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STUDIES ON INSECTICIDE RESISTANCE IN MOSQUITOES[1]

W. MATHIS, H. F. SCHOOF, AND R. W. FAY

Recently the World Health Organization has prepared kits and described a field procedure for checking the possible occurrence of insecticide resistance in larvae or adults of various mosquitoes. The kits are available from the Division of Environmental Sanitation of WHO and adult kits will be furnished gratis unless demands are too high while the larvae kits will be available at a nominal cost.

The larvae test kits contain carefully prepared ethanol concentrates of DDT, dieldrin and BHC and permit rapid preparation of a series of test concentrations for the larval studies. The adult kits contain tubes for holding the mosquitoes prior to and after exposure to insecticide residues and tubes for use during the exposure period. The test residues of various concentrations of DDT or dieldrin are in the form of Risella-oil impregnated papers commercially prepared in Switzerland. All equipment needed for the tests is included in the kit which is about the size of a small suitcase.

With these kits a base line response of a given population of mosquitoes is established in the field. By means of subsequent routine tests any changes in the population response to the insecticide can be detected. With a base line properly obtained the investigator can eliminate or accept physiological resistance to the insecticide as a possible cause of control failures. In addition the base line and subsequent tests provide estimates as to the possibilities of insecticide resistance appearing in the operational program. The present paper discusses the type of results obtained with the adult testing kit in studies with laboratory and field populations of various mosquitoes.

Evaluations with laboratory strains of mosquitoes showed essentially straight line log dosage — probit mortality relationships. In tests with field strains however the straight line relationship was not always present, but rather S-shaped curves appeared with a central plateau in which there was not an increase in mortality with increases in dosage. Tests with the Savannah laboratory strain of *Anopheles quadrimaculatus* using impregnated papers with dieldrin concentrations of 0.1, 0.2, 0.4, 0.8 and 1.6 percent gave mortalities of 8, 0, 20, 100 and 100 percent respectively. These values plotted as a log dosage — probit mortality graph illustrate a straight line response with a very steep slope indicating a high degree of homogeneity in the laboratory strain. Comparable tests were made with the Lynah field strain of *A. quadrimaculatus* and dieldrin concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 4.0 percent gave mortalities of 44, 64, 75, 78, 80, 87 and 94 percent respectively. When the log dosage — probit mortality is plotted for these values an S-shaped curve is encountered in which a plateau response or essentially no increase in mortality occurred

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

between the values for 0.4 percent dieldrin and then the curve started to swing up again.

In proposing reasons for these different types of response, the differences between field populations and laboratory populations of mosquitoes must be considered. A mosquito population from an insectary colony reared by a standard technique tends to become homogeneous. This selection toward homogeneity occurs because all of the population, all of the individuals in the strain, are reared in the same conditions. When held free from any insecticide exposure the individuals are still selected by other factors in the rearing technique. When insecticides are used as a selection factor, each individual may be exposed to the same chance of contacting insecticides. In this way the next generation is produced by adults all of whom have had similar exposures. In field populations, however, such circumstances do not generally exist. Many individuals may be exposed to insecticides and undergo some selection, others may completely escape insecticide contacts, yet these two segments of a population combine in the field to produce the next generation. In this way mixed populations with regard to insecticide resistance may appear in the field.

The straight line log dosage — probit mortality relationship assumes populations in which the individuals making up the population differ by just small degrees from one another so that there is no break in the line of response to the insecticide. Insecticidal resistance is a characteristic however in which the resistant individuals may differ from the susceptible individuals by a degree of considerable magnitude. When part of a test population consists of resistant individuals, and part of susceptible individuals, there may be an area in the response curve of the population where all susceptible individuals are eliminated and then a break in response until the resistant individuals start to be affected.

The following mathematical model assumes a field population consisting of two segments, one susceptible, the other resistant. The susceptible segment shows the following straight line mortality response to dosages of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 percent concentrations, namely 4, 56, 97, 100 100, 100 and 100 percent mortalities respectively. The resistant segment likewise shows a straight line mortality response of 0, 0, 0, 1, 34, 93 and 100 percent mortalities respectively to the above concentrations. With a mixed population of 80 percent susceptible — 20 percent resistant individuals the response would be 4, 46, 78, 81, 87, 98 and 100 percent mortality for the above concentrations. In the interval between 0.2 and 0.8 percent concentration there is less mortality response and then the resistant segment of the population begins to be affected. This produces an S-shaped curve, the flattened area occurring essentially at the mortality level comparable to the percentage of susceptible individuals in the population.

In order to demonstrate this in the laboratory two strains of *A. quadrimaculatus* were used as a laboratory strain, susceptible to dieldrin

and a field strain in which 95 percent of the individuals showed a marked dieldrin resistance. With exposure to dieldrin concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 4.0 percent the susceptible strain gave 8, 0, 20, 100, 100, 100 and 100 percent mortalities and the resistant strain gave 4, 5, 4, 3, 4, 4, and 4 percent mortalities respectively. Mixtures of these two strains were made in a big cage and random samples were removed for resistance evaluations. With a strain mixture of 80 percent susceptible — 20 percent resistant the mortality values were 0, 0, 15, 79, 83, 80 and 85 percent respectively to the above concentration showing a plateau at the 80 percent level. With a strain mixture of 40 percent susceptible — 60 percent resistant the mortality values were 1, 7, 21, 51, 54, 45 and 46 percent respectively, showing a plateau response at the 50 percent level.

The resistance levels of field populations may be further complicated by dispersion patterns of a given species since the adults reproducing the next generation in a study area may represent individuals developed within the study area combined with adults migrating from areas within one flight range of the study area. As long as no control occurs, the adults migrating into the study area may not differ markedly from those leaving the study area. No control may be applied in the study area but if within one flight range insecticide selection is occurring on a population then the insects invading the study area may be different from those leaving the area. The result in the study area is a mixed population with eggs for the next generation coming from adults under no selection and from adults which have been selected.

In determining the base line for a field strain of *Culex fatigans* using 2½ hour exposures to concentrations of 0.2, 0.4, 0.8, 1.6, 3.2 and 4.0 percent dieldrin the following mortality values were obtained: 13, 25, 54, 44, 45 and 52 percent respectively. The presence of the plateau response at the 50 percent mortality level indicated that further selection with dieldrin would result in a highly resistant strain of the species. By increasing the exposure period to 6 hours and the dieldrin to 8 percent concentration 80 percent mortality was obtained, in the initial parent selection. Survivors were mated and the F_2, F_3 and F_4 generations have given mortalities of 44, 26 and 16 percent respectively using 6 hour exposures to the 8 percent dieldrin concentration.

In summary tests with a field kit for determining possible insecticide resistance in adult mosquitoes have resulted in two types of base lines (a) straight line log dosage — probit mortality values and (b) S-shaped curves for log dosage — probit mortality values. The latter type may have a plateau interval in which mortality does not increase with increased dosages. This may indicate mixed populations of susceptible and insecticide resistant individuals.