

a 62360

22

WHO/Insecticides/141

WHO/Mal/345

2 May 1962

ORIGINAL: ENGLISH

A FIELD TRIAL IN UGANDA OF A NEW METHOD
FOR DETERMINING THE IRRITABILITY
OF MOSQUITOS TO INSECTICIDES

(Preliminary report)

by

A. Coluzzi;^a J. R. Cullen;^b J. de Zulueta^c

The finding during the last years that failures to interrupt malaria transmissions may not always be due to either poor spraying or to the development of physiological resistance has again focused the attention of malariologists and entomologists on problems of mosquito behaviour. Renewed efforts have been made to assess the irritability of mosquitos to insecticides, a factor which in toxicants like DDT is of paramount importance, and in 1960 a provisional irritability test method was proposed by the WHO Expert Committee on Insecticides.¹ Experience in the laboratory and in the field showed that the method was not entirely satisfactory due mainly to the activity observed in the control mosquitos, brought into contact with filter-papers impregnated only with risella oil. The test papers in this method were filter-papers treated with a 2 or a 4% solution of DDT in risella oil.

A. & M. Coluzzi recently proposed a new method for the determination of the irritability of mosquitos embodying some of the features of the WHO provisional method such as the use of the plastic conical exposure chambers and the treated papers originally supplied by WHO for the determination of susceptibility and resistance in adult mosquitos. Full details of the new method may be found in

^a Deputy Director, Malaria Institute, Rome

^b WHO Staff Member, Uganda Malaria Eradication Pilot Project

^c Division of Malaria Eradication, WHO, Geneva

a separate report to WHO² and it may be sufficient to explain here that it differs from the WHO provisional method in two main points: (i) the use of small vials 2 x 1 inches where the mosquitos to be examined are placed before the test for a half-an-hour conditioning period; and (ii) the placing of the conical exposure chambers in black painted wooden boxes where the source of illumination becomes the treated filter-paper itself placed against a piece of ground glass. The method has been tried in Italy in the Malaria Institute, Rome, and proved satisfactory - consistent results being obtained with several anopheline species with practically no flights in the controls. In view of these encouraging results, the Environmental Health Division, WHO, Geneva, arranged for one of us (A.C.) to travel to Uganda for one month in order to carry out, in co-operation with the staff of the Uganda Eradication Pilot Project, a series of trials to assess the value of the new method under typical field conditions. The results of these trials are the subject of the present report.

METHODS

The tests were carried out in two places: (i) the small laboratory of the Malaria Project at Kihihi, Kigezi District, situated less than 1° south of the equator, at 3700 feet above sea-level. The laboratory is built of mud and wattle and mosquito screening. Temperature and humidity cannot be controlled, but light can be varied by the raising or lowering of blinds over the mosquito screening. (ii) The Government Rest House at Bwera, Toro District, a simple wooden bungalow, was also used in some of the tests. There was of course no temperature and humidity control, but the light could be adjusted to a certain extent by means of curtains. Bwera is 95 miles from Kihihi, almost exactly on the equator, at an altitude of 3800 feet.

The mosquitos tested were: (1) the Kisumu strain of A. gambiae maintained in the insectary at Kihihi; (2) a strain of Aedes aegypti from the East African Virus Research Institute, Entebbe, also maintained at the Kihihi insectary; (3) wild-caught A. gambiae from the Katojo area, five miles from Bwera; and (4) wild-caught A. pharoensis from Kayanja, a small fishing village on the shores of Lake Edward, 10 miles from Bwera. Both the wild-caught A. gambiae and A. pharoensis came from untreated areas where residual insecticides have not been used. Tests with the colony strains of A. gambiae and Aedes aegypti were carried out at the Kihihi laboratory and the wild-caught A. pharoensis and A. gambiae at the Bwera Rest House.

Females from the colony strains were blood-fed each day two hours before the beginning of the test and were left for a minimum of half-an-hour in the conditioning vials. In Bwera only wild-caught females having recently fed (stages II and III of sella) were used in our tests. Mosquitos were released directly from the conditioning vials into the conical exposure chambers and, after the 15-minutes testing period, were removed with a bent sucking-tube and placed in paper cups for 24-hours mortality counts.

Temperature and humidity readings were made at the beginning and at the end of each testing period and light intensity was measured also before and after testing with a Weston photometer applied against the conditioning vials. In the course of the trials, however, it was found that light readings made through the exposure chambers were more satisfactory but the light measurements given in Table I of this report are all readings made through the conditioning vials.

RESULTS

Tables I and II summarize the results obtained on a total number of 356 mosquitos. As can be seen, in spite of the considerable variation experienced in environmental conditions, and more particularly in light conditions, the number of flights in the control mosquitos remained very low, so that for practical purposes all the activity seen in the test mosquitos can be attributed to the irritant effect of the DDT. Mosquitos released into the exposure chambers after their initial conditioning period fly in most cases directly to the filter-papers and, in the case of control papers, rest on them until the end of the 15 minutes' observation period or, if the papers are impregnated with DDT, until they feel the irritant effect of the insecticide. This represents a clear improvement over the provisional WHO method where there were a good number of flights during the first few minutes.

The chosen period of observations of 15 minutes proved to be adequate in our experiments. A glance at Table I will show that with all the species tested the mean number of take-offs either decreased or levelled off before the end of the 15 minutes.

Morning and afternoon tests did not yield, in general, significantly different results, in spite of the changes in temperature, humidity and light conditions. There was an occasion, however, when an afternoon storm produced a drop of temperature of over 5° and a drop in illumination from 8 to 0.8 foot candles, as well as an increase in relative humidity from 62 to 74%. These sudden changes were associated with a marked diminution of activity in the A. gambiae (Kisumu strain) being examined and this points to the need to carry out irritability tests in the field under conditions as uniform as possible. To adjust temperature and humidity may not always be easy but light conditions can in most cases be controlled by means of blinds or curtains.

Concerning the irritability shown by the various strains examined, A. gambiae proved by far the most irritable mosquito in our series and this applies to both the colony strain and the wild-caught A. gambiae. The average number of flights was slightly higher in the colony strain but since the environmental conditions were different and since, in all probability, the age of the mosquito was also different it would be difficult to draw any conclusion in this case. We may mention here that an attempt to secure a sufficient number of A. gambiae females from the DDT-sprayed area in North Kigezi failed, due to the prevalent dry conditions, which have reduced almost to nil the A. gambiae population in that area.

The results obtained with A. gambiae are very similar to those obtained by two of us (J.R.C. and J. de Z.) with the Kisumu strain maintained in our insectary and with wild-caught females from the treated area in North Kigezi, using the WHO provisional method and working at the insectary of Kihiki.³ It was found then that, using 4% DDT papers as in the present observations, the average number of flights for the Kisumu strain was 25.5 per mosquito and for the North Kigezi strain 21.8. In the present series the average number of take-offs for the Kisumu strain was 33.3 and for the wild-caught A. gambiae from the Katojo unsprayed area 27.9.

Regarding A. pharoensis, the results obtained before at Kihiki³ with material from North Kigezi were markedly different, the average number of take-offs with 4% DDT papers being 16.2 per mosquito - instead of the 3.9 found in the present series. To what this difference can be attributed is not clear to us, but comparative tests with the two methods may reveal whether the difference is in fact due to a different response to DDT in the two strains or, as is more likely, to a difference due to the method employed.

Aedes aegypti (E.A.V.R.I. strain) had also been tested before at Kihikihi with the provisional WHO method³ and here again marked differences in the results were observed, the average number of flights being 2.6 per mosquito and, in the present series, 10.3. In this case too, judgement must be reserved until comparative trials with the two methods are carried out.

CONCLUSIONS

From what has been said already while discussing our results, it must have been noticed that we hesitate to draw any conclusions from the small differences observed between the two strains of A. gambiae tested; also that we hesitate to interpret the differences found between our present results and those obtained earlier with the WHO provisional method. This is due to the fact that, although the new method appears to be much more satisfactory than the provisional WHO method, the results obtained must, nevertheless, be influenced to a considerable extent by the prevailing conditions of temperature, humidity and illumination and, if wild-caught females are used, their variable age must also influence the results.

From our past and present experience in the testing of irritability, we believe that when tests are carried out in the field only marked differences between species or strains should be considered as having any significance. For a better assessment of irritability we recommend use of the new method in the laboratory, under controlled humidity, temperature and light conditions; also to use females of the same age and in the same feeding conditions. If this is not feasible the tests should be carried out under conditions as uniform as possible. Light in particular should be adjusted by means of blinds or curtains to values between 2 and 15 foot candles (the photometer applied to the exposure chambers) and reflected light should be reduced to a minimum.

REFERENCES

1. World Health Organization, Tenth report of the Expert Committee on Insecticides (1960) Wld Hlth Org. techn. Rep. Ser. 191
2. Coluzzi, M. (1961) Essai d'elaboration d'une procédure expérimentale pour déterminer l'irritabilité des moustiques adultes aux insecticides (Unpublished report to WHO)
3. Cullen, J. R. & de Zulueta, J. (1962) Observations on the irritability of mosquitos to DDT in Uganda; WHO/Mal document

TABLE I. NUMBER OF TAKE-OFFS

Species	Insecticide tested	Number of mosquitoes exposed	Number of take-offs in each minute															Temp. °C Max. Min.	Light in foot candles	Rel. Hum. (%)	24 hours mortality (%)	Range in No. of take-offs			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					Max.	Min.		
<i>A. gambiae</i> Kihhi colony (Kisumu strain)	DDT 4%	100	9	20	32	42	71	110	180	249	298	348	366	426	396	397	389				20.0		140	0	
		mean	0.09	0.2	0.32	0.42	0.71	1.10	1.80	2.49	2.98	3.48	3.66	4.26	3.96	3.97	3.89								
	Control	50	2	1	1	1	0	0	0	0	0	0	0	0	1	0	0	6				2.0		2	0
		mean	0.04	0.02	0.02	0.02	0	0	0	0	0	0	0	0	0.02	0	0	0.12							
<i>A. gambiae</i> Katojo, unsprayed zone	DDT 4%	36	4	5	10	22	23	52	74	95	103	94	110	98	114	104	1003				44.4		121	2	
		mean	0.11	0.14	0.28	0.61	0.64	1.44	2.05	2.64	2.86	2.64	2.61	3.05	2.72	3.17	2.88								
	Control	10	1	1	4	0	1	0	0	0	0	0	0	0	0	0	0	7				0.0		5	0
		mean	0.1	0.1	0.4	0	0.1	0	0	0	0	0	0	0	0	0	0	0.7							
<i>A. pharoensis</i> Kayanja, unsprayed zone	DDT 4%	58	0	4	6	11	11	15	11	14	17	22	16	23	26	24	29	229				1.7		35	0
		mean	0	0.07	0.1	0.19	0.19	0.26	0.19	0.24	0.29	0.38	0.27	0.39	0.45	0.41	0.50	3.94							
	Control	27	0	0	0	0	1	2	0	0	0	0	0	0	0	1	0	4				0.0		3	0
		mean	0	0	0	0	0.04	0.07	0	0	0	0	0	0	0	0.04	0	0.148							
<i>Aedes aegypti</i> Kihhi colony (E.A.V.R.I. strain)	DDT 4%	50	12	41	63	64	59	59	47	49	30	25	21	15	14	9	8	516				0.0		38	1
		mean	0.24	0.82	1.26	1.28	1.18	1.18	0.94	0.98	0.60	0.50	0.42	0.30	0.28	0.18	0.16	10.32							
	Control	25	5	5	1	1	1	0	1	1	2	0	0	0	0	0	0	17				0.0		3	0
		mean	0.2	0.2	0.04	0.04	0.04	0	0.04	0.04	0.08	0	0	0	0	0	0	0.68							

The purpose of the WHO/Mal Series of documents is three-fold:

- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

The issue of a paper in this series does not therefore constitute formal publication and a paper so issued may, with the agreement of the author and WHO, be published in a WHO periodical or elsewhere.

Authors alone are responsible for views expressed in signed articles. The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.