EVALUATION AND STANDARDIZATION OF SEVERAL BIOASSAY METHODS FOR INSECTICIDE RESIDUE ANALYSIS

by

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STATEMENT BY AUTHOR

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INTRODUCTION

Principles of Bioassay: Bioassay is the measurement of the response of a living organism to some chemical or other substance which has biological activity. The main principle of its use is the quantitative detection of such a substance by observing certain criteria with respect to biological activity. This response may be affected by temperature, light intensity, age, sex, biology of the test organism and by individual and species variation. This makes it necessary to standardize a method of carrying out each step in the bioassay system. The factors to be considered are: the biological nature of the test material, presentation of the chemical to the organism, selection of "The Analytical Reagent" (insects) in a standard way for every test, and interpretation and evaluation of data.

Biological assay has been employed for about fifty years as a means of detecting and standardizing drugs and other substances which exhibit biological activity. During the last two decades bioassay has also been put to use in evaluating insecticides. At first it was used as a method for evaluation of toxicity and sensitivity and for screening insecticides for effectiveness. This led to the use of bioassay for quantitative analysis of insecticide residues.

Chemical methods of analysis are still a standard procedure for analysis for most insecticides. Bioassay methods are valuable, however, for quantitative detection of residues of new insecticides for which chemical methods have not been developed, and for screening insecticides.

Available methods of chemical analysis for some insecticides may be so specific that not all the active residue metabolites or oxidation products present are detected. In contrast, bioassay methods detect these other biologically active products without the highly specialized instruments or large investment of time required for chemical methods.

The rapid development of synthetic organic insecticides led to the development of bioassay methods suitable for their quantitative analysis. The nonspecificity of bioassay makes it applicable to new problems of analysis of biologically active materials such as insecticides. Variations in exposure methods and individual and species variations are still problems that must be coped with. The proper choice of a method of bioassay for an insecticide may determine whether or not the results are of value. There is a tendency to set bioassay apart from the other methods. When all the criteria used for a proper analytical technique are observed in bioassay, it can be considered along with gravimetric, colorimetric and other methods as an accepted method of analysis.

At first the use of bioassay was limited to finding relative toxicity and to screening insecticides. Entomologists and insect toxicologists then adapted it for residue analysis and for evaluation of new insecticides. The basic standards and laboratory techniques used in chemical analysis work are also used for bioassay so that the value of bioassay is comparable in this respect to chemical methods. Bioassay is now regarded as one of the standard methods of analysis.

The mechanism of bioassay of insecticides involves exposing the test organism to known amounts of the toxicant in an exact and reproducible manner. The resultant per cent mortality for each dose level is then recorded and used as a reference standard. Unknown samples are run concurrently and the results determined by comparison with the standard.

The criteria used for evaluation and recording of bioassay data vary with the method of exposure, the test organism used, the insecticide under consideration and the facilities available. Examples of such criteria are loss of mobility, inhibition of growth, morbidity, death, and latency of response to the insecticide. Important aspects of a bioassay system are the accuracy and sensitivity with which a toxicant can be detected, and the reproducibility of results. The ease and rapidity of obtaining results are important considerations.

Comparison of Bioassay and Chemical Methods: As a rule bioassay methods are not as specific as chemical analytical methods, but the sensitivity in certain cases is greater. Comparison of amounts of toxicant determined by colorimetric and bioassay methods (Hartzell, et al, 1954) resulted in higher values for colorimetric than for bioassay methods. Impurities in the extracts caused background absorption resulting in higher values for colorimetric methods. Masking by lipids lowered the rate of insecticidal action in bioassay systems. This may be eliminated in analyses of insecticide residues by newer methods for purification of tissue extracts.

Bioassay can be as reliable as chemical methods of analysis as shown by data reported by Laug (1946) and Nolan and Wilcoxon (1950) from the use of both methods on the same samples. The validity of a new method of bioassay may be established by comparison to a standard chemical method and vice versa. This has made the use of bioassay methods in most laboratories a standard method of analysis used along with chemical analysis.

Some laboratories may regard bioassay as an alternate or as a supplemental tool for residue analysis until a suitable chemical method is established. It is difficult to place bioassay as an analytical tool in its proper perspective with other methods because the "analytical reagents" used in bioassay (insects) are not kept in bottles on a shelf.

Bioassay of mixed residues can present a problem, but in comparison with chemical methods bioassay may prove superior if the components of the mixture are unknown. It would be difficult to use a chemical method since there would not be a response from all the toxicants present.

Statistical analysis: Rectification of mortality data through improved statistical methods of analysis (Bliss, 1935) has facilitated more accurate and rapid (Wilcoxon, 1949) evaluation of experimental bioassay data. Bliss devised the method of probit-analysis which consists of plotting the dose as its logarithm against the dose effect in probability units or probits. The resulting dose-effect function is a straight line instead of a sigmoid curve. This aids in the evaluation of bioassay data and improves the accuracy of the

statistical analysis. Statistical methods have since been expanded and their use elaborated in some instances to a point too involved for use by the non-statistician. However, the absolute importance of statistical evaluation is even more essential and involved for bioassay than for chemical analytical methods.

SURVEY OF BIOASSAY METHODS

Numerous methods of bioassay have been devised, but not all can be regarded as equally useful nor reliable. The many methods have been developed according to the convenience, experience and facilities available and can be classified into three general groups.

Aqueous exposure: Mosquito larvae used as test insects (Bushland, 1951) were effective, but took 24 to 48 hours to obtain results. A more rapid and sensitive method (Burchfield et al, 1952) with mosquito larvae used the photomigration of the larvae as the criterion for detecting small amounts of parathion by the use of Aedes aegypti larvae (Nolan and Wilcoxon, 1950) permitted detection of .002 parts per million of this organic phosphate.

The brine shrimp Artemia salina, was used in detecting as little as 0.01 ppm of methoxychlor, DDT, lindane, chlordane, toxaphene, endrin and Bayer 17147 (Michael et al, 1956). Another microcrustacean, Daphnia, was used to detect parathion, DDT, and BHC with sensitivities comparable to those obtained with the brine shrimps and mosquito larvae (Wasserburger, 1952). By using Daphnia Anderson (1944) detected one part per billion of DDT in Lake Erie water. Kocher et al (1953) were able to determine quantities of less than 1 milligram of insecticide per kilogram of vegetables with a greater precision than was possible by other methods. Specific movements of Daphnia after being influenced by various contact insecticides made it possible to distinguish the chlorinated hydrocarbons from the organic phosphates (Pfaff, 1955). Goldfish

(Gersdorff, 1930) were used successfully in toxicity tests of rotenone. Later tests using Guppies (Lebistes reticulatus) to detect rotenone from Derris and Lonchocarpus roots (Pagan, 1948), and DDT (Pagan and Hageman, 1950) detected these toxicants in amounts as low as 0.025 ppm.

Direct Feeding: This method consists of presenting the toxicant, mixed in with plant or animal tissue, to the test insect. There are two ways by which the direct feeding method may be used: (1) The tissue to be analyzed may be macerated and presented in a standard way to the test insect. By this method of presentation sublethal amounts of a toxicant are not detected. (2) By first extracting the tissue to be analyzed and concentrating the extract, the sublethal amounts of toxicant can be detected. This second mode of presentation consists of mixing the concentrated extract with an acceptable food material, and presenting it to the test insect. Several factors may influence the results of this method, and limit its use. In most cases the test insect has been the house fly or the pomace fly. These insects feed primarily on sugars in the toxicant media, and only the insecticide which is on or near the sugars will be picked up by test insects. It has been shown that some insecticides have an affinity for fats (Laug, 1946). Thus insecticides not associated with sugars may not be ingested, and toxic effects would only be by fumigation or contact through the tarsi. These factors lower the sensitivity of this method.

A microbioassay method consisting of direct feeding of insects on milk containing the insecticide (Sun and Sun, 1953) made it possible to detect minute amounts of five insecticides. Sun and Pankaskie (1954)

exposed pomace flies to macerated tissues and were able to detect 0.1 ppm of aldrin or dieldrin in a variety of fruits and vegetables. Direct feeding of pomace flies on custard pumpkin to which aldrin had been added (Fisher and Smallman, 1954) was used to assay this and other insecticides. A similar method, with modifications, is being used successfully at Cornell University (Dewey, 1958).

Concentrations of parathion lethal to the rice stem borer were determined by allowing third and fifth instar larvae to feed directly on a reed wick which absorbed artificial nutrients and the toxicant (Hashizume, 1954). The artificial medium contained sugars, casein, yeasts and minerals disolved in water. Concentrations of parathion causing 100% morbidity or mortality within 24 hours were less than 0.5 ppm. This coincided with results obtained by other workers in laboratory and field experiments.

Frawley et al (1952) devised a procedure for the biological assay of insecticides by oral administration to house flies. An ether extract of the sample containing the insecticide was evaporated to dryness on sugar and then dissolved in water and fed to the flies. This technique obviates the meticulous purification of sample extracts, which is frequently necessary for other bioassay procedures. This method has some of the drawbacks of other direct feeding methods.

Film Exposure: The more obvious procedure in bioassaying insecticides is to expose the test insect to a film of the insecticide. Numerous ways may be devised with regard to type of exposure chamber, type of test insect and time of exposure to insecticides. Laug (1946) first used biological assay in quantitative determination of insecticide residues. He evaporated extracts of DDT in Erlenmeyer flasks within which

houseflies were exposed to the residues for 24 to 48 hours.

A bioassay method for determining aldrin (Dahm and Pankaskie, 1949) consisted of exposing uniformly sampled lots of adult houseflies to a residue of the toxicant for 48 hours and observing the resultant mortalities. Corn oil (20 milligrams per jar) was added to the standards to increase pickup, prevent evaporation and to approximate the fatty or waxy residues obtained from solvent extractions from animal or plant tissues. Dahm and Pankaskie advocate that the area of toxicant to which the flies were exposed was not of significance. The range of LD_{50} for aldrin in their tests was 1.1 to 1.66 micrograms per gram of flies. The control replicates never exceeded 3% mortality.

Small shell vials were used by Hoskins and Messenger (1950) for evaluating toxicity of organic insecticides and bioassaying insecticide residues in plant and animal tissue. This method differed from that of Dahm and Pankaskie by being based on the per cent of the exposure chamber surface covered by the film, and by a discrete instead of a continuous exposure. Inclusion of a small amount of light oil increased the uniformity of deposit (Hoskins et al, 1952) over the surface area of the vial. This enabled a higher percentage of the toxicant to be picked up by the test insect.

Sun and Sun (1953) outlined four different methods of evaluating data and of determining insecticidal residues: (1) A "Ranking Method" for a more rapid and simple way of analyzing for the presence or absence of insecticidal residues; (2) An LD_{50} method for calculating traces of insecticidal residue quantitively; (3) An interpolation method for evaluating small sublethal amounts of insecticidal residues; and (4) An inter-

polation method for determining larger amounts of insecticidal residues. The methods all consisted of a continuous exposure of houseflies for 24 to 48 hours in standard size jars. In all cases a comparable blank extract was used to make up standards.

METHODS AND MATERIALS

This paper is a comparison and evaluation of methods which show promise of becoming analytical bioassay tools. Four different methods of bioassay were used in the analysis of six different insecticides.

Method I: This is the method devised by Hoskins and Messenger (1950) and consists of exposing female houseflies (Musca domestica) to the toxicant in small shell vials, which were completely coated with insecticide. These exposure chambers were flat bottomed shell vials 50 mm. long with an outside diameter of 23 mm. The vials were altered by heating with an oxygen torch to form a lip on the inside. This was necessary in order to prevent the liquid from spilling while the vial was being rolled on its side. The rolling evenly distributed the toxicant on the entire inside surface.

A measured amount of the toxicant was pipeted into the vial and allowed to evaporate to about one milliliter; one milliliter of a petroleum-ether solution of oil was then added. This solution was prepared by adding two milliliters of mineral oil to a liter of petroleum ether. The purpose of the oil was to afford a more even distribution of the toxicant in the vial and to increase the amount of toxicant picked up by the insects. It also approximates the constituent waxes, fats and oils of the extracts and prevents losses of the insecticide during and subsequent to the evaporation of the solution. After all the solutions were in the vials, they were rolled in a stream of warm air to evaporate the solvents. This left the residue evenly distributed on the inner surface. Twenty-

five female house flies of an age group of three to six days were anesthetized with carbon dioxide then counted and placed in holding vials to revive. Completely revived, flies were introduced into each exposure vial, and the vial covered with a piece of cheese cloth held on by a rubber band. The vials were then maintained at approximately 80° Fahrenheit for 60 minutes and agitated and rotated every 15 minutes. After one hour the flies were transferred to holding jars, which contained a vial of water and a cube of sugar. Mortalities were counted after 18 to 24 hours.

Method II: This method is a modification of the first method. Male flies were used instead of female flies. Only one to four days old flies were used because male flies are more sensitive to insecticides and their use produces high and unreliable mortality curves after the fourth day.

Method III: This method consisted of exposing mixed male and female flies continuously over a period of 18 to 48 hours in wide mouth pint jars. The toxicant and the oil solution were pipeted into jars and allowed to evaporate to dryness. This left the residue deposited on the bottom of the jars. Approximately 50 flies (male and female) were then transferred into the jars. They were provided with food and water by inverting a small vial of water with a cotton plug onto the wire screen lid and placing a cube of sugar on the lid. This allowed the flies to feed through the wire screen during the time for which they were exposed. Mortalities were recorded after 18 to 48 hours.

Method IV: This method is similar in principle to Method III, but a different insect and exposure chamber were used. Mixed male and female pomace flies (Drosophila melanogaster) were continuously exposed

in petri dishes for from 18 to 48 hours. This method restricted ventilation in the chambers. Flies were anesthetized for counting, immediately before transferring to the petri dishes, with carbon dioxide or exposure for from five to ten minutes in the freezing compartment of a refrigerator. The toxicant and oil solution were pipeted into the bottom half of the petri dish and evaporated to dryness. A short dental cotton plug 20mm long was soaked in 25% sucrose solution and squeezed sufficiently so that the solution did not flow. This was placed at one end of the dish prior to introducing 50 pomace flies. It was necessary to incubate the flies in the dark to obtain satisfactory results. (Pomace flies are positively phototaxic and tend to spend the majority of the incubation time crawling on the surface facing the light.). A tightly sealed cardboard box was inverted over the petri dishes to exclude light.

The following six insecticides were tested, using preparations formulated from stock samples provided by the manufacturers for research purposes. Acetone was used as the solvent in each case.

DDT; 2,2-bis-(p-chlorophenyl)-1,1, 1-trichloroethane.

Lindane; gamma isomer of 1,2,3,4,5,6,-hexachlorocyclohexane.

Toxaphene; octachlorocamphene

Dieldrin; 1,2,3,4,10,10-hexachloro-6, 7-epoxy-1,4a,5,6,7,8,8a,-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene.

Malathion; 0,0-dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate

Guthion; 3,4-detobenzotriazine-methyl-0, 0-dimethyldithiophosphate.

Statistics: Data collected were first recorded in each case as the dose in micrograms per exposure chamber relative to the dose effect

expressed as per cent mortality. The dosages were then converted to logarithms and the per cent mortalities converted to probit units for use in a log-probit method of analysis (Bliss, 1935). All computations were made by an I B M computer at the Computing Center of the University of Arizona to facilitate calculations and reduce possibilities for error.

The complete method of statistical analysis used for the data in this paper, together with an example illustrating its applications is presented in Appendix B.

The analysis of the data centers on the dependence of one variable on another, known as regression. In toxicology the lethal effects of a drug or chemical are described as the regression of the mortality, expressed as per cent kill, on the dose. The variable mortality, hereafter, referred to as Y, is a function of the independent variable, dosage, referred to as X. The uses of regression are varied. In some cases one may wish to learn if Y is dependent on X, and if so, to get a measure of the relationship. At other times it may be necessary to predict Y from X, or vice versa. The present purpose was (1) to determine the differences in error after the effects of the doses on the mortalities had been tabulated and (2) to determine the sensitivity of the test in relation to the ED_{50} values. This error was calculated by the equation:

$$s^2_{y \cdot x} = \frac{Sy^2 - b\,(Sxy)}{(n - 2)}$$

where Sy^2 is the sum of the squares of the deviations of the dose-effect, b is the sample regression coefficient, and Sxy is the sum of the products of the deviations of the dose and dose-effect.

Further calculations to test for the exact fit of the regression line may be made if necessary. However, by fitting a line geometrically, one can come significantly close to the line fitted by least mean squares. A more accurate method of estimating the error variance is the method of probit analysis as given by Finney (1952). It was found that the estimate of error for the two methods (that of Finney and the regression method used in this paper) was close. Therefore the regression method was used for the present study, since it is much quicker and simpler to calculate.

EXPERIMENTAL RESULTS

Results obtained for each individual test are tabulated in Appendix A. The means for the results in Appendix A were calculated for each method of bioassay and are tabulated separately for each insecticide in Table 1. From these mean standard deviations and LD_{50} values it is evident that there is a wide variation in per cent error and the sensitivities of methods with regard to the different insecticides.

The sensitivity of a method will refer to the mean LD_{50} of that method throughout the paper and the reliability will refer to the standard deviation. Methods with lower LD_{50} values have a higher sensitivity and those with lower standard deviations are more reliable. The expression "LD_{50}" refers to micrograms of insecticide per exposure chamber required to kill 50 per cent of a population.

An overall comparison of the mean standard error for each insecticide shows that the continuous exposure method (Method III) is generally the one with least error, with the exception of the results obtained on malathion. All results obtained from the bioassay of malathion showed a high percentage of error. The standard error at the LD_{50} level by the continuous exposure method was 20%. This indicates that the per cent mortalities with an LD_{50} dose may vary from 30 to 70% from one test to another. Guthion, which is also an organic phosphate, exhibited only a 9.4% error by this same method. This method is the most reliable for the bioassay of Guthion. As an illustration of this, the order of mean standard deviation for Guthion is: 9.4, 10.4, 14.1, and 19.1 per cent for methods III, I, II and IV respectively.

Bioassay of dieldrin, toxaphene, lindane and DDT, as well as Guthion, by method III showed a difference of two to eight per cent error between this method and the other methods. In all cases method III had the lowest per cent standard error, and was more reliable.

The standard error of the results from the small vial method using male Musca domestica (Method II) is as follows: 11.8, 10.4, 9.0, 12, and 14.1 per cent for toxaphene, malathion, dieldrin, Guthion and DDT respectively. Three tests were run by this method on lindane, but all the mortalities were less than two per cent and were not recorded.

Results of bioassay by the small vial method using female flies (Method I) resulted in standard errors of 9, 12.2, 10.3, 12.2, 17.8, and 10.4 respectively.

Bioassay by the Drosophila method (Method IV) showed consistently lower percentages of error with exception of its use on DDT. Standard errors for this method were 6.5, 7.9, 9.4, 14.5, 8.7 and 19.1 for toxaphene, malthion, dieldrin, lindane, Guthion and DDT respectively. This method is relatively quick and easy to carry out but may present problems when its sensitivity is considered.

Sensitivities of the four methods generally follow a pattern in reverse to the standard error for all six insecticides, with some exceptions. The most sensitive method is the Drosophila method, followed closely by the small vial method using male house flies. However, in the case of DDT, the sensitivity of the Drosophila method is the lowest of the four methods, but the standard error is also low. Bioassay by the Drosophila method is very sensitive in comparison to all the other methods, but there is also a loss of reliability. The reverse is true for the

continuous exposure method. This refers to a very general case and cannot be applied to every insecticide, as illustrated by the comparison of data from the bioassay of malathion and lindane by all four methods. The sensitivity of Method IV was high (1.17 micrograms) with a low standard error (7.9%) for malathion, while the other methods showed a much lower sensitivity (20.36, 2.94, and 15.89 micrograms) together with a high percentage of error (20, 10.4, and 12.2%). For lindane, the sensitivity of Method IV was intermediate (0.49 micrograms) and had a per cent error of 14.5%; Method I had a higher sensitivity (0.39 micrograms) with a 12.2% error; Method III had a sensitivity of 0.53 micrograms and a 14.4% error. Lindane data for the small vial method using male flies were not recorded since they were not significant.

Sensitivities for Method I were 12.14, 15.89, 0.31, 3.77 and 1.73 micrograms for toxaphene, malathion, dieldrin, lindane, Guthion and DDT respectively. Similar tests using Method II had sensitivities of 12.36, 2.94, 0.22, 2.11 and 2.03 for toxaphene, malathion, dieldrin, Guthion and DDT respectively.

Representative test data taken from Appendix A have been plotted on log-probit paper for three insecticides evaluated by all four methods (figures 1-4). These figures illustrate the relative degree of toxicity of each insecticide, the relative sensitivity and error of the methods used and the slopes of the dosage-mortality curves. These figures are also included to demonstrate the standard method of plotting tabular data for evaluation and to estimate the LD_{50} values. In actual laboratory bioassay of an unknown sample, the raw data are plotted on log-probit paper and the dosage-mortality curve fitted geometrically. This is then compared with a standard curve and the values for the unknown

sample determined. If the data are homogeneous, this dosage mortality curve usually agrees with the curve determined by statistical methods. The difference is not usually significant enough to warrant the use of statistics to obtain an exact fit of the regression line. It takes less time and effort to evaluate a data plotted in this way than to tediously compare tabular data. Estimation of the LD_{50} value for each set of data is relatively simple by interpolation from the line on the graph.

Comparison of lines fitted by sight shows that the LD_{50} values do not differ significantly from values determined by statistical methods. As an illustration, the LD_{50} values for DDT by the Drosophila method are 7.4 (figure 4) and 7.5 micrograms for the visual estimate and the statistical calculation, respectively. Other examples are the LD_{50} values for dieldrin of 0.26 (figure 4) and 0.26 in the same respective order as the first example. Whenever the points of the data are scattered, the test is obviously difficult to evaluate, the regression line will be difficult to fit by sight and use of some statistical fitting process is preferable. An example of this is the line for Guthion in figure 4, which has an LD_{50} of about 1.3 micrograms when fitted by eye. However, the LD_{50} value determined by statistical methods is 0.68 micrograms.

Obviously a set of data with a wide dispersal of per cent mortalities at more than one dose level has a low reliability. Such a test should be repeated or a better method of bioassay substituted.

Other methods should be substituted if the first has failed to produce favorable results, even with the utmost care in technique. Such a practice is time consuming and may not be fruitful. If information is available as to which method is generally more reliable or

sensitive it may save needless experimentation with different methods with an unknown sample.

The variations between methods are, in part, a measure of their different capabilities for applying equal amounts of insecticide to each of the flies within a single exposure chamber. The variation between insecticides is a reflection of differences of such properties as volatility, solubility, and rate of penetration.

A laboratory should have at its disposal more than one method of bioassay for use in residue analysis and should standardize the methods that are to be used.

Table 1. Comparison of Standard Deviation and LD_{50} values of methods used in bioassay of six insecticides.

Method *	No. of Tests Conducted	Mean Standard Deviation about the LD_{50}		Mean LD_{50} in micrograms
		probits	per cent	
		a. Toxaphene		
I	3	.2273	9.0	12.14
II	3	.3002	11.8	12.36
III	6	.1680	6.7	16.28
IV	4	.1625	6.5	9.94
		b. Malathion		
I.	4	.3108	12.2	15.89
II	4	.2647	10.4	2.94
III	4	.5267	20.0	20.36
IV	5	.2007	7.9	1.17
		c. Dieldrin		
I	4	.2603	10.3	0.31
II	5	.2263	9.0	0.22
III	4	.1947	7.7	0.29
IV	4	.2375	9.4	0.29

Table 1. (concluded)

Method *	No. of tests conducted	Mean Standard Deviation about the LD_{50}		Mean LD_{50} in micrograms
		probits	per cent	
		d. Lindane		
I	6	.3103	12.2	0.39
II	3	-	-	-
III	3	.3692	14.4	0.53
IV	4	.3712	14.5	0.49
		e. DDT		
I	6	.4628	17.8	3.77
II	4	.3047	12.0	2.11
III	3	.2447	9.7	6.42
IV	6	.2205	8.7	14.43
		f. Guthion		
I	4	.2637	10.4	1.73
II	4	.3620	14.1	2.03
III	3	.2388	9.4	3.37
IV	5	.4985	19.1	0.86

* Method I is the small vial method using female houseflies; Method II is the small vial method using male houseflies; Method III is the continuous exposure method using mixed male and female houseflies; and Method IV is the Drosophila method.

CONCLUSIONS

The continuous exposure method using mixed male and female houseflies is the most feasible method to use for the least percent error, and rapidity and ease of conducting the bioassay. Although this method has the least error and is easier and more rapid, in some cases these do not compensate for the loss in sensitivity. Another drawback to this method is that its reliability decreases with an increase in the age of the flies. The flies should be of an age group of not more than four days. If houseflies are over four days old it is better to use the small vial method, employing only female flies. This latter method is more time consuming but is more reliable for older flies.

The standard error for Method II was too high for all the insecticides tested. This method is not very reliable and should be used only in cases where reproducibility of results is not necessary. It is one of the more sensitive methods and may be used to detect the presence or absence of minute quantities of insecticides, but not ordinarily for quantitative determinations.

The method employing Drosophila melanogaster is the most sensitive for all insecticides tested. This, in conjunction with its relatively low percent of standard error, makes it the method of choice for the bioassay of toxaphene, malathion, and dieldrin. This method shows a high sensitivity for the bioassay of Guthion, but also shows a high percent of error. This would eliminate its use for this insecticide. For DDT this method has a low percent error and a low sensitivity. Due to the low sensitivity, it is not a good method for bioassay of DDT.

The small vial method using female flies is the best all around method for the detection of small amounts of insecticides since its standard error and sensitivity are intermediate. The fact that it is more time consuming may rule it out in many instances when speed is important or when a more sensitive method is desired.

The Drosophila method is preferable when speed, accuracy and a high sensitivity are required of a bioassay. For tests requiring high sensitivity, with no regard for reproducibility and in cases where only male houseflies are available, the small vial method using male flies may be used. The continuous exposure method using mixed male and female houseflies, provided flies of the required age group are available, is the best method to use when speed, accuracy and a high sensitivity are necessary.

Figure 1. Bioassay by the Small Vial Method (♀)

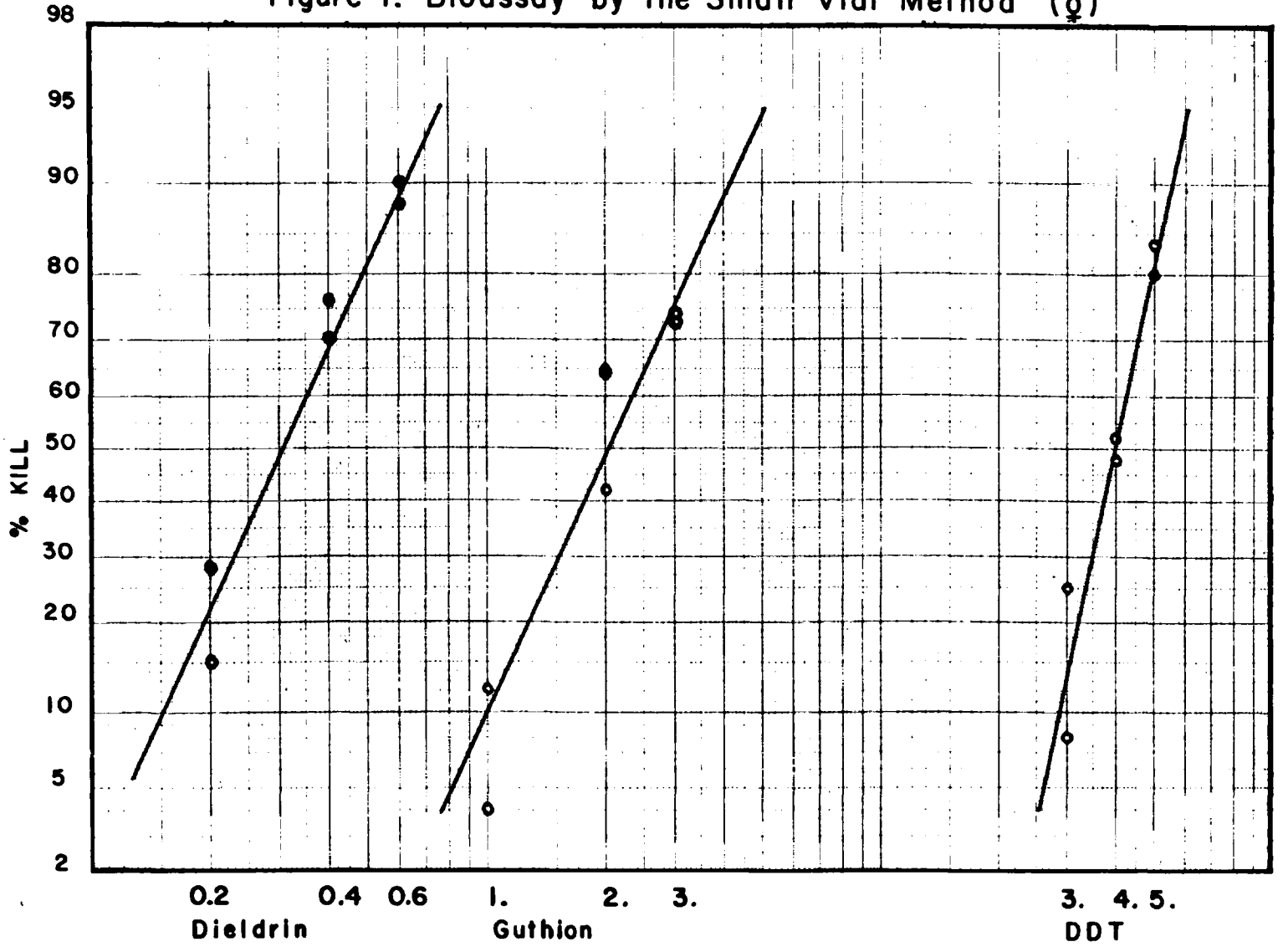


Figure 2. Bioassay by the Small Vial Method (♂)

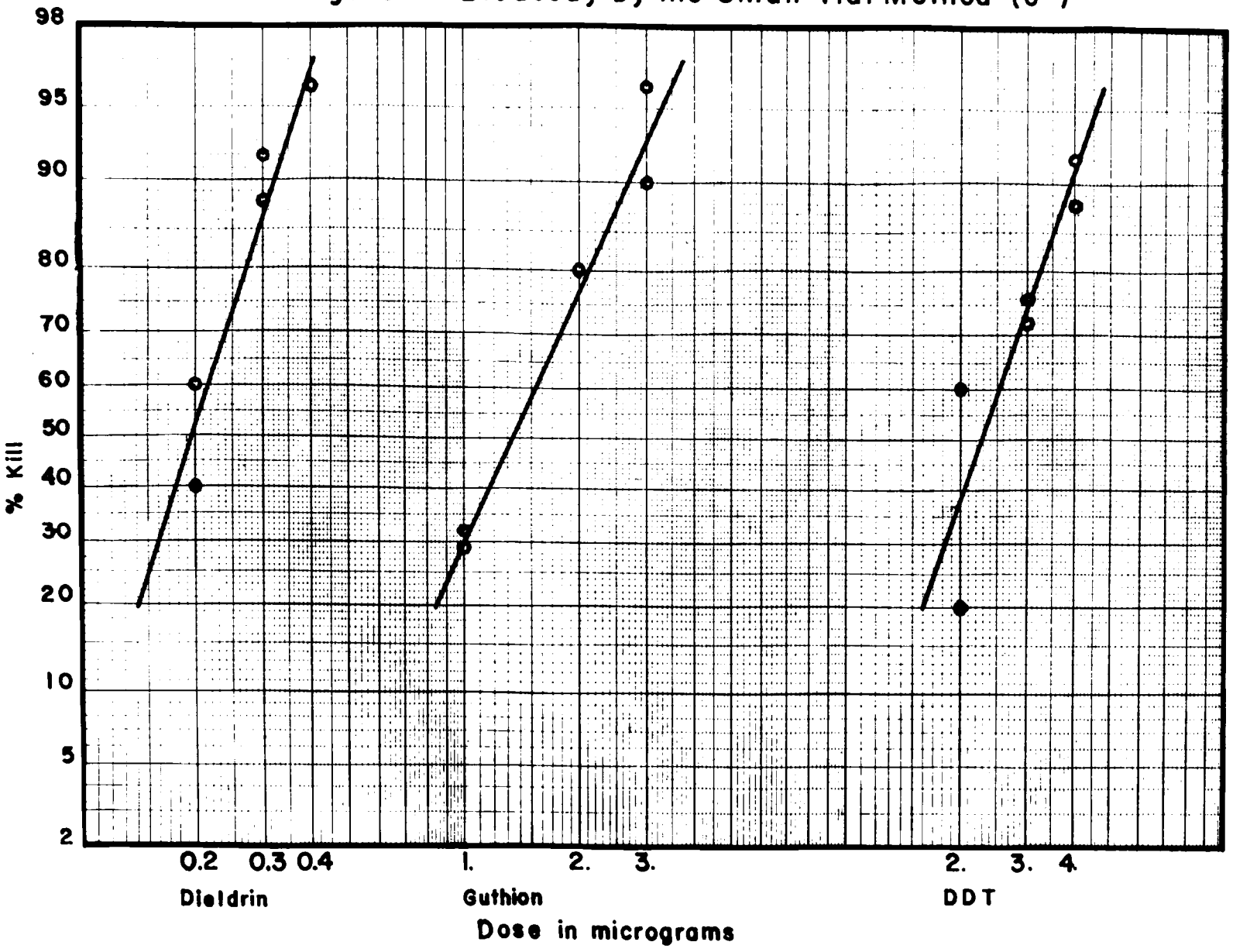


Figure 3. Bioassay by the Continuous Exposure Method (♂ & ♀)

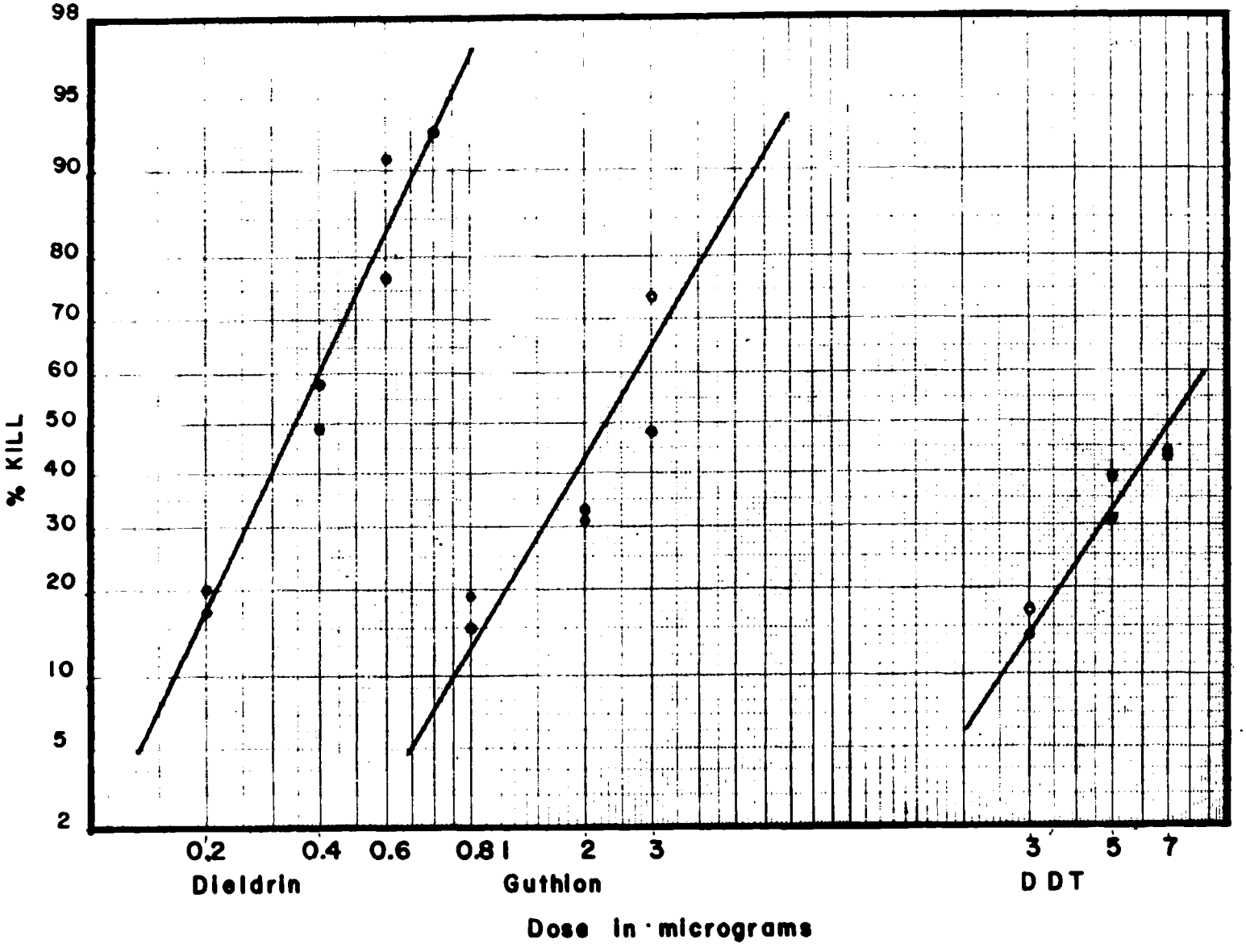
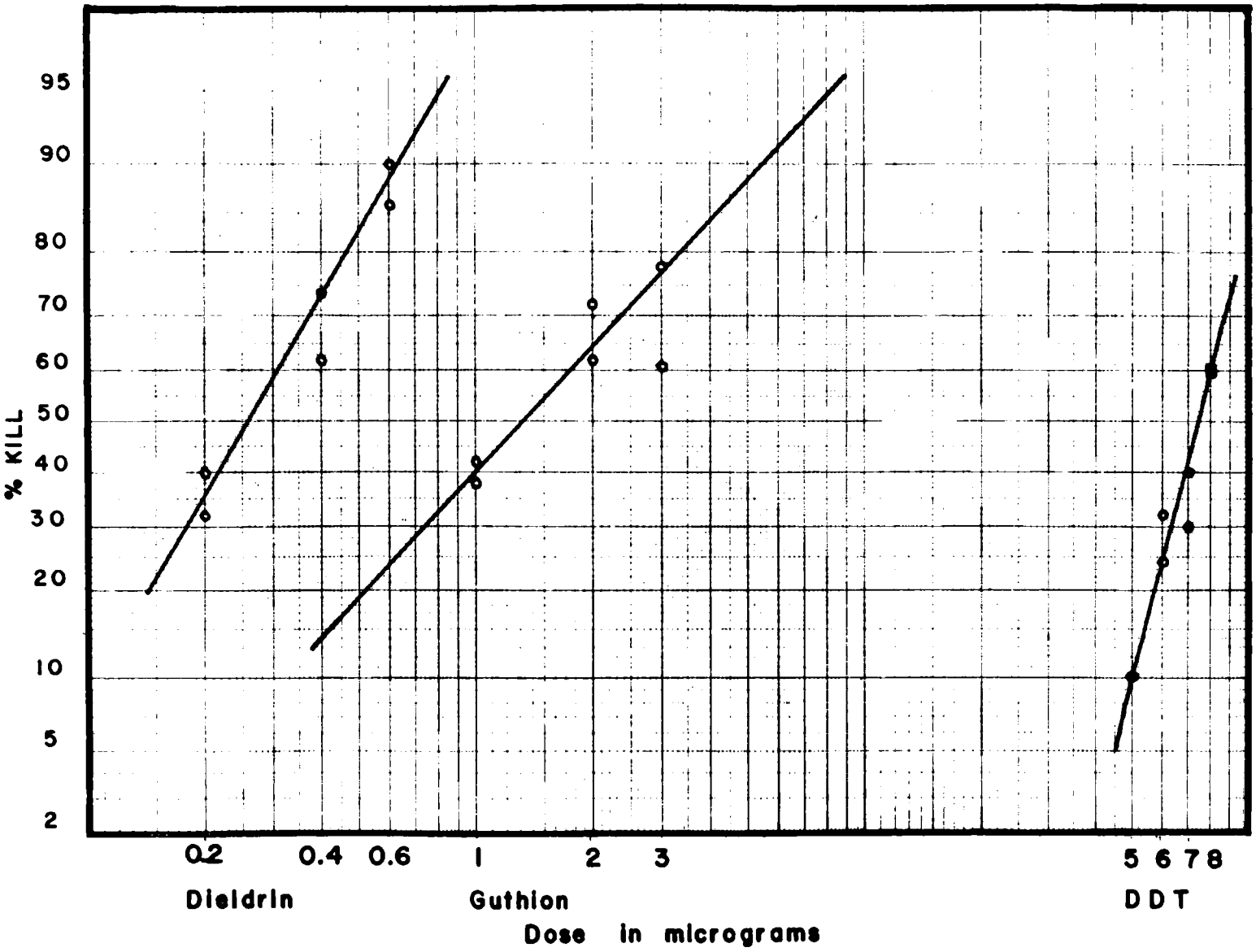


Figure 4. Bioassay by the Drosophila Method



APPENDICES

APPENDIX A.

DETAILED TABULAR DATA

Table 2. Bioassay results of Lindane by Method I

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
1					.40	.3605
	0.3	0.4771	24	4.2937		
	0.3	0.4771	13	3.8736		
	0.5	0.6990	59	5.2275		
	0.5	0.6990	84	5.9945		
	0.7	0.8451	96	6.7507		
2					.378	.2720
	0.2	0.3010	04	3.2493		
	0.4	0.6021	54	5.1004		
	0.4	0.6021	54	5.1004		
	0.6	0.7782	84	5.9945		
	0.6	0.7782	95	6.6449		
3					.659	.2958
	0.3	0.4771	04	3.2493		
	0.5	0.6990	16	4.0055		
	0.5	0.6990	12	3.8250		
	0.7	0.8451	62	5.3055		
	0.7	0.8451	64	5.3585		
	0.9	0.9542	80	5.8416		
	0.9	0.9542	80	5.8416		
4					.132	.2905
	0.3	0.4771	84	5.9945		
	0.3	0.4771	92	6.4051		
	0.5	0.6990	95	6.6449		

Table 2. (concluded)

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
5					.187	.5407
	0.2	0.3010	37	4.6681		
	0.2	0.3010	72	5.5828		
	0.4	0.6021	88	6.1750		
	0.4	0.6021	96	6.7507		
6					.593	.1024
	0.5	0.6990	24	4.2937		
	0.5	0.6990	28	4.4172		
	0.7	0.8451	73	5.6128		
	0.7	0.8451	75	5.6745		
	0.9	0.9542	92			
	0.9	0.9542	95	6.6449		

Table 3. Bioassay results of Lindane by Method IV.

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
7	48 hour exposure using mixed flies				.350	.1157
	0.2	0.3010	18	4.0846		
	0.2	0.3010	21	4.1936		
	0.4	0.6021	56	5.1510		
	0.4	0.6021	61	5.2793		
	0.6	0.7782	75	5.6745		
	0.6	0.7782	83	5.9542		
8	48 hour exposure using mixed flies				.514	.1931
	0.2	0.3010	11	3.7735		
	0.2	0.3010	15	3.9636		
	0.4	0.6021	35	4.6147		
	0.4	0.6021	37	4.6681		
	0.6	0.7782	49	4.9749		
	0.6	0.7782	68	5.4677		
9	48 hour exposure using mixed flies				.893	.9512
	0.4	0.6021	08	3.5949		
	0.4	0.6021	17	4.0458		
	0.6	0.7782	32	4.5323		
	0.6	0.7782	41	4.7725		
	0.8	0.9031	39	4.7207		
	0.8	0.9031	40	4.7464		
10	48 hour exposure using males				.154	.1897
	0.2	0.3010	60	5.2533		
	0.2	0.3010	64	5.3585		
	0.4	0.6021	83	5.9542		
	0.4	0.6021	90	6.2816		
	0.6	0.7782	91	6.3408		
	0.6	0.7782	96	6.7507		

Table 3. (continued)

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
11	48 hour exposure using females				.291	.2607
	0.2	0.3010	14	3.9197		
	0.2	0.3010	32	4.5323		
	0.4	0.6021	70	5.5244		
	0.4	0.6021	71	5.5535		
	0.6	0.7782	95	6.6449		
	0.6	0.7782	96	6.7507		
12	48 hour exposure using males				.230	.5719
	0.2	0.3010	24	4.2937		
	0.2	0.3010	60	5.2533		
	0.4	0.6021	71	5.5534		
	0.4	0.6021	88	6.1750		
13	48 hour exposure using females				.362	.2821
	0.2	0.3010	16	4.0055		
	0.2	0.3010	24	4.2937		
	0.4	0.6021	45	4.8743		
	0.4	0.6021	50	5.0000		
	0.6	0.7782	75	5.6745		
	0.6	0.7782	88	6.1750		
14	48 hours exposure using mixed flies				.319	.4433
	0.2	0.3010	26	4.3567		
	0.2	0.3010	28	4.4172		
	0.4	0.6021	57	5.1764		
	0.4	0.6021	82	5.9154		
	0.6	0.7782	64	5.3585		
	0.6	0.7782	86	6.0803		
	0.8	0.9031	66	5.4125		
	0.8	0.9031	68	5.4677		

Table 3. (concluded)

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
15	48 hour exposure using mixed flies				.375	.1529
	0.2	0.3010	22	4.2278		
	0.2	0.3010	26	4.3567		
	0.4	0.6021	45	4.8743		
	0.4	0.6021	48	4.9498		
	0.6	0.7782	70	5.5244		
	0.6	0.7782	74	5.6433		
	0.8	0.9031	82	5.9184		
	0.8	0.9031	88	6.1750		

Table 4. Bioassay results of Lindane by Method III.

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
16	24 hours of exposure using four day old flies.				.666	.8236
	0.3	0.4771	12	3.8250		
	0.3	0.4771	27	4.3872		
	0.5	0.6990	29	4.4466		
	0.5	0.6990	37	4.6681		
	0.7	0.8451	50	5.0000		
	0.7	0.8451	51	5.0251		
	0.9	0.9542	70	5.5244		
17	24 hours of exposure using four day old flies.				.496	.1063
	0.2	0.3010	2	2.9463		
	0.2	0.3010	2	2.9463		
	0.4	0.6021	28	4.4172		
	0.4	0.6021	31	4.5041		
	0.6	0.7782	60	5.2533		
	0.6	0.7782	66	5.4125		
	0.8	0.9031	88	6.1750		
	0.8	0.9031	89	6.2265		
18	48 hours of exposure using four day old flies				.457	.1933
	0.2	0.3010	4	3.2493		
	0.2	0.3010	6	3.4452		
	0.4	0.6021	32	4.5323		
	0.4	0.6021	32	4.5323		
	0.6	0.7782	68	5.4677		
	0.6	0.7782	68	5.4677		
	0.8	0.9031	90	6.2816		
	0.8	0.9031	93	6.4758		
19	24 hours of exposure using five day old flies.				.442	.1688
	0.2	0.3010	23	4.2612		
	0.2	0.3010	21	4.1936		
	0.4	0.6021	38	4.6945		
	0.4	0.6021	44	4.8490		
	0.6	0.7782	59	5.2275		
	0.6	0.7782	71	5.5534		

Table 4. (concluded)

Test No.	Dose ug	Log of 10x Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
20	48 hours of exposure using five day old flies				.393	.2360
	0.2	0.3010	28	4.4172		
	0.2	0.3010	24	4.2937		
	0.4	0.6021	38	4.6945		
	0.4	0.6021	44	4.8490		
	0.6	0.7782	70	5.5244		
	0.6	0.7782	76	5.7063		

Table 5. Bioassay results of DDT by Method I.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
29						
	3	0.4771	39	4.7207	3.188	.2027
	3	0.4771	52	5.0502		
	4	0.6021	61	5.2793		
	4	0.6021	73	5.6128		
	5	0.6990	79	5.8064		
	5	0.6990	87	6.1264		
30	2	0.3010	8	3.5949	3.798	.7395
	2	0.3010	13	3.8736		
	3	0.4771	8	3.5949		
	3	0.4771	8	3.5949		
	4	0.6021	27	4.3872		
	4	0.6021	62	5.3055		
	5	0.6990	81	5.8779		
	5	0.6990	96	6.7507		
31	3	0.4771	4	3.2493	6.864	.3852
	3	0.4771	8	3.5949		
	4	0.6021	4	3.2493		
	4	0.6021	12	3.8250		
	5	0.6990	28	4.4172		
	5	0.6990	36	4.6415		
32	2	0.3010	4	3.2493	3.627	.7002
	2	0.3010	12	3.8250		
	3	0.4771	20	4.1584		
	3	0.4771	28	4.4172		
	4	0.6021	17	4.0458		
	4	0.6021	88	6.1750		
	5	0.6990	77	5.7388		
	5	0.6990	92	6.4051		
33						
	3	0.4771	8	3.5949	3.956	.2685
	3	0.4771	25	4.3255		
	4	0.6021	48	4.9498		
	4	0.6021	52	5.0502		
	5	0.6990	80	5.8416		
	5	0.6990	84	5.9945		

Table 5. (Concluded).

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
34						
	1	0.0000	9	3.6592	1.198	.4808
	2	0.3010	4	3.2493		
	3	0.4771	4	3.2493		
	3	0.4771	19	4.1221		
	4	0.6021	14	3.9197		
	4	0.6021	24	4.2937		
	5	0.6990	36	4.6415		
	5	0.6990	44	4.8490		

Table 6. Bioassay results of DDT by Method II

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
21					1.511	.1072
	3	0.4771	87	6.1264		
	3	0.4771	91	6.3408		
	4	0.6021	96	6.7507		
	4	0.6021	96	6.7507		
	5	0.6990	100			
	5	0.6990	100			
22					2.54	.4598
	1	0.0000	4	3.2493		
	2	0.3010	17	4.0458		
	3	0.4771	57	5.1764		
	4	0.6021	91	6.3408		
23					2.27	.3977
	2	0.3010	20	4.1584		
	2	0.3010	60	5.2533		
	3	0.4771	72	5.5828		
	3	0.4771	76	5.7063		
	4	0.6021	88	6.1750		
	4	0.6021	92	6.4051		
24					2.122	.2543
	1	0.0000	8	3.5949		
	2	0.3010	32	4.5323		
	2	0.3010	48	4.9498		
	3	0.4771	80	5.8416		
	3	0.4771	84	5.9945		
	4	0.6021	84	5.9945		
	4	0.6021	88	6.1750		

Table 7. Bioassay results of DDT by Method III.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
25	24-hour exposure of 6-day-old flies				5.393	.2908
	2	0.3010	4	3.2493		
	2	0.3010	16	4.0055		
	2	0.3010	18	4.0846		
	2	0.3010	19	4.1221		
	4	0.6021	28	4.4172		
	4	0.6021	40	4.7467		
	4	0.6021	47	4.9247		
	4	0.6021	49	4.9749		
	6	0.7782	45	4.8743		
	6	0.7782	51	5.0251		
	6	0.7782	52	5.0502		
	6	0.7782	61	5.2793		
26	24-hour exposure of 4-day-old flies.				7.853	.1104
	3	0.4771	14	3.9197		
	3	0.4771	17	4.0457		
	5	0.6990	31	4.5041		
	5	0.6990	39	4.7207		
	7	0.8451	43	4.8236		
	7	0.8451	44	4.8490		
27	48-hour exposure of 6-day-old flies.				4.002	.2056
	2	0.3010	11	3.7735		
	2	0.3010	21	4.1936		
	2	0.3010	23	4.2612		
	2	0.3010	24	4.2937		
	4	0.6021	49	4.9749		
	4	0.6021	50	5.0000		
	4	0.6021	51	5.0251		
	4	0.6021	59	5.2275		
	6	0.7782	60	5.2533		
	6	0.7782	64	5.3585		
	6	0.7782	64	5.3585		
	6	0.7782	79	5.8064		

(cont'd)

Table 7. Bioassay results of DDT by Method III. (concluded)

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
28	24-hour exposure of 5-day-old flies				6.019	.3331
	4	0.6021	27	4.3872		
	4	0.6021	29	4.4466		
	5	0.6990	61	5.2793		
	6	0.7782	40	4.7467		
	6	0.7782	49	4.9749		

Table 8. Bioassay results of DDT by Method IV.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
35	48-hour exposure using mixed flies.				12.212	.2242
	6	0.7782	8	3.5949		
	6	0.7782	18	4.0846		
	6	0.7782	20	4.1584		
	8	0.9031	18	4.0846		
	8	0.9031	24	4.2937		
	8	0.9031	30	4.4756		
	10	1.0000	36	4.6415		
	10	1.0000	36	4.6415		
	10	1.0000	48	4.9498		
36	24-hour exposure using mixed flies.				10.19	.4129
	6	0.7782	2	2.9463		
	6	0.7782	14	3.9197		
	8	0.9031	16	4.0055		
	8	0.9031	36	4.6415		
	10	1.0000	46	4.8996		
	10	1.0000	48	4.9498		
37	48-hour exposure using mixed flies.				7.543	.1754
	5	0.6990	10	3.7184		
	6	0.7782	24	4.2937		
	6	0.7782	32	4.5323		
	7	0.8451	30	4.4756		
	7	0.8451	40	4.7467		
	8	0.9031	60	5.2533		
	8	0.9031	60	5.2533		
38	48-hour exposure using mixed flies.				10.17	.2284
	6	0.7782	18	3.5949		
	6	0.7782	22	4.2278		
	6	0.7782	22	4.2278		
	8	0.9031	29	4.4466		
	8	0.9031	30	4.4756		
	8	0.9031	36	4.6415		
	10	1.0000	44	4.8490		
	10	1.0000	46	4.8996		
	10	1.0000	58	5.2019		

(cont'd)

Table 8. Bioassay results of DDT by Method IV. (Concluded)

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
39	48-hour exposure using mixed flies.				14.77	.2181
	6	0.7782	20	4.1584		
	6	0.7782	10	3.7184		
	8	0.9031	32	4.5323		
	8	0.9031	30	4.4756		
	10	1.0000	30	4.4756		
	10	1.0000	30	4.4756		
40	24-hour exposure using mixed flies.				31.75	.0640
	5	0.6990	10	3.7184		
	5	0.6990	12	3.8250		
	10	1.0000	24	4.2937		
	10	1.0000	20	4.1584		
	15	1.1761	30	4.4756		
	15	1.1761	32	4.5323		

Table 9. Bioassay results of Guthion by Method I.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
41					1.331	.3193
	1	0.0000	33	4.5601		
	1	0.0000	50	5.0000		
	2	0.3010	60	5.2533		
	2	0.3010	62	5.3055		
	3	0.4771	64	5.3585		
	3	0.4771	87	6.1264		
42					2.167	.3112
	1	0.0000	4	3.2493		
	1	0.0000	12	3.8250		
	2	0.3010	42	4.7981		
	2	0.3010	64	5.3585		
	3	0.4771	73	5.6128		
	3	0.4771	74	5.6433		
43					1.825	.1272
	1	1.0000	8	3.5949		
	1.5	1.1761	29	4.4466		
	2.0	1.3010	64	5.3585		
	2.5	1.3979	75	5.6745		
44					1.622	.2971
	1	0.0000	16	4.0055		
	1	0.0000	0	0.0000		
	2	0.3010	52	5.0502		
	2	0.3010	78	5.7722		
	3	0.4771	90	6.2816		
	3	0.4771	91	6.3408		

Table 10. Bioassay results of Guthion by Method II.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
45					2.957	.6463
	1.0	0.0000	56	5.1510		
	1.0	0.0000	60	5.2533		
	2.0	0.3010	60	5.2533		
	2.0	0.3010	84	5.9945		
	3.0	0.4771	24	4.2937		
	3.0	0.4771	76	5.7063		
46					2.345	.3217
	1	0.0000	12	318250		
	1	0.0000	32	4.5323		
	2	0.3010	35	4.6147		
	2	0.3010	40	4.7467		
	3	0.4771	56	5.1510		
	3	0.4771	71	5.5534		
47					1.304	.1737
	1	0.0000	29	4.4466		
	1	0.0000	32	4.5323		
	2	0.3010	80	5.8416		
	2	0.3010	80	5.8416		
	3	0.4771	90	6.2816		
	3	0.4771	96	6.7507		
48					1.507	.3065
	1	0.0000	8	3.5949		
	1	0.0000	24	4.2937		
	2	0.3010	80	5.8416		
	2	0.3010	80	5.8416		
	3	0.4771	96	6.4051		
	3	0.4771	92	6.7507		

Table 11. Bioassay results of Guthion by Method III.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
49	48-hour exposure				3.771	.1063
	2	0.3010	19	4.1221		
	2	0.3010	21	4.1936		
	3	0.4771	38	4.6945		
	3	0.4771	42	4.7981		
	4	0.6021	47	4.9247		
	4	0.6021	57	5.1764		
50	24-hour exposure				2.562	.3054
	1	0.0000	15	3.9636		
	1	0.0000	19	4.1221		
	2	0.3010	31	4.5041		
	2	0.3010	33	4.5601		
	3	0.4771	48	4.9498		
	3	0.4771	74	5.6433		
51	24-hour exposure				3.803	.3049
	1	0.0000	5	3.3551		
	1	0.0000	6	3.4452		
	2	0.3010	12	3.8250		
	2	0.3010	13	3.8736		
	3	0.4771	40	4.7467		
	3	0.4771	52	5.0502		

Table 12. Bioassay results of Guthion by Method IV.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
52	48-hour exposure				.6814	.7485
	0.5	0.6990	37	4.6681		
	0.5	0.6990	45	4.8743		
	1.0	1.0000	50	5.0000		
	1.0	1.0000	87	6.1264		
	1.5	1.1761	88	6.1750		
	1.5	1.1761	90	6.2816		
	2.0	1.3010	92	6.4051		
	2.0	1.3010	96	6.7507		
53	48-hour exposure				.3834	.7586
	0.5	0.6990	8	3.5949		
	0.5	0.6990	58	5.2019		
	1.0	1.0000	76	5.7063		
	1.0	1.0000	84	5.9945		
	1.5	1.1761	78	5.7722		
	1.5	1.1761	80	5.8416		
	2.0	1.3010	92	6.4051		
	2.0	1.3010	94	6.5548		
54	24-hour exposure				1.099	.2181
	0.5	0.6990	22	4.2278		
	0.5	0.6990	27	4.3872		
	1.0	1.0000	38	4.6945		
	1.0	1.0000	42	4.7981		
	1.5	1.1761	62	5.3055		
	1.5	1.1761	72	5.5828		
	2.0	1.3010	61	5.2793		
	2.0	1.3010	78	5.7722		
55	48-hour exposure				.9188	.4093
	0.5	0.6990	15	3.9636		
	0.5	0.6990	34	4.5875		
	1.0	1.0000	42	4.7981		
	1.0	1.0000	51	5.0251		
	1.5	1.1761	52	5.0502		
	1.5	1.1761	86	6.0803		
	2.0	1.3010	88	6.1750		
	2.0	1.3010	92	6.4051		

(cont'd)

Table 12. Bioassay results of Guthion by Method IV. (concluded)

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
56	48-hour exposure				1.213	.3580
	0.5	0.6990	10	3.7184		
	0.5	0.6990	16	4.0055		
	0.8	0.9031	12	3.8250		
	0.8	0.9031	20	4.1584		
	1.5	1.1761	50	5.0000		
	1.5	1.1761	72	5.5828		
	2.0	1.3010	77	5.7388		
	2.0	1.3010	87	6.1264		

Table 13. Bioassay results of Toxaphene by Method I.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
57						
	10	1.0000	28	4.4172	12.86	.1456
	10	1.0000	24	4.2937		
	15	1.1761	64	5.3585		
	15	1.1761	60	5.2533		
	20	1.3010	88	6.1750		
	20	1.3010	92	6.4051		
58						
	10	1.0000	4	3.2493	15.54	.2984
	10	1.0000	8	3.5949		
	15	1.1761	30	4.4756		
	15	1.1761	36	4.6415		
	20	1.3010	87	6.1264		
	20	1.3010	88	6.1750		
59						
	5	0.6990	7	3.5242	8.03	.2379
	5	0.6990	8	3.5949		
	10	1.0000	84	5.9945		
	15	1.1761	95	6.6449		
	15	1.1761	96	6.7507		

Table 14. Bioassay results of Toxaphene by Method II.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
60						
	5	0.6990	0	0.0000	10.16	.3268
	10	1.0000	36	4.6415		
	10	1.0000	60	5.2533		
	15	1.1761	88	6.1750		
	15	1.1761	92	6.4051		
61						
	5	0.6990	16	4.0055	6.761	.1131
	5	0.6990	16	4.0055		
	10	1.0000	88	6.1750		
	10	1.0000	92	6.4051		
	15	1.1761	100	------		
	15	1.1761	100	------		
62						
	5	0.6990	3	3.1192	20.16	.4608
	5	0.6990	10	3.7184		
	10	1.0000	23	4.2612		
	10	1.0000	23	4.2612		
	15	1.1761	25	4.3255		
	15	1.1761	25	4.3255		

Table 15. Bioassay results of Toxaphene by Method III.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
63	Testing with three day old flies for 24 hours.					
	10	1.0000	4	3.2493	23.9	.1702
	10	1.0000	9	3.6592		
	15	1.1761	16	4.0055		
	15	1.1761	22	4.2278		
	20	1.3010	38	4.6945		
	20	1.3010	39	4.7207		
64	Testing with three day old flies for 48 hours.					
	10	1.0000	43	4.8236	11.23	.1396
	10	1.0000	41	4.7725		
	15	1.1761	63	5.3319		
	15	1.1761	75	5.6745		
	20	1.3010	83	5.9542		
	20	1.3010	79	5.8064		
65	Testing with five day old flies for 24 hours.					
	10	1.0000	10	3.5242	17.86	.1326
	10	1.0000	7	3.7184		
	15	1.1761	40	4.7467		
	15	1.1761	41	4.7725		
	20	1.3010	62	5.2019		
	20	1.3010	58	5.3055		
66	Testing with five day old flies for 48 hours.					
	10	1.0000	39	4.7207	11.83	.1090
	10	1.0000	38	4.6945		
	15	1.1761	60	5.2533		
	15	1.1761	70	5.5244		
	20	1.3010	84	5.9945		
	20	1.3010	85	6.0364		
67	Testing with four day old flies for 24 hours					
	5	0.6990	18	4.0846	14.25	.2195
	5	0.6990	21	4.1936		
	10	1.0000	24	4.2937		
	10	1.0000	34	4.5875		
	15	1.1761	51	5.0251		
	15	1.1761	59	5.2275		
	20	1.3010	57	5.1764		
	20	1.3010	71	5.5534		

Table 15. (concluded)

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
68	Testing with 5 day old flies for 48 hours.					
	5	0.6990	15	3.9636	7.858	.2323
	5	0.6990	34	4.5875		
	10	1.0000	66	5.4125		
	10	1.0000	67	5.4399		
	15	1.1761	82	5.9154		
	15	1.1761	84	5.9945		
	20	1.3010	90	6.2816		
	20	1.3010	96	6.7507		
69	Testing with four day old flies for 24 hours.					
	10	1.0000	45	4.8743	9.984	.1410
	10	1.0000	55	5.1257		
	15	1.1761	64	5.2585		
	15	1.1761	68	5.4677		
	20	1.3010	71	5.5534		
	20	1.3010	80	5.8416		
70	Testing with five day old flies for 24 hours.					
	5	0.6990	11	3.7735	10.98	.2012
	5	0.6990	20	4.1584		
	10	1.0000	38	4.6945		
	10	1.0000	51	5.0251		
	15	1.1761	60	5.2533		
	15	1.1761	69	5.4959		
	20	1.3010	74	5.6433		
	20	1.3010	85	6.0364		
71	Testing with four day old flies for 48 hours.					
	5	0.6990	11	3.1192	10.12	.4016
	5	0.6990	20	4.1584		
	10	1.0000	53	5.0753		
	10	1.0000	40	4.7467		
	15	1.1761	75	5.6776		
	15	1.1761	83	5.9542		
72	Testing with four day old flies for 24 hours.					
	5	0.6990	9	3.6592	20.69	.1435
	5	0.6990	0	0.0000		
	10	1.0000	19	4.1221		
	10	1.0000	24	4.2937		
	15	1.1761	35	4.6147		
	15	1.1761	44	4.8490		

Table 16. Bioassay results of Toxaphene by Method IV.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
73	Testing with Drosophila for 48 hours.					
	5	0.6990	21	4.1936	7.697	0.0943
	5	0.6990	27	4.3872		
	10	1.0000	66	5.4125		
	10	1.0000	70	5.5244		
	15	1.1761	84	5.9945		
	15	1.1761	86	6.0803		
74	Testing with Drosophila for 48 hours.					
	10	1.0000	50	5.0000	9.679	.2066
	10	1.0000	52	5.0502		
	15	1.1761	94	6.5548		
	15	1.1761	94	6.5548		
	20	1.3010	98	7.0537		
	20	1.3010	100	-----		
75	Testing with Drosophila for 24 hours.					
	5	0.6990	0	0.0000	13.53	.3006
	5	0.6990	5	3.3551		
	10	1.0000	24	4.2937		
	10	1.0000	28	4.4172		
	15	1.1761	47	4.9247		
	15	1.1761	72	5.5828		
76	Testing with Drosophila for 48 hours.					
	5	0.6990	4	3.2493	10.78	.1337
	5	0.6990	6	3.4452		
	10	1.0000	42	4.7981		
	10	1.0000	52	5.0502		
	15	1.1761	74	5.6433		
	15	1.1761	74	5.6433		
77	Testing with Drosophila for 48 hours					
	5	0.6990	26	4.3567	11.61	.2156
	5	0.6990	12	3.8250		
	10	1.0000	40	4.7467		
	10	1.0000	38	4.6945		
	15	1.1761	66	5.4125		
	15	1.1761	62	5.3055		

Table 17. Bioassay results of Malathion by Method I.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
78						
	10	1.0000	16	4.0055	13.5	.3657
	10	1.0000	24	4.2937		
	15	1.1761	48	4.9498		
	15	1.1761	60	5.2533		
	20	1.3010	84	5.9945		
	20	1.3010	96	6.7507		
79						
	15	1.1761	18	4.0846	19.99	.3587
	20	1.3010	40	4.7467		
	25	1.3979	60	5.2533		
80						
	2	0.3010	22	4.2278	4.87	.2457
	2	0.3010	25	4.3255		
	4	0.6021	46	4.8996		
	4	0.6021	48	4.9498		
	6	0.7782	48	4.9498		
	6	0.7782	52	5.0502		
	8	0.9031	64	5.2585		
	8	0.9031	75	5.6745		
	10	1.0000	56	5.1510		
	10	1.0000	83	5.9542		
81						
	15	1.1761	4	3.2419	25.22	.2731
	20	1.3010	16	4.0055		
	20	1.3010	32	4.5323		
	25	1.3979	40	4.7467		
	25	1.3979	56	5.1510		

Table 18. Bioassay results of Malathion by Method II.

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
82						
	1	1.0000	8	3.5949	3.33	.2218
	1	1.0000	9	3.6592		
	1.5	1.1761	12	3.8250		
	1.5	1.1761	16	4.0055		
	2.0	1.3010	22	4.2278		
	2.0	1.3010	39	4.7207		
83						
	0.5	0.6990	17	4.0458	4.438	.2525
	0.5	0.6990	19	4.1221		
	1.5	1.1761	17	4.0458		
	1.5	1.1761	29	4.4466		
	2.0	1.3010	30	4.4756		
	2.0	1.3010	37	4.6681		
	2.5	1.3979	44	4.8490		
	2.5	1.3979	53	5.0753		
84						
	1	1.0000	24	4.2937	2.272	.2073
	1	1.0000	32	4.5323		
	2	1.3010	39	4.7207		
	2	1.3010	40	4.7467		
	3	1.4771	56	5.1510		
	3	1.4771	69	5.4959		
85						
	2	1.3010	64	5.3585	1.722	.3773
	2	1.3010	69	5.4959		
	2.5	1.3979	68	5.4677		
	2.5	1.3979	92	6.4051		
	3	1.4771	90	6.2816		
	3	1.4771	96	6.7507		

Table 19. Bioassay results of Malathion by Method III.

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
86	Testing with five day old flies for 24 hours.					
	10	1.0000	2	2.9463	35.72	.1104
	10	1.0000	4	3.2493		
	15	1.1761	8	3.5949		
	15	1.1761	9	3.6592		
	20	1.3010	18	4.0846		
	20	1.3010	20	4.1584		
	25	1.3979	31	4.5041		
87	Testing with five day old flies for 24 hours.					
	5	0.6990	3	3.1192	16.91	.4648
	5	0.6990	13	3.8736		
	10	1.0000	12	3.8250		
	10	1.0000	20	4.1584		
	15	1.1761	70	5.5244		
88	Testing with five day old flies (new standard)					
	2	0.3010	5	3.3551	16.97	1.5198
	2	0.3010	6	3.4452		
	5	0.6990	6	3.4452		
	5	0.6990	13	3.8736		
	10	1.0000	22	4.2278		
	10	1.0000	12	3.8250		
	20	1.3010	70	5.5244		
89	Testing with five day old flies (using new dilution of standard)					
	20	1.3010	100	-----	11.85	.0252
	20	1.3010	69	5.4959		
	25	1.3979	78	5.7722		
	25	1.3979	74	5.6433		
	30	1.4771	83	5.9542		
	30	1.4771	79	5.8064		

Table 20. Bioassay results of Malathion by Method IV

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
90	24 hour exposure.					
	1	0.0000	24	4.2937	1.28	.1913
	1	0.0000	36	4.6415		
	1	0.0000	48	4.9498		
	1	0.0000	48	4.9498		
	2	0.3010	68	5.4677		
	2	0.3010	70	5.5244		
	2	0.3010	70	5.5244		
	2	0.3010	72	5.5828		
	3	0.4771	78	5.7722		
	3	0.4771	80	5.8416		
	3	0.4771	84	5.9945		
	3	0.4771	86	6.0803		
91	48 hour exposure.					
	1	0.0000	32	4.5323	1.116	.1667
	1	0.0000	42	4.7981		
	1	0.0000	48	4.9498		
	1	0.0000	50	5.0000		
	2	0.3010	80	5.8416		
	2	0.3010	82	5.9154		
	2	0.3010	82	5.9154		
	2	0.3010	86	6.0803		
	3	0.4771	90	6.2816		
	3	0.4771	94	6.5548		
	3	0.4771	94	6.5548		
	3	0.4771	96	6.7507		
92	24 hour exposure.					
	1	0.0000	16	4.0055	1.666	.2197
	1	0.0000	34	4.5875		
	2	0.3010	56	5.1510		
	2	0.3010	64	5.3585		
	3	0.4771	78	5.7722		
	3	0.4771	80	5.8416		

Table 20. (Concluded)

Test No.	Dose ug	Log Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
93	48 hour exposure.					
	1	0.0000	44	4.8490	1.046	.3430
	1	0.0000	52	5.0502		
	2	0.3010	74	5.6433		
	2	0.3010	82	5.9154		
	3	0.4771	80	5.8416		
	3	0.4771	96	6.7507		
94	24 hour exposure.					
	1	0.0000	66	5.4125	1.1727	.1900
	1	0.0000	78	5.7722		
	2	0.3010	90	6.2816		
	2	0.3010	92	6.4051		
95	24 hour exposure					
	1	0.0000	28	4.4172	1.353	.2722
	1	0.0000	30	4.4756		
	2	0.3010	68	5.4677		
	2	0.3010	76	5.7063		
	3	0.4771	94	6.6449		
	3	0.4771	98	7.0537		
96	48 hour exposure.					
	1	0.0000	66	5.4125	1.289	.6684
	1	0.0000	78	5.7722		
	2	0.3010	92	6.4051		
	2	0.3010	98	7.0537		
	3	0.4771	98	7.0537		
	3	0.4771	100	8.7190		
97	24 hour exposure.					
	0.4	0.6021	2	2.9463	1.147	.1315
	0.4	0.6021	4	3.2493		
	0.6	0.7782	14	3.9197		
	0.6	0.7782	15	3.9636		
	1.0	1.0000	38	4.6945		
	1.0	1.0000	40	4.7467		

Table 21. Bioassay results of Dieldrin by Method I

Test No.	Dose ug	Log of 10x Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
98					.3385	.4077
	0.1	0.0000	4	3.2493		
	0.2	0.3010	8	3.5949		
	0.3	0.4771	43	4.8236		
	0.4	0.6021	55	5.1257		
	0.5	0.6990	84	5.9945		
99					.2708	.2204
	0.1	0.0000	4	3.2493		
	0.2	0.3010	26	4.3567		
	0.3	0.4771	48	4.9498		
	0.4	0.6021	75	5.6745		
	0.5	0.6990	92	6.4051		
100					.3316	.1118
	0.2	0.3010	18	4.0846		
	0.3	0.4771	44	4.8490		
	0.4	0.6021	67	5.4399		
	0.5	0.6990	73	5.6128		
101					.3016	.1873
	0.2	0.3010	15	3.9636		
	0.2	0.3010	28	4.4172		
	0.4	0.6021	70	5.5244		
	0.4	0.6021	76	5.7063		
	0.6	0.7782	89	6.2265		
	0.6	0.7782	90	6.2816		

Table 22. Bioassay results of Dieldrin by Method II.

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
102						
	0.1	0.0000	4	3.2493	.2410	.0435
	0.1	0.0000	0	0.0000		
	0.3	0.4771	68	5.4677		
	0.3	0.4771	68	5.4677		
	0.5	0.6990	92	6.4051		
	0.5	0.6990	92	6.4051		
103						
	0.2	0.3010	40	4.7467	.1956	.2771
	0.2	0.3010	60	5.2533		
	0.3	0.4771	88	6.1750		
	0.3	0.4771	92	6.4051		
	0.4	0.6021	96	6.7507		
104						
	0.2	0.3010	60	5.2533	.1576	.1264
	0.2	0.3010	66	5.4125		
	0.3	0.4771	84	5.9945		
	0.3	0.4771	88	6.1750		
	0.5	0.6990	96	6.7507		
105						
	0.2	0.3010	4	3.2493	.2584	.1873
	0.2	0.3010	8	3.5949		
	0.3	0.4771	80	5.8416		
	0.3	0.4771	84	5.9945		
106						
	0.2	0.3010	4	3.2493	.2553	.4971
	0.2	0.3010	24	4.2937		
	0.3	0.4771	84	5.9945		
	0.3	0.4771	88	6.1750		
	0.4	0.6021	96	6.7507		
	0.4	0.6021	96	6.7507		

Table 23. Bioassay results of Dieldrin by Method III.

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
107	Testing with six day old flies for 24 hours.					
	0.2	0.3010	33	4.5601	.308	.2092
	0.3	0.4771	50	5.0000		
	0.4	0.6021	57	5.1764		
	0.5	0.6990	62	5.3055		
	0.6	0.7782	85	6.0364		
	0.7	0.8451	87	6.1264		
108	Testing with five day old flies for 24 hours.					
	0.2	0.3010	17	4.0458	.3469	.2400
	0.2	0.3010	20	4.1584		
	0.4	0.6021	49	4.9749		
	0.4	0.6021	58	5.2019		
	0.6	0.7782	77	5.7388		
	0.6	0.7782	91	6.3408		
	0.8	0.9031	93	6.4758		
109	Testing with six day old flies for 24 hours.					
	0.2	0.3010	60	5.2533	.1765	.1014
	0.3	0.4771	80	5.8416		
	0.4	0.6021	88	6.1750		
	0.5	0.6990	95	6.6449		
	0.6	0.7782	98	7.0537		
110	Testing with five day old flies for 24 hours.					
	0.2	0.3010	20	4.1584	.3476	.2282
	0.3	0.4771	41	4.7725		
	0.4	0.6021	70	5.5244		
	0.5	0.6990	72	5.5828		
	0.6	0.7782	68	5.4677		
	0.7	0.8451	80	5.8416		
111	Testing with five day old flies for 48 hours.					
	0.2	0.3010	36	4.6415	.2494	.2338
	0.3	0.4771	56	5.1510		
	0.4	0.6021	81	5.8779		
	0.5	0.6990	74	5.6433		
	0.6	0.7782	77	5.7388		
	0.7	0.8451	82	5.9154		

Table 24. Bioassay results of Dieldrin by Method IV.

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
112	24 hour exposure.					
	0.2	0.3010	22	4.2278	.2779	.1224
	0.2	0.3010	32	4.5323		
	0.4	0.6021	74	5.6433		
	0.4	0.6021	76	5.7063		
	0.6	0.7782	92	6.4051		
	0.6	0.7782	94	6.5548		
113	48 hour exposure.					
	0.2	0.3010	42	4.7981	.2083	.1780
	0.2	0.3010	50	5.0000		
	0.4	0.6021	84	5.9945		
	0.4	0.6021	92	6.4051		
	0.6	0.7782	96	6.7507		
	0.6	0.7782	96	6.7507		
114	24 hour exposure.					
	0.2	0.3010	10	3.7184	.3432	.1244
	0.2	0.3010	16	4.0055		
	0.4	0.6021	60	5.2533		
	0.4	0.6021	67	5.4399		
	0.6	0.7782	87	6.1264		
	0.6	0.7782	88	6.1750		
115	24 hour exposure.					
	0.2	0.3010	10	3.7184	.2669	.5265
	0.2	0.3010	54	5.1004		
	0.4	0.6021	75	5.6745		
	0.4	0.6021	86	6.0803		
	0.6	0.7782	92	6.4051		
	0.6	0.7782	96	6.7507		
116	24 hour exposure.					
	0.2	0.3010	32	4.5323	.2669	.1769
	0.2	0.3010	40	4.7467		
	0.4	0.6021	62	5.3055		
	0.4	0.6021	74	5.6433		
	0.6	0.7782	86	6.0803		
	0.6	0.7782	90	6.2816		

Table 24. (Concluded)

Test No.	Dose ug	Log of 10X Dose	Dose effect as percent mortality	Dose effect in probit units	LD_{50} ug	Standard deviation in probits
117	48 hour exposure.					
	0.2	0.3010	50	5.0000	.2132	.2870
	0.2	0.3010	52	5.0502		
	0.4	0.6021	72	5.5828		
	0.4	0.6021	74	5.6433		
	0.6	0.7782	96	6.7507		
	0.6	0.7782	96	6.7507		

APPENDIX B

DETAILED STATISTICAL METHOD

Key to Symbols:

X = the individual dose levels in log units.

Y = the response to each dose in probit units.

S = the symbol signifying a summation.

b = the sample regression coefficient: = $\frac{Sxy}{Sx^2}$

a = the mean of the mortalities corresponding to a deviation of zero in the dose levels.

n = the number of observations in the test.

SX = the summation of all the doses.

SY = the summation of all the dose effects.

SX^2 = the summation of the squares of the doses.

SY^2 = the summation of the squares of the dose effects.

SXY = the summation of the dosage times the dose effect.

Sxy = sum of the products of the deviations of both parameters X and Y.

Sx^2 = sum of the squares of the deviations of X.

Sy^2 = sum of the squares of the deviations of Y.

Procedure

A. The data.

1. List the actual doses used, and the percent effects then convert the doses to logarithms (X) and the per cent effects to probit units (Y).

2. When some of the doses in a test are less than one and designated in decimals, multiply all the doses in that test by ten before

converting into logarithms.

B. Calculations of functions.

1. Calculate the summations SX, SY, SX^2, SY^2, and SXY.

2. To calculate the functions Sxy, Sx^2, and Sy^2, the following equations are used.

$$Sxy = SXY - \frac{(SX)\ (SY)}{n}$$

$$Sx^2 = SX^2 - \frac{(SX)^2}{n}$$

$$Sy^2 = SY^2 - \frac{(SY)^2}{n}$$

3. Calculate the sample regression coefficient, b as:

$$b = \frac{Sxy}{Sx^2}$$

4. Calculate the function, a as:

$$a = \frac{SY}{n} - b\ \frac{(SX)}{n}$$

5. From the above two calculations the LD_{50} is arrived at by the equation:

$$Y = a + bX \text{ and } X = \frac{Y - a}{b}$$

where Y is the 50% mortality point, then substituting the probit unit for 50% (5) in place of Y in the equation solve for X which would be the dose required for a 50% effect, and equivalent to the LD_{50}.

6. To determine the error variance and standard deviation for the regression equation the following procedure is used:

$$s^2_{y.x} = \frac{Sy^2 - b\ (Sxy)}{(n - 2)}$$

$$s_{y.x} = \sqrt{s^2_{y.x}}$$

Where $s^2_{y.x}$ is the variance and $s_{y.x}$ is the standard deviation or error.

Sample Problem

Solution of the Dose-effect curve of Dieldrin by Method IV

Dose in micrograms	Dose in micrograms x10x	log dose	Dose effect as Mortality	Dose effect in probit units
0.2	2.0	.3010	22	4.23
0.2	2.0	.3010	32	4.53
0.4	4.0	.6021	74	5.64
0.4	4.0	.6021	76	5.71
0.6	6.0	.7782	92	6.41
0.6	6.0	.7782	94	6.55

Step 1: SX=3.3626 SY=33.07

SX^2 = 2.1174 SY^2=186.82

SXY = 19.556

Step 2:

$$Sxy = SXY - \frac{(SX)\,(SX)}{n} = 19.556 - \frac{(3.3626)\,(33.07)}{6} = 1.0235$$

$$Sx^2 = SX^2 - \frac{(SX)^2}{n} = 2.1174 - \frac{(3.3626)^2}{6} = .2329$$

$$Sy^2 = SY^2 - \frac{(SY)^2}{n} = 186.82 - \frac{(33.07)^2}{6} = 4.5492 \text{ or } 4.55$$

Sample Problem (Concluded)

Step 3:

$$b = \frac{Sxy}{Sx^2} = \frac{1.0235}{.2329} = 4.40$$

$$a = \frac{(Sy)}{n} - \frac{b\,(Sx)}{n} = \frac{33.07}{6} - 4.40\ \frac{(3.3626)}{6} = 3.05$$

Step 4:

$LD_{50} = Y = a + bX$. If Y is 50% kill then Y = 5.0000 probit units; therefore $5 = a + bX$ and $X = \frac{5 - 3.05}{4.40} = 0.44318$. The antilog of 0.44318 is 2.77; therefore to convert back to the original dose level divide this by 10: 2.77/10 = 0.2777 = the LD_{50}

Step 5:

$$s^2_{y.x} = \frac{Sy^2 - b\,(Sxy)}{(n-2)} = \frac{4.55 - 4.40\ (1.0235)}{6 - 2} = 0.01165$$

$s_{y.x}$ = 0.01165 probit units.

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