VARIANCES IN RESPONSE ENCOUNTERED WHEN KUN STRAIN, MALE, INSECTICIDE-SUSCEPTIBLE HOUSEFLIES WERE USED TO DETERMINE THE POTENCY OF MALATHION ON DIFFERENT DAYS

by

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INTRODUCTION AND REVIEW OF LITERATURE

Variation in responses of insects to a stimulus is unavoidable as every entomologist knows. A knowledge of the type and extent of this variance is necessary in the design and analysis of experiments.

Bliss and Beard (1935) in a study of variation of response to gases of the adult milkweed bug (Oncopeltus fasciatus, Dall.) stated that there can be two sources of variation in susceptibility. The first he called "static" which would cause certain individuals to maintain their susceptibility. The second, "dynamic variation", may change the susceptibility of the individual very quickly. These two types of variation were also discussed by Clark (1933). Dynamic variations may be caused by several factors. Rai, et al. (1956) found that malathion was more toxic to houseflies (Musca domestica, Linn.) as the temperature was increased from 63° to 75° F.. Davidow and Hagan (1955) stated that the toxicity of a compound may vary from species to species of insect and with environmental conditions. They suggested that sex, age, weight, and state of nutrition may affect susceptibility. Intraspecies differences, differences which normally occur in a population, may have a marked effect on results obtained in acute toxicity studies. Yamasaki and Ishii (1957) found that variation in susceptibility can be introduced by species variation, sex, stage of growth as well as temperature and moisture conditions.

Nagasawa (1957) studied the effects of diet on knockdown and length of adult life of houseflies. He also found that male houseflies were more susceptible to p,p'- DDT powder than were the females. Sun and Sun (1953) found that young house flies are more susceptible than older ones. Gunther, et al., (1958), in studies of toxicities of 44 compounds related to diphenylmethane, found variation in replications which were repeated at different times. "Agreement among replicates in a given series of tests was excellent, but variations among the averages of series repeated at different times were considerable and amounted to as much as 600% in the LT_{50} values and 1000% in the LD_{50} values." The confused flour beetle, (Tribolium confusum Div.), was used as the biological indicator.

Finney (1952) described different types of bioassays. One type, "indirect assay based upon quantal ('all or nothing') responses", has a unique characteristic. The variance of any per cent mortality can be calculated according to a formula discussed by Wadley (1948). The variance of $p = \frac{pq}{N}$, when p represents the proportion affected, q equals (1-p) and N the number of individuals in the test. Wadley (1948) stated that this variance of a proportion (σ_p^2) can be increased by a number of factors. These include environmental changes, but the most important are real changes in resistance of successive lots of insects. Real differences in susceptibility, over and above the true variance, is common among such

groups.

It is apparent, then, that one source of variation, the variance of p (σ_p^2) is proportional to p (Finney, 1952). Hence, Goulden, (1956) discussing probit analysis, stated that the variance of Y, the dependent variable, at all levels of X, the independent variable, is not equal. He stated that σ_p^2 is greatest when p=q=0.5 and decreases to a minimum when p=0.0, p=1.0. This variance must be considered in the analysis of experiments. Fitting a regression formula through data involves making the assumption of homoscedasticity (Snedecor and Cochran, 1957). Goulden (1956) stated that this is definitely not the case in quantal type assays.

Several methods of analysis, disregarding heterogeneity of variance, have been suggested. Wadley (1948) stated that the analysis of variance could be used when N is large or when means of several percentages are used. Berkson (1950) tabulated LD_{50}'s calculated by eight different methods and compared them. He stated that for practical importance differences among them are quite small. Finney (1952) recognized that one of the oldest and most widely used principles for the estimation of unknown parameters is the method of least squares. In this method, the estimates are taken as those values which minimize the sum of squares of the differences between the observation and the corresponding theoretical values or expectations.... For quantal responses, minimization of the quantity $\Sigma\left[N(p-\bar{P})^2\right]$ has no merits as a

method of estimation: it takes no account of the fact that the variance of p (σ_p^2) depends on p, so that large deviations from p can occur much more easily in the region of p=q=0.5 than at the extremes. If the method is modified and based upon a weighted sum of squares, with the reciprocals of the variances as weights, the quantity to be minimized becomes $\Sigma \left[\frac{N(p-\bar{P})^2}{pq} \right]$; the least squares method is then identical with that of minimum chi square (χ^2).

Bartlett's test of homogeneity, which tests the hypothesis: $\sigma_1^2 = \sigma_2^2 = \cdots = \sigma_n^2$ is outlined by Snedecor and Cochran (1957) for both equal and unequal subclass numbers. Another such test is in general $\chi^2_{n-1} = \frac{\Sigma \chi^2}{\frac{pq}{N}}$ when N is equal for each per cent response (Wadley 1948). Snedecor and Cochran (1957) gave a method of χ^2 for unequal numbers. If the number in a group (N), the number affected (X), the per cent affected (p), and the product (pX) are tabulated, chi-square is $100 \left[\frac{\Sigma(pX) - \bar{p} \cdot \Sigma(X)}{\bar{P}(100-\bar{p})} \right]$ with a-1 degrees of freedom, where a is the number of groups.

Houseflies are known to vary in response to a toxicant when various conditions are changed. However, when the age, sex, food, and rearing conditions are held relatively constant other variables may cause different lots of houseflies to respond differently. Lots represent different groups of houseflies which are of the same genetic population but from different parents.

Variation from lots to lots was tested to determine if this variance was greater than the characteristic variance of quantal responses. The knowledge gained may be used in designing and evaluating future experiments making critical comparisons between insecticides.

MATERIALS AND METHODS

Materials

KUN strain houseflies (Musca domestica L.) were used in this study. These were originally obtained from Kansas University and have been maintained in various laboratories without exposure to any insecticide for 15 to 20 years.

Flies for these tests were reared in the laboratory according to CSMA procedure, except for slight modifications. A description of the rearing procedure is given by Rai, et al. (1956).

Malathion used for testing was obtained from the American Cyanamid Company, Stamford, Connecticut and was 99.6 per cent pure. Glass-distilled acetone was used for dilutions and cleaning glassware. The insecticide (malathion and acetone) was applied by a micro-device designed and described by Roan and Maeda (1953).

The houseflies were conditioned and held during the test in a constant temperature cabinet. The temperature in the 6.2 cubic foot box was held at 76 $\pm$ 2 degrees Fahrenheit during the test. Circulation within the chamber

was provided by a fan. A detailed description of the test procedure was described by Rai, et al. (1956).

Methods

A complete block design was used to study the response of male houseflies to an insecticide. Days were used as blocks with repetitions of six levels of insecticide appearing six times in each block. The levels of insecticide (expressed in ug./ml. per fly) were 0.40, 0.35, 0.30, 0.25, 0.20, and 0.00 with the 0.00 level as a measure of natural mortality. Four day old male houseflies were used in the tests.

Shortly before treatment, samples of malathion were weighed and five dilutions prepared. Test solutions were prepared each test day.

Each day, six repetitions of 50 flies each were tested at each level of insecticide. A control, for each repetition, which received no insecticide but an equal exposure to carbon dioxide, was maintained. In this manner any mortality due to factors other than the insecticide and acetone could be tabulated.

A minimum of carbon dioxide was used to anesthetize the flies while sexing and treating. All parts of the dilution and treatment apparatus were thoroughly washed and rinsed with acetone before and after their use. The syringe and needle were rinsed with glass-distilled acetone after

completing treatment with each level. They were then dried with a stream of air. The syringe was rinsed in the test solution, then filled for treatment. The first few drops of insecticide from the syringe were discarded to free the syringe from air bubbles.

Individual treatment was accomplished by placing the needle to the mesosternum of each fly and applying the insecticide. The treated fly was immediately placed in a recovery jar and a second fly treated. When 50 flies were in a jar, cheesecloth was placed over the mouth of the jar, and held fast by a rubber band. Recovery jars were then placed on the bottom shelf of the constant temperature cabinet.

Following each test the recovery jars were rinsed with acetone, scrubbed with hot detergent water, rinsed with acetone again and allowed to dry. Before reuse, each jar was rinsed with acetone a third time and allowed to dry. Cheesecloth and food were discarded after each test.

Flies remained in the constant temperature cabinet for 24 hours following the application of the insecticide. They were then removed and counts of surviving insects made.

The average weight of a particular group of houseflies was obtained by weighing a random group of 50 flies. This average weight was used to calculate the LD_{50} estimates for each repetition.

RESULTS

This experiment was begun June 1, 1958. It was designed to measure the variance of a bioassay with quantal response when repetitions are conducted over a six week period. Actual testing was done every week from June 5 through July 23.

Response was measured by counting fly mortality in each jar of 50 male houseflies. Each day six repetitions of 50 male flies at six levels of toxicant were performed. One level served as a control.

Table 1. Average per cent mortality of houseflies at five concentrations of malathion and a control on six dates.

Day	Levels of malathion in micrograms per milliliter acetone					
	.40	.35	.30	.25	.20	Control
6-18-58	83	73	45	45	25	.0
6-25-58	94	95	86	83	46	1.0
7- 2-58	95	89	83	60	16	1.0
7- 9-58	98	91	78	74	54	3.0
7-16-58	94	89	76	53	37	.0
7-23-58	96	91	72	59	30	.0

Table 1 illustrates that there are differences in mortalities at different levels of toxicant. This is expected, for the assigned levels were pretested to insure covering a minimum of 50 per cent of the mortality range

with each test. It is also evident that within a concentration there is variation in response from day to day. Control mortality was less than three per cent on all days.

It was found in the literature that such measures as "dead or alive" are quantal in nature and have a variance which is subject to change as the proportion affected changes. It is possible to test for differences in variances at each average p by using Bartlett's test of homogeneity (Snedecor and Cochran, 1957). Corrected chi square by Bartlett's test was 34.82.

Chi square exceeds the tabled value at P = .005. From this evidence it was concluded that s^2 is different for different p. In Table 3 is given the average per cent mortality at each level of toxicant. Heterogeneity of variance would be expected from such data, for the variance of p (estimated by s^2) would be greatest when P = 0.5 and a minimum when p = 1 or 0. This is obvious when p and the corresponding s^2 are compared to other values.

It is also noticed that s^2 is generally quite large. The estimates of s^2 for p = 77, 62, and 35 are especially large.

It is apparent that the estimates of s^2 are biased upward by day to day differences. It is possible to separate the day to day effects and obtain an unbiased estimate of s^2, by the analysis of variance.

A one-way analysis of variance was calculated for each

level of insecticide. By a variance component analysis (Table 2) an estimate of σ_e^2 was obtained, a similar form is given by Snedecor and Cochran (1957).

Table 2. Component analysis for one-way analysis of variance with degrees of freedom.

Source of Variation	D/F	Expectation of mean square	Estimates
Days	5	$\sigma_e^2 + \lambda K_R^2$	$s_e^2 + 6K_R^2$
Within Days	30	σ_e^2	s_e^2

By the one-way analysis of variance each average p has an unbiased estimate of s_e^2 (Table 3).

Table 3. Comparison of s^2 and σ_e^2 for five average p with σ_p^2.

p	* σ_p^2	s^2	s_e^2
93	17.0172	41.2825	21.5323
88	29.0987	73.6445	28.8889
77	53.9589	237.6730	62.5445
62	65.2570	208.2635	46.0222
35	62.8190	213.6250	59.0222

* $\sigma_p^2 = \frac{pq}{N}$

Scheffe' (1956), and Ramachandran (1958) gave a $\chi^2_{n-1} = \frac{(n-1)s^2}{\sigma^2}$ where $\angle(n-1)s^2\rfloor$ is equal to

$$\sum_{i=1}^{N_i} (X_i - \bar{x})^2.$$

Using σ_p^2 as an estimate of σ^2 and $35(s^2)$ as Σ_i^2 all corresponding χ^2 values are greater than the tabular value at P = .05. This is evidence at P = .05 that s^2 is too large to be called an estimate of σ_p^2. There is additional variability included in the estimate, s^2, which overshadows the variance of p, (σ_p^2).

If a similar test, using s_e^2 as an estimate of s^2, the formula becomes:

$$\chi^2_{n-1} = \frac{(n-1)\, s_e^2}{\sigma_p^2}.$$

Scheffé (1956) stated that s_e^2 is an unbiased estimate.

Chi square values calculated on the basis of s_e^2 when compared to the tabular value proved to be non-significant at P = .05. There is evidence that the effects of days bias the estimate of s^2 upward, since at P = .05 s_e^2 is a reliable estimate of σ_p^2, the true variance of p.

By the formula $(\frac{pq}{n})$, the variances of percentages differ as the per cent (p) takes on different values. These differences are shown to be significant by Bartlett's test of homogeneity. The variance of the per cent when estimated by s^2 is too large to be recognized as an estimate of σ_p^2. By removing the variance caused by day to day differences, a second estimate of σ_p^2 is found, s_e^2.

This "within" variance, s_e^2, is a reliable estimate of σ_p^2 as shown at P = .05 with the chi-square test. It was apparent that the effects of day to day differences were present and of important magnitude.

It has been shown that s_e^2 is an estimate of σ_p^2. Another variance component remains, k_R^2, which is the variance which estimates the day to day variation at a particular level of toxicant. It might be expected that this estimate, k_R^2, would be constant at each level; i.e. the effects of days could be measured equally well at any level.

Using a general formula given by Bross (1950) it is shown that $k_R^2 = \frac{\text{Among Ms} - \text{Within Ms}}{\lambda}$: where λ is a coefficient assigned to k_R^2 in the component analysis. Thus $k_R^2 = \frac{\text{Among Ms} - \text{Within Ms}}{6}$. Values of k_R^2 are shown in Table 4.

Table 4. Estimates of k_R^2 for five average per cent mortalities and intraclass correlation.

Average per cent mortality	k_R^2	Intraclass correlation I
93	19.71	.4779
88	54.55	.6538
77	204.32	.7656
62	189.28	.8044
35	180.04	.7531

Following the method given by Bross (1950) for setting a confidence limit on k_R^2, it was found that,

$P(6.9532 \leq k_R^2 \leq 83.5645) = .90$. Since this confidence interval does not include all estimates of k_R^2, it is apparent the k_R^2 is not a constant from level to level. For some reason day effects are greater when the proportion affected is near 0.5. This is beyond the scope of these investigations.

Intraclass correlations calculated for each average p, show that good reproducibility exists within each level for each lot of houseflies.

From Bartlett's test of homogeneity it was found that the variance of p changed, as expected, at different levels of toxicant. Finney (1952) has shown that a method of analysis which minimizes the quantity $\Sigma \left[\frac{n(p-\pi)^2}{pq} \right]$ takes account of different variances of p. Such a method of analysis, which fits a straight line through observed values of p for each level of concentration, is termed probit analysis.

To expedite computation the probit analysis was done by the IBM 650 Data Processing Machine. The program used for the analysis was written by Dr. R. R. Sokal[1] of Kansas University.

Results obtained from this analysis included estimates of Beta and the LD_{50}, with 5 per cent fiducial limits. The

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[1] Received by personal communication.

estimated mortality (Y_1) for each concentration (X_1) was also computed so that graphing could easily be accomplished.

By coding the individual doses in terms of micro-grams per gram body weight, the computed ED_{50} values are actually LD_{50} values.

The probit analysis was computed for each individual repetition and the mean of all 36 repetitions. The hypothesis: = 0 was tested at P = .05, as was heterogeneity chi square.

Of special interest was the LD_{50}, as it is a measure of the amount of toxicant to create a 50 per cent mortality among the individuals tested. It is a measure of susceptibility or the ability of a group to tolerate the toxicant.

It is known that an estimate of a parameter is approximately normally distributed (Mood, 1950) and such measurements can be studied by the analysis of variance. By using the functional analysis of variance described by LeClerg(1957) it can be determined by testing orthogonal polynomials if a graphic relationship can be used to explain the response.

Table 5. Functional analysis of variance of LD_{50} values determined on six equally spaced days.

Source of variation	D/F	MS	F	Statistical Significance
Days	5	23.90	22.36	.01
Linear	1	45.03	42.12	.01
Quadratic	1	25.76	24.10	.01
Cubic	1	19.72	18.45	.01
Quartic	1	4.65	4.35	.05
Remainder	1	24.55	22.96	.01
Within Days	30	1.07		

From Table 5 it can be seen that the test to test effects on the LD_{50} were significant at P = .01. This leads to the rejection of the H_o of no difference, and the acceptance of the H_a that the day to day effects are different. An attempt to find a polynomial which would describe the day to day effects proved futile. After fitting the quartic polynomial, a significant remainder was left. It is concluded that day to day variation in LD_{50} values are not systematic. However some tendency for a graphic trend is present as is indicated by significant mean squares for linear, quadratic, and cubic. The quartic polynomial accounted for very little variation (3.9%) even though its' mean square was significant at P = .05.

The LD_{50} estimate of June 18 was the greatest and is different from the other estimates. The mean LD_{50} for all tests is 21.19 ug/gram fly. $P(19.89 \leq LD_{50} \leq 22.27) = .95$ is the probability statement based on .05 fiducial limits.

Figure 1 illustrates the differences among extreme regression for six repetitions when pooled. The LD_{50} for June 25 is 11.63 ug/gram fly, while 24.83 ug/gram fly was the LD_{50} for June 18. They are compared to the average for all tests.

The mean LD_{50} estimate for all tests, 21.19 ug/gram fly, is compared to other pooled estimates in Table 6. Variation in LD_{50} estimates is shown by comparing them to

21.19 ug/gram fly. Estimates ranged from 55 per cent to 117 per cent of the mean estimate.

Table 6. Pooled LD_{50} estimates from six tests compared to the LD_{50} estimate for all tests.

Date tests performed	Pooled LD_{50} estimate	Pooled estimates as a per cent of 21.19 ug/gram fly
June 18	24.83	117.18
25	11.63	54.88
July 2	21.08	99.48
9	23.00	108.54
16	22.15	104.53
23	16.14	76.17

Comparisons among the estimate of 21.19 ug/gram fly and estimates collected by other workers using similar techniques and KUN strain males are shown in Table 7.

Table 7. Estimates of LD_{50} for pure (99.6%) malathion on houseflies by various workers.

Name	Dates	LD_{50} estimate	Insect
L. Rai	1956	20.83	Male KUN housefly
G. Ware	1957	16.89	Male KUN housefly
G. Krause	1958	21.19*	Male KUN housefly

*contains effects of acetone.

Estimates of β were obtained from the probit analysis. Each estimate was tested at $P = .05$ by a t test, the null

EXPLANATION OF PLATE I

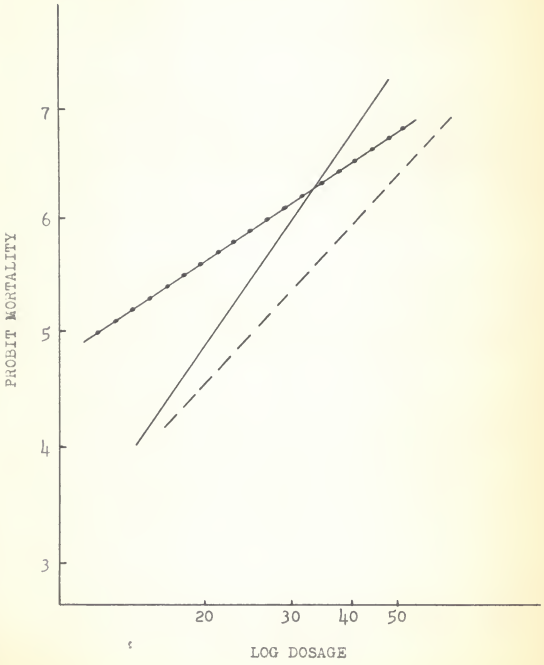
Pooled probit regression of tests performed June 18, 1958 and June 25, 1958 compared to average for all tests (LD_{50}).

—·—·—·—·—·—·—·—·— June 25, 1958.

— — — — — — — — — June 18, 1958.

_____ All tests.

PLATE I



hypothesis was H_o: $\beta = o$. In all cases this hypothesis was rejected.

All tests were conducted using the same population of houseflies. To determine the homogeneity of tolerances, the β estimates were separated by the analysis of variance and Duncan's new multiple range test (LeClerg, 1957). Graphic trends were tested by using the functional analysis of variance and testing individual degrees of freedom.

Table 8. Analysis of variance of Beta estimates with individual degrees of freedom.

Source of variation	D/F	M.S.	SIG
Repetitions	5	11.3532	.01
Linear	1	4.7392	ns
Quadratic	1	3.2623	ns
Cubic	1	15.6271	.01
Quartic	1	2.5981	ns
Other	1	30.4823	.01
Within Repetitions	30	1.3302	

The analysis of variance shows that differences exist among estimates of Beta, taken on different days. However, the analysis of individual degrees of freedom shows that time trends explain only a small per cent (46.3%) of the variability in the Beta estimates.

Table 9. Duncan's new multiple range test, separation of average estimates.

Day	6/18	7/9	6/25	7/16	7/23	7/2
Ranked means	5.38	5.42	6.17	6.54	7.36	9.01
*Significance						

*means underlined are not significantly different at P = .05.

Duncan's mean separation test (Table 9) shows that the estimate of July 2 was higher than all others. This indicates that the homogeneity of tolerance among the test group was greatest on this date. Lower Beta estimates on other dates indicate heterogeneity of tolerance within test groups. Estimates collected on June 25, July 16, and 23 were not significantly different. The second highest estimate 7.36 was different from the two lowest estimates.

DISCUSSION

Variation in response was studied by several statistical methods. It was found that quantal type responses have a variance which is characteristic to binomially distributed data. However, other variables also affect the response of male houseflies.

Characteristic to a binomial distribution is the fact that the variance of p is greatest when it is near 0.5, and diminishes as it approaches 1 or 0. Bartlett's test of homogeneity shows that the variance of different percentages are significantly different at the .005 level of probability.

A chi-square test suggests that lot to lot differences are great enough to prevent the recognition of the true variance σ_p^2.

Differences in response from lot to lot were significant. There was no discernable trend of response over equally spaced days which would describe the change in susceptibility. LD_{50} estimates from pooled data from six daily repetitions, compared to the LD_{50} estimate for 36 repetitions range from 54.9 to 117.18 per cent of the mean estimate.

The mean LD_{50} estimate calculated by pooling all 36 repetitions was 21.19 ug/gram fly. This compares with 20.83 ug/gram fly found by Lallan Rai in 1956 using similar techniques and KUN strain males.

It was found by the analysis of variance and a component analysis that the variance due to days (lots of houseflies) was significant at P = .05. Intraclass correlations show there was greater reproducibility within days than among days.

The greatest source of variation beyond that of the binomial, was that of k_R^2. The binomial variance cannot be minimized beyond $\frac{pq}{N}$, therefore sample size is determined by the variance of days.

Various numbers of lots to be sampled to be within a specified difference of the LD_{50} with two levels of proba-

bility are given in Table 10. It is noticed that N becomes very large as d becomes small.

Table 10. Probability of an estimate being within a per cent of the LD_{50} (malathion) when sampling various numbers of lots of KUN male houseflies.

d as a per cent of LD_{50}	Lots of houseflies (N)	
	P = .90	P = .95
18.9	6	8
16.5	7	10
15.5	8	11
14.3	9	12
13.4	10	14
11.4	13	18
9.6	18	24
7.7	30	45
6.1	40	60
3.0	120	120

This is evidence that sampling days (N) is more useful and efficient in determining a reliable estimate of the LD_{50} than large numbers of repetitions within days. Repetitions within days should be limited to the necessary number required to obtain an estimate of the response on that day.

Relative efficiency was calculated according to a method found in Cochran and Cox (1957). It was found that a randomized complete block design, with days as blocks has a relative efficiency of 1.96 compared to a completely random design. This may be interpreted to mean that approximately one half as many tests need to be run to obtain an equally reliable estimate when the effects of

days are removed.

Table 11. Number of lots of houseflies to be sampled to be within d=4 ug/gram fly, at various levels of α and β.

P of type I error α	P of type II error β	Lots to detect d
.05	.3	19
.05	.2	25
.01	.3	30
.01	.2	36

The effect of power of the test (1-β) upon the number of lots to be sampled is illustrated in Table 11. Within each level of α the necessary number of lots to detect a difference of 4 ug/gram fly is greater when the power of the test is increased. It is shown (Table 10) that when d is approximately 4, N is 8 at P = .95, when the type II error is considered the required N, to detect d = 4 when P = .05, β = .3, is 19. The effects of power are very great.

Estimates of Beta were found to differ from week to week. Duncan's multiple range test shows that the Beta estimate found on July 2 was significantly higher than all others. Estimates collected on June 6, July 9, June 25, and July 16 did not differ.

Homogeneity of tolerance was more evident on July 2. Groups tested on other dates showed greater heterogeneity

than did this group. Four of the six lots tested showed basically the same degree of susceptibility to the toxicant.

SUMMARY

Variation in response of a laboratory-reared KUN strain of insecticide susceptible, four-day-old, male houseflies to malathion applied topically was studied.

Several statistical methods were used to detect and measure variation due to various sources. It was found that there were two sources of variation. The first was heterogeneity between per cent mortalities. This was expected and its significance was detected by Bartlett's test of homogeneity. It was found that this within type of variance was greatest when the proportion affected approached 0.5 and was less at higher proportions.

Using a component analysis it was found that a greater source of variation, that attributed to lot to lot of houseflies, was also present. This second type of variation, among lots, must be considered in obtaining reliable estimates of insecticidal potency.

The effect of the second source of variation upon sample size is discussed. A randomized complete block design is suggested to determine estimates of parameters. Days (lots of insects) represent blocks and their effects can be removed to gain efficiency over a completely random design.

Estimates of the LD_{50} and Beta were determined by the probit analysis. Estimates of these parameters were found to differ when they were determined from different lots of houseflies. The pooled estimate of the LD_{50} is comparable to estimates found by other workers at this Station.

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VARIANCES IN RESPONSE ENCOUNTERED WHEN KUN STRAIN, MALE, INSECTICIDE-SUSCEPTIBLE HOUSEFLIES WERE USED TO DETERMINE THE POTENCY OF MALATHION ON DIFFERENT DAYS

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The effects of variances encountered when bioassays, involving quantal data, are used to determine potency of insecticides were studied.

Variation in response of insects to insecticides have been reported by several workers. Davidow, et al., (1955) Yamasaki and Ishii, (1955) and Sun and Sun (1953) described various circumstances known to cause variation in response. Gunther, et al., (1958) found greater reproducibility within repetitions than among repetitions done on different days; they used Tribolium confusum Div. as an indicator.

The KUN strain, male, 4-day-old houseflies, reared for many years in the laboratory according to the standardized CSMA procedure and treated topically with malathion carried in acetone, was used to determine response to the insecticide, malathion. Each experiment was conducted each Tuesday between June 18 and July 23, 1958.

Several statistical methods were used to detect and measure variation due to various sources. It was found that there were two sources of variation. The first was heterogeneity between per cent mortalities. This was expected and its significance was detected by Bartlett's test of homogeneity. It was found that this within-type of variance was greatest when the proportion affected approached 0.5 and was less at higher proportions.

Using a component analysis it was found that a greater source of variation, that attributed to lot to lot of

houseflies, was also present. This second type of variation, among lots, must be considered when obtaining reliable estimates of potency.

The effect of the second source of variation upon sample size is discussed. A randomized complete block design is suggested to determine estimates of parameters. Days (lots of insects) represent blocks and their effects can be removed to gain efficiency over a completely random design.

Estimates of the LD_{50} and Beta were determined by the probit analysis. Estimates of these parameters were found to differ when they were determined from different lots of houseflies. The pooled estimate of the LD_{50} is comparable to estimates found by other workers at this Station.