

WORLD HEALTH
ORGANIZATIONORGANISATION MONDIALE
DE LA SANTÉWHO/Mal/85 ✓
15 May 1953

ORIGINAL: ENGLISH

The Secretary of the Expert Committee on Malaria
has the honour to communicate hereunder
the following note:

RECENT EXPERIMENTS ON POSSIBLE RESISTANCE TO DDT
BY ANOPHELES ALBIMANUS IN PANAMA

by

Dr. Harold TRAPIDO
Gorgas Memorial Laboratory, Panama

The term "resistance" has been used in recent years to mean several different things. The chlorinated hydrocarbon residual insecticides have been shown to be of variable effectiveness against different species of anophelines, depending on the habits and behaviour and on the intrinsic susceptibility of these insects to the toxicants. In addition, one species may initially respond in different ways in different places, as in the case of Anopheles darlingi. In British Guiana this species is reported to have been virtually eradicated from a large coastal area by DDT residual house spraying alone (Giglioli, 1951), while in parts of Brazil this technique has been of very much more limited effectiveness (Pinotti, 1951).

In this communication the writer confines himself to the particular point of whether or not there is conclusive evidence that intensive use of DDT over a period of years has or has not brought about a change in the response of anophelines to this toxicant, with particular reference to Anopheles albimanus. Such changes (true acquired resistance) are now well known in the house fly and in several culicine mosquitoes. What evidence do we have that this has or is happening in anophelines?

One great difficulty in attempting to assess the situation has been the lack of a simple standard technique for measuring the MLD or LD₅₀ of the newer

insecticides against larval and adult stages of mosquitoes. There has been more work of this sort done with larvae than adults, probably at least in part since it is easier to arrange a continuous contact between larvae and toxicant in a liquid medium. The work of various authors, summarized in part by Trapido (1951) indicates that there exist significant differences in the initial susceptibility of various anophelines to DDT. While there may be hidden pitfalls in the method of exposing anopheline larvae to acetone or alcoholic suspensions of DDT in water, to establish MLDs, the problem of readily exposing workable numbers of adult anophelines to uniform deposits of DDT have been greater. The series of papers by Hadaway and Barlow have clearly illustrated the importance of such factors as the nature of the surface, the physical state of the toxicant, the size and form of the deposited crystals (or particles in the case of wetttable powders) in determining the mortality rate.

The writer has recently reported on a change observed in the effectiveness of DDT residual house spraying for the control of Anopheles albimanus at two experimental villages on the Chagres River in Panama (Trapido, 1952). One of these villages had been sprayed twenty times over a period of eight years and the other thirteen times in five years. Comparison of house catches following spraying in 1952 with those after the early sprayings in the period 1945 to 1947 demonstrated that there was no longer the drastic reduction in numbers of albimanus, as had been initially observed, nor did there appear to be a selective mortality of engorged albimanus. The large reduction in the proportion of albimanus which are successful in becoming engorged with blood was an effect that persisted. It was considered possible that this apparent change might be due to either acquired physiological resistance to DDT, or the selection of a population hyperirritable to DDT, or a combination of these factors. It was necessary at this point to measure the possible contribution of each of these factors in the laboratory. Since the publication of this paper, experiments to measure possible physiological resistance have been completed, and the results are given here.

A simple technique suitable for measuring the toxicity of DDT to adult anophelines, which provides for the contact of the insects with the toxicant in a

uniform and easily duplicated physical state was suggested to the writer by Dr. James Busvine of the London School of Hygiene and Tropical Medicine. This consists of dissolving the DDT in a nonvolatile vehicle such as clear mineral oil, diluting this with ether, and applying the resultant solution to filter paper disks. The ether evaporates off rapidly and the filter paper disks which are then uniformly impregnated with DDT in mineral oil may be used after ten or fifteen minutes. In practice, one ml of the DDT-mineral oil solution was diluted with three ml of ether and this dripped onto filter paper disks 125 mm in diameter, supported on a bed of pins. After permitting the ether to evaporate, a tangent cut three cm from the edge of filter paper was made, and the paper then rolled and inserted into shell vials 25 x 80 mm. Approximately thirty blood-engorged mosquitoes were then introduced into each paper-lined vial by means of an aspirating tube, the tube was withdrawn and the paper projecting over the edge of the shell vial crimped to imprison the mosquitoes in the paper-lined vial. Only the bottom of the vial, 25 mm in diameter, was an untreated surface, and it was found that the mosquitoes preferred to rest on the filter paper walls rather than the small glass surface at the bottom of the test vials. Following exposure the mosquitoes were released into clean holding cages, and the mortality subsequently determined at 24 hours. Various dilutions of pure p,p' DDT in mineral oil were tried in calibration runs, using a stock colony of Anopheles albimanus which had never been exposed to DDT. It was found that 0.5 per cent DDT with an exposure time of 12 minutes produced a kill approximating 90 per cent after 24 hours.

While this technique does not expose the mosquitoes to the sort of dry films they would experience in the field, it does assure that the same sort of insecticide contact is duplicated in all tests, and the consistency of the results obtained on different trials confirmed the validity of the test method. The space in which the mosquitoes are confined is small enough so that fairly uniform contact with the treated surfaces was maintained throughout the exposure period. This method is intended, of course, only to measure the ability of the mosquitoes to survive contact with the toxicant, not their ability to detect or avoid it.

Following this standard procedure, blood-engorged albimanus from four

sources were exposed. In all 2122 mosquitoes were used in these tests.

1. Stock Colony. - These albimanus are from the colony established at the Gorgas Memorial Laboratory in 1938, and have never had contact with DDT or any other chlorinated hydrocarbon insecticide.
2. Exposed Colony. - In 1948 the stock colony of albimanus was split and one portion was exposed to DDT as a dry deposit out of acetone solution for a period sufficient to produce a mortality of approximately 80 per cent. The progeny of the survivors were exposed in this manner through more than 70 generations until 1952.
3. Santa Rosa Strain. - The village of Santa Rosa had been sprayed 14 times with five per cent DDT in kerosene between 1947 and 1953, when engorged female albimanus from a horse-baited stable trap in the centre of the village were obtained for these tests.
4. Gatuncillo Strain. - Gatuncillo had been sprayed with the same toxicant 21 times between 1944 and 1953. For these tests albimanus taken in the same manner as those from Santa Rosa were used.

Exposure of blood engorged Anopheles albimanus to 0.5 per cent p,p' DDT
in mineral oil.

(Exposure period of 12 minutes)

| Source of Mosquitoes | No. of Replications | Per Cent Mortality After 24 hours | |
|----------------------|---------------------|-----------------------------------|---------------------------------|
| | | Mineral Oil (Control) | 0.5 Per Cent DDT in Mineral Oil |
| Stock | 12 | 5 | 92 |
| Exposed | 8 | 3 | 85 |
| Santa Rosa | 6 | 4 | 95 |
| Gatuncillo | 10 | 6 | 97 |

These results indicate clearly that despite the contact of the wild albimanus population with DDT over a period of years, these mosquitoes now have essentially

the same susceptibility to DDT as a laboratory colony with a history of no contact with DDT. Similarly the stock colony strain which had been exposed to DDT for more than 70 generations shows no significant decrease in susceptibility. It can only be concluded that any differences in the effectiveness of DDT residual house spraying for the control of albimanus must be due to a behaviour change, and not to any change in the intrinsic toxicity of DDT to this mosquito.

Literature Cited

GIGLIOLI, George

1951 Eradication of Anopheles darlingi from the inhabited areas of British Guiana by DDT residual spraying. J. Nat. Malaria Soc., 10:142-161.

PINOTTI, Mario

1951 The nation-wide malaria eradication programme in Brazil. J. Nat. Malaria Soc., 10:162-182.

TRAPIDO, Harold

1951 The toxicity of DDT to Anopheles claviger (Meigen) in Sardinia and on the Italian mainland. J. Nat. Malaria Soc., 10:266-271.

TRAPIDO, Harold

1952 Modified response of Anopheles albimanus to DDT residual house spraying in Panama. Amer. J. Trop. Med. & Hygiene, 1:853-861.