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KINETIC RESPONSE OF MOSQUITOS TO CHEMICALS

by

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1. INTRODUCTION

Kennedy (1947) made early observations on the excitant and repellent effects of sublethal contacts with DDT on adult mosquitos. The term "excito-repellency" to describe this aspect of the action of DDT gained some currency, but recently the more general term "irritability" has been used (de Zulueta 1959). Kennedy's experiment consisted in the exposure of groups of mosquitos to papers treated with acetone solutions of DDT and to untreated papers. They were confined in a glass box one wall of which was lined with treated paper. Their behaviour was recorded over a series of time intervals, in terms of the number settled on the paper at the end of each period, and the number of departures from the paper during each period. The mean duration of the resting period under each set of conditions was thus estimated.

Recently a rather similar arrangement has been used in the World Health Organization "Provisional instructions for determining the irritability of adult mosquitos to insecticides" (WHO 1959). In the first part of this test single mosquitos are held in a plastic exposure chamber over impregnated paper and, after a settling period, the time to take-off is measured. The second part consists of counting the number of take-offs by groups of mosquitos similarly confined over a 15-minute period, again after an initial settling period.

The results of trials of this type quite clearly show that certain insecticides, especially DDT, do have a pronounced effect on mosquito activity, increasing it markedly over that shown by untreated controls. This can be observed in the course of susceptibility tests by the standard WHO method, the activity in the

tubes with DDT contrasting with the peaceful behaviour of the controls. In some species strains showing hyperactivity in their response to DDT as compared with normal strains have been reported (Brown 1958), having apparently developed as a result of selection by field use of the insecticide. This phenomenon has been named behaviouristic resistance.[1]

In both the irritability test and the susceptibility test, however, there is the common factor of forced contact, and the numerical response is measured in terms of mean duration of resting on the treated surface. In the field, the factor governing survival or disease is rather the duration of flight and the distance travelled than the frequency of take-off, and the present experiments were designed to study this more directly. In the course of them it was hoped to throw some light on some other points, including the following:

(a) Is the effect a general systematic one, or due to a specific avoidance reaction?

(b) How long do the effects take to develop, and how long do they persist?

(c) How is the irritability and enhanced activity due to insecticides related to the phenomenon of repellency?

The requirement for study of these points is a means of estimating the degree of kinetic or locomotor activity exhibited by mosquitos during and after stimulation by insecticidal and repellent substances. An objective method for the study of the activity of insects under different conditions of nutrition was devised by Brown & Evans (1960). The apparatus consisted of a series of boxes connected by stemless conical glass funnels. Blowflies (Phormia) were introduced into box 1; the funnels gave a bias toward movement through to box 4. The spontaneous activity of flies in different nutritional states was compared on a basis of passages percent. of possible passages in a given time, the intervals used being 5, 15 and 60 minutes. Adaptation of this method for adult mosquitos requires a much longer time interval, since they are far less active than blowflies, but by running trials overnight, it was found possible to measure spontaneous and induced activity and to show clear differences in the amount of activity under different conditions.

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[1] Editorial note. "The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication" is discussed by R. C. Muirhead-Thomson (1960) in Bull. Wld Hlth Org. 22, 721.

2. APPARATUS

A wooden box lined with clean white filter paper was subdivided into four boxes each 12 cm x 12 cm in base-area and 15 cm high, these dimensions being chosen to allow the walls to be covered with WHO standard size insecticide impregnated papers. The 12 x 15 cm cardboard partitions between the boxes were each pierced with a rectangular opening 7.5 cm x 5 cm into which was fitted a card baffle narrowing to a slot 6 cm x 0.6 cm. The whole was closed by a perspex lid pierced with holes for loading, and covered by a dark paper hood. (See Appendix)

3. BIOLOGICAL MATERIAL AND METHOD

Bloodfed females, four days old, from the Lagos colony of susceptible Anopheles gambiae were used in batches of twenty. Since they were fed in the morning, the normal activity cycle was 12 hours out of phase; except while being handled, however, they were kept in the dark; temperature was kept constant at 27°C, so that the activity shown may be regarded as that of the resting phase after a blood-meal.

Insecticide susceptibility in the colony, as measured by the WHO standard method, was:

DDT	:	LC_{50} 0.5%
Dieldrin	:	LC_{50} 0.05%
Malathion	:	LC_{50} 0.77%

For comparison, and also to assess the possibility of using the method as a means of differentiating between the relative irritability of different strains or species, a short series of trials was also run using Culex fatigans of the susceptible Lagos strain. Like most strains of the species this one is only moderately susceptible to DDT. Conditions were the same as those for A. gambiae. Neither colony had any history of previous selection by insecticides.

Three types of experiment were conducted. The first type was what may be termed "facultative exposure", resembling what must occur in the field, in that exposure is terminated either on the knockdown of the insect or by its escape to an untreated resting-place, in this case box 2. The second and third types were forced

exposures outside the apparatus, followed (immediately or after a holding-period) by measurements of continued activity when the samples were placed in the insecticide-free apparatus. Standard WHO impregnated papers were used for all exposures. The types and time-intervals were as follows:

Exposure		Holding-period	Observation-period(s) (measurement of activity)
Type	Container		
(a) facultative	box 1	nil	5 hrs (2-7 p.m.) plus 13 hrs (7 p.m. - 8 a.m.)
(b) 1-hr forced	WHO-test cylinder	nil	5 hrs (2-7 p.m.) or 13 hrs (7 p.m. - 8 a.m.)
(c) 1-hr forced	WHO-test cylinder	5 hrs (2-7 p.m.)	13 hrs (7 p.m. - 8 a.m.)

Temperature during the trials was controlled at 78-80°F. Activity was estimated by scoring the number of passages achieved from one box to the next, as follows:

mosquitos remaining in box 1 (n_1) ..... zero
moving to box 2 (n_2) 1
moving to box 3 (n_3) 2
moving to box 4 (n_4) 3

The activity shown was expressed as a percentage of the total possible activity, using the formula:

$$\frac{(n_2 + 2n_3 + 3n_4) \times 100}{(n_1 + n_2 + n_3 + n_4) \times 3} = \text{percentage activity}$$

Different sizes of connecting baffle were tested until the dimensions described were standardized. The object was to ensure that a fair number of passages occurred, and that the majority of passages were in one direction. Trials were therefore carried out to determine the proportion of passages with and without stimulation by contact with 1% DDT in box 1, with the baffles in normal and reversed direction. They were carried out in parallel with mosquitos from the same batch, and extended over 18 hours, with an observation at 5 hours. Results were as in Table 1.

There was some variation between batches, but in all cases forward movement was from twice to four times as great as reverse, and movement with DDT-exposure was twice to four times as great as without it. In the case of forward movement the difference between activity of survivors and decessors was great, as might be expected since those able to move more freely had less contact with the insecticide. It will also be seen that while the forward movement was twice the reverse, mortality was less by only one third. The movement observed was therefore assumed to be at random biased only by the direction of the baffles.

The chemicals tested were DDT, dieldrin, malathion, DMP and Deet; also Baytex - O-O dimethyl - O-4 - (methyl mercapto) 3-methyl phenyl thiophosphate.

4. EXPERIMENTS WITH A. GAMBIAE

4.1 Effects of facultative exposures

The results of two distinct series of trials are summarized in Table IIa. In the first series batches of 20 mosquitos were put into box 1 which was lined with treated paper and the activity estimated after 5 hours (2 p.m. to 7 p.m.), before much mortality had occurred, and again at 18 hours (2 p.m. to 8 a.m.). In the second series the same procedure was followed, but only 13 hours of activity were estimated (7 p.m. to 8 a.m.).

Mortality with malathion was 40% in 13 hours, rising to 64% at 18 hours; activity for the first five hours was reduced below the level in the controls, but later rose to equal the latter. Mortalities with dieldrin followed a similar course, but the degree of activity was greater than that in the controls throughout. With DDT this effect was even more marked. As might be expected, with all the insecticides activity in decessors was less than in survivors, or in other words the more active insects survived, but in the control groups the decessors had been more active than the survivors. It will be noted that the mortality due to DDT was less than that due to the other insecticides; later trials with forced exposures (Tables III and IV) showed a reversal of this result. This indicates that both chlorinated hydrocarbons have stimulant or irritant effects, while malathion has a depressant effect in the early stages of intoxication. Table IIb summarizes the results of a similar trial

with Baytex and the two repellents DMP and Deet; activity being measured after 5 and 18 hours of continuous exposure. Both repellents are insecticidal, Deet more so than DMP. All three caused increased activity in the early stages but over the longer period only Deet induced any increased activity.

4.2 Effects of forced exposures followed by release into the apparatus

In the next series of trials exposures of one hour to the chemicals (6 p.m. to 7 p.m.) were followed by release of the mosquitos into an untreated box 1, subsequent activity being recorded after 13 hours (7 p.m. to 8 a.m.). Results are shown in Table III, and indicate that the early inhibition of activity caused by malathion is followed by a period of increased movement. Dieldrin and DDT both caused increased movement as compared with the controls. This with the foregoing trial indicates that all three insecticides cause an increase in activity; and that this is non-directional, and cannot be regarded as a negative taxis, though the result in the field, as in the first trial, would be to shorten the period of contact. DDT provokes the increased locomotive activity at once, dieldrin after a latent period, and malathion after a preliminary period of inhibition.

A similar series was conducted with the two repellents, DMP and Deet with a lower concentration of DDT for comparison. In this case the one-hour exposures took place from 1 p.m. to 2 p.m. and activity was estimated at 5 hours (2 p.m. to 7 p.m.) and 18 hours (2 p.m. to 8 a.m.). The results in Table IIIb show that the repellents act in a similar way to DDT so far as induction of activity is concerned, in that most of the movement occurs in the first five hours after exposure. The repellents were also to some extent insecticidal, but in the case of DMP the survivors were more active, while with Deet they were less active than those which died. It may be noted here that the effect of stronger concentrations of both these chemicals is a rapid knockdown of the mosquitos, which is followed by recovery if they are at once removed from contact.

These results contrast with those given in Table IIb, where facultative exposure to the repellents did not result in any marked increased activity except in the early stages.

4.3 Forced contact followed by a resting period before measurement of activity

In these tests the mosquitos were given one hour of forced contact followed by a resting period of five hours duration, with measurement of activity between 7 p.m. to 8 a.m. Results of two series of trials are summarized in Table IVa and IVb. In this case the variation between batches was much more marked than in previous trials, as may be seen by the wide difference between activity in the two control series. In general it seems that enhanced activity due to DMP, Deet and dieldrin was maintained more than five hours after exposure, while with DDT and malathion it returned to the normal level.

A strong indication of a different type of phenomenon is shown by a comparison of the results in Tables IV and IIIb. After the insects had been in the insecticide-free apparatus for five hours, their activity between the sixth and eighteenth hours was in all cases reduced to a low level (between 2% and 5% following exposure to chemicals; 15% in the controls). But when the first five hours were spent as a holding-period outside the apparatus, the activity within the apparatus in the subsequent 13 hours was at a much higher level (between 26% and 64% following exposures; 27% and 43% in the controls).

In view of these differences it is necessary to treat with reserve any levels of activity measured from the time the mosquitos are first placed in the apparatus, since they may represent the combined effect of activation by the chemical and activation by the box itself. To eliminate the latter, some initial period of habituation to the box would be necessary.

5. EXPERIMENTS WITH CULEX FATIGANS

The trials with this species followed the course of the first A. gambiae experiments, namely facultative exposure (in box 1) with freedom to escape. Table V summarizes the results. With malathion these much less active insects did not show the reduction of activity observed in A. gambiae. With dieldrin there was a latent period during which activity was little more than in the controls, followed by an active period, while with DDT activity was raised at once, and later died down, just as with A. gambiae.

6. DISCUSSION

In the case of A. gambiae all the chemicals tested stimulate some increase in kinetic activity. With DDT, Baytex and the repellents, this is marked from the start of exposure, but dieldrin shows a latent period when activity is not much raised, and malathion produces decreased activity in the early stages of intoxication. Mosquitos removed from contact with DDT and malathion show a period of enhanced activity, followed by a phase where their activity returns to normal: the other materials produce more lasting effects. It is interesting that the repellents appear to act as rather inefficient insecticides, with an action akin to that of DDT, producing high levels of kinetic activity but low mortalities. The fact that exposure to DDT produces relatively much higher mortalities than the other insecticides when exposure is forced, but much lower ones when exposure is facultative, underlines the importance of the irritability and enhanced activity with which the insect responds to this chemical. Since most of our estimates of the relative effectiveness of insecticides are at present derived from experiments conducted under conditions of forced contact, it may well be that further study of the results of facultative contact in the laboratory might explain some of the less satisfactory performances of DDT in the field.

The possibility of utilising the apparent reduction of activity in the early stages of malathion intoxication to enhance the mortality from other insecticides in a mixture by increasing the period of contact is a point which requires further investigation.

Culex fatigans shows similar reactions with DDT and dieldrin to those of the Anopheles, but the decrease in activity with malathion is either not shown or is very short-lived. It seems possible that the method used in these trials for estimating the kinetic response of insects to chemicals may, with further modification to deal with different species, have fairly wide uses. The usual methods of comparing the efficiency of residues under conditions of forced contact clearly cannot tell the full story, and the fate subsequent to exposure of insects which escape cannot fail to be of interest. The different patterns of activity shown by the two species tested indicate also that the method may play a part in detecting strains or species showing "behaviouristic resistance".

7. SUMMARY

An apparatus is described and figures composed of a series of boxes connected by baffles which allows the kinetic activity of flying insects to be measured. A method of calculating an index of activity is used, based on the percentage of the total possible activity; this could be applied to samples of any mosquito population. Exposure to chemicals can be either continuous during the period of measurement or at intervals before. DDT, Baytex and the repellents DMP and Deet produce an immediate response in the form of increased activity which is most intense and dies down soonest with DDT, in the mosquitos Anopheles gambiae and Culex fatigans. Dieldrin causes increased activity after a latent period in both species; malathion leads to an initial reduction in activity in A. gambiae but not in C. fatigans. It is suggested that experiments of this type may produce useful information as to the mode of action of residual insecticides additional to that obtained from conventional test methods.

8. ACKNOWLEDGEMENTS

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9. REFERENCES

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TABLE I. RATES OF PASSAGE OF MOSQUITOS THROUGH BAFFLES IN FORWARD AND REVERSE DIRECTIONS, WITH AND WITHOUT STIMULUS FROM DDT

(mean of five replicates of 20)

Setting of baffles	Percentage of possible activity (see text)				Percentage mortality
	in 5 hours	in 18 hours			
		Overall	survivors	decessors	
Forward (blank)	24	35	35	0	0
Reverse (blank)	6	10	10	0	2
Forward (DDT 1.0%)	47	61	66	44	39
Reverse (DDT 1.0%)	24	29	33	26	58

TABLE IIa. PERCENTAGE OF POSSIBLE ACTIVITY SHOWN BY A. GAMBIAE UPON FACULTATIVE EXPOSURE TO VARIOUS INSECTICIDES, AND MORTALITY FROM SUCH EXPOSURE

(means of five replicates of 20; series (a) observed after 5 and 18 hours in the apparatus, series (b) after 13 hours only)

Chemical	Overall percent. of possible activity in period			Percent. activity in survivors and decessors and percentage mortality over 13 & 18 hours (a) or 13 hours (b)		
	5 hours	13 hours	18 hours	activity of survivors	activity of decessors	percent. mortality
Malathion 0.25%	(a) 5	-	30	42	24	64
	(b) -	32	-	38	24	40
Dieldrin 0.1%	(a) 31	-	51	87	41	68
	(b) -	42	-	50	36	58
DDT 0.5%	(a) 35	-	44	62	16	39
	(b) -	72	-	77	26	9
Control	(a) 21	-	28		66	6
	(b) -	28	-	26	50	5

TABLE IIb. (AS IIa, SERIES (a))

Chemical	Percentage activity in period		activity of survivors	activity of decessors	percent. mortality
	5 hours	18 hours			
DMP 3%	12	30	31	29	9
Deet 3%	19	39	39	38	35
Baytex 0.125%	29	33	59	27	82
Control	6	33	33	16	2

TABLE IIIa. ACTIVITY AND MORTALITY IN A. GAMBIAE OVER 13 HOURS AFTER 1 HOUR OF FORCED EXPOSURE OUTSIDE THE APPARATUS

(means of 5 replicates of 20)

Chemical	Overall percent. of possible activity	Percent. activity in survivors	Percent. activity in decessors	Percent. mortality
Malathion 0.25%	49	52	41	15
Dieldrin 0.1%	54	58	42	30
DDT 1.0%	36	38	34	58
Control	33	32	47	4

TABLE IIIb. ACTIVITY AND MORTALITY IN A. GAMBIAE OVER 5 AND 18 HOURS AFTER 1 HOUR OF FORCED EXPOSURE OUTSIDE THE APPARATUS

(means of 5 replicates of 20)

Chemical	Overall percent. of possible activity		Percent. activity in survivors, 18 hours	Percent. activity in decessors, 18 hours	Percent. mortality, 18 hours
	5 hours	18 hours			
DMP 3%	37	41	50	36	30
Deet 3%	52	57	62	48	38
DDT 0.25%	44	46	55	38	56
Control	19	34	32	55	8

TABLE IVa. ACTIVITY AND MORTALITY IN A. GAMBIAE OVER 13 HOURS AFTER 1 HOUR OF FORCED EXPOSURE AND A FIVE-HOUR HOLDING-PERIOD OUTSIDE THE APPARATUS

(mean of 5 replicates of 20)

Chemical	Overall percent. of possible activity	Percent. activity in survivors	Percent. activity in decessors	Percent. mortality
DMP 3%	64	62	74	17
ENT 22542 3%	52	51	54	29
DDT 0.25%	42	40	46	27
Control	43	44	33	5

TABLE IVb. (AS IVa)

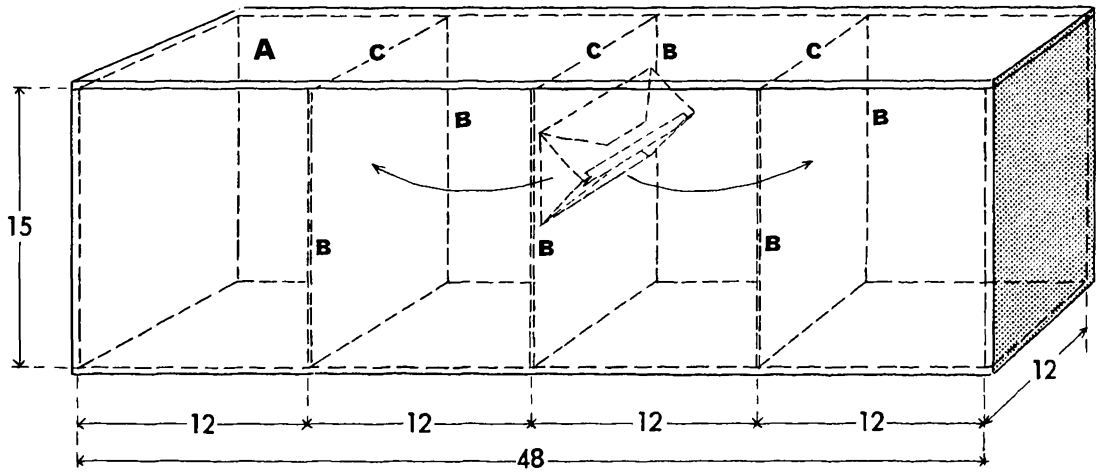
Dieldrin 0.1%	43	35	51	31
Malathion 0.25%	26	24	33	21
Control	27	27	20	6

TABLE V. ACTIVITY AND MORTALITY IN *CULEX FATIGANS* UPON FACULTATIVE EXPOSURE TO VARIOUS INSECTICIDES

(means of 3 replicates of 40; series (a) observed after 5 and 18 hours in apparatus, series (b) after 13 hours only)

Chemical	Overall percent. of possible activity in period			Percent. activity in survivors and decessors and mortality over 18 hours (a) or 13 hours (b)		
	5 hours	13 hours	18 hours	activity of survivors	activity of decessors	percent. mortality
Malathion 1%	(a) 22	-	31	31	31	62
	(b) -	25	-	36	14	52
Dieldrin 0.05%	(a) 2.7	-	37	33	38	85
	(b) -	33	-	47	32	91
DDT 4%	(a) 32		38	50	13	33
	(b) -	39	-	49	12	26
Control	(a) 0.6	-	10	10	0	0
	(b) -	5	-	5	0	0

APPARATUS FOR MEASURING KINETIC RESPONSE OF MOSQUITOS TO CHEMICALS ON FACULTATIVE EXPOSURE, AND FOLLOWING EXPOSURE

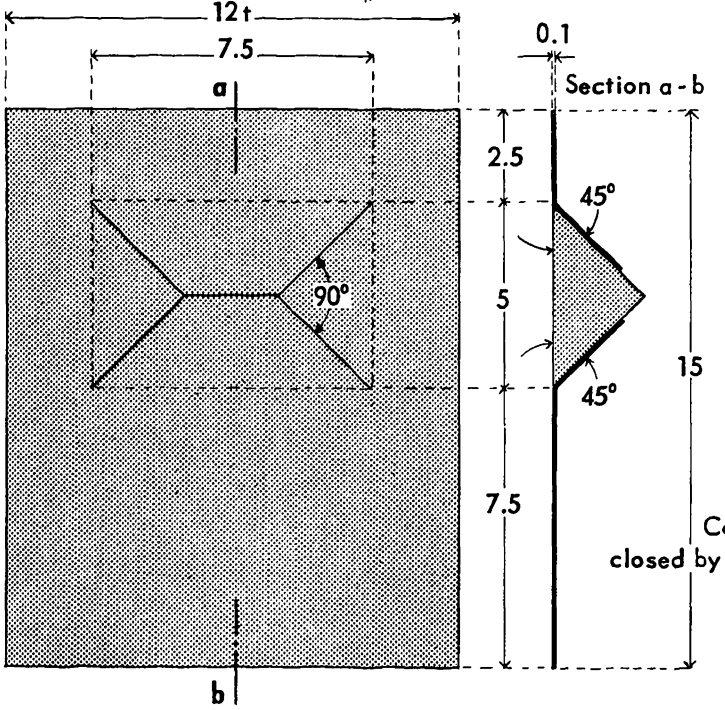


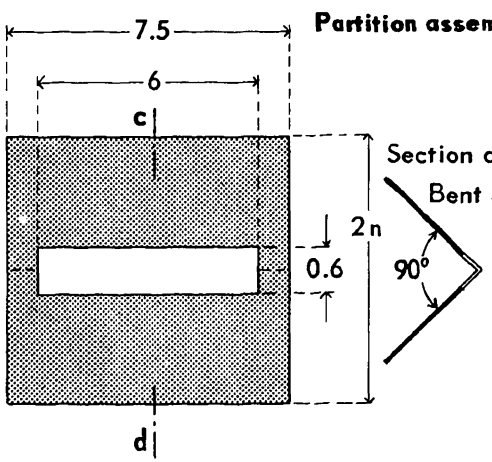
Box assembly

1/4 scale isometric

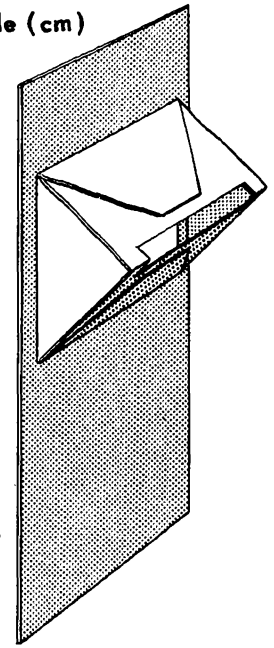
Internal dimensions (cm)

Demountably assembled by wire staples





Partition assembly 1/2 scale (cm)



Constructed in soft-wood of any convenient thickness (t); closed by a Perspex lid (12 + 2t) × (48 + 2t) with loading aperture A.

BB: Sawcut grooves for partitions

CC: Partitions

$n = \sqrt{12.5}$ cm

12 +: slightly oversize to engage grooves BB

WHO 1260