies, 24.1 per cent, compares with that for 50 flies, 21.3 per cent (exposure time was 48 hours). Using 50 flies the variation between the highest and lowest replicate was 54 as compared to 70 when 20 flies were exposed to 2 ppm endrin. For 2.5 ppm, the average mortality was 6.6 per cent when 20 flies were exposed and 38 per cent when 50 flies were exposed. The differences between the highest and lowest replicates at 2.5 ppm when exposing 20 flies was 16 and when exposing 50 flies it was 15 per cent.

Dosage-effect data for dieldrin and endrin in canned pumpkin using the Culture Tube Method with wood stakes were not plotted on logarithmic-probability paper because of the extreme variation in results.
Table 19
DOSAGE-MORTALITY DATA FOR DIELDRIN IN PUMPKIN
(Culture Tube Method - Wood Stakes)
22.5 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>58.0</td>
<td>35</td>
</tr>
<tr>
<td>1</td>
<td>39.1</td>
<td>45</td>
</tr>
<tr>
<td>1.5</td>
<td>70.0</td>
<td>30</td>
</tr>
<tr>
<td>1.75</td>
<td>58.3</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>82.5</td>
<td>55</td>
</tr>
</tbody>
</table>
Table 20

DOSAGE-MORTALITY DATA FOR DIELDRIN IN PUMPKIN

(Culture Tube Method - Wood Stakes)

23 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>32.5</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>56.6</td>
<td>35</td>
</tr>
<tr>
<td>1.5</td>
<td>77.3</td>
<td>20</td>
</tr>
<tr>
<td>1.75</td>
<td>78.3</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>24.1</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 21

DOSAGE-MORTALITY DATA FOR ENDRIN IN PUMPKIN

(Culture Tube Method - Wood Stakes)

48 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.8</td>
<td>5</td>
</tr>
<tr>
<td>0.75</td>
<td>5.8</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>29.1</td>
<td>70</td>
</tr>
<tr>
<td>1.5</td>
<td>25.0</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>17.5</td>
<td>95</td>
</tr>
</tbody>
</table>
Table 22

DOSAGE-MORTALITY DATA FOR ENDRIN IN PUMPKIN

(Culture Tube Method - Wood Stakes)

48 Hrs. Exposure at 25°C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.8</td>
<td>5</td>
</tr>
<tr>
<td>1.5</td>
<td>15.0</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>24.1</td>
<td>70</td>
</tr>
<tr>
<td>2.5</td>
<td>6.6</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>80.0</td>
<td>55</td>
</tr>
</tbody>
</table>
An assay for dieldrin in frozen snap beans was carried out using the Culture Tube Method with wood stakes. The average per cent mortalities increased with increased dosage as expected in toxicological work, which was not the strict case in pumpkin. The mean per cent mortalities for 0.75, 1, 1.5 and 2 ppm concentration levels were 34.1, 46.6, 69.1 and 90.0, respectively.

The mortality differences between the highest and lowest replicates for 0.75, 1, sample plus 1, 1.5 and 2 ppm were 30, 50, 35, 40 and 30, respectively. The WSD value, which indicates the per cent mortality range between the highest and lowest replicates, was a high value, \( \pm 24.6 \). The difference between the sample plus 1 ppm and 1 ppm in the standard series was 13.4 per cent. Taking dilution into consideration, the residue value lies between 0 and 1.11 ppm (See Table 23).
Table 23

DOSAGE-MORTALITY DATA FOR DIELDRIN IN FROZEN SNAP BEANS

(Culture Tube Method - Wood Stakes)

21 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>0.75</td>
<td>34.1</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>46.6</td>
<td>50</td>
</tr>
<tr>
<td>Sample + 1 ppm</td>
<td>60.0</td>
<td>35</td>
</tr>
<tr>
<td>1.5</td>
<td>69.1</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>90.0</td>
<td>30</td>
</tr>
</tbody>
</table>

WSD = ± 24.6

The difference between the sample plus 1 ppm and 1 ppm in the standard series is stated to be

\[(60.0 - 46.6) ± 24.6 = 13.4 ± 24.6 = -11.2 \text{ to } 38.0 \text{ per cent}\]

The sample plus 1 ppm may be as low as 48.8 per cent and as high as 98.0 per cent, these values correspond to 1.00 and >2.00 ppm. Considering dilution, the residue lies between 0 and >1.11 ppm.
Graph 19

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN FROZEN SNAP BEANS

(Culture Tube Method - Wood Stakes)

21 Hrs. Exposure at 25°C.

Per Cent Mortality - Probit Units

Concentration - ppm

Log Scale
The wood stake technique was abandoned because of the marked variation between the high and the low replicates for most of the concentration levels and the unpredictable response to insecticide concentration.

The wood stakes used in the Culture Tube Method were the plain type or untreated type. The plain type of stake supposedly has not received any treatment which would tend to preserve the wood when put to their conventional use in the soil as plot markers in vegetable gardening. If a plain stake is treated, the compounds used in such an operation may have an effect on the fruit fly mortality in the biological assay.

Each stake should be considered as biological material. Each stake tends to vary in physical properties. From stake to stake, varying amounts of water and acetone-insecticide solution may be absorbed from the portions of prepared test material when they are placed and spread over the surface of the stake. By examining the end section of a stake after the test material had been spread over its one surface, an indication of the depth of the penetration of the juices of the test material could be observed.

Apparently the addition of a piece of dead wood (although still a biological tissue) to the assay system, comprising a live test organism and macerated vegetable tissue, seems to be responsible for the variable results.
2. The Stainless Steel Strip Modification

Next, strips of stainless steel sheet, the size of the wood stakes, were used in the Culture Tube Method with pumpkin. Stainless steel was chosen because it is not a biological material and is therefore not a source of variation under the conditions of the test. Also, stainless steel strips are easy to clean and are resistant to rust.

The differences in per cent between the highest and lowest replicates (6 replicates at each dosage level for 0.375, 0.5, 0.6 and 0.75 ppm were 20, 25, 20 and 30, respectively for dieldrin. The average per cent mortalities for the above 4 concentration levels were 20.8, 49.1, 62.5 and 71.6, respectively (See Table 2h).

The highest dieldrin level in the standard series for canned pumpkin employing the Culture Tube Method (stainless steel strips) was used as the lowest concentration for a similar study using the Modified Sun Method. A concentration range of 0.375 to 0.75 ppm was needed to produce a standard curve for the Culture Tube Method with stainless steel strips (See Table 2h), whereas the higher concentration range of 0.75 to 1.75 ppm was necessary to produce a standard curve employing the Modified Sun Method (See Table 5).
### Table 24

**DOSAGE-MORTALITY DATA FOR DIELDRIN IN PUMPKIN**

(Culture Tube Method - Stainless Steel Strips)

24 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.375</td>
<td>20.8</td>
<td>20</td>
</tr>
<tr>
<td>0.5</td>
<td>49.1</td>
<td>25</td>
</tr>
<tr>
<td>0.6</td>
<td>62.5</td>
<td>20</td>
</tr>
<tr>
<td>0.75</td>
<td>71.6</td>
<td>30</td>
</tr>
</tbody>
</table>
Graph 20

DOSAGE - MORTALITY CURVE FOR DIELDRIN IN PUMPKIN

(Culture Tube Method - Stainless Steel Strips)

24 Hrs. Exposure at 25°C.

Per Cent Mortality - Probit Units

x - Standard Series

Concentration - ppm

Log Scale
In preliminary studies with dieldrin using the Culture Tube Method (stainless steel strips), different numbers of the assay organism, *D. melanogaster*, were exposed to concentrations of 0.375, 0.5, 0.6 and 0.75 ppm (See Table 25). The differences in per cent between the highest and lowest replicates exposing 10 flies were 60, 60, 20 and 50; exposing 20 flies were 20, 25, 20 and 30; and exposing 50 flies were 2, 2, 4 and 20, respectively.

It can be seen in Table 25, that the mortality tends to decrease as the number of flies exposed per replicate increases at the lower concentrations. The lowest level of dieldrin, 0.375 ppm, brought about approximately 50 per cent kill when 10 flies were exposed to this concentration, but only about 20 per cent when 20 flies were exposed and less than 1 per cent when 50 flies were exposed. When employing 50 flies per replicate, there was little mortality produced when the flies were exposed to 0.375, 0.5 and 0.6 ppm dieldrin. After 48 hours exposure using 50 flies, 63.3, 83.0 and 91.3 per cent mortalities, respectively were recorded for these concentrations.

For a given number of fruit flies and exposure time, the total amount of toxicant present must be above a certain level to produce a desired mortality. The amount of toxicant present in the pumpkin at the 0.375 ppm concentration was great enough to kill almost 50 per cent when 10 flies were exposed, but not enough to produce 1 per cent kill when 50 flies were exposed per replicate. This situation is known as the crowding effect.

Fisher and Smallman (19) state that there is a maximum number of flies that can be exposed to a unit of treated tissue. They found
that when the number reaches 18 to 20 flies per replicate, the quantity of insecticide rather than the concentration becomes critical.

Thus the data of Fisher and Smallman agree with the data presented herein i.e. that a population of 20 flies per tube gave the most satisfactory kill of flies at low concentrations of dieldrin
### Table 25

**DOSAGE-MORTALITY DATA FOR DIELDRIN IN PUMPKIN USING DIFFERENT NUMBERS OF ASSAY ORGANISMS**  
(Culture Tube Method - Stainless Steel Strips)

<table>
<thead>
<tr>
<th>Dieldrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 flies (23 Hrs.)</td>
<td>20 flies (24 Hrs.)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.375</td>
<td>46.6</td>
<td>20.8</td>
</tr>
<tr>
<td>0.5</td>
<td>55.0</td>
<td>49.1</td>
</tr>
<tr>
<td>0.6</td>
<td>66.6</td>
<td>62.5</td>
</tr>
<tr>
<td>0.75</td>
<td>76.6</td>
<td>71.6</td>
</tr>
</tbody>
</table>
When endrin was tested with the Culture Tube Method (stainless steel strips) with canned pumpkin, lower concentrations were used to produce the dosage-effect curve than when the Modified Sun Method was employed. The endrin concentrations used in the Culture Tube Method were 0.75, 1, 1.25 and 1.5 ppm (See Table 26); and 0.75, 1, 1.5 and 2 ppm in the Modified Sun Method (See Table 6).

The differences in per cent between the highest and lowest replicates for 0.75, 1, 1.25 and 1.5 ppm endrin were 20, 40, 30 and 15; respectively.
Table 26

DOSAGE-MORTALITY DATA FOR ENDRIN IN PUMPKIN

(Culture Tube Method - Stainless Steel Strips)

22.5 Hrs. Exposure at 25° C.

<table>
<thead>
<tr>
<th>Endrin ppm</th>
<th>Average Per Cent Mortality (6 Replicates)</th>
<th>Difference in Per Cent Between Highest and Lowest Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>16.6</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>49.1</td>
<td>40</td>
</tr>
<tr>
<td>1.25</td>
<td>68.3</td>
<td>30</td>
</tr>
<tr>
<td>1.5</td>
<td>86.6</td>
<td>15</td>
</tr>
</tbody>
</table>
Graph 21

DOSAGE - MORTALITY CURVE FOR ENDRIN IN PUMPKIN

(Culture Tube Method - Stainless Steel Strips)

22.5 Hrs. Exposure at 25° C.
V. Discussion

A. Evaluation of Six Organic Insecticides Employing the Modified Sun Method

Six organic insecticides - malathion, demeton, perthane, Strobane, dieldrin and endrin - were evaluated by the direct exposure - macerated tissue type of assay. The Modified Sun Method was used to find dosages that would give a range of mortalities between 30 and 85 percent, and which when plotted on logarithmic-probability paper would approximate a straight line. If a standard curve cannot be conveniently prepared, a quantitative determination by biological means cannot be made. Four of the 6 compounds were eliminated from further consideration after the standard curves were determined.

In 6 attempts with malathion it was not possible to prepare a standard curve with 100 dosages. The extreme variation between replicates for the different dosage levels is explained by the fact that organic phosphates break down when in a water system due to hydrolysis. Malathion was not assayed by the direct exposure - macerated tissue technique, because of this variation.

Demeton, perthane and Strobane in pumpkin under the given conditions had relatively low toxicities for fruit flies. Therefore, small residues of these compounds could not be detected using this type of biological assay. The LD$_{50}$ value for demeton in 22 hours at 25$^\circ$ C. was approximately 9 ppm. The ID$_{50}$ value for perthane in 15 hours at 25$^\circ$ C. was approximately 200 ppm. For Strobane, the ID$_{50}$
value in pumpkin in 48 hours exposure at 25° C. was approximately 27 ppm.

Dieldrin and endrin were the two compounds which were deemed satisfactory for assay. Dosage-mortality curves for dieldrin and endrin would be reproduced from day to day. The 30 to 85 per cent mortality range was obtained with concentrations from 0.75 to 1.75 ppm for dieldrin and 0.75 to 2 ppm for endrin. Dieldrin and endrin had high toxicities for fruit flies compared to the other compounds, and therefore the detection of small residual quantities of these insecticides is feasible. The differences in per cent mortality between the highest and lowest replicates did not tend to vary as much as for some of the other compounds.

B. Consideration of the Assay Results of the Modified Sun Method with Respect to the Newly Established Tolerances

The tolerance established for dieldrin by the Food and Drug Administration was 0.1 ppm. When a tolerance is published for endrin, it probably will be 0.1 ppm because endrin is the stereoisomer of dieldrin and it is thought to be similar in activity. Tolerances are based mainly on mammalian toxicity and the sensitivity of the available methods of estimation.

Considering the above, it can be stated that endrin treated frozen lima beans and dieldrin treated frozen snap beans contain more toxicant than the legal tolerance. For endrin treated frozen lima beans the residue values were stated to lie between 0.22 and 1.11 ppm in the first assay and between 1.11 and >1.11 ppm in the second assay (See Table 27). The residue values for dieldrin treated
frozen snap beans lie between 0.11 and >0.64 ppm in the first assay and 0.10 and >1.11 ppm in the second assay (See Table 27). The lower concentration value given for the residue values of endrin treated frozen lima beans and the dieldrin treated frozen snap beans were equal to or greater than 0.1 ppm. If the samples had been drawn from commercial packs, then the packs should not be placed in the usual marketing channels because they would be liable to seizure by the FDA authorities.

When the residue value lies between zero and some concentration greater than 0.1 ppm, that sample may also contain an amount of the pesticide above the established tolerance. For the first assay of dieldrin treated canned lima beans the residue value lies between 0 and 0.30 ppm. The residue value lies between 0 and 0.20 ppm in the second assay of the endrin treated canned lima beans. The residue values were stated to lie between 0 and >0.83 ppm in the first assay and between 0 and 0.64 ppm for the second assay of dieldrin treated frozen lima beans. The residue value lies between 0 and 0.11 ppm in the second assay of endrin treated frozen snap beans (See Table 27). If adequate control samples of canned and frozen lima beans and frozen snap beans had been available, assays might have been run on samples with added known amounts of dieldrin or endrin. In this way the statistical results of assays of known toxicant concentration could be compared to the statistical results of assays of unknown toxicant levels, thereby clarifying the idea of the residue value lying between two concentrations.

Tukey's Method of statistical analysis is ideal for biological
assay data because it more adequately takes into account the variation between replicates for each concentration involved in the assay (WSD value) and the difference between the mean mortalities of the sample plus the insecticide increment and the corresponding concentration of the standard series, than does Wilcoxon's Ranking Method (59) as used by Sun (53).
Table 27

SUMMARY OF RESULTS FOR ASSAYS USING THE MODIFIED SUN METHOD AND STATISTICALLY EVALUATED BY TUKEY'S METHOD

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Residue Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Canned Lima Beans</strong></td>
</tr>
<tr>
<td>7</td>
<td>0 and 0.30 ppm</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0 and 0.07 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0 ppm</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0 and 0.20 ppm</td>
</tr>
<tr>
<td></td>
<td><strong>Frozen Lima Beans</strong></td>
</tr>
<tr>
<td>11</td>
<td>0 and &gt;0.83 ppm</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0 and 0.64 ppm</td>
</tr>
<tr>
<td></td>
<td><strong>Frozen Snap Beans</strong></td>
</tr>
<tr>
<td>13</td>
<td>0.22 and &gt;1.11 ppm</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.11 and &gt;1.11 ppm</td>
</tr>
<tr>
<td>15</td>
<td><strong>Frozen Snap Beans</strong></td>
</tr>
<tr>
<td></td>
<td>0.11 and 0.64 ppm</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.10 and &gt;1.11 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.20 and 0.54 ppm</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0 and 0.11 ppm</td>
</tr>
</tbody>
</table>
By using Wilcoxon's Ranking Method to treat the data, it is possible to determine whether small differences between dosage levels (treatments) are statistically significant at a probability of 0.05 (See Table 28). If the treatments differ significantly (Chi-square test for the comparison of several treatments) then a test is carried out to determine if there is a significant difference between the sample plus the insecticide increment and the corresponding dosage of the standard series (test for unpaired replicates). Essentially the Ranking Method is a rapid but not highly precise means of testing for significant differences between treatments.

For canned lima beans, there were no significant differences between the sample plus 1 ppm toxicant and 1 ppm in the standard series. This would be expected, after the samples had been held in storage for over one year. These results correspond to the results as analyzed by the Tukey Method (See Table 27), because for each assay the residue value was stated to lie between two concentrations which constituted a narrow range. The calculated results by the Tukey Method indicate in themselves the variation encountered in the individual assay.

A small residue, 0.07 ppm, was significant in the second assay of dieldrin in frozen lima beans. This value is under the 0.1 ppm tolerance established for dieldrin on certain horticultural commodities.

Large residues of endrin, 0.67 and 0.66 ppm were found in frozen lima beans. This high concentration would warrant seizure by the Food and Drug Administration if marketed. These calculated
results (Ranking Method) again do not indicate the variation which actually occurred. As previously stated a large difference in percent between the highest and lowest replicates for the 2.5 ppm dosage level in the standard series was recorded. The residue values as calculated by Tukey’s Method indicate this variation in the results when the residues are stated to lie between 0.22 and >1.11 ppm and 1.11 and >1.11 ppm. According to either method of statistical analysis, it can be stated that the residue is greater than 0.1 ppm endrin (the probable tolerance).

It would seem that lima beans would be free of residue, because of protection afforded by the pod from spray and dust treatments. In the pilot plant operation the beans were shelled by hand. The toxicant at least in part was probably transferred from the pods to the beans by the hands of the shellers. A similar situation probably occurs in commercial operations. In a commercial operation, the beans coming out of a viner might possibly be contaminated. A short time after the operation of the viner is begun, the viner could become contaminated with toxicant from the vines and pods.

Small residues were found in the frozen snap bean samples. In the first assay for dieldrin, 0.13 ppm dieldrin residue was detected. In the first assay for dieldrin treated frozen snap beans, 0.18 ppm toxicant was detected. The same deductions may be made if the calculated differences (Tukey’s Method) are examined. The dieldrin residue value in the first dieldrin assay was stated to lie between 0.11 and 0.64 ppm and the endrin residue value in the first endrin assay was stated to lie between 0.20 and 0.54 ppm.
By both methods of analysis, the samples would be liable to seizure if these were part of a commercial pack.

By analyzing the data for the second assay for dieldrin residue in frozen snap beans by Tukey's Method (See Table 27) the variation in the replicates as well as the estimated limits of the residue value itself are indicated (0.10 and >1.11 ppm). If this sample was from a commercial pack it would be liable to seizure by the FDA authorities.

By the Ranking Method of analysis, it can only be stated that there was no significant difference between all the treatments and that there was no difference between the sample plus 1 ppm and 1 ppm in the standard series at a probability level of 0.05.

When the residues are expressed as values lying between two concentrations as by the Tukey Method of statistical analysis, both the estimated residue and the variation of the replicates are indicated. By using the Ranking Method, it becomes a case of testing for significant difference between two treatments, at a certain probability level.
<table>
<thead>
<tr>
<th>Table No.</th>
<th>T Value at 0.05 Level</th>
<th>Experimental Value</th>
<th>Significant Residue or not Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned Lima Beans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>34.5</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>33</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>45</td>
<td>Not sign.</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>39</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Frozen Lima Beans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>35</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>22</td>
<td>Sign. 0.07</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>21</td>
<td>Sign. 0.67</td>
</tr>
<tr>
<td>14</td>
<td>27</td>
<td>21</td>
<td>Sign. 0.66</td>
</tr>
<tr>
<td>Frozen Snap Beans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>27</td>
<td>26.5</td>
<td>Sign. 0.13</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>27</td>
<td>28</td>
<td>Not sign</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>27</td>
<td>23.5</td>
<td>Sign. 0.18</td>
</tr>
<tr>
<td>18</td>
<td>27</td>
<td>30.5</td>
<td>Not sign</td>
</tr>
</tbody>
</table>
C. Comparison of the Modified Sun Method and the Culture Tube Method
(Stainless Steel Strips)

The Culture Tube Method using stainless steel strips, although not perfected in the current study, has several advantages over the Modified Sun Method.

Although the flies are counted and anesthetized in a continuous flow of carbon dioxide in both methods, there is one obvious disadvantage in the Modified Sun Method. In this method the flies are swept into an aluminum foil container which is placed on the surface of the test material. The flies recover from the anesthesia and are immediately in contact with the toxic material. In the Culture Tube Method, the anesthetized flies are counted out directly into the test container, where they are allowed to recover before the toxic material is introduced. This feature is a distinct advantage. If all the flies in one test tube do not recover (recovery is usually a rapid process), it can be set aside and another test tube substituted. In the Modified Sun Method if some of the flies fail to recover in a short time, the test container must be opened, the aluminum cup and the live and dead flies removed, and then another cup with anesthetized flies introduced.

The counting procedures in the Culture Tube Method are easier than in the Modified Sun Method. The live flies are counted out in both cases with the aspirator gun. The flies are drawn directly into the test tubes in the Culture Tube Method, whereas they are first drawn into a small vial, transferred to the aluminum cup and then the cup placed on the surface of the macerated vegetable material.
It is less difficult to count the number of dead flies in the test tubes in the Culture Tube Method than it is in the 4 oz. jars used in the Modified Sun Method. The jars must be continuously turned to make the count. The imperfections of the glass in the jars also increases the counting difficulties in the Modified Sun Method.

The test tubes and stainless steel strips used in the Culture Tube Method are easier to wash and decontaminate than the 4 oz. narrow mouthed jars used in the Modified Sun Method. Also, handling test tubes and cotton stoppers is a less difficult procedure than fastening squares of cheesecloth over the narrow mouthed jars.

Since the number of flies used in the Culture Tube Method is less than the number used in the Modified Sun Method, it is not necessary to rear and maintain so many active fruit fly cultures.

In the evaluation work with dieldrin and endrin in pumpkin using the Culture Tube Method, lower concentrations were employed to produce a standard curve than in the Modified Sun Method. In this work, the seemingly low standard series values that Pankaskie and Sun (45) suggest for most plant materials, 0.1, 0.2 and 0.3 ppm are more closely approached by the Culture Tube Method than the Modified Sun Method. Possibly the Culture Tube Method could be developed into the more sensitive of the two methods.

The Culture Tube Method is less time consuming than the Modified Sun Method. It takes less time for fly counting and for handling and washing equipment. As each procedure involves direct exposure of the fruit flies to the macerated tissue, the time required for the blending technique is the same for both methods.
D. General Discussion and Role of Bioassay

Most biological assay techniques are laborious. If an accurate rapid chemical test is available, it should be used to detect the residue present in processed plant material.

For many of the hydrocarbon type compounds with toxic properties, analytical methods have been published, but the perfection of these methods has been slow. Generally, the hydrocarbons are inert, and this property has hindered the development of utilizable colorimetric tests.

The Schechter-Haller Method (46) and various modifications for the analysis of DDT and analogs of DDT have been used extensively although there are certain definite limitations. Methods of analysis for aldrin and dieldrin and their isomers have been recently published but have only limited use. Danish and Lidov (17) have published a colorimetric method for estimating small amounts of aldrin (not specific, other pesticidal compounds interfere).

Gunther et al. (26) published a preliminary report on the colorimetric detection of dieldrin. In developing a color test for dieldrin the difficulty lies in cleanly opening the epoxide portion of the molecule. The chemistry of the reaction which they used is complicated and obscure with the result that the optimum conditions for the production of color must be derived empirically. Dieldrin is heated with p-nitro phenol in this procedure. The closely related isomers of dieldrin (endrin, aldrin and isodrin) do not respond to the color reaction.

Chlorinated hydrocarbon residues are often estimated by a
total chlorine or organic chloride determination. The data accrued with such non-specific methods must be interpreted with caution. A chlorine or chloride determination may be indicative of the amount of Cl present but not of the amount of intact residual compound. The spraying or dusting history of the crop involved must be known. If two chlorinated compounds have been used for control, it is not possible by these methods to estimate the constituent parts of the residue. If two compounds have been employed, the analysis may be carried out, and the effective residue may be expressed in terms of one of the compounds present.

Sun (53) points out that due to the continuous discovery and development of new insecticides, an accurate chemical method of analyzing a few micrograms of a new toxicant, especially in the presence of a large amount of interfering substances, is usually not available within a short period of time for practical applications on residue problems. Therefore, for the new compounds and for some of the widely employed pesticides, biological assay may be the only method of estimating toxic residues. For dieldrin and its related isomers, biological assay is the most widely accepted method of estimation.

The advantages of bioassay are that it may be the only available method of estimation of toxic residue and also may be used as a comparative or supplementary method for an accepted chemical procedure. Biological assay is not a specific method for one insecticide, therefore the history of the control practices for the horticultural crop must be known. Several compounds are often used during the course of the growing season because many of the insecticides are
specific for a limited number of pests, hence one compound will not be as effective as 2 or 3 compounds, i.e., ovotran (p-chlorophenyl p-chlorobenzensulphonate) for mites, DDT for codling moth, and fermate (ferric dimethyldithiocarbamate) for scab. If a determination is made on a crop which has been treated with more than one pesticide during the course of its growing season, the combined effective residue may be estimated as the compound which is used in the standard series (reference compound). This is generally not a useful procedure in most cases unless the compounds are similar in toxicity and structure.

In the recent publication of tolerances (54) it is stated that where residues from two or more chemicals in the same class are present on a fruit or vegetable the tolerance for the total of such residues shall be the same as that for the chemicals having the lowest numerical tolerance in this class. Therefore, by using the compound with the lowest tolerance for the preparation of the standard curve and also in the addition procedure, the combined residue could be estimated in terms of pesticides having the lowest numerical tolerance in the given class.

In biological assay, there tends to be a masking effect. This effect occurs when the test insect is directly exposed either to an evaporated extract or to macerated tissue. When an extraction process is used, the extract consists of the toxicant and other compounds which were leached out by the solvent. These sticky, waxy materials mask the toxicant to some degree.

The presence of inherent plant materials may cause mortality.
These substances affect the assay in the same way the insecticides do, in that the small quantities give no appreciable mortality in the checks but they do give the small detectable increment mortality when a known amount of toxicant is added.

The factor which affects biological assay the most is contamination. Every effort must be made to eliminate all sources of contamination. Since very small concentrations are being estimated, the smallest amount of toxicant may greatly affect the results. All equipment should be thoroughly washed and heated at high temperatures for a prolonged period. Laboratory benches, the worker's hands, and equipment which cannot be decontaminated by heat may be sources of contamination.

Sun and Pankaskie (51) suggested that fruit flies only be employed to determine minute quantities of a toxicant which cannot be detected by house flies, because fruit flies are more sensitive to toxicants and therefore are more susceptible to insecticidal contamination.

Biological assay is an old method of estimation in pharmacology for many drugs of plant or animal origin. Since many organic insecticides have come into wide use before chemical methods for their analysis have been developed, biological assay according to Hoskins and Messenger (32), has been an emergency recourse. The importance from the public health and legal viewpoints of minute residual traces of insecticides in foodstuffs, especially since the pesticidal tolerances have been established, has made quantitative determination imperative.
VI. Summary

Six insecticides, malathion, demeton, perthane, Strobane, dieldrin and endrin were evaluated by the Modified Sun Method, using D. melanogaster as the assay organism. This procedure was followed to establish dosage levels that would give a range of mortalities between 30 and 85 per cent and which would approximate a straight line when plotted on logarithmic-probability paper. Mortalities were recorded at various time intervals until the dosage concentrations gave mortalities in the desired range. This study eliminated malathion, demeton, perthane and Strobane from further consideration. Extreme variation between replicates and the difficulty of locating dosage points between the 30 and 85 per cent mortality range, eliminated the organic thiophosphate malathion. Demeton (large variations between replicates for each dosage, also), perthane and Strobane had relatively low toxicities for the fruit fly, therefore, small residues could not be detected using this method. The ID$_{50}$ values at 25°C for demeton, perthane and Strobane were approximately 9 ppm in 22 hours, 200 ppm in 15 hours and 27 ppm in 48 hours, respectively. The stereoisomers, dieldrin and endrin, were found suitable for assay by the method of placing the test insect in direct contact with macerated tissue.

The fruit flies were reared on a corn meal-molasses-agar media at 25°C. Six replicates, using 50 flies each in the Modified Sun Method and 20 flies each in the Culture Tube Method, were prepared for each insecticide level. The flies were from a mixed population and varied from 6 to 30 hours in age.
The concentration of acetone-insecticide solutions and the dosage levels in the test materials were expressed in parts per million on a weight-weight basis.

Assay determinations were made for dieldrin and endrin in canned lima beans, frozen lima beans and frozen snap beans, using the Modified Sun Method. A small increment of toxicant, a concentration that brought about between 40 and 60 per cent mortality in the standard series, was added to the treated sample. The difference between the sample plus the increment of toxicant and the selected level in the standard series, is the mortality which is due to the unknown effective residue.

The dosage-mortality curves were plotted using the simplified method of Litchfield and Wilcoxon (39). Tukey's method of statistical analysis (56) for several groups of measurements was employed. For each assay the residue value was stated to lie between two insecticide concentrations.

For dieldrin treated samples of canned lima beans (1953 crop stored in cans 12 months) the residue values were stated to lie between 0 and 0.30 ppm and 0 and 0.07 ppm, for frozen lima beans (1954 crop after 3 months) 0 and >0.83 ppm and 0 and 0.64 ppm, and for frozen snap beans (1954 crop after 3 months) 0.11 and 0.64 ppm and 0.10 and >1.11 ppm. For endrin treated samples of canned lima beans (1953 crop after 12 months in cans) the residue values were stated to lie between 0 ppm and 0 and 0.20 ppm, for frozen lima beans (1954 crop after 3 months) 0.22 and >1.11 ppm and 1.11 and >1.11 ppm, and for frozen snap beans (1954 crop after 3 months)
0.20 and 0.54 ppm and 0 and 0.11 ppm. Thus the legal tolerance of
dieldrin on canned and frozen lima beans and frozen snap beans can be
determined by means of fruit fly bioassay (Modified Sun Method).

Dosage-effect curves were prepared for dieldrin and endrin in
pumpkin using the Culture Tube Method. Pumpkin was spread over wood
stakes and stainless steel strips. When stainless steel strips
(a non-biological material) were used, there were smaller differences
in per cent mortality between the highest and lowest replicates for
each level.

The 30 to 85 per cent mortality range was brought about by a
lower dosage of dieldrin and endrin blended in pumpkin in the Culture
Tube Method using stainless steel strips (0.375 to 0.75 ppm for
dieldrin and 0.75 to 1.5 ppm for endrin) than in the Modified Sun
Method (0.75 to 1.75 ppm for dieldrin and 0.75 to 2 ppm for endrin).

Mortality data were recorded in all cases after certain in-
tervals of time and until the fruit fly mortalities fell into the
30 to 85 per cent range. Since this was not a comparative toxi-
city study, exposure time was a variable.
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Autobiography

I, Gerald Deland Gernon, Jr., was born in Champaign, Illinois, June 10, 1926. I received my primary education in Champaign, Illinois. After being graduated from University High School, Urbana, Illinois in May of 1943, I attended the University of Illinois for one year. I served in the United States Navy from June 1944 to June 1946.

In 1949 I received the baccalaureate degree from the College of Liberal Arts and Science, University of Illinois with a major in chemistry. I was graduated in 1951 from the University of Illinois with the degree Master of Science in Food Technology.

In 1952, I was appointed Research Assistant by the Department of Horticulture, Ohio Agricultural Experiment Station. In 1953, I was appointed Research Fellow, Institute of Nutrition and Food Technology, The Ohio State University, specializing in Food Technology as related to horticultural products, while fulfilling the requirements for the degree Doctor of Philosophy.