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ASSESSMENT OF THE TOXICITY TO ANOPHELES GAMBIAE¹
OF THE FUMIGANT INSECTICIDE DICHLORVOS (D.D.T.)

by

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The insecticide O,O-dimethyl O,2,2-dichlorovinyl-phosphate, known as dichlorvos, has been studied in experimental huts at Nagugu Out-station as part of the World Health Organization programme of testing and developing new insecticidal compounds. The mode of action of dichlorvos is novel compared with that of the residual contact insecticides, since it is brought into contact with mosquitos as a vapour that diffuses from a plastic dispenser. The studies have thus required several kinds of investigation to determine fumigant as well as incidental surface effects of the insecticide. The results presented in this paper are of trials made between February 1961 and May 1962.

METHODS

Three principal types of dispenser were used. In the first, produced on an experimental basis by Ciba Ltd. Basle, Switzerland, the insecticide in plasticiser solution is contained in a plastic tube and diffuses through the walls of the tube. Three individual modifications of this type were used, A1 with a discharge tube 10 cm x 1.5 cm diameter, B2 with a shorter tube (5 cm x 1.5 cm) and C3 with a long tube (10 cm x 1.5 cm) and containing a modified liquid composition of 70% dichlorvos, 20% epoxy stabilizers and 10% dibutyl phthalate. (Some physical determinations have been made on five other models of this dispenser resembling the C3 modification but differing in the quality of the polyvinyl chloride discharge tube, its wall thickness and the

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shape of its section.) The second and third types have the insecticide dissolved in solid matrices. The one, furnished by the United States Public Health Service Technical Development Laboratories, Savannah, Georgia, is a 200 gm-wax cylinder, 15 cm x 4 cm diameter, containing 25% dichlorvos, 56% Montan wax and 19% dibutyl phthalate. The other, produced by Shell Chemical Company, United States of America, is an annular cylinder 14 cm long x 3.5 cm containing 30% dichlorvos in polyvinyl chloride plus plasticizer. From these two the dichlorvos diffuses out of the solid solution from the walls of the cylinder.

The experimental huts have been described by Rapley (1961) the volume of air-space within the huts being approximately 500 cu. ft. One dispenser was hung in each hut in a central position and half-way between the eaves and the ridge of the roof. In one hut (No. 20) three Savannah dispensers were used so that the decrease in vapour concentration occurring with age of dispensers could be studied more fully.

The kills of naturally entering A. gambiae were calculated from the daily counts of dead mosquitos on the floor plus the numbers in the window traps that died within 24 hours. The catching techniques have been described by Hocking and others (1960). Over-all mortalities were assessed in experimental huts with roofs lined with sorptive mud as well as in huts with grass roofs, in view of the roof-resting habits of A. gambiae (Smith 1962).

Biological assessments were made of the distribution of fumigant effect of dichlorvos in huts by placing or suspending 22 small cages (3 inches by 3 inches by 6 inches) containing A. gambiae, in various places within the huts and in the window traps. A wind vane and an anemometer, near the huts, gave records of wind direction and speed during the fumigation tests. The secondary effect of residual toxicity by contact with surfaces of the huts was studied by suspending pieces of palm-mat, a foot square, and discs of sorptive mud, in four experimental huts at eave level, and removing them once a week to an insecticide-free building for bio-assay tests. The mosquitos used for fumigation and bio-assay tests were blood-fed A. gambiae caught in local houses.

The daily rate of loss of weight from the various dispensers, which was partly related to the loss of dichlorvos, was determined in the laboratory at Arusha, and in experimental huts. The air in the experimental huts was sampled at intervals to determine the concentration of vapour. This air was drawn through a pair of spined bubblers (Neal, E. & Perry, B. J., 1959), standing at the midpoint of the floor and joined in series by spherical joints, at 10 litres per minute using the pump and control equipment described by Johnstone, D. R. (1961). In the hut No. 20 experiment with three dispensers, air was sampled at two points viz. (a) middle of the floor, about one foot above the surface as above, and (b) approximately five feet above the centre of the floor and two feet below the dispensers. In order to minimize loss of vapour from solution in the 25 ml water placed in each bubbler these were cooled in ice water and carry-over was then restricted to about 20 per cent. The dichlorvos in solution was determined by the resorcinol method developed by Ciba Ltd, and some samples were also examined by the cholinesterase method of Michel, H. O. (1949).

The blood enzyme levels for the "sleepers" in huts containing dichlorvos dispensers, were checked over a period of six months. This was done by means of a portable blood-testing unit in which the pseudo-cholinesterase level is measured by the change in pH when blood is mixed with acetylcholine (shown by the colour of an indicator).

RESULTS

1. Over-all mortalities in naturally entering mosquitos

The monthly over-all mortalities produced in naturally entering A. gambiae are summarized in Table 1. In huts with grass roofs, all dispensers gave satisfactorily high kills in the first month, but only the two Ciba dispensers with long discharge tubes (A1 and C3) gave high kills during the second month of operation. There was no indication that the type of wall surface alone - plywood, non-sorptive or sorptive mud - influenced the kills in the huts with grass roofs. Over-all mortalities were however lower in huts with roofs lined with sorptive mud, and only the Savannah type

gave a satisfactorily high kill during the first month. The lower over-all mortalities caused by the Ciba dispensers during the first month of operation, compared with the Shell and Savannah types, was explained by the chemical findings. These showed that the Ciba dispensers did not become fully operative until after they had been opened for a week, whereas the others were immediately operative after removal of their plastic covers.

Table 2 shows the weekly over-all mortalities given by different types of dichlorvos dispenser in huts with grass and mud-lined roofs. During the first week the three types of Ciba dispenser gave lower mortalities than the Savannah and Shell types. High floor counts in huts with grass roofs showed that the greater proportion of mosquitos entering the huts died before leaving, whereas in huts with mud-lined roofs high window trap mortalities and low floor counts showed that, even at similar over-all mortalities, most mosquitos died after entering the window trap.

2. Fumigation tests

Average mortalities of 31% and 37% were obtained from fumigation tests in three grass-roofed and three mud-roofed huts, indicating that the vapour toxicities were similar in both types of hut. The results of 16 fumigation tests are summarized in Table 3 and show that there was a great variation in kill in different parts of the hut. The highest mortality was inflicted level with the dispenser and the lowest kill was in the window trap during the first month. In the second month of operation the lowest kill was at floor level thereby showing that dichlorvos vapour was not reaching the floor in more than very small concentrations.

The kill in the huts appeared to be influenced by the strength of the wind. The mean mortalities of several tests suggested that high mortalities were inflicted when there was a gentle wind outdoors (see Table 4.). Similarly, the results of certain tests indicated that wind direction affected the mortality in different parts of the hut, but the general picture was of highest mortalities in the section of the hut diametrically opposite the doorway.

3. Bio-assays on different types of surface

The average mortalities from bio-assays in four huts containing dichlorvos dispensers, are summarized in Table 5. The results show that the palm-mat and mud plaques acquired toxicity and that there was thus a residual contact effect in a hut containing a dichlorvos dispenser. The results also indicate that the matting was more toxic than the mud plaques, thereby suggesting that in the huts with a low atmospheric concentration of dichlorvos the mud plaques were able to absorb and degrade the insecticide, thus reducing its activity.

4. The daily losses in weight (L_t gm) of the various dispensers are expressed by the relationships:

Ciba A1	$\log_{10} L_t = 1.603 - 1.586 \log_{10} t$	where $t = 15-100$ days
Ciba B2	$L_t = 0.234 - 0.112 \log_{10} t$	where $t = 10- 80$ days
	or $\log_{10} L_t = 0.216 - 0.973 \log_{10} t$	where $t = 20-150$ days
Ciba C3	$L_t = 0.655 - 0.340 \log_{10} t$	where $t = 10- 70$ days
	or $\log_{10} L_t = 0.941 - 1.221 \log_{10} t$	where $t = 20-150$ days
Ciba IV	$L_t = 0.706 - 0.402 \log_{10} t$	where $t = 10- 40$ days
	or $\log_{10} L_t = 0.514 - 0.940 \log_{10} t$	where $t = 10-100$ days
Ciba V	$L_t = 0.749 - 0.425 \log_{10} t$	where $t = 10- 40$ days
	or $\log_{10} L_t = 0.628 - 1.048 \log_{10} t$	where $t = 10-100$ days
Ciba VI	$L_t = 0.745 - 0.425 \log_{10} t$	where $t = 10- 40$ days
	or $\log_{10} L_t = 0.687 - 1.084 \log_{10} t$	where $t = 10-100$ days
Ciba VII	$L_t = 0.625 - 0.372 \log_{10} t$	where $t = 10- 35$ days
	or $\log_{10} L_t = 0.247 - 0.878 \log_{10} t$	where $t = 10-100$ days
Ciba VIII	$L_t = 0.677 - 0.410 \log_{10} t$	where $t = 10- 35$ days
	or $\log_{10} L_t = 0.231 - 0.856 \log_{10} t$	where $t = 10-100$ days
Savannah	$L_t = 0.210 - 0.100 \log_{10} t$	where $t = 1-100$ days
Shell	$\log_{10} L_t = 0.547 - 0.381 \log_{10} t$	where $t = 1-150$ days

Table 6 shows some arbitrarily selected experimental figures for the daily loss in weight of dispensers compared with those calculated from the above equations, which were derived from the best fit line to the complete data. During the early life of the dispenser the log time against loss in weight gives the more satisfactory fit but later the log time x log weight loss is the better.

All dispensers are sensitive to marked changes in temperature and humidity, the solid Savannah and Shell types being more so than the Ciba. In higher temperatures and drier atmospheres greater losses were observed than on cool, humid days, possibly because moisture was absorbed by the dispenser due to highly hygroscopic dimethyl phosphate which is a breakdown product of dichlorvos. The Shell dispenser particularly shows wide scatter about the above line.

5. The vapour concentrations found in the various huts are given in the tables and those from hut No. 20 (three dispensers) can be expressed by $\log_{10} C_t = -0.703 - 0.549 \log_{10} t$ where C_t is the concentration after t days, in μgm dichlorvos per litre of air. Experimental data as compared with the concentration in air calculated from this relation are given in Table 7.

DISCUSSION

The studies have shown that the fumigant insecticide dichlorvos is toxic to A. gambiae entering experimental huts. A satisfactory mortality of over 75% was not maintained for more than two months in grass-roofed huts with a single dispenser or with three dispensers, and average mortalities never exceeded 75% after the first month in huts with mud-lined roofs. Chemical findings from the hut with three Savannah dispensers showed that there was a great initial loss of dichlorvos from these dispensers, followed by low concentrations in the hut for a long period but not sufficiently toxic to maintain high mortalities after two months. If the weekly over-all percentage mortalities of free entering mosquitos are plotted on log/probit paper against the weekly mean vapour concentration as obtained from the relationship in paragraph 5 above, (see Table 8) an approximately linear dose/response curve is obtained with LC_{50} at 0.02 $\mu\text{gm/litre}$ and LC_{95} at 0.04 $\mu\text{gm/litre}$. (LC denotes

lethal concentration of dichlorvos in the air of the hut for 50% and 95% over-all mortalities.) A significant feature of this line is its low slope, which indicates that small variations in the vapour concentration cause large differences in mosquito mortality.

In other hut experiments it has not been possible to devise similar relationships between concentration and mortality, although it is possible to state that the higher ranges of over-all mortalities occur between limits of 0.03 and 0.02 $\mu\text{gm/litre}$ air, and the lower ranges of over-all mortalities between 0.02 and 0.01 $\mu\text{gm/litre}$ air. The relationship between vapour concentration and overall mortality is not a simple linear one in the higher ranges of over-all mortality; in the lower ranges the very small changes in concentration of insecticide that account for the differences in over-all mortalities were not detected by the limited amount of chemical sampling performed.

The mean daily weight losses for Ciba-type dispensers placed in the experimental huts follow the same curve as that determined more sensitively in Arusha laboratory (see paragraph 4 above). It has not been possible however to relate these weight losses to the vapour concentrations determined, again probably because of insufficient data. It has been possible to derive from plots of log daily loss against probit over-all mortalities (Table 2) the values of LL_{50} and LL_{95} (lethal daily weight loss for 50% and 95% mortalities). From the mean daily weight losses shown for huts 5 and 20 in Table 9 it will be seen that the rates of dispensing for the two huts are similar, yet the LL_{50} and LL_{95} for hut 5 (about 60 and 200 mgm/day respectively) with the mud roof are higher than the corresponding figures of about 40 and 100 mgm/day for hut 20 with the grass roof. With other pairs of huts containing the other types of dispenser similar results were obtained and the over-all mortalities in the huts with mud-lined roofs were some 10% lower than in huts with grass roofs. The results of bio-assay tests suggest that the lower over-all mortalities in huts with mud roofs may be in part due to some sorption of the insecticide by the mud surface. A possible explanation of the higher proportion of dead mosquitos in the window traps of huts with mud-lined roofs than in huts with grass roofs, was that mosquito behaviour was affected by dichlorvos. The results of studies on this subject confirm the suggestion and are described in a separate paper.

The mathematical relationship for the daily rate of loss of dichlorvos from the dispensers given in paragraph 4 above can be used, when related to a satisfactory level of over-all mosquito mortality, to predict the useful life of new dispensers under the same conditions. Seventy-five per cent. over-all mortality can be regarded as such a satisfactory level and from the experiment using huts 5 and 20 a value for the LL_{75} of about 70 mgm/day can be derived. The predicted lives for various dispensers are tabulated in Table 10, together with the experimental values abstracted from Table 2. The agreement is sufficiently close to give confidence in the predicted values for the five dispensers Ciba IV-VIII.

The results of fumigation tests show that the dichlorvos vapour is unevenly distributed in a hut. It would seem that at low wind speeds the vapour is less stratified than when it is calm, but is in still sufficient concentrations to inflict high mortalities. At higher wind speeds the over-all concentration of the vapour was possibly reduced below lethal concentrations in a larger section of the hut. The results of fumigation tests also show that exposure periods of two hours give mortalities which are fair indication of the magnitude of over-all mortalities in an experimental hut, and indeed the values of LL_{50} and LL_{95} from hut 20 mentioned above are similar to corresponding figures derived from fumigation test data.

No inimicable effects of the dichlorvos vapour on the "sleepers" in the huts were detected, as indicated by blood pseudo-cholinesterase levels.

Finally, it is concluded that the lethal action of dichlorvos vapour is complicated by the dual effect of fumigant and contact actions, both of which are imperfectly understood. The fumigant effect varies in different parts of the hut; and to what extent adsorption, absorption and degradation affect the toxicity of the different surfaces is unknown. Since even in the presence of three dispensers, over-all mortalities fell to a low level by the third month, it follows that only when there is an improvement in the technique of dispensing the insecticide will a longer fumigant effect be practicable.

SUMMARY

1. The toxicity of the insecticide O,O-dimethyl O.2,2-dichlorovinyl phosphate, known as dichlorvos (DDVP) was assessed by means of field trials with experimental huts.
2. Different types of dispenser, produced on an experimental basis by different organizations, maintained satisfactorily high over-all mortalities of 75% or over for one or two months. Over-all mortalities were about 10% higher in the huts with grass roofs than in huts with roofs lined with mud.
3. Weekly over-all percentage mortalities plotted on log/probit paper against weekly mean vapour concentration gave an LC_{50} at 0.02 $\mu\text{gm/litre}$ and an LC_{95} at 0.04 $\mu\text{gm/litre}$.
4. The results of fumigation tests and of chemical determinations of vapour concentration indicated that vapour toxicity was similar in huts with grass and mud-lined roofs. The results of bio-assay tests showed that there was a secondary residual contact effect, and indicated that the lower over-all mortalities in huts with mud roofs might be in part due to some sorption and degradation of the insecticide by the mud surface.
5. No inimicable effects of the dichlorvos vapour on the "sleepers" in the huts were detected, as indicated by blood pseudo-cholinesterase levels.

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TABLE 1. OVER-ALL MORTALITIES IN A. GAMBIAE
 ENTERING EXPERIMENTAL HUTS CONTAINING
 DICHLORVOS DISPENSERS

Type of dispenser	No. of huts examined	Per cent. 24-hour mortality with, in parenthesis, the number of <u>A. gambiae</u> caught		
		Months after treatment		
		0-1	1-2	2-3
<u>Single dispenser</u>			<u>Grass roofs</u>	
Ciba (A1)	4	72 (2312)	77 (1619)	53 (657)
Ciba (B2)	4	74 (785)	62 (554)	-
Ciba (C3)	1	83 (341)	72 (352)	-
Savannah	3	85 (1328)	39 (1437)	-
Shell	1	85 (732)	41 (1419)	-
Control	2	27 (801)	13 (1311)	9 (161)
			<u>Mud roofs</u>	
Ciba (A1)	2	62 (803)	53 (898)	-
Ciba (C3)	1	66 (2036)	56 (3319)	-
Savannah	1	75 (1077)	40 (1509)	-
Control	1	23 (450)	16 (1002)	-
<u>Three dispensers</u>			<u>Grass roofs</u>	
Savannah	1	93 (204)	84 (139)	55 (93)

TABLE 3. THE EFFECT OF THE SITE OF CAGED MOSQUITOS
 IN EXPERIMENTAL HUTS ON KILLS INFLICTED BY DICHLORVOS

Site of caged mosquitos	Per cent. mortalities	
	Months	
	1	2
Ridge level	54	27
Level with dispenser	93	53
Eaves	71	41
Just below window trap level	80	40
Floor	80	22
In window trap	52	28

TABLE 4. MEAN MORTALITIES IN FUMIGATION TESTS AT DIFFERENT
 RANGES OF WIND SPEED OUTDOORS

Per cent. mortalities after 2 hours exposure			
Calm	Up to four feet/second	Over 4 feet to 5 feet/second	Over 5 feet/ second
95	69	27	54
58	89	15	68
69	83	50	29
10	73	14	54
		44	
		80	
		73	
Average 58	78	43	51

TABLE 5. PER CENT. MORTALITIES IN BIO-ASSAYS ON PIECES OF PALM MAT AND MUD PLAQUES PREVIOUSLY SUSPENDED IN HUTS WITH DICHLORVOS DISPENSERS

	Age of dispensers in days					
	18	24	32	42	51	57
<u>Palm mats</u>						
Huts with dispensers						
Exp. time in hours	2	2	2	2	4	4
Per cent. mortality	73	76	79	19	40	61
<u>Control</u>						
Exp. time in hours	2	2	2	2	4	4
Per cent. mortality	9	0	0	0	0	0
<u>Mud plaques</u>						
Huts with dispensers						
Exp. time in hours	2	2	2	2	4	4
Per cent. mortality	55	29	54	18	19	19
<u>Control</u>						
Exp. time in hours	2	2	2	2	4	4
Per cent. mortality	0	16	0	0	0	0

TABLE 6. EXPERIMENTAL AND CALCULATED DAILY LOSS IN WEIGHT OF DISPENSERS

Dis- penser type	Time after opening days									
	1	10	15	20	30	40	60	80	100	150
Ciba A1										
Exp.obs.	-	-	576	199	195	142	63	38	27	-
Calc.(ii)	-	1040	547	347	183	115	61	39	27	-
B2:										
Exp.obs.	-	111	112	85	65	37	34	42	17	.11
Calc.(i)	-	122	103	88	69	55	35	21	-	-
Calc.(ii)	-	-	-	90	60	45	31	23	19	.11
C3:										
Exp.obs.	-	212,182	221,217	214,205	161,160	113,127	49,48	37,38	32,34	36
Calc.(i)	-	315	255	213	153	111	51	10	-	-
Calc.(ii)	-	-	-	225	137	97	59	41	32	19
IV:										
Exp.obs.	-	280	260	190	80	96	89	56	21	-
Calc.(i)	-	304	233	183	112	62	-	-	-	-
Calc.(ii)	-	375	256	194	134	102	70	53	43	-
V:										
Exp.obs.	-	225	248	185	85	105	68	52	23	-
Calc.(i)	-	324	249	196	121	68	-	-	-	-
Calc.(ii)	-	380	249	185	120	89	58	43	34	-
VI:										
Exp.obs.	-	231	261	198	86	110	69	51	23	-
Calc.(i)	-	320	245	192	117	64	-	-	-	-
Calc.(ii)	-	404	259	189	122	89	58	42	33	-
VII:										
Exp.obs.	-	184	196	135	48	66	59	51	23	-
Calc.(i)	-	253	188	142	76	29	-	-	-	-
Calc.(ii)	-	234	164	127	89	69	49	38	31	-
VIII:										
Exp.obs.	-	185	201	137	52	74	64	50	23	-
Calc.(i)	-	267	195	144	71	20	-	-	-	-
Calc.(ii)	-	237	168	131	93	73	51	40	33	-
Savannah:										
Exp.obs.	150	110	80	60	90	40	36	30	30	15
Calc.(i)	210	110	92	80	62	40	32	20	10	0
Shell:										
Exp.obs.	548	111	-	70	78	89	30	96	130	53
Calc.(ii)	352	118	-	91	78	70	60	53	49	42

The calculated losses in weight denoted by (i) are obtained from log t x loss relationship and those denoted by (ii) from log t x log loss equations.

TABLE 7. MEAN CONCENTRATION OF DICHLORVOS IN THE ATMOSPHERE OF HUT 20 CONTAINING THREE SAVANNAH DISPENSERS

Days after starting	Expected determination concentration $\mu\text{gm/litre}$	Concentration in $\mu\text{gm/litre}$ calculated from equation
2	0.127	0.132
7	0.082	0.068
14	0.056	0.047
22	0.045	0.036
32	0.023	0.030
51	0.022	0.023
58	0.019	0.021
72	0.020	0.019
86	0.019	0.017
113	0.010	0.015

TABLE 8. OVER-ALL % MORTALITY IN HUT 20
CONTAINING THREE SAVANNAH DISPENSERS AND THE INTERPOLATED DICHLORVOS
CONCENTRATION IN THE AIR DERIVED FROM DATA IN TABLE 7.

Weeks after start	% Mortality	Interpolated vapour concentration at mid-week, $\mu\text{gm/litre}$
1	98	0.100
2	86	0.054
3	100	0.041
4	87	0.034
5	70	0.030
6	95	0.027
7	89	0.024
8	84	0.022
9	62	0.021
10	66	0.020
11	35	0.019
12	30	0.018
13	36	0.017
14	9	0.0165
15	44	0.016

TABLE 9. CONCENTRATION OF DICHLORVOS VAPOUR AND MEAN DAILY LOSS IN WEIGHT OF DISPENSERS COMPARED WITH OVER-ALL MORTALITIES OF NATURALLY ENTERING MOSQUITOS IN HUTS AND MORTALITIES IN FUMIGATION TESTS

Date of observation	Hut type	Dispenser type	Age of dispenser in days	Dichlorvos $\mu\text{gm/litre}$ air	Mean daily loss in weight	Fumigation tests		Over-all mort. % in huts on days of obs.
						% mort.	Exposure in hours	
4.12.61	Grass/sorptive	C3	7	0.010	-			100
13.12.61			16	0.014	0.107			100
21.12.61			24	0.011	0.078			76
10. 1.62			44	0.013	0.072	44	2	59
22. 1.62			56	0.011	0.056	10	2	81
4.12.61	Sorptive/sorptive	C3	7	0.021				83
13.12.61			16	0.015	0.102			100
21.12.61			24	0.016	0.090			90
10. 1.62			44	0.021	0.070			39
22. 1.62			56	0.015	0.067	73	2	95
6.12.61	Sorptive/sorptive	S	9	0.021				94
15.12.61			18	0.009				64
3. 1.62			37	0.017			4	0
16. 1.62			50	0.013				31
24. 1.62			58	0.012				88
6.12.61	Grass/sorptive	S	9	0.029				93
15.12.61			18	0.016				49
3. 1.62			37	0.029				77
24. 1.62			58	0.014			80	2

C3 denotes Ciba C3 type

S denotes Savannah type

TABLE 10. THE USEFUL LIVES OF DISPENSERS PREDICTED AND OBSERVED

Dispenser type	Predicted life	Experimental life to 75% mortality in grass-roofed huts
CIBA A1	55	50
CIBA B2	25-30	40
CIBA C3	50	50
SAVANNAH	25	25
SHELL	40	30
CIBA IV	40-60	
CIBA V	40-50	
CIBA VI	40-50	
CIBA VII	30-40	
CIBA VIII	30-40	

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