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LABORATORY EXPERIMENTS ON THE IRRITABILITY OF ANOPHELES ATROPARVUS AND ANOPHELES STEPHENSI TO DDT

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ABSTRACT

Two strains of *Anopheles atroparvus,* one resistant and one susceptible to DDT and two of *Anopheles stephensi,* one resistant and one susceptible to DDT were tested for their irritability to DDT. Various laboratory investigations of the effects of insecticides on mosquito behaviour and their irritability were carried out.

In these series of experiments a comparison was made between the results obtained by a tentative method proposed in 1960 by the WHO Expert Committee on Insecticides in which the mosquitoes are confined over the treated surface in a small plastic chamber and in a large cage. Under the large cage conditions DDT-resistant *A. stephensi* were more readily irritated by the insecticide than a susceptible strain of the same species.

INTRODUCTION

Investigations during the past few years in the field and in the laboratory showed that failure of antimalaria-campaigns is not always due to poor spraying or development of physiological resistance but may also be due to the irritation of mosquitoes by the insecticide (Trapido, 1952). Presence of irritability in mosquitoes makes them fly away from houses without sufficient contact with sprayed surfaces. Therefore the irritability of mosquitoes with regard to several insecticides (but especially DDT) is one of the problems in malaria eradication.

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A higher degree of irritability than normal has been termed "behaviouristic resistance". It should be borne in mind, however, that the consequence of increased irritability is a lower mosquito mortality but does not involve any increase in tolerance to the insecticide WHO, 1963).

Laboratory experiments have been carried out on the connection between resistance and susceptibility to insecticides and irritability in *A. stephensi* and the same connection between resistance, and susceptibility and irritability in *A. atroparvus.*

In all the irritability tests WHO 4% DDT impregnated papers were used unless stated otherwise, when papers were prepared in the laboratory by the Busvine-Nash method.

MATERIALS AND METHODS

A series of observations on the irritability of mosquitoes to DDT were carried out in the Laboratory of Parasitology, University of Leiden, Netherlands, 1964. The methods involved were as follows:

a) **WHO Bioassay method:**

The instructions given in the report of the WHO Expert Committee on insecticides (WHO, 1960) and in Cullen & de Zulueta (1962) were used as a basis for the experiments. Transparent conical exposure chambers as used in the WHO bioassay test were mounted vertically over the exposure paper on a sheet of hardboard by means of rubber bands. Impregnated 4% DDT and 2% DDT papers and the non-impregnated control papers were used as exposure surfaces.

Two-day-old mosquitoes were blood fed for the test. Well-gorged, undamaged females were isolated, one per paper cup and kept in darkness. Exposures were made in the morning between 09.00 and 13.00 hours. The mosquitoes were tested in a light intensity of approximately 3 foot-candles and this light was filtered through transparent conical.

Mosquitoes were introduced in to the chambers individually. In this series of experiments 3 minutes were allowed as a settling period before the number of take-offs in the following 15 minutes was counted, using hand-tally counters.

the vertical walls after which they were caught in a transparent plastic box with dimensions 15 x 15 x 15 cm. In another series of tests the mosquitoes were caught in a WHO susceptibility test tube with a circular hole. In both series of experiments the holes were in a slide-unit with an easily movable slide. Throughout this series the top of the cage was covered by a glass plate.

The number of mosquitoes per experiment was 25 and the duration of one test was 15 minutes.

All the experiments were performed at 24°C and 75% relative humidity.

The mosquitoes used were:

Anopheles atroparvus Van Thiel, 1927 (Ranskapelle susceptible strain and Mosna resistant strain); *Anopheles stephensi* Liston, 1901 (Delhi susceptible strain and Iran DDT and dieldrin resistant).

The susceptibility of *A. atroparvus* and *A. stephensi* to DDT was as follows:

a)	*A. atroparvus*		
	strain	*Lc50*	*Lc90*
	Ranskapelle (susceptible)	1.8% DDT	5.0% DDT
	Mosna (resistant)	>4	
b)	*A. stephensi*		
	strain		
	Delhi (susceptible)	1.0% DDT	2.1% DDT
	Iran (resistant)	>4	

A. Results of investigation by WHO bioassay method

1. *A. atroparvus,* susceptible strain, using Busvine and Nash impregnated papers. Average number of take-offs per mosquito per 15 minutes on 4% DDT impregnated paper 20.1.
   Total number of mosquitoes observed: 24.
2. *A. atroparvus,* resistant strain, using Busvine and Nash impregnated papers. Average number of take-offs per mosquito per 15 minutes on 4% DDT impregnated paper 21.1.
   Total number of mosquitoes observed: 24.
3. *A. atroparvus,* susceptible strain, WHO impregnated papers used. Average number of take-offs per mosquito per 15

1.3.
Total number of mosquitoes (susceptible strain) observed: 24.

During the whole series, control tests were run on clean papers. Number of take-offs in these control tests per mosquito per 15 minutes was 0.8 and 1.2 (Table 2).

5. *A. stephensi,* resistant to DDT and dieldrin.
Average number of take-offs per mosquito per 15 minutes on 4% DDT impregnated paper 8.8.
Total number of mosquitoes observed: 50.
6. *A. stephensi,* susceptible strain.
Average number of take-offs per mosquito per 15 minutes on 4% DDT impregnated paper 9.2.
Total number of mosquitoes observed: 50.

Average number of take-offs per mosquito per 15 minutes in control tests with these strains of *A. stephensi* 0.4 and 0.1 respectively (Table 3).

B. Results of Investigations with a large cage

Although it should be admitted that there were only rather weak reasons to postulate a correlation between susceptibility and irritability, there was a good reason for serious doubt whether the bioassay method would yield much information as to what happens when mosquitoes leave DDT treated surfaces after irritation by DDT.

It was thought that more reliable results would be obtained by giving the mosquitoes an actual opportunity to escape from a cage.

Preliminary experiments had to be done in order to obtain information on the influence on the escape rate of:

a) The shape of the hole (circular hole and rectangular slit).
b) The illumination.
   1. Constant dim fluorescent light from above and very dim diffuse light from a 15 watt bulb placed at a distance.
   2. Cage and escape box about equally illuminated and cage alone covered by a black hood.
c) The time of the day (afternoon experiments and evening experiments).

Significant escape from the large cage occurred under conditions in which the glass plate on top of the cage was covered by the black hood and when the plastic escape box was directly exposed to the light in the room

A. stephensi, resistant strain

When the large cage and escape box was illuminated with a fluorescent light the observed escape rate was between 5.0% and 6.0%

(Table 4). This rate increased to between 22.8% and 63% when the large cage was covered with a black hood and the escape box exposed to fluorescent light (Tables 5–6). The observed escape rate was then 17.5% (Table 7).
Total number of mosquitoes used: 850.
In control tests the escape rate was 0.0%–11.4%.
Total number of mosquitoes used: 802.

A. stephensi, susceptible strain

When the large cage was covered with a black hood and the escape box exposed to fluorescent light the observed escape rate was 0.5%. This rate was nil for the large cage covered with black hood and escape box in dim light (Tables 6–7).
Total number of mosquitoes used: 325.
In control tests the escape rate was nil.
Total number of mosquitoes used: 200.

A. atroparvus, resistant strain

A peculiar difference in behaviour between *A. stephensi* and *A. atroparvus* made it necessary to modify the method followed in testing with the large cage. With *A. atroparvus* mosquitoes the escape rate did not exceed 2.0% using 4.0% DDT impregnated paper. It was also observed that the distribution of the resting mosquitoes on the walls was quite dissimilar in both species: *A. stephensi* was fairly equally distributed on the surface, whereas *A. atroparvus* congregated towards the upper parts of the cage.

CONCLUSIONS

Irritation of mosquitoes by DDT in a confined space like the bioassay chambers used in these experiments is caused by direct contact with the treated surface, not by vapour.

Strains of mosquitoes known to differ in their susceptibility to DDT and in their degree of avoidance of a DDT treated surface did not show any significant difference in numbers of take-offs on comparison in paired series of tests with the plastic chamber technique originally recommended by WHO for testing irritability.

Judged by the number of take-offs in the WHO bioassay method there seems to be no difference between the strains but there is if the rate of escaping from a large cage is taken as the criterion.

In the two strains of *A. stephensi* examined, a difference in susceptibility to DDT is definitely accompanied by a difference in irritability. The resistant strain was more irritated than the susceptible under test conditions where escape of the mosquito was possible.

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Table 1

Comparison of irritability between susceptible and resistant strains of *A. atroparvus**, individual exposure

Strain	Concentration	No. of anopheles tested	No. of take-offs in 15 minutes				
			Average no. of take-offs per anoph.	Standard error	Variance	Range in No. of take-offs per anopheles	
						Min.	Max.
(1) Susceptible	2% DDT	24	15.1	1.5	57.8	0	28
	4% DDT	24	20.1	1.9	84.8	5	43
	Control	24	1.9	0.6	7.6	0	9
(2) Resistant	2% DDT	24	17.1	1.6	63.5	7	36
	4% DDT	24	21.1	2.1	102.5	5	48
	Control	24	0.8	0.2	1.3	0	3
(3) Susceptible	4% DDT	24	17.2	1.5	57.5	6	41
	Control	24	1.4	0.2	1.2	0	4

* Series No. 1 and 2 with Busvine & Nash method impregnated paper and No. 3 with WHO standard impregnated paper.

Table 2

Irritability of *A. atroparvus* to DDT when direct contact of mosquito with treated surface was prevented by intervening gauze, Individual exposure

Strain	Concentration	No. of anopheles tested	No. of take-offs in 15 minutes				
			Average no. of take-offs per anoph.	Standard error	Variance	Range in No. of take-offs per anopheles	
						Min.	Max.
(1) Susceptible	4% DDT	30	2.4	0.7	15.7	0	12
	Control	30	0.8	0.3	2	0	5
(2) Susceptible	4% DDT	24	1.3	0.5	5	0	7
	Control	24	1.2	0.4	3.5	0	6

* Series Nos. 1 and 2 are shown in the experiments at a distance of 5 mm and 10 mm from impregnated papers respectively.

Table 3
Comparison of irritability between susceptible and resistant strains of *A. stephensi*

Strain	Concentration	No. of anopheles tested	No. of take-offs in 15 minutes				
			Average no. of take-offs per anoph.	Standard error	Variance	Range in No. of take-offs per anopheles	
						Min.	Max.
Resistant	4% DDT	50	8.8	0.6	20.9	1	20
	Control	50	0.4	0.1	0.97	0	4
Susceptible	4% DDT	50	9.2	0.5	11.8	4	18
	Control	50	0.1	0.04	0.1	0	1

Table 4

Large cage and escape box in fluorescent light (moon light)*

Concentration	Time Hours	Species	Strain	Exposure time (min.)	Type of opening	No. of anopheles in the		Total	% escaped
						large cage	escape box		
4% DDT	15.00–18.00	*A. stephensi*	resistant	15	rectangular	94	6	100	6
Control	15.00–18.00	*A. stephensi*	resistant	15	"	200	2	202	1
4% DDT	21.00–23.00	*A. stephensi*	resistant	15	"	95	5	100	5
Control	21.00–23.00	*A. stephensi*	resistant	15	"	125	0	125	0
4% DDT	15.00–18.00	*A. atroparvus*	resistant	15	"	100	0	100	0
Control	15.00–18.00	*A. atroparvus*	resistant	15	"	144	2	146	1.4
4% DDT	21.00–23.00	*A. atroparvus*	resistant	15	"	98	2	100	2
Control	21.00–23.00	*A. atroparvus*	resistant	15	"	175	0	175	0

* The opening not closed for control, but closed for DDT exposure in initial 2 minutes.

Table 5

Large cage covered with black hood and escape box in fluorescent light*

Concentration	Time Hours	Species	Strain	Exposure time (min.)	Type of opening	No. of anopheles in the		Total	% escaped
						large cage	escape box		
4% DDT	21.00–23.00	*A. stephensi*	resistant	15	rectangular	37	63	100	63
Control	21.00–23.00	*A. stephensi*	resistant	15	"	155	20	175	11.4
4% DDT	15.00–18.00	*A. atroparvus*	resistant	15	"	99	1	100	1
Control	15.00–18.00	*A. atroparvus*	resistant	15	"	123	0	123	0
4% DDT	21.00–23.00	*A. atroparvus*	resistant	15	"	99	1	100	1
Control	21.00–23.00	*A. atroparvus*	resistant	15	"	187	13	200	6.5

* The opening not closed for control, but closed for DDT exposure in initial 2 minutes.

Table 6

Large cage covered with black hood and escape box in fluorescent light*

Concentration	Time Hours	Species	Strain	Exposure time (min.)	Type of opening	No. of anopheles in the		Total	% escaped
						large cage	escape box		
4% DDT	15.00–18.00	*A. stephensi*	resistant	15	rectangular	270	80	350	22.8
Control	15.00–18.00	*A. stephensi*	resistant	15	"	149	1	150	0.7
4% DDT	15.00–18.00	*A. stephensi*	susceptible	15	"	199	1	200	0.5
Control	15.00–18.00	*A. stephensi*	susceptible	15	"	100	0	100	0

Table 7

Large cage covered with black hood and escape box in dim light.*

4% DDT	15.00–18.00	*A. stephensi*	resistant	15	"	165	35	200	17.5
Control	15.00–18.00	*A. stephensi*	resistant	15	"	150	0	150	0
4% DDT	15.00–18.00	*A. stephensi*	susceptible	15	"	125	0	125	0
Control	15.00–18.00	*A. stephensi*	susceptible	15	"	100	0	100	0

* The opening for control and DDT exposure both were closed in initial 2 minutes.