Notes

Some Effects of Dichlorvos in Huts on the Behaviour of Mosquitos in Southern Nigeria *

by M. BAR-ZEEV, *Project Leader, WHO Insecticide Testing Unit, Lagos, Nigeria* [a]

Experiments were carried out in a village in southern Nigeria for the purpose of determining whether dichlorvos (dimethyl 2,2-dichlorovinyl phosphate), impregnated in a flat, solid, plastic-type dispenser containing 20% active ingredient [b] has a repellent effect on naturally entering mosquitos in treated huts. The same type of dispenser had previously been tested by the WHO Insecticide Testing Unit (unpublished) and similar types by Gratz, Bracha & Carmichael [c] to determine their toxic effect on mosquitos. The present tests were carried out in ordinary African huts in a village near the laboratories of the WHO Insecticide Testing Unit, as well as in experimental huts, specially built for the purpose, in the same village.

Experimental and native huts

The two experimental huts were similar and located about 3 m apart. They were each 3 m square, with walls 1.6 m high. These walls were built of mud coated with a thin layer of cement on the outside and inside. The roof, made of corrugated iron sheet, tapered towards the middle where the height of the hut reached 2.9 m. The edge of the roof projected from the walls to a distance of 30 cm and this projection was painted black to prevent reflected light from passing into the hut through the eaves, which were 10 cm wide. On the east side was a window (70 cm × 70 cm) for placing a window-trap, with a wooden shutter. On the south side was a wooden door (1.5 m × 84 cm). In order to prevent ants or other insects from removing dead mosquitos from the floors, the floors were cemented and covered with heavy oil (about 2.5 cm deep). On top of this was placed a wooden floor standing on wooden strips (5 cm thick); the wooden floor was thus elevated about 5 cm from the cement floor. Its edge was about 2.5 cm from the walls of the hut, thus making a continuous gap which the ants could not cross. This method was found efficient; it was tested on several occasions by placing a number of dead mosquitos on this floor in the evening; they were all recovered intact the next morning.

The ordinary African huts, which were also used in these tests, are built of mud with a corrugated, galvanized-iron (" tin ") roof. A typical hut consists of a corridor with three rooms on each side. Rooms may have a ceiling of raffia-palm leaf mat, or raffia-palm mid-rib, or none at all. A full description of similar huts has been given by Gratz, Bracha & Carmichael.[c] One room in each of the two African huts (one treated, and the other untreated) was used in these tests.

Materials and methods

The tests were carried out in two stages. In the first, mosquitos were caught by suction tubes continuously from 11 p.m. to 6.30 a.m. as soon as observed in the treated and untreated huts. They were caught mostly when at rest on the walls but also when biting or attempting to bite. Collections were grouped at intervals of two-and-a-half hours, and the mosquitos classified as to species, and whether fed, unfed or gravid. Previous tests by the WHO Insecticide Testing Unit (unpublished) had shown that *Anopheles gambiae* and *Anopheles hargreavesi*, the two predominant anophelines in this area, usually begin to enter the huts of the villages, as well as to bite, after 11 p.m.

In the second stage, window-traps designed to catch out-going mosquitos were used. These traps consisted of Terylene netting on a 30-cm cubical wooden frame, the entrance side being drawn into a

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[a] Permanent address: Israel Institute for Biological Research, Ness-Ziona, Israel.

[b] Produced and supplied by Shell Chemical Company, New York.

[c] Gratz, N. G., Bracha, P. & Carmichael, A. (1963) *Bull. Wld Hlth Org.*, **29**, 251-270.

TABLE 1
NUMBER OF CULICINES ENTERING TREATED AND UNTREATED HUTS THROUGHOUT THE NIGHT

Time of collection	African huts [a]		Experimental huts [a]	
	Treated	Untreated	Treated	Untreated
11.00 p.m.	46	53	8	49
11.00-1.30 a.m.	100 (31)	145	109 (23)	148
1.30-4.00 a.m.	69 (27)	140	123 (25)	170
4.00-6.30 a.m.	56 (29)	132	77 (30)	125
Total dead and alive	358	470	395	492
Total dead and alive minus figures for 11.00 p.m.	312	417	387	443

[a] Numbers dead on the floor are shown in parentheses.

truncated cone with a 2.5 cm diameter hole at its apex, which was 2.5 cm from the opposite face of the trap. The traps represented the only source of light through which mosquitos will usually try to escape. The window-traps were replaced every two hours between 11 p.m. and 7 a.m. in order to determine the rate of egress of naturally entering mosquitos every two hours throughout the night from a treated and an untreated hut.

The first-stage tests were carried out alternately, one week in the experimental huts and the following week in the African huts, from June to the end of October, making a total of 13 tests in the African huts and 11 in the experimental huts. Those of the second stage were carried out in the experimental huts only, twice or three times a week from November to the middle of May, making a total of 61 tests.

Four men were engaged in these tests, two in the treated and two in the untreated huts. In the African huts, the two rooms used (one in the treated hut, the other in the untreated hut) were occupied only by the above-mentioned four men on the nights of testing; they entered the rooms a little before 11 p.m. The rest of the hut was usually occupied by the hut-owners, who generally went to sleep inside the hut by about 9 p.m. On the other non-testing nights these rooms were usually occupied by some of the hut-owners. The experimental huts were occupied only by the above-mentioned four men and only during the test period (11 p.m. to 7 a.m.).

In order to prevent any possible bias, the following procedures were used. The men in the treated and those in the untreated huts were interchanged in each test. In the experimental huts, as well as in the African huts, the treated and untreated huts were interchanged after each test in the first-stage experiments and once a week in the second-stage experiments, by removing the dispensers from the treated huts and placing them in the corresponding untreated huts. Thus, the huts which were treated in one test or for a week, as the case may be, were untreated in the following test or week, and *vice versa*. This was done in order to counteract any possible differences, especially in the African huts, between the treated and the untreated. Once a week, caged houseflies were exposed in the treated huts for eight hours. When the 24-hour mortality dropped to below 70% (houseflies are much more resistant to dichlorvos vapours than mosquitos), the old dispensers were replaced by new ones. This renewal of dispensers took place about every three weeks.

The dispensers were placed in the treated huts at a height of about 2.7 m and at the rate of one dispenser per 21 m^3, similar to the ratio used by Gratz, Bracha & Carmichael.[c] In the experimental huts, one dispenser was used, since the volume of the experimental hut was about 19.8 m^3.

First-stage experiments. At the time these tests were carried out (June-October), the number of *A. gambiae* and *A. hargreavesi* was unfortunately extremely low. The results of the first stage are, therefore, based only on culicines. Over 90% of these are a mixture of *Taeniorynchus (Mansonioides) africanus* and *Taeniorynchus (Mansonioides) uniformis*. The results are given in Table 1. Although the numbers of mosquitos collected at 11 p.m. are given in the table, this collection should not be considered in the final results, since some of the mosquitos that had entered before 11 p.m. might have

TABLE 2
NUMBER OF MOSQUITOS OBTAINED IN THE WINDOW-TRAPS OF EXPERIMENTAL HUTS AT VARIOUS PERIODS OF THE NIGHT

Time of removing window-traps	Treated hut [a]		Untreated hut [a]	
	A. gambiae	Culicines	*A. gambiae*	Culicines
1.00 a.m.	60 (11.5)	332 (11.1)	97 (16.6)	538 (12.5)
3.00 a.m.	182 (34.9)	730 (24.4)	234 (39.9)	1 036 (24.1)
5.00 a.m.	171 (32.8)	831 (27.8)	116 (19.8)	1 036 (24.1)
7.00 a.m.	88 (16.9)	611 (20.4)	97 (16.6)	1 095 (25.5)
Alive in hut after 7.00 a.m.	21 (4.0)	489 (16.3)	42 (7.2)	587 (13.7)
Dead on the floor after 7.00 a.m.	45	237	0	3
Total number in window traps	501	2 504	544	3 705
Total number alive	522	2 993	586	4 292
Total number alive and dead	567	3 230	586	4 295

[a] Percentage of total alive shown in parentheses.

been killed by that time in the huts treated with the insecticide. (Mosquitos killed before 11 p.m. were not counted since the floor sheets were laid down when the test started—namely, at 11 p.m.—and therefore the figures do not represent the true number of mosquitos entering before that time in the treated hut compared with the untreated.) It is interesting, however, to note (Table 1) that a relatively low number of culicines was found at 11 p.m. in both the treated and untreated huts, indicating that the great majority entered the huts after that time. It also indicates that most of the culicines which had entered the huts on the previous night did not remain there on the following night.

It can be seen from Table 1 that the number of culicines entering the treated African hut was 25% less than that in the untreated hut. Similarly, the number entering the treated experimental hut was 13% less than that in the untreated experimental hut. This indicates that while there may have been some deterrent effect it was, in any case, not a pronounced one.

Second-stage experiments. Since culicines were found to be entering the huts more or less regularly throughout the night, it would be expected that if dichlorvos vapour had a repellent effect, and if window-traps (to trap outgoing mosquitos) were changed at intervals throughout the night, more culicines would be found in the window-traps of the treated hut, taking into consideration, however, that in a treated hut there may be a total of about 13%-25% fewer culicines, owing to a possible deterrent effect as observed in previous tests (Table 1), as well as owing to mortality in a treated hut.

The second-stage experiments were carried out in the two experimental huts only (one treated, and the other untreated). The window-traps in these huts were the only area through which light could pass. None could pass through the eaves, owing to the projection of the roof, which, as already mentioned, was also painted black. In spite of this, a number of mosquitos may have escaped through the eaves. It was assumed, however, that the numbers in the window-traps reflected the exodus of mosquitos from the two huts. By the time the tests of the second stage started, at the beginning of November, the number of *A. gambiae* had increased substantially (but not that of *A. hargreavesi*), and the former species was therefore included in these tests. The results are given in Table 2. It can be seen that the number of culicines in the window-traps of the treated, as compared with the untreated, hut was lower (25%), corresponding to the generally lower number of culicines entering treated huts observed in the previous tests (Table 1) as well as to mortality (8%) in the treated hut.

These second-stage experiments, while supporting the evidence of the first-stage tests that dichlorvos had a moderate deterrent effect on the culicines seeking to enter, indicated that it had no repellent effect once they had in fact entered.

It is of interest to note, from the percentage of the total live culicines obtained in the window-traps at

TABLE 3
AVERAGE PERCENTAGES OF FED, UNFED AND GRAVID MOSQUITOS IN THE WINDOW-TRAPS

Mosquitos	Treated hut	Untreated hut
A. gambiae:		
Fed	19	19
Unfed	68	74
Gravid	13	7
Culicines:		
Fed	5	7
Unfed	74	74
Gravid	21	19

various times during the night (Table 2), that a lower proportion left the huts between 11 p.m. and 1 a.m. than at subsequent periods, and that during these later periods their egress was more or less regular. It is also of interest to note that the great majority of culicines (84% of the total alive in the treated and 86% of the total in the untreated) had already left the huts by 7 a.m.

As to *A. gambiae* obtained in the window-traps at various times during the night (Table 2), it can be seen that the total number (alive and dead) in the treated and in the untreated hut is quite similar. It can also be seen that the proportions of the total numbers alive in the window-traps at the various periods of the night are quite similar in the treated and untreated huts. It appears, therefore, that, as with culicines, dichlorvos at the dosage used did not exert any repellent effect on the *A. gambiae* that had entered the treated hut.

It is interesting to note that the lowest numbers of *A. gambiae* in the window-traps were obtained between the hours of 11 p.m. to 1 a.m. and 5 a.m. to 7 a.m., indicating that most of them left the huts between 3 a.m. and 5 a.m. Also, as in the case of culicines, the majority of *A. gambiae* (96% of the total alive in the treated and 93% in the untreated) left the huts before 7 a.m.

The percentages of fed, unfed and gravid mosquitos obtained in the window traps are given in Table 3. There is no difference in these percentages between the treated and untreated huts, indicating that the dichlorvos had no effect on the gonotrophic conditions of fed, unfed and gravid *A. gambiae* or culicines, nor therefore did it inhibit them from feeding indoors. It is an interesting fact that most of the culicines, as well as *A. gambiae*, obtained in the window-traps were unfed. These results are similar to those obtained in window-traps in an untreated village (control) when various insecticides were tested on a village scale.[d]

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[d] Bar-Zeev, M. & Bracha, P. (1965) *Bull. Wld Hlth Org.*, 33, 461-470.

A New Virus Infection of Mosquito Larvae

by JAROSLAV WEISER, *Laboratory of Insect Pathology, Institute of Entomology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia*

The probable existence of a lethal virus infection (polyhedrosis) in mosquitos was first reported in 1963 by the Californian investigators Kellen, Clark & Lindegren,[a] who observed the presence of tetragonal intranuclear inclusion bodies in moribund fourth-instar larvae of *Culex tarsalis* Coquillett. These investigators have since detected another virus, with a particle size of 175 mμ, but full details of this finding have not yet been published (Kellen, personal communication, 1965). A similar virus infection of mosquito larvae has recently been observed in Blatná, Bohemia, Czechoslovakia.

In the spring of 1964, studies of aedine mosquitos in south-western Bohemia led to the discovery of

[a] Kellen, W. R., Clark, T. B. & Lindegren, J. E. (1963) *J. Insect Path.*, 5, 98.