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CRITERIA AND MEANING OF TESTS WITH THE WHO ADULT MOSQUITO KITS<sup>1</sup>

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Use of the WHO test procedure for adult and larval anopheline mosquitos involves the assumption that a range of dosage and length of exposure that give low to complete kill of typical susceptible strains of a given species will provide a baseline to which the response of any other population may be compared. The tests are quite arbitrary in the sense that the possible range of dosage is dependent upon the length of exposure and the period thereafter when mortality is evaluated, and would be quite different if these conditions of the test were altered markedly.

In practical use, several difficulties have been encountered, among which may be mentioned:

- (a) No species in even the most natural conditions is invariable in its response and hence the baseline varies with many conditions such as season of the year, nutrition, period since a blood-meal, stage of egg development, age, etc. Conditions during a test will also affect the results, e.g., temperature, intensity of light or any other factor influencing activity of the test insects.
- (b) Populations under control measures, or any other selection process, often give response lines differing not only in position but in shape from the characteristic straight log dosage-probit (ld-p) lines of the reference

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population. A frequent situation is a marked bending towards higher dosages above a certain mortality. If the line becomes horizontal and continues so to the highest dosage used, it is obvious that only a portion of the total group can be killed by the procedure and the survivors may be called resistant. This situation is very common with dieldrin and offers no difficulty in interpretation.

With DDT the bending of the ld-p line usually is less pronounced and first noticed only at high mortalities. Since the location of points is less certain in this region because of the method of plotting, an ambiguity is almost certain to arise. A number of suggestions have been made to assist the experimenter who often works in primitive surroundings with only the kits furnished by WHO.

(a) In 1960 the following criteria were suggested: (i) no increase in  $LC_{100}$  indicates continued susceptibility; (ii) an increase in  $LC_{100}$  but no significant survival after one hour's contact with 4% DDT papers or 1.6% dieldrin papers is called "intermediate condition"; (iii) if at least half the individuals survive one hour's contact (as judged 24 hours later) with 4% DDT papers or 1.6% dieldrin papers, the strain is resistant. (WHO/Mal/266, Rev.1; WHO/Insecticides/117, 1960)

(b) These criteria were modified to allow for differences in response to DDT and dieldrin as follows.

<u>Category</u>	<u>Response to DDT</u>	<u>Response to dieldrin</u>
Susceptible	$LC_{100}$ close to normal baseline value (usually 4% x 1 hour) for the species; occasional survivor permitted.	As for DDT: for most species $LC_{100}$ is 0.4% x 1 hour.
Intermediate	Substantial portion but < 50% survive the baseline $LC_{100}$ . $LC_{50}$ usually close to baseline value.	As for DDT; but < 50% survive 1.6% x 1 hour.
Resistant	50% or more survive 4% x 1 hour (or whatever the $LC_{100}$ baseline is for the species).	50% or more survive 1.6% x 1 hour.

(Slightly abbreviated from WHO/Mal/325; WHO/Insecticides/129, 1961)

(c) Patel et al. (1961) suggest the following for A. culicifacies females:

- (i) greater than 75% mortality from one hour's exposure to 2% DDT papers indicates susceptibility;
- (ii) 20% to 75% mortality, moderate tolerance;
- (iii) less than 20% mortality, resistance.

(d) A slight variation applicable also to A. culicifacies was suggested by Samnotra (1961):

2% DDT paper	mortality > 50%	susceptible
2% DDT paper	mortality < 50%	intermediate
4% DDT paper	mortality < 50%	resistant

One hour exposure in all cases.

(e) Criteria referring particularly to A. culicifacies and A. annularis were adopted by the Indian Entomologists' Conference of the National Malaria Eradication Programme in 1961:

Tolerance - mortality less than 80% with 2% DDT papers but more than 50% with 4% DDT papers;

Resistance - less than 50% mortality with 4% DDT papers for 1 hour (Krishnamurthy, 1962).

(f) In Indonesia, when A. sundaicus or A. aconitus are not completely killed by 1 hour's exposure to 4% DDT or 1.6% dieldrin paper, the experiment is repeated using 2 or 4 hours' exposure (Muir, 1962). If mortality is then complete, the condition is called vigour tolerance; if 95% or less, it is resistance.

(g) The most elaborate criteria have been proposed by Davidson (1962). These depend upon the use of "discriminating dosages", i.e., certain concentrations of the DDT or dieldrin papers which he has found to eliminate more or less completely the susceptible, or the susceptible and intermediately

resistant members of a test group, in the latter case leaving only the most highly resistant. Since different concentrations or periods of exposure would be used with different species, this method in its full development is suited for determination of the complete make-up of a population rather than mere detection of resistance.

The use of so many criteria by different experimenters is proof that the results are often difficult to interpret. This arises in part from the nature of the ld-p line, which in theory can be straight only if the distribution of sensitivity within a tested group follows the normal distribution. Resistance to insecticides has been shown in an ever-increasing number of species to be genetically determined and the single factor operative in most cases gives rise to three genotypes whose differences in sensitivity are displayed in the corresponding phenotypes. If two or even three genotypes differing in sensitivity are present in a group of insects, the resulting ld-p line cannot be straight but must have inflections or horizontal regions corresponding to the percentages of the group contributed by each genotype.

In the case of female anopheline mosquitos, it has been shown by tests with the respective genotype that the three are well separated in their responses to dieldrin so that "discriminating dosages" can be used to (a) kill all susceptibles leaving hybrids plus resistant, and (b) kill susceptibles plus hybrids leaving only resistant. Thus the make-up of any group can be determined without ambiguity. Any inheritable property, in this case insecticide resistance, which behaves in this manner is often characterized as "dominant".

In contrast, among anophelines and many other insects as well, the susceptible and hybrid genotypes differ to a lesser extent and the ranges of response to the insecticide overlap. Hence, there can be no true discriminating dosage, though an approximate one that kills all the susceptibles but only a few of the hybrids is sometimes used. The term "recessive" resistance is often used to denote such cases of overlapping susceptibilities. In any test including the two genotypes, the ld-p line cannot be straight but must have an inflection corresponding to the

percentage of susceptibles present. The mortality caused by any given dosage will be less than that for the susceptible genotype only. The reduction in mortality is a function of the percentage of hybrids present but the relation is complex, and the effect varies with the dosage used and the slope of the ld-p line for each genotype.

The following calculations illustrate the response of a group consisting of 80% susceptibles ( $LC_{50} = 0.3\%$ ), 19% hybrids ( $LC_{50} = 3.0\%$ ) and 1% homozygous resistants (which are not affected within the dosage range). The slope of both ld-p lines through the experimental points is 3.0 (probits/log unit of dosage). These data are typical of A. culicifacies females. The mortality of each genotype is the product of the mortality at each dosage as shown by the ld-p line multiplied by the percentage of the genotype in the group. Total mortality is the sum.

(1) Dosage	(2) Mortality of ss	(3) x 0.80	(4) Mortality of sr**	(5) x 0.20	(6) Total mortality	(7) Columns (2)-(6)
0.1	8.0	6.4	0	0	6.4	1.6
0.2	30.0	24.0	0	0	24.0	6.0
0.3	50.0	40.0	0	0	40.0	10.0
0.5	75.0	60.0	2.0	0.4	60.4	14.6
0.7	86.5	68.8	5.0	1.0	69.8	16.7
1.0	94.0	75.2	12.0	2.4	77.6	16.4
2.0	99.0	79.2	38.5	7.7	86.9	12.1
3.0	100.0	80.0	59.0	11.8	91.8	8.2
4.0	100.0	80.0	72.5	14.5	94.5	5.5

\* ss = susceptible genotype

\*\* sr = hybrid genotype

It may be seen from column (7) that the largest decrease in mortality occurs with a dosage near 1%. However, the difference is still large at 2% which kills nearly all the susceptibles. This doubtless is the reason why several experimenters

have suggested the 2% dosage for distinguishing what has been called intermediate condition, moderate tolerance, and similar terms. If the test group contains a larger portion of the homozygous resistant genotype, all mortality will be reduced further, especially at the 4% dosage which for this reason is especially useful for detecting the presence of a high degree of resistance in an appreciable part (e.g., 10-15%) of a mosquito population.

The above discussion may be summarized by stating that the small decrease in mortality often found with 2% and 4% papers, which has sometimes occasioned much alarm among mosquito control officials, usually indicates only that several percentages of hybrids are present. In the case of dieldrin, this foreshadows a rapid increase in the proportion of uncontrollable individuals since the hybrids are almost totally immune to any practicable spraying. But with DDT, the hybrids are almost as easily killed and a long interval may pass before the appearance of sufficient homozygous resistant to affect control. If these have some biological disadvantage such as low fertility, short life, etc., there may never be a serious effect upon practical control. It may be noted that, whenever any genotype has such a handicap, the Hardy-Weinberg formula ( $ss + 2 sr + rr = 1$ ) is not valid and the composition of a population cannot be evaluated from the use of one discriminating dosage. It can be determined, however, from the location of inflections in the full ld-p line. The practice of expressing the status of mosquitos in a region at a certain time in terms of the genotype ratios is a step in the right direction, provided it is based upon adequate tests.

Recently the development of DDT resistance in A. culicifacies in the Panchmahals District of Gujerat State, India, has been examined critically by Luen & Shalaby (1962). The most interesting feature is that some 10 years' use of DDT preceded the finding of resistance among A. culicifacies females. This is not really surprising since intensive spraying was begun only in 1958 when the Malaria Eradication Programme was launched. Previously the small fraction of hybrids in the population escaped detection by the tests. Also, avoidance of treated surfaces or quick departure from them reduced the severity of selection.

A comprehensive survey (Hamon & Garrett-Jones, 1963) of the resistance situation for both dieldrin and DDT on the part of A. sacharovi, A. stephensi, A. culicifacies, A. sundaicus and A. gambiae in their respective regions has indicated that the gradually increasing intrusion of resistant individuals into the populations often is due to agricultural use of these insecticides. The process is of course quickened by intensive spraying in the Malaria Eradication Programme. It is unfortunate that in many malarious regions there has been little contact between agricultural and public health workers and programmes.

Some conclusions and recommendations.

1. The present adult kit and method of use are satisfactory but a 3% DDT paper would be useful to reinforce conclusions based upon results with the 2% paper.
2. A comparison of the effects of 1 and 2 hour exposure should be made to determine if the latter gives more information on the presence of hybrid and homozygous resistant genotypes.
3. Quantitative data on the effects of differences in temperature during and subsequent to exposure should be accumulated since this may have a several-fold effect upon the dosage required for a given percentage mortality, such as the  $LC_{50}$ .
4. More data are needed upon the seasonal variation in sensitivity of all genotypes in order to compare results obtained at different times of the year.
5. The sensitivity characteristics of the hybrid, especially the DDT-resistant one, should be determined for anopheline and other species not yet studied.
6. The resistant genotype should be studied for biological handicaps since such information will be of use in predicting the speed and extent of development of high resistance and its loss upon cessation of control.
7. Of greatest importance is quantitative information on the relation between the results with test kits and the effectiveness of practical spraying, with its complications due to irritability of mosquitos in the presence of spray deposits and the differing endo- and exophilic habits of the various species.

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