METHOD FOR THE FIELD DETERMINATION OF SUSCEPTIBILITY LEVELS OF ADULT MOSQUITOES [1]

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During the early use of residual insecticides against mosquito vectors, the control of the insects and the reduction of diseases such as malaria were spectacular. More recently, however, mosquito control efforts in many areas have become increasingly difficult because of the development of insecticide-resistant populations. Since unsatisfactory control also can arise from inadequate insecticide applications, defective chemicals, or the action of environmental factors on the residues, it is important to determine the correct cause for the operational failure. The need for a suitable field technique as a means of determining the susceptibility level of the vector population to the toxicant involved soon became obvious. Consequently, in 1956, studies were begun to develop a kit which would contain all essential equipment for conducting tests in any locality, would be compact enough for a person to carry easily, and would be durable under tropical climatic conditions. Efforts were made to incorporate the more important features of existing test methods (Fay *et al.*, 1953; Busvine and Nash, 1953), and so far as possible produce a method having simplicity, sensitivity, accuracy, and reproducibility under field conditions.

MATERIALS AND METHOD. The complete test kit (Figure 1) is contained in a sturdy marine-plywood carrying case (14½" by 22" x 6"). It includes the following items: 2 plastic aspirator tubes; 30 wire-screen-top threaded-base plastic tubes (125 mm. long by 44 mm. diameter) with 10 marked red for use as exposure tubes and 20 unmarked for use as holding tubes; 30 screw-cap metal bases equipped with sliding metal closures; 30 metal spring clips; 16 impregnated papers each of five DDT-Risella oil concentrations, of six dieldrin-Risella oil concentrations and of Risella oil only; and miscellaneous auxiliary items such as untreated papers, rubber bands, cotton, forceps, record forms, graph paper, and instructions. The test papers [2] (Whatman No. 1 or Schleicher-Schull 2043A 120 mm. x 150 mm.) are commercially impregnated to give residual deposits of 360 mg./100 sq. cm. of 0.25, 0.5, 1.0, 2.0, and 4.0 percent DDT in Risella oil and of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 percent dieldrin in Risella oil.

In a typical test sequence, the adults of the desired species are aspirated from natural resting sites and 25 to 30 specimens are placed in a single holding tube (Figure 2, steps 1 and 2. Subsequently, these specimens are transferred to an exposure tube fitted with a treated paper. To do so, the metal bases of a holding and an exposure tube are placed together, the metal slides are opened, and the mosquitoes are gently blown into the exposure tube (Figure 2, steps 3 and 4) which is placed in an upright position. At the end of a 1-hour exposure period, the mosquitoes are returned to a holding tube, lined with an untreated paper, by reversing the transfer procedure described above. Paper is substituted as a cover for the holding tube and a cotton pad soaked in sugar solution is placed on the screened end of the tube (Figure 2, steps 5 and 6). At the end of 24 hours, the specimens incapable of resting on the sides of the tube are counted as dead, and the percentage mortality is calculated.

In the application of the kit for resistance

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia.

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determinations, an initial baseline of response of a given species of mosquito to various concentrations of DDT or dieldrin is established. Subsequent routine checks then are made periodically to determine any change in the response of the species. The method does not reflect variations in the behavioristic response of the insects, but measures their ability to survive contact with the test residues for a specific time under certain conditions. Since these conditions do not reflect the actual exposure of the insect to field control application, the method should not be used to compare the field effectiveness of different insecticides. Essentially, it is designed to detect physiological resistance levels of sufficient magnitude to hamper control operations.

In determining the base line of susceptibility values, mosquito populations should be sampled from several locations in the program area and processed as follows for each insecticide:

(a) Run one test (20–25 specimens) with each insecticidal concentration and with a Risella-oil control, using a 1-hour exposure period. If these tests provide the desired mortalities—one concentration giving less than 50 percent mortality and one (or preferably two) giving complete mortality—proceed to step (c).

(b) If the initial tests do not provide the desired mortality levels noted in step (a), shorter exposure periods of 15 or 30 minutes, or longer exposure periods of 2, 4, or 8 hours should be tried. If suitable values are then obtained, proceed to step (c). If 8-hour exposures to the highest concentrations fail to produce essentially complete mortality, the species may be assumed to have a level of resistance. How-

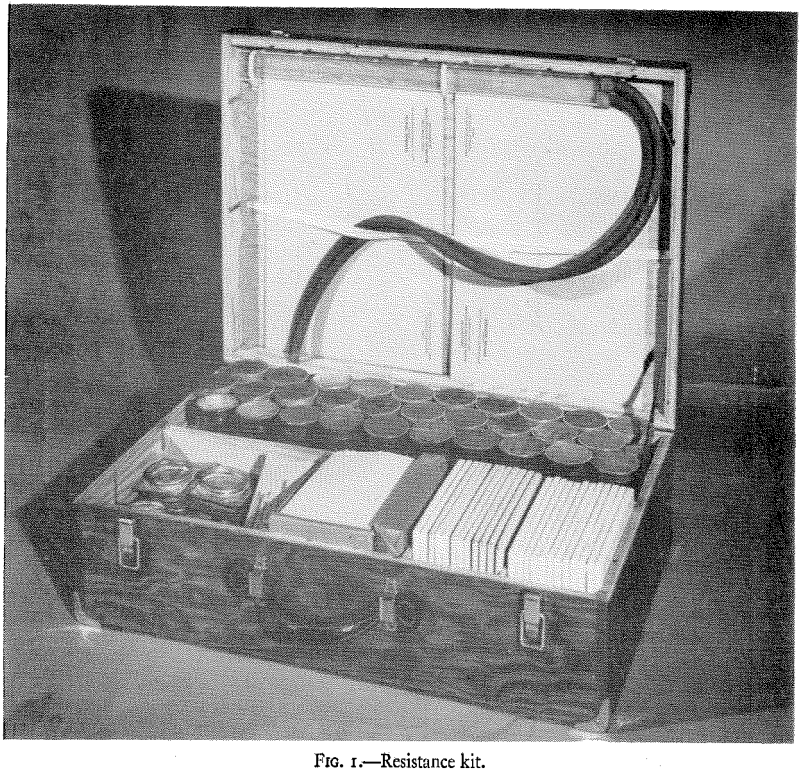

FIG. 1.—Resistance kit.

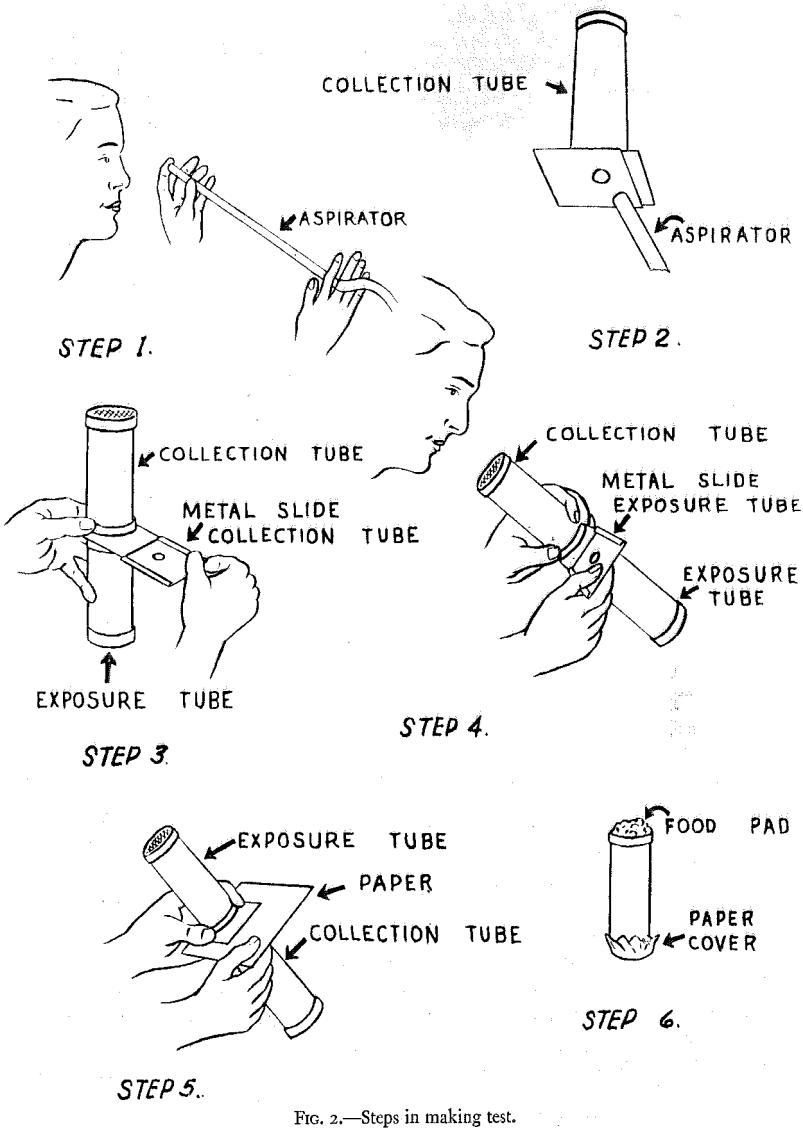

FIG. 2.—Steps in making test.

ever, these results should be verified as in step (c).

(c) Using the concentrations giving the desired mortalities from steps (a) or (b), run four additional replicates of each of these dosages, including a Risella-oil control with each test series. The average mortalities of the five replicates from step (c) + (a) or (b) are taken as the values for establishment of the base level of susceptibility. The lowest insecticidal concentration which showed complete mortality is chosen for the subsequent routine concentration in step (d). If the average

mortality of the five replicates at the highest concentration is at least 95 percent, this concentration may be used in step (d).

(d) After the establishment of the base level of susceptibility, five replicates at the routine concentration chosen in step (c) are run at suitable intervals to detect any change in the response of the field population. If complete mortality again is obtained, no change in susceptibility is indicated. If survivors occur, they may result from seasonal or environmental changes or may indicate physiological resistance. These possibilities are distinguished in step (e).

(e) Run two replicates at each of the concentrations used in step (c). If the results show (1) approximately the same mortalities for concentrations which gave less than 95 to 100 percent mortality in step (c), and (2) mortalities of less than 100 percent for concentrations which originally gave complete mortality, the change is indicative of physiological resistance in the mosquito population. This should be confirmed by a second series of replicates. If the results show a lower mortality for all test concentrations, the change is indicative of a seasonal or environmental variation in response. These types of response are illustrated in Figure 3.

DISCUSSION. The application of the test procedure to field conditions assumes that at least three test concentrations will be available for obtaining the baseline, one giving less than 50 percent mortality and one (or preferably two) giving complete mortality. However, with populations already resistant, such is not possible, e.g., with a strain of *Aedes aegypti* from Cucuta, Colombia, no mortality was obtained even with the maximum DDT

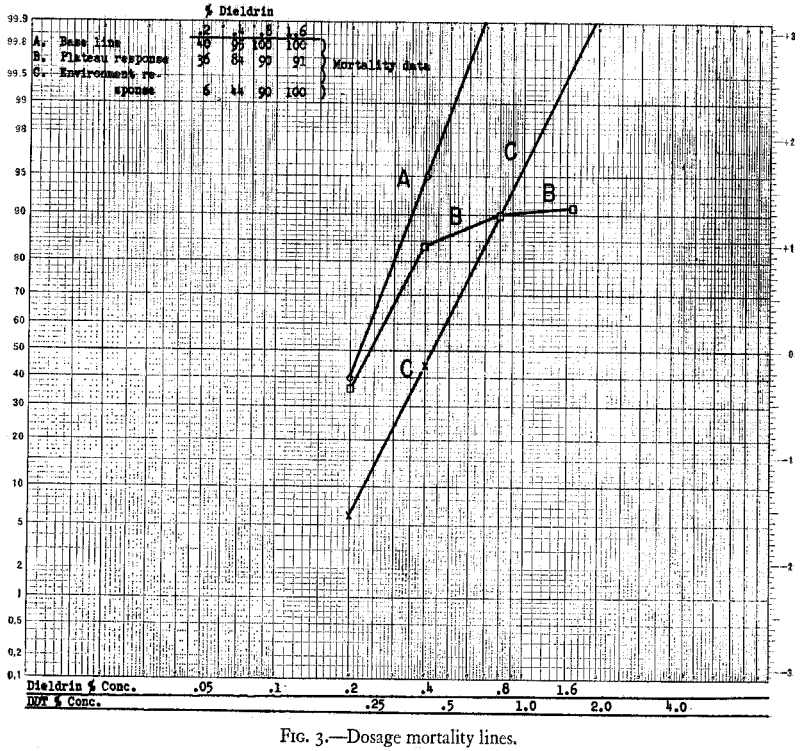

FIG. 3.—Dosage mortality lines.

concentration, thus indicating a high level of resistance to that toxicant. In tests with DDT-Risella oil papers against a local field strain of *Aedes taeniorhynchus*, the highest concentration (4 percent) gave 96, 98, and 99 percent 24-hour mortalities after 1-, 2-, and 4-hour exposures, respectively.

TABLE 1.—Twenty-four-hour mortalities[a] (percent) of adult female *Culex quinquefasciatus* with 90- and 150-minute exposures to dieldrin-Risella oil impregnated papers

Conc. dieldrin (percent)	Mortality (percent) with exposure of	
	90-min.	150-min.
0.1	2	0
0.2	2	14
0.4	16	25
0.8	33	53
1.6	45	42
3.2	33	44
4.0	29	61

[a] Mortality values represent the means of two replicates each.

Since complete mortality was not quite obtained even with the 4-hour exposure period, the investigator has to accept the possibility of a low proportion of resistant individuals in the population, and proceed on the basis of the survival of occasional individuals, and the use of 95 percent mortality as a recheck value.

The method also assumes that the mortality plotted in probit units on log-dosage graph paper will be essentially a straight line function. This assumption has been true with laboratory strains and with certain field strains. In other field strains, however, such as *Culex quinquefasciatus* (*fatigans*) (Table 1), there was a break in the mortality response to increased dosage which resulted in a plateau-type response. In a field strain of *Aedes taeniorhynchus* from Savannah, 4-hour exposure to 0.2, 0.4, 0.8, and 1.6 percent dieldrin-Risella oil papers gave 12, 33, 39, and 39 percent 24-hour mortalities, respectively. Here the mortality data indicate partially resistant populations, and a straight line response of probit mortality to log dosage no longer occurs.

The basic concept of a straight line relationship between probit mortality and log dosage assumes a homogeneous population in which the individuals differ from each other in their response in a continuous fashion. In an essentially completely susceptible strain, or in a highly selected resistant strain, this concept may still apply. All the individuals in laboratory strains are held under essentially equal conditions and tend to become uniform in their response to whatever selective factors are present. In field strains, however, the same selective factors are not exerted on the entire populations, and greater variation prevails. Marked differences were shown to dieldrin-Risella oil papers by a laboratory and a field strain of *Anopheles quadrimaculatus* (Table 2). The field strain is composed of a mixed population of approximately 75 percent susceptible and 25 percent resistant individuals. The susceptible portion of the field strain in this case is more susceptible than the laboratory strain. Between the susceptible and resistant individuals of a mixed population, there apparently is a decided break in the mortality response with an increase of exposure time. As shown in the mathematical model (Figure 4), the susceptible portion of the population may respond in a linear fashion following the line S, whereas the resistant portion of

TABLE 2.—Twenty-four-hour mortalities[a] (percent) of adult female *A. quadrimaculatus* of an insectary (Savannah) and of a field (Lynah) strain with 90-minute exposures to dieldrin-Risella oil impregnated papers

Conc. dieldrin (percent)	Mortality (percent)	
	Savannah	Lynah
.1	8	44
.2	0	64
.4	20	75
.8	100	78
1.6	100	80
3.2	—	87
4.0	—	94

[a] Mortality values represent the means of eight replicates each.

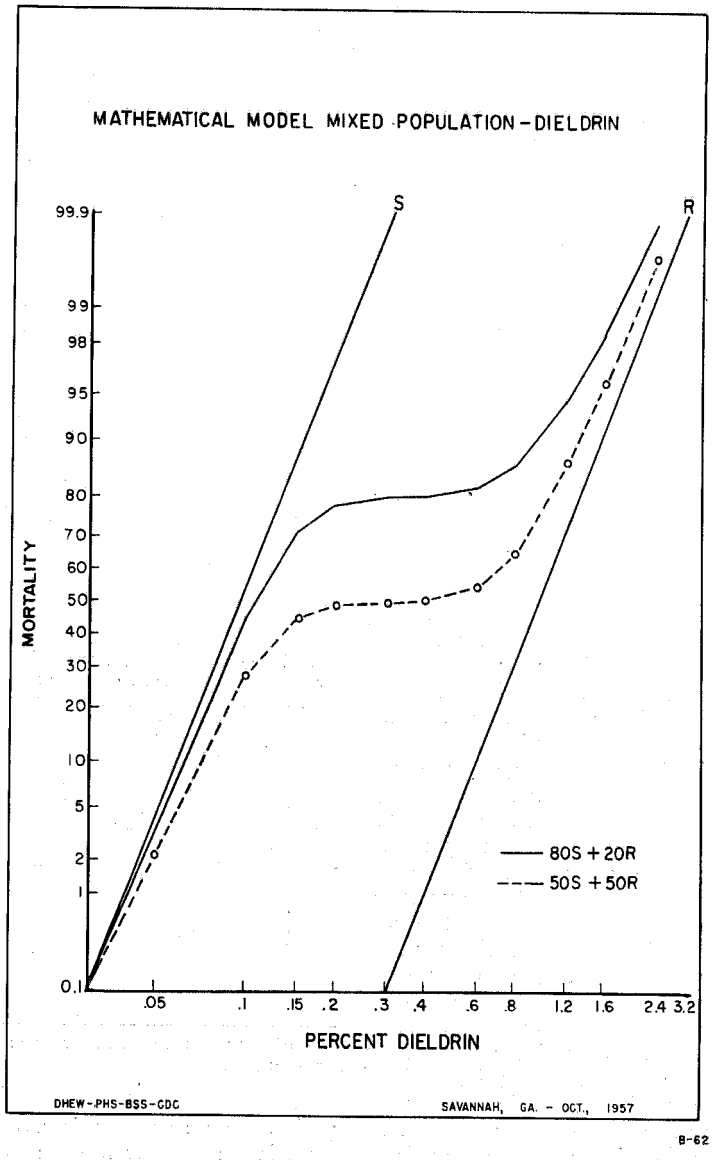

FIG. 4.—Mathematical model mixed population-dieldrin.

the population respond along the line R. Mixed populations of 80 percent susceptible and 20 percent resistant, or of 50 percent susceptible and 50 percent resistant, would follow the curved lines shown with a plateau effect occurring at approximately the proportion of the mixed populations. The plateau effect becomes more marked if the differences between susceptible and resistant individuals are greater.

This type of response was demonstrated in the laboratory using a mixture of a susceptible (S) and a dieldrin-resistant (C) strain of *A. quadrimaculatus*. Twenty-five adults removed from mixed populations containing known proportions of each strain were exposed to dieldrin-Risella oil test papers. The resultant values (Figure 5) show a plateau effect at approximately the expected levels with some irregularities which probably arose from the fact that the samples did not contain true proportions of the mixed population, or from the percentage (3–5) of susceptible individuals which existed in the resistant strain.

A field population prior to exposure to chemical measures may show a linear response of probit mortality to log dosage and still contain a small undetected segment of resistant individuals since only a small portion of the total adult population is being sampled. With the institution of control measures the susceptible population may be greatly reduced (particularly apparent if specimens are captured inside treated dwellings). Thus, the ratio of resistant to susceptible individuals in the population is increased and the resistant portion of the population now may be evident and cause the mortalities to fall below 100 percent. This drop in mortality levels indicates a definite resistance potential in the population.

The usefulness of this resistance detection method lies equally on the manner and accuracy followed in conducting the tests, and on the interpretation which is given the results. The method should not be expected to provide a consistent narrow range of mortalities, since even under controlled conditions the variation in mortalities of laboratory-reared specimens in replicated tests varies as much as 30 to 35 percent. Consequently, the average mortality of 4 to 5 tests is the assessment index to be considered.

Of the components in the kit, the impregnated papers theoretically offer the greatest chance of variability. However, extended studies of these papers indicate that:

(a) Preparation of the test residues by a single commercial source appears to be the best guarantee of uniformity. Papers prepared commercially at three different impregnation dates over a 1-year period gave comparable results.

(b) Under repeated tests with four individual 4 percent DDT papers, a series of fifty-five 60-minute exposures with each paper showed a mean mortality of 88 percent (range 67–100 percent). A series of thirty-three 90-minute exposures with each of four 0.8 percent dieldrin papers showed a mean mortality of 93 percent (range 62–100 percent). The current recommendation is that each paper be used about 20 times.

(c) Two papers each of 4 percent DDT-Risella oil and of 0.8 percent dieldrin-Risella oil which were tested weekly over a one-year period maintained their effectiveness. It is recommended that papers may be used for one year from the date of impregnation.

(d) The deposits of both toxicants remained stable under 80° F. storage for at least 1 year, but at 115° F., the DDT-treated papers showed some loss of effectiveness after 2 months of storage.

(e) The Risella-oil base itself does not have any measurable action on test mosquitoes and mortality is a function of the insecticide only.

From these data, it is apparent that preparation, storage, and usage in the time periods and condition specified have little effect upon the reliability of the impregnated papers.

In the interpretation of data, the investigator must rely on the general guide

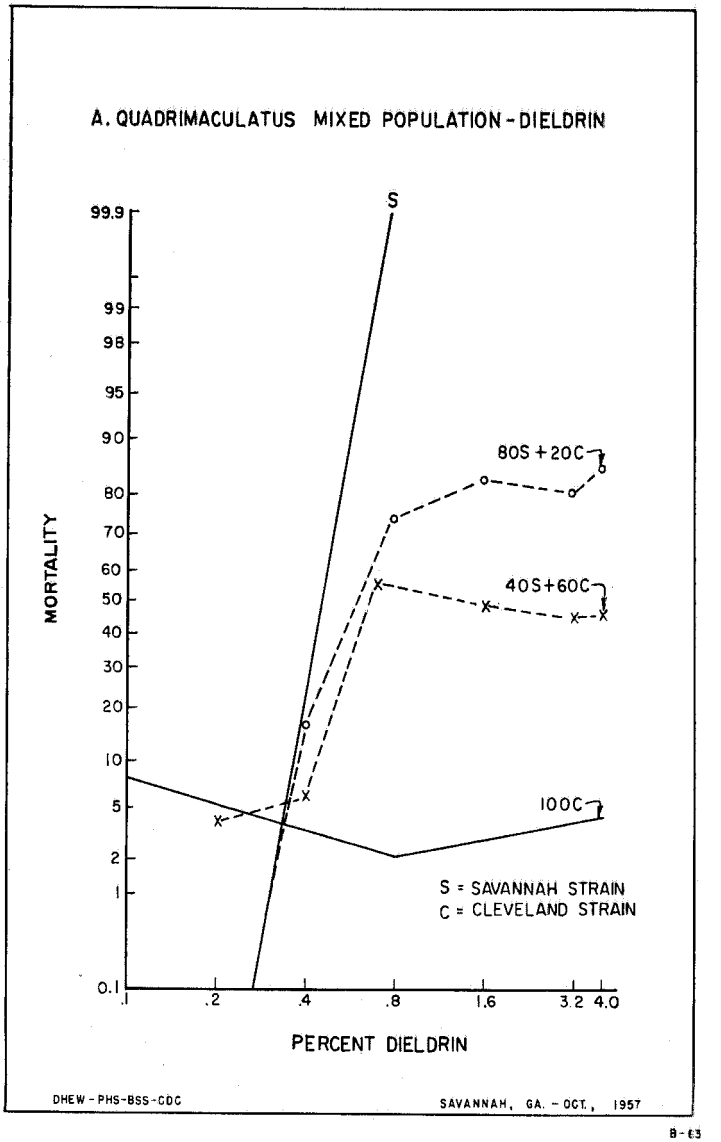

FIG. 5.—*A. quadrimaculatus* mixed population-dieldrin.

lines previously indicated. Completely susceptible or highly resistant strains are readily differentiated, but those falling between the extremes may sometimes be difficult to assess. The chief criterion for resistance concerns the appearance of a plateau response to an increase in insecticide concentration. Where such evidence appears, its relationship to the failure or success of the program must be interpreted by the field personnel familiar with the control area.

Thus, the kit data establish whether or not a vector population is developing physiologic resistance to the toxicant employed. The influence of such findings must be weighed against other factors (*i.e.*, mosquito indices in dwellings, parasite rates) to obtain an overall evaluation of resistance as to its effect upon the eradication program.

In addition to detecting resistance development in vector populations under treatment, the present method is of value in giving an insight into the potential of a vector population becoming resistant to a specific toxicant prior to the initiation of control operations. Thus, while the method is not designed and should not be used to compare the relative residual efficacy of compounds, it may be employed to detect the presence of a population segment which is resistant to certain toxicants but not to others. Based on the genetic shift in populations under selection, the chances of critical resistance development in a species would appear to be greater in a population containing the genes responsible for this characteristic than in those from which the genes were absent.

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