Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1959, **20**, 757-776

INSECTICIDE RESISTANCE IN ANOPHELINES IN EASTERN SAUDI ARABIA

R. L. PEFFLY

Entomologist, Arabian American Oil Company, Dhahran, Saudi Arabia

SYNOPSIS

In 1953, the fifth year of a malaria control programme with DDT in Eastern Saudi Arabia, the resting of *Anopheles stephensi* on recently sprayed surfaces suggested the development of resistance in this species to DDT, and this suspicion has been confirmed by tests carried out from 1955 to 1958.

DDT was replaced by dieldrin in 1955 and malaria rates, which had been rising, were again reduced. No dieldrin-resistance has been found in local *A. stephensi* strains, and no *A. stephensi* have been collected from treated villages since the wide use of dieldrin.

A. pulcherrimus, *A. coustani* var. *tenebrosus*, *A. fluviatilis* and *A. sergenti* have proved susceptible to DDT, but the first two have developed resistance to dieldrin. *A. pulcherrimus* was rarely collected before and during the use of DDT but has become increasingly frequent since the introduction of dieldrin. Its possible role in transmitting malaria in the area is discussed.

Egg measurements and ratios on several series of eggs obtained from locally collected *A. stephensi* fall within the limits of the range set for *A. stephensi mysorensis*, not previously recorded outside India.

In his comprehensive ten-year study of malaria in the Eastern Province of Saudi Arabia, Daggy (1957, 1959) describes how the prevalence of this serious disease was effectively reduced by timely annual applications of DDT residual sprays. This control programme was successful for five years; but during the sixth and seventh years of DDT spraying, malaria increased to an alarming level and was again a serious problem in this area. From this malariometric evidence and from entomological observations, development of DDT-resistance by the vector *Anopheles stephensi* was suspected. A switch to dieldrin in the eighth, ninth, and tenth years again reduced malaria rates to the lowest ever in 1957.

Entomological observations throughout these years were aimed primarily at *Anopheles stephensi*. The preferred daytime resting-sites of *A. stephensi* are walls and ceilings of palm-thatched structures. They also rest on walls and palm-mat ceilings of mud-plastered stables and houses. There are about equal numbers of these two types of building in the villages in the Eastern Province. Shelters in gardens are usually palm-thatched. Daggy reported

that before village-wide DDT sprays were applied, as many as 500-600 blood-filled female *A. stephensi* could be collected with a suction tube from a square yard of wall surface in palm-thatched structures within a 15-minute period. The first oasis-wide application of DDT was made in Qatif in the autumn of 1948 and no *A. stephensi* were found resting on treated walls and ceilings for 12 months. In the autumn of 1954, the seventh year of the programme, *A. stephensi* were collected from walls and ceilings of village and garden stables and houses that had been treated with a 2.0-2.5 g/m^2 deposit of DDT several weeks before. Throughout the following twelve months, *A. stephensi* were collected in considerable numbers in most of the treated villages where routine mosquito surveys were made. This species was particularly abundant in two villages, al-Ajam and Safwa, where DDT sprays had been applied every year since 1948 (Fig. 1).

At this time, several somewhat isolated garden dwellings in the Qatif oasis were treated with 0.25 g/m^2 dosages of dieldrin. *A. stephensi* did not reappear in hand-caught collections from these locations for four or five months. Dieldrin was first applied in the entire Qatif oasis in 1955 at the rate of 0.45 g/m^2. Since that time with annual dieldrin sprays applied at the rate of 0.7 g/m^2, no *Anopheles stephensi* have been collected from any of the villages in this oasis. The same is true since 1956 for the larger al-Hasa oasis, 160 km south of Qatif. The only *A. stephensi* collected in these areas have been in garden shelters outside the villages.

Information in this paper on resistance of *A. stephensi* to DDT is based on tests run with mosquitos collected from al-Mutairifi, a village in al-Hasa; from a small garden, Shrafiyah, not far from al-Mubarraz in al-Hasa; from an isolated garden, 'Ain al-Saih, on the shores of the Persian Gulf south of Dhahran; from a garden area west of Dammam, and from a garden on Tarut island (Fig. 1 and 2). No *A. stephensi* have been collected in al-Mutairifi since it was sprayed with dieldrin in the autumn of 1956. The *A. pulcherrimus* tested were collected from three locations in the Qatif oasis, the villages of Safwa and al-Ajam and from Shamaliyah, a garden near the village of Saihat. The *A. coustani* var. *tenebrosus* tested were from al-Mutairifi, Safwa and Shamaliyah. The *A. fluviatilis* were from 'Ain al-Saih, Dammam and Shamaliyah.

Insecticide applications were not identical in all areas of the Eastern Province during this ten-year period. The malaria control programme was originally a co-operative effort between the Saudi Arabian Government and the Arabian American Oil Company. Government approval and funds were not always obtained in time for spraying to produce the greatest benefit. Since 1956, the Saudi Arabian Government has assumed full responsibility for this programme and spraying has been done regularly.

The two villages of Safwa and al-Ajam, separated from each other and from the main part of the Qatif oasis by 5 km or more, were used as experimental villages and were sprayed every year. The spray histories of these

FIG 1. PORTION OF EASTERN SAUDI ARABIA

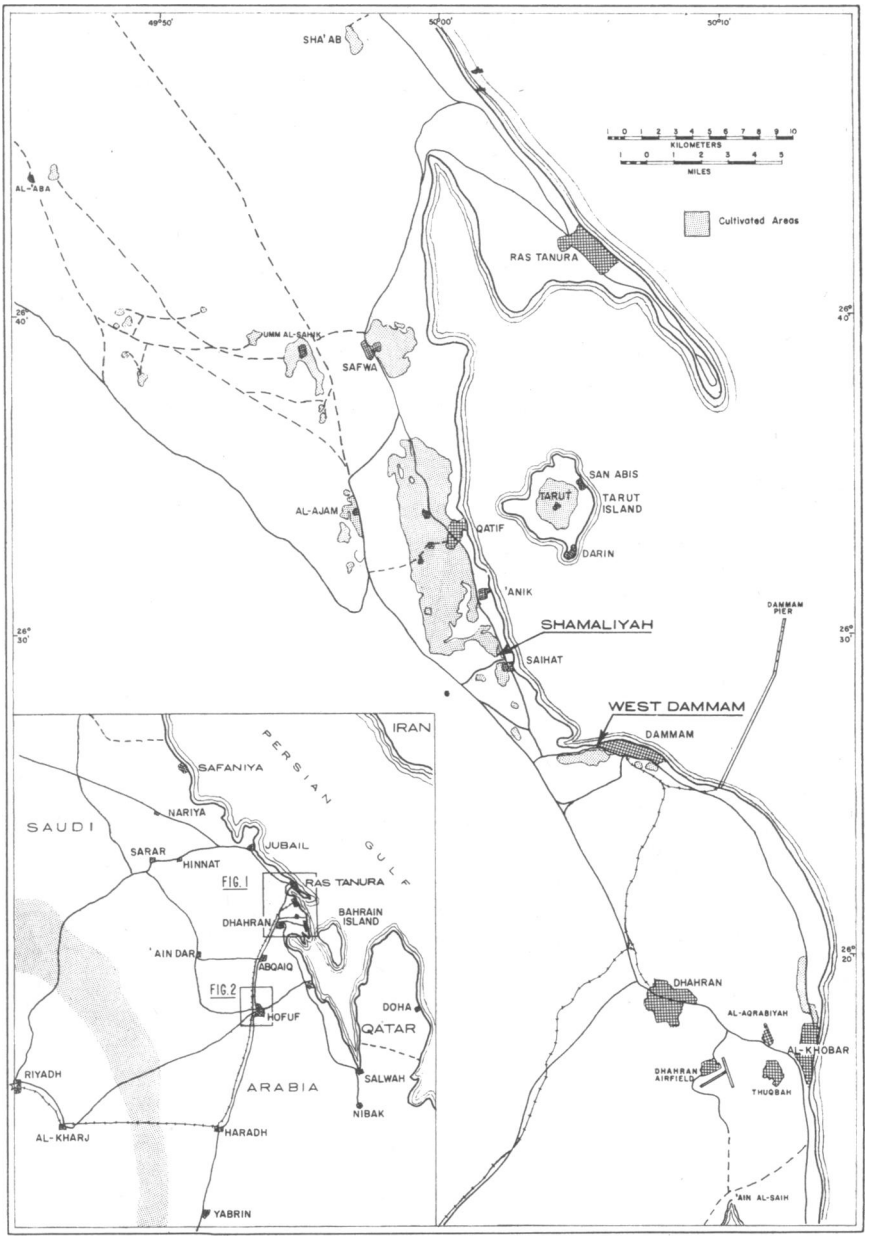
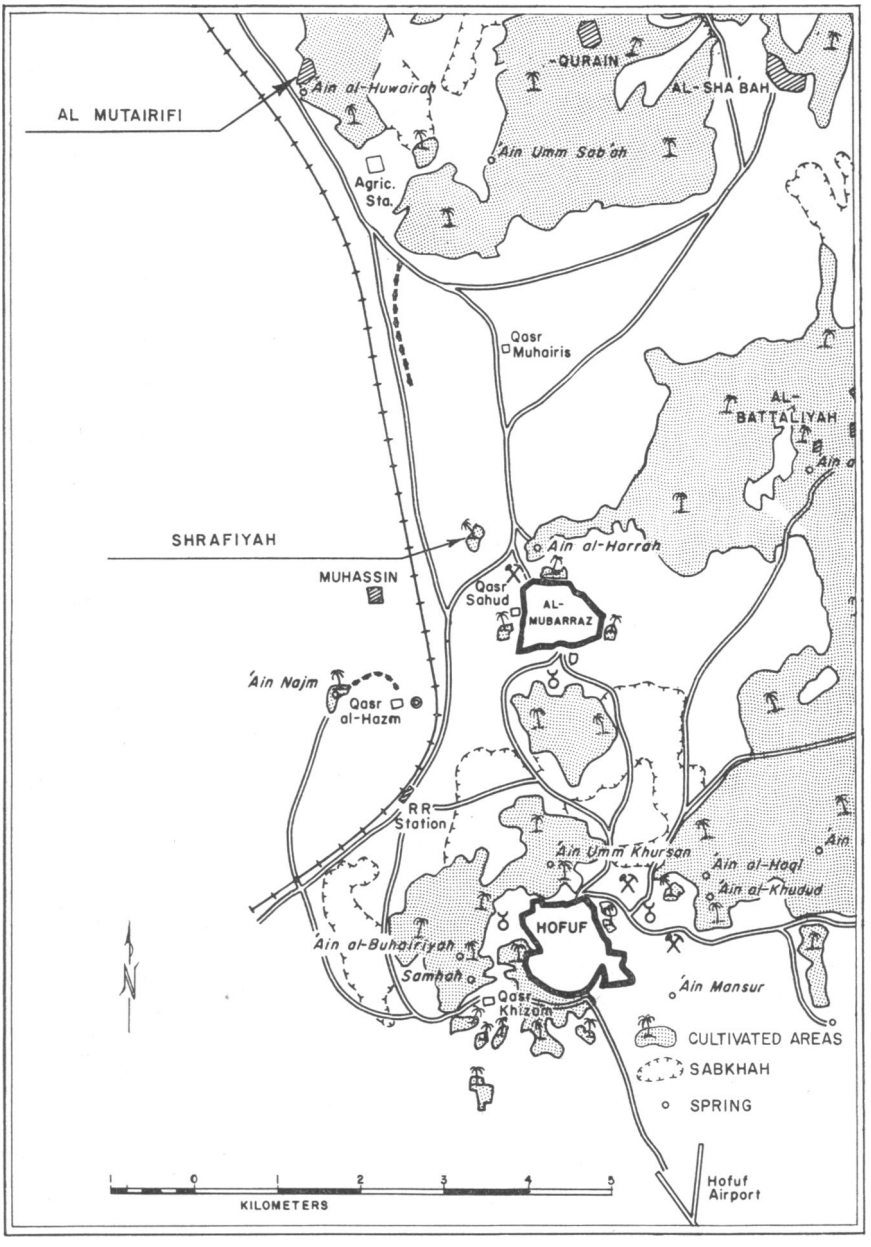


FIG. 2. PORTION OF OASIS OF AL-HASA



and the other locations are shown in Table 1. The general area in which the Shamaliyah garden is located was sprayed with dieldrin in the autumn of 1956 and of 1957, but the agricultural workers who live there claim that no spray was applied to their dwellings where mosquitos were collected in either of these years. The Shrafiyah garden had been sprayed with dieldrin in the spring of 1955 and in the autumn of 1957. The owner of this garden says that it was not sprayed before 1955, nor was it sprayed in 1956.

TABLE 1. SPRAY SUMMARY OF LOCALITIES IN EASTERN SAUDI ARABIA FROM WHICH MOSQUITOS WERE COLLECTED FOR INSECTICIDE TESTS*

YEAR	AL-AJAM & SAFWA	DAMMAM & TARUT	AL-MUTAIRIFI	SHRAFIYAH	SHAMALIYAH	'AIN AL-SAIH
1948	DDT [a]	DDT	-	-	DDT	U
1949	DDT	DDT	DDT	DDT?	DDT	N
1950	DDT	NO SPRAY				S
1951	DDT	DDT	DDT	DDT?	DDT	P
1952	DDT	DDT	-	-	DDT	R
1953	DDT	DDT	DDT	DDT?	DDT	A
1954	DDT	DDT	-	-	DDT	Y
1955	Dieldrin [b]	Dieldrin	DDT (Spring)	Dieldrin (Spring)	Dieldrin	E
1956	Dieldrin [c]	Dieldrin	Dieldrin	Dieldrin?	Dieldrin?	D
1957	Dieldrin	Dieldrin	Dieldrin	Dieldrin	Dieldrin?	-

* All spraying was done in the autumn except where otherwise indicated.
[a] DDT dosage from 1948 through 1954: 2.0–2.5 g/m².
[b] Dieldrin dosage in 1955: 0.45 g/m².
[c] Dieldrin dosage in 1956 and 1957: 0.7 g/m².

After dieldrin had been first used in 1954 on an experimental basis in palm-thatched and mud-plastered buildings in several gardens in the Qatif oasis, *Anopheles pulcherrimus* and *A. coustani* var. *tenebrosus* began appearing in greater numbers in hand-caught collections from these dieldrin-treated surfaces. This trend continued from 1955 through 1958.

Tests to determine the status of resistance of *A. stephensi* were begun in November 1955 when Mr G. Davidson of the Ross Institute of Hygiene and Tropical Medicine briefly visited this area as a WHO consultant. The mimeographed report [1] of his visit has been quoted frequently during the last two years in various papers on anopheline resistance. Testing was

[1] Unpublished WHO document MH/AS/17.56

TABLE 2. EGG MEASUREMENTS OF *A. STEPHENSI* FROM 'AIN AL-SAIH AND TARUT, SAUDI ARABIA, COMPARED WITH THOSE OF *A. STEPHENSI* VAR. *MYSORENSIS* FROM INDIA AS REPORTED BY SWEET AND CO-AUTHORS (1938)

STRAIN	LENGTH (MICRONS)		WIDTH INCLUDING FLOATS (MICRONS)		LENGTH OF FLOAT (MICRONS)		NUMBER OF RIDGES ON ONE SIDE OF FLOAT		PROPORTION OF LENGTH COVERED BY FLOATS	
	MEAN	STANDARD DEVIATION	MEAN	STANDARD DEVIATION	MEAN	STANDARD DEVIATION	MEAN	STANDARD DEVIATION	MEAN	STANDARD DEVIATION
SWEET & RAO	476	24	160	12	218	20	13	1.2	0.46	0.03
♀1 'AIN AL-SAIH 138 Eggs	487	19	151	9	208	21	12.3	0.85	-	-
♀2 'AIN AL-SAIH 127 Eggs	463	15	159	9	218	13	12.9	0.88	-	-
♀3 'AIN AL-SAIH 127 Eggs	462	14	154	9	212	13	13.1	0.62	0.46	0.09
♀♀1, 2, 3 'AIN AL-SAIH 392 Eggs	471	18	154	9	212	14	12.8	0.87	0.45*	-
MIXTURE TARUT 115 Eggs	471	17	153	16	228	20	12.8	0.91	0.48*	-

* These proportions were determined from means of egg length and float length.

continued in 1956, 1957 and 1958 on *A. stephensi*, *A. pulcherrimus* and *A. coustani*. Occasional specimens of *A. fluviatilis* and *A. sergenti* were also included in some tests.

The question which race of *Anopheles stephensi* occurs in Eastern Saudi Arabia arose during the course of these studies. References from India (Sweet et al., 1938; Krishnan, 1954) indicate that egg measurements are a reliable means of separating *A. stephensi mysorensis* from *A. stephensi stephensi*.

Measurements of egg lengths, float lengths, egg widths, and observations of the number of ridges on the floats were made on several series of eggs obtained from mosquitos collected at 'Ain al-Saih and at Tarut. A total of 507 eggs was examined. All measurements and ratios as shown in Table 2 fell well within the limits of the range of *A. stephensi mysorensis* as set by Sweet et al. (1938). The distribution records of Mattingly & Knight (1956) do not show *A. stephensi mysorensis* to be present in Saudi Arabia. This is the first record of its existence in this country.

Methods

All mosquitos used in our tests were collected in the field and most were blood fed before collection. Collection sites usually were cow and donkey stables, but occasionally collections were made in human sleeping-quarters. Various collecting methods were employed, but usually collection was done with aspirators. Donkey-baited Magoon and dawn traps and window traps placed outside openings of heavily infested rooms before dusk were less successful in catching *A. stephensi* than the hand-collection method. In certain locations the animal-baited traps collected *A. coustani* and *A. pulcherrimus*.

At first mosquitos were held in wire-screened cages of one cubic foot capacity, but there was always a rather high mortality by the time they reached the laboratory. Later the mosquitos were kept in unwaxed paper cups covered with nylon net on which was placed a cotton pad moistened with sugar solution. A much higher percentage of mosquitos survived the transport in the cups than in the larger screened cages. Tests were run in the field if the temperature was high and the distance from headquarters was over one hour by car, but most often tests were run in the laboratory. Tables 3, 4, 5, and 6 indicate the places where the tests were conducted and the mean outside and laboratory temperatures. When tests were run in the field, the cups were put in cardboard boxes so placed in the Arab dwellings that ants could not destroy the mosquitos during the 24-hour post-exposure holding-period.

The Busvine & Nash (1953) technique of impregnating filter papers in triplicate or quadruplicate with a range of concentrations of DDT or

TABLE 3. MORTALITY OF *A. STEPHENSI* 24 HOURS AFTER 1 HOUR'S EXPOSURE TO FILTER PAPER IMPREGNATED WITH DDT OR DIELDRIN BY BUSVINE & NASH TECHNIQUE

DDT

LOCALITY	DATE	TESTED IN LAB	TESTED IN FIELD	MEAN TEMP.°F OUT	MEAN TEMP.°F IN	0.5 % TOTAL	0.5 % % DEAD	1.0 % TOTAL	1.0 % % DEAD	1.5 % TOTAL	1.5 % % DEAD	2.0 % TOTAL	2.0 % % DEAD	4.0 % TOTAL	4.0 % % DEAD	6.0 % TOTAL	6.0 % % DEAD	CHECK TOTAL	CHECK % DEAD	MLC%
'AIN AL-SAIH	NOV 1955	x		68	75	15	87	19	95	22	91	21	95	8	100	3	100	-	-	< .5
''	OCT 1956	x		75	74	-	-	37	16	-	-	39	51	36	97	36	100	44	0	1.8
AL-MUTAIRIFI	NOV 1955	x		74	75	19	5	20	20	22	18	32	25	44	23	53	70	-	-	4.2
''	SEP 1956		x	89	-	-	-	87	7	-	-	120	13	131	19	132	36	165	6	8.0
DAMMAM	OCT 1956		x	75	-	15	33	13	38	-	-	83	37	81	54	82	60	82	0	3.2
''	AUG 1957		x	97	-	11	0	56	7	-	-	88	31	96	47	92	80	148	5	3.4
SHRAFIYAH *	SEP 1957		x	95	-	27	38	21	62	-	-	22	73	23	89	-	-	25	16	0.7
TARUT	MAY 1958		x	83	-	64	16	66	24	-	-	61	15	59	29	-	-	64	2	>4.0

DIELDRIN

						0.025 %		0.05%		0.1 %		0.2 %		0.40%						
'AIN AL-SAIH	NOV 1955	x		68	75	-	-	-	-	16	94	15	100	-	-			-	-	<0.1
''	OCT 1956	x		75	74	-	-	41	39	42	48	40	97	41	100			44	0	0.08
AL-MUTAIRIFI	NOV 1955	x		74	75	-	-	31	0	20	20	17	59	-	-			-	-	.17
''	SEP 1956		x	89	-	-	-	128	43	125	80	134	97	131	100			165	6	0.06
DAMMAM	OCT 1956		x	75	-	-	-	28	18	30	53	29	69	29	97			82	0	0.1
''	AUG 1957		x	97	-	-	-	115	24	120	59	122	95	78	100			127	6	0.08
SHRAFIYAH *	SEP 1957		x	93	-	63	12	59	28	67	43	61	93	66	96			44	32	0.09
TARUT	MAY 1958		x	83	-	90	11	87	12	83	41	82	68	55	100			73	8	0.14

* Mortality corrected by Abbott's formula.

dieldrin was used. Stock solutions of 4% DDT and 0.4% dieldrin in Risella oil were provided by the World Health Organization. After the mosquitos (no more than 6 per vial) had been exposed for one hour to the treated filter papers they were transferred to unwaxed paper cups covered with nylon net and provided food from cotton pads soaked in honey or sugar solutions. Mortality records on females only were made after 24 hours. Species of these field-collected mosquitos had to be determined at the close of the tests. Tests were repeated from four to six times. It was not always possible to follow this procedure because in certain localities mosquito populations were not sufficiently numerous. Median lethal concentrations were determined by the simplified graphic method developed by Litchfield & Wilcoxon (1949).

Anopheles stephensi

DDT tests

Susceptible strains. Two *A. stephensi* strains were susceptible to DDT since both had median lethal concentrations (MLC's) equal to or less than the 1.6% of the London susceptible colony as reported by Davidson (1958).

Shelters at the isolated garden at 'Ain al-Saih, 9 km from the nearest treated area, have never been sprayed in a malaria control programme. In November 1955, this strain had an MLC of less than 0.5% DDT. When retested in October 1956 it had an MLC of 1.8% (Table 3). Since the numbers tested were small in 1955, the 1956 MLC probably does not represent a true increase but only a better measure of the true level of susceptibility of this population.

By 1957, the general area in which the Shrafiyah garden is located had been sprayed three times with DDT and twice with dieldrin. The owner of this particular garden, however, says that his buildings were sprayed only twice, the first time in 1955 and the second time in 1957. If this is true, no DDT has ever been used there but dieldrin has been used twice. This seems to be borne out by the test results. With an MLC of 0.7% DDT it appears that this strain had not been exposed to selection pressure by DDT. This isolated garden is about 1 km from the town of al-Mubbarraz. With the limited flight range of *A. stephensi*, it is possible that an " island " of susceptibles could remain in an unsprayed area even though the general area has been repeatedly treated with residual insecticide.

DDT-resistant strains. Three *A. stephensi* strains tested were considered to be resistant to DDT since they all had MLC's of this substance of over 3.%.

Al-Mutairifi, by the autumn of 1955, had been sprayed four times with DDT. At that time the MLC of DDT to this strain was 4.2% (shown

TABLE 4. MORTALITY OF *A. PULCHERRIMUS* 24 HOURS AFTER 1 HOUR'S EXPOSURE TO FILTER PAPER IMPREGNATED WITH DDT OR DIELDRIN BY BUSVINE & NASH TECHNIQUE

DDT

LOCALITY	DATE	TESTED IN		MEAN TEMP.° F		0.5 %		1.0 %		2.0 %		4.0 %		6.0 %		CHECK		MLC%
		LAB	FIELD	OUT	IN	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	
AL-AJAM	OCT 1956		x	78	-	-	-	41	26	39	54	31	84	41	95	41	0	1.8
"	MAR 1958	x		68	78	-	-	115	5	111	34	114	86	53	98	116	0	2.4
SAFWA	DEC 1957	x		69	72	39	0	44	3	45	40	48	100	-	-	54	7	2.2
SHAMALIYAH	JAN 1958	x		57	71	50	12	52	19	101	48	29	90	-	-	43	2	1.7

DIELDRIN

					0.025%		0.05%		0.1 %		0.2 %		0.4 %				
AL-AJAM	OCT 1956	x	78	-	-	-	41	5	36	22	31	16	37	32	34	0	0.76
SAFWA	DEC 1957	x	69	72	12	8	19	5	20	0	18	0	7	0	12	0	>0.4

					0.5 %		1.0 %		2.0 %		4.0 %						
AL-AJAM *	FEB 1958	x	68	78	24	0	84	2	86	8	84	24	-	-	87	1	8.2(X2)
SHAMALIYAH *	JAN 1958	x	57	71	24	4	25	16	15	20	15	13	-	-	20	5	>4.0(X2)

* 2-hour exposure

as 5.0% in Davidson's preliminary mimeographed report of 1955). When retested in the autumn of 1956, before it was sprayed with dieldrin, the MLC of DDT had almost doubled, to reach 8.0%. Lower pre-test temperatures may have been the prime factor influencing these differences in results as well as continued exposure to aging DDT residues.

By 1956, the garden near Damman had been sprayed six times with DDT and once with dieldrin. By 1957, one more dieldrin application had been made in this area. Despite repeated exposure to DDT, the MLC's of DDT to the Damman garden strain were only 3.2% and 3.4% in 1956 and 1957 respectively.

By the spring of 1958, Tarut had been sprayed six times with DDT and three times with dieldrin. When tested in 1958, this strain had an MLC of over 4.0% DDT.

Dieldrin tests

All the *A. stephensi* strains tested were susceptible to dieldrin, with MLC's ranging from 0.08% to 0.17%. This range is close to the MLC of 0.13% as determined by Davidson (1958) for a London susceptible colony and within the range for other susceptible anophelines as shown by Busvine (1956b).

Anopheles pulcherrimus

DDT tests

Three strains of *A. pulcherrimus* were tested locally. All were from sprayed areas, but all were considered to be susceptible to DDT, having MLC's within or close to the range of other susceptible anophelines (0.2%-2.0%) as reported by Busvine (1956b).

Two of the strains were from the villages of Safwa and al-Ajam, which had been sprayed annually since 1948. By October 1956, seven sprays of DDT and two of dieldrin had been applied. By December 1957 and March 1958, an additional application of dieldrin had been made. The al-Ajam strain was tested in October 1956 and March 1958. The MLC of DDT increased slightly from 1.8% to 2.4% during this time (Table 4). Tests run in December 1957 showed the Safwa strain to have an MLC of more than 1.0% but less than 4.0% DDT, which we still consider in the susceptible range for this species.

The third *A. pulcherrimus* strain was from Shamaliyah, which is located in an area that had had six DDT sprays and two dieldrin sprays by January 1958. At that time the MLC of DDT to this strain was 1.7%.

Dieldrin tests

No basic data are to hand for *A. pulcherrimus* susceptible populations in relation to dieldrin, but other anophelines tested with dieldrin have

TABLE 5. MORTALITY OF *A. COUSTANI* 24 HOURS AFTER 1 HOUR'S EXPOSURE TO FILTER PAPER IMPREGNATED WITH DDT OR DIELDRIN BY BUSVINE & NASH TECHNIQUE

DDT

LOCALITY	DATE	TESTED IN	MEAN TEMP.° F	0.5 %		1.0 %		2.0 %		4.0 %		6.0 %		CHECK		MLC%
		LAB. FIELD	OUT IN	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	
AL-MUTAIRIFI	SEP 1957	x	94 77	9	100	10	100	10	100	9	100	-	-	8	13	<0.5
''	MAY 1958*	x	81 73	49	21	43	46	46	79	22	100	-	-	51	10	1.0
SAFWA	SEP 1957*	x	94 70	71	11	74	48	71	73	72	99	-	-	68	18	1.1
''	JAN 1958	x	61 71	96	1	95	8	96	11	97	76	-	-	96	4	2.9
SHAMALIYAH	JAN 1958	x	55 72	47	6	50	16	79	29	43	84	-	-	42	0	2.0

DIELDRIN

				0.025 %		0.05%		0.1 %		0.2 %		0.4 %				
AL-MUTAIRIFI	SEP 1957	x	94 77	7	0	4	20	6	50	6	84	6	100	8	13	0.093
SAFWA	SEP 1957*	x	94 70	74	18	74	9	73	9	73	6	73	5	76	11	>0.4
''	DEC 1957	x	71 72	20	0	12	0	12	0	12	0	24	0	12	8	>0.4
SHAMALIYAH	JAN 1958	x	55 72	27	15	24	17	24	0	23	22	24	21	24	17	>0.4

						0.5 %		1.0 %		2.0 %		4.0 %				
SAFWA **	JAN 1958	x	55 72	-	-	48	4	48	6	48	13	48	46	48	2	5.1(X2)
SHAMALIYAH **	JAN 1958	x	55 72	-	-	26	27	20	20	20	60	23	56	20	10	2.3(X2)
AL-MUTAIRIFI	MAY 1958	x	81 73	-	-	48	29	48	37	51	35	47	79	47	4	1.8

* Mortality corrected by Abbott's formula.
** 2-hour exposures

ranges of from 0.004% to 0.29% for unexposed populations (Busvine, 1956b). *A. pulcherrimus* from al-Ajam when tested in October 1956 appeared to be tolerant to dieldrin. Tests with the standard 0.05%-0.4% concentrations produced only low mortalities. By extending the log-dosage probit mortality line an MLC of 0.76% was extrapolated. In February of 1958, one dieldrin application later, this range of concentrations (0.05%-0.4%) produced no more mortality than that which occurred among the check mosquitos. Tests were then made by exposing the mosquitos for two hours to concentrations of dieldrin ranging from 0.5% to 4.0%. By extending the log-dosage probit mortality line produced with these concentrations and exposures, an MLC of 8.2% was extrapolated, suggesting high resistance.

The Shamaliyah strain of *A. pulcherrimus* was also tested with the higher dieldrin concentrations and the two-hour exposure period. Mortalities of 4%, 16%, 20%, and 13% were produced respectively by the 0.5%, 1.0%, 2.0% and 4% dieldrin concentrations. The log-dosage probit mortality line fitted to these figures was so steep that an MLC was not determined. It is obvious, however, that the MLC of dieldrin to this strain was well over 4.0%, indicating a high degree of resistance to dieldrin.

The Safwa strain of *A. pulcherrimus* was tested in December 1957 only with one-hour exposures to the lower series of dieldrin concentrations. One mosquito was killed in each of the lower concentrations (0.025% and 0.05%) but there was no mortality at the highest concentration of 0.4%. The MLC of dieldrin to this strain was therefore over 0.4%, indicating that this strain is also relatively resistant to dieldrin when compared with known base data for other anophelines.

Anopheles coustani var. *tenebrosus*

DDT tests

In September 1957, a few *A. coustani* var. *tenebrosus* were collected in a donkey-baited dawn trap set up at al-Mutairifi. All DDT concentrations produced complete mortality so the MLC was less than 0.5%. In May 1958 when this strain was retested the MLC was found to be 1.0% (Table 5).

The Safwa strain was tested in September 1957 and again in January 1958. The MLC increased from 1.1% to 2.9% during this time. This difference was probably due to the lower outdoor temperature that prevailed in January.

The Shamaliyah strain was also tested in January 1958. This strain had an MLC of 2.0% DDT.

These MLC's, with the exception of that of the Safwa strain in January, were no higher than the highest MLC shown by Busvine (1956b) for

TABLE 6. MORTALITY OF *A. FLUVIATILIS* 24 HOURS AFTER 1 HOUR'S EXPOSURE TO FILTER PAPER IMPREGNATED WITH DDT OR DIELDRIN BY BUSVINE & NASH TECHNIQUE

DDT

LOCALITY	DATE	TESTED IN		MEAN TEMP.° F		0.5 %		1.0 %		2.0 %		4.0 %		6.0 %		CHECK		MLC%
		LAB	FIELD	OUT	IN	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	TOTAL	% DEAD	
'AIN AL-SAIH	NOV 1955	x		68	75	6	100	3	100	3	100	4	100	3	100	-	-	<0.5
DAMMAM	OCT 1956		x	83	-	-	-	-	-	12	75	14	100	13	100	12	0	<2.0
SHAMALIYAH	JAN 1958*	x		56	72	36	62	26	95	15	100	8	100	-	-	20	15	0.43?

DIELDRIN

						0.025%		0.05%		0.1 %		0.2 %		0.4 %				
'AIN AL-SAIH	NOV 1955	x		68	75	-	-	2	100	6	100	-	-	-	-	-	-	<0.05
DAMMAM	OCT 1956		x	83	-	-	-	6	83	10	90	10	100	13	100	10	0	<0.05
SHAMALIYAH	JAN 1958*	x		56	72	30	23	20	67	11	100	12	100	13	100	13	39	<0.05

* Mortality corrected by Abbott's formula.

susceptible strains of anophelines. No specific data are available for non-exposed populations of *A. coustani* var. *tenebrosus* for more direct comparisons. With the data at hand, we feel there is no evidence for DDT-resistance in this species at present.

Dieldrin tests

In September 1957, when only a few specimens of the al-Mutairifi strain were tested, it appeared that this strain was susceptible to dieldrin with an MLC of 0.09%. When retested in May 1958, after a dieldrin spray in the autumn of 1957, the MLC had increased twentyfold to 1.8%.

The Safwa strain was tested in September and December 1957, with the standard 0.025%-0.4% range of concentrations. Only low mortalities were produced in September and none was produced in December. When exposed for two hours to the higher concentrations (0.5%-4.0%) of dieldrin an MLC of 5.1% resulted.

When the Shamaliyah strain was tested for one hour with the low series of dieldrin concentrations low mortalities were produced. When exposed for two hours to the higher series of concentrations an MLC of 2.3% dieldrin resulted.

These *A. coustani* var. *tenebrosus* strains with MLC's of dieldrin over 0.4% are all considered resistant to dieldrin when compared with normal susceptible anopheline strains whose MLC's range from 0.004% to 0.29% (Busvine, 1956b). So far, no specific dieldrin data are available for *A. coustani* from non-exposed populations.

Anopheles fluviatilis

Anopheles fluviatilis was never collected in great numbers. The tests in which this species was included showed it to be susceptible both to DDT and to dieldrin (Table 6) when compared with general ranges known for other susceptible anophelines and with results reported from India (Sharma et al., 1957), which showed *A. fluviatilis* from an unsprayed area to have an MLC of 0.3% DDT and of 0.22% dieldrin.

DDT tests

The MLC of DDT to the Damman strain was less than 2.0%. The Shamaliyah strain had an MLC of 0.43% DDT. The 'Ain al-Saih strain appeared to be even more susceptible, with an MLC considerably less than 0.5% DDT.

Dieldrin tests

These same strains tested with dieldrin all had MLC's less than 0.05%, indicating no resistance. The strain from 'Ain al-Saih appeared to be the most susceptible to this insecticide also.

Anopheles sergenti

Anopheles sergenti was also included in some of the tests with Shamaliyah mosquitos but in even smaller numbers than *A. fluviatilis*.

Basic data elsewhere indicate an MLC of less than 0.5% DDT from untreated areas.[1] Our results with DDT and dieldrin seem to show this species to be susceptible to both insecticides. The high mortality in the checks makes any more definite conclusions impossible with these limited data (Table 7).

TABLE 7. MORTALITY OF *A. SERGENTI* FROM SHAMALIYAH 24 HOURS AFTER 1 HOUR'S EXPOSURE TO FILTER PAPER IMPREGNATED WITH DDT OR DIELDRIN BY BUSVINE & NASH TECHNIQUE

DDT			Dieldrin		
concentration (%)	number tested	percentage killed	concentration (%)	number tested	percentage killed
0.5	19	79	0.025	13	39
1.0	7	100	0.05	5	40
2.0	11	64	0.1	2	100
4.0	2	100	0.2	2	100
			0.4	2	100
Controls	13	43	Controls	9	67

Discussion

Busvine (1956a) points out that if certain conditions of testing, such as age of mosquitos, their state of nutrition and testing temperature, are not standardized, only very gross types of resistance can be demonstrated. The conditions under which these tests were done were not controlled. Attempts to rear *Anopheles stephensi* were unsuccessful and it was necessary to depend upon the prevalence of the mosquitos and the ability of technicians to collect them in the field. Since all specimens were field-collected, their age was unknown. Most of them had fed previously, but not all of them. Tests were run at various times during the year and it can be noted from Tables 3-6 that there is a rather wide range of mean temperatures throughout the year. Thus the pre-testing temperature of the mosquitos was not controlled. Sometimes tests were run under outdoor shade conditions; usually however, they were run indoors in air-conditioned rooms. With such essential testing conditions uncontrolled, the MLC's calculated

[1] *Information circular on the resistance problem, No. 13* (unpublished WHO document)

from the test results obtained can be considered rough approximations only.

Busvine (1956b) refers to a tenfold DDT-resistance of *A. stephensi* from a sprayed area in Eastern Saudi Arabia as compared with a strain from an unsprayed area. This was based on Davidson's 1955 preliminary report of 0.5% as the MLC for a susceptible strain and 5.0% for a resistant strain. On the basis of more extensive testing, Davidson (1958) refers to a threefold to sixfold resistance of strains from sprayed areas as compared with the same strain from the unsprayed area. These estimates were based on preliminary unpublished information obtained in 1955 and 1956. Subsequent testing in 1956 and 1957 produced results that further modify the degree of resistance of these strains as discussed below.

When the higher of two MLC's of DDT (1.8%) to the unsprayed 'Ain al-Saih strain is used as the base, the resistance of the al-Mutairifi strain is four times greater. that of the Damman strain is less than two times greater, and that of the Tarut strain is more than two times greater. If the lower MLC of the 'Ain al-Saih strain (less than 0.5%) or that of the strain from Shrafiyah were used as the base, the MLC's of the resistant strains would be approximately six to twelve times greater. Even though twofold to fourfold resistance does not appear to be of a gross type, in *Anopheles stephensi* this level of resistance is high enough to reduce the effectiveness of DDT as a malaria control measure.

Tests were run at various times of the year, but temperature differences seem to have had no consistent bearing on certain differences of results. The al-Mutairifi and Damman strains both were collected and tested under similar conditions in two consecutive years. The MLC of DDT to the al-Mutairifi strain doubled from November 1955 to September 1956, but the MLC of the Damman strain remained the same from October 1956 to August 1957. The increase in the MLC of the al-Mutairifi strain in 1955 and 1956 could have been due to the higher temperatures in 1956.

The number of previous sprayings seem to have had little effect on the degree of resistance developed in the various *A. stephensi* strains. The Damman strain, with six DDT sprays, was less resistant than the al-Mutairifi strain, which had had half as many DDT sprays. The MLC's of the 'Ain al-Saih and al-Mutairifi strains increased from November 1955 to September and October 1956. The increase shown by the 'Ain al-Saih strain could not have been due to further selection by an additional application of DDT since it was not sprayed during this time.

However, in al-Mutairifi some further selection may have been exerted from November 1955 to September 1956 by the deposit of DDT that had been applied in March 1955. This deposit was eight months old when the 1955 tests were run and eighteen months old by September 1956.

One would expect that if specimens had been tested from al-Ajam and Safwa, sprayed every year for seven years, MLC's in 1954 would probably

have shown more resistance to DDT than the other resistant strains. Malariometric evidence, available in 1953 and 1954, showed that DDT was failing to control malaria in these two villages. Unfortunately for the purpose of this study, no *A. stephensi* could be collected in these two villages in the autumn of 1955 after dieldrin had been applied there, nor have any been collected there since that time.

The low MLC's of dieldrin to this species, entomological observations, recent malaria survey data, and hospital records of clinical malaria strongly indicate that in this area of Saudi Arabia dieldrin can be expected to keep malaria carried by *A. stephensi* under control. Strains that have been tested during two consecutive years since dieldrin has been used do not give any indication that dieldrin resistance is developing. But it is likely that strains tested since 1955 have been taken from sites that may have been missed during spraying operations. This is conceivable because they are all places somewhat isolated from the more populous villages. Also the complete absence of *A. stephensi* (in our collections) from dieldrin-sprayed villages makes it seem likely that the pockets of *A. stephensi* that have been found occur only because these places were not sprayed. Another indication that our collections may have been from unsprayed buildings is that some of these collections contained *A. fluviatilis* and *A. sergenti.* Tests with only a few specimens indicated that both of these species were very susceptible to both insecticides.

While DDT was being used for malaria control, *A. pulcherrimus* was collected only occasionally and when present in collections was not numerous. Since dieldrin has been used on a large scale, *A. pulcherrimus* has become the predominant anopheline in many localities. Boyd (1949) states that *A. pulcherrimus* rests in partially lighted quarters in human habitations, cattle sheds or outhouses. According to a statement of Christophers & Shortt referred to by Macan (see Leeson et al., 1950), the presence of *A. pulcherrimus* would be much more readily detected than that of *A. stephensi*, because *A. stephensi* is of a much more retiring habit. The present numerical superiority of *A. pulcherrimus* over *A. stephensi* is possibly related to one change in the environment—the use of dieldrin.

There is no complete agreement among various workers on the role of *A. pulcherrimus* in malaria transmission. The dominant opinion is that it is unimportant as a malaria vector. On the other hand, there are references which point out that it may be a vector. Smart (1956) bases his record that *A. pulcherrimus* is a vector of minor importance in Iraq and Iran on a personal communication from Mer in 1945. Boyd (1949) states that *A. pulcherrimus*, when exceedingly numerous, becomes a very dangerous malaria carrier; it is able to maintain a severe endemic even in the absence of efficacious vectors. He further states that it is considered to be a vector in Central Asia, where it has been found to be naturally infected, and also in Sind, India. It is considered to be an important vector in the Caucasus

and Iraq, but it probably plays no important part as a vector in other parts of India. Boyd also refers to the work of Simanin in the USSR. This worker successfully demonstrated experimental stomach infection with *Plasmodium falciparum* but reported no salivary gland infection. Horsfall (1955) states that data are too few to determine the status of this species in relation to plasmodia. Dissections done in India showed no infections, but Covell in 1944 reported that the form occurring in the southern USSR is a significant vector of human plasmodia.

Even though there is not complete agreement on the role of *A. pulcherrimus* in the transmission of malaria, there is enough evidence for those who are responsible for malaria control to be watchful. The fact that dieldrin seems to be highly ineffective against this species may permit it to develop in numbers sufficient to make it a factor in malaria transmission in Eastern Saudi Arabia. The solution, however, would seem to be simple: the use of DDT again would reduce this DDT-susceptible species to the insignificant level it was at before dieldrin was used.

There is general agreement among authors against *A. coustani* var. *tenebrosus* as a malaria vector. It is short-lived and highly zoophilic, so, therefore, does not enter the reservoir of human plasmodia. It, however, is of interest that this species is also resistant to dieldrin but susceptible to DDT.

Mohan (1955) reported that he had raised a DDT-resistant strain of *A. fluviatilis* in India. No MLC values were given. Resistance was first evidenced after 29 generations and increased only slightly by the 47th generation. He stated that resistance was late in appearing and equally slow in building up but was nevertheless clear-cut. There is a feeble indication that strains of *A. fluviatilis* from the sprayed gardens at Damman and Shamaliyah may be slightly more resistant to DDT and dieldrin than the strain from 'Ain al-Saih. The number of specimens tested was so low, however, that this extremely feeble " resistance " should arouse no serious concern.

ACKNOWLEDGEMENTS

I am indebted to Dr R. H. Daggy, under whose direction these studies were made possible. The assistance at different times of S. Saliba, L. Francis, G. Hashim, M. Khoury and I. Anani in field collections and tests is acknowledged.

RÉSUMÉ

En 1953, cinquième année du programme de lutte contre le paludisme par le DDT dans l'est de l'Arabie Saoudite, la présence d'*Anopheles stephensi* sur des surfaces soumises à des pulvérisations de DDT quelques semaines seulement auparavant laissait à penser que cette espèce était en train de devenir résistante au DDT. Dans le même temps,

les cas de paludisme augmentèrent. Des tests préliminaires pratiqués en 1955 en utilisant la technique de Busvine-Nash confirmèrent cette hypothèse de même que d'autres tests effectués de 1956 à 1958. La concentration létale moyenne (CLM) de DDT pour une souche d'*Anopheles stephensi* provenant d'une région non soumise à des pulvérisations était inférieure à 0,5%. La CLM pour des souches provenant de régions traitées variait entre 3 et 8%.

Après le remplacement du DDT par la dieldrine en 1955, les cas de paludisme diminuèrent à nouveau. Les souches locales d'*A. stephensi* n'ont pas acquis de résistance à la dieldrine; la CLM de cet insecticide pour les souches sensibles au DDT, de même que pour les souches résistantes, variait entre 0,06% et 0,17%. La CLM d'une souche sensible provenant de Londres qui était de 0,13% correspondait bien à ces chiffres. Aucun *A. stephensi* n'a été recueilli dans les villages traités depuis que la dieldrine a été utilisée dans l'ensemble des oasis. Les spécimens utilisés pour les tests furent recueillis dans des abris de jardins isolés qui avaient pu être oubliés au cours des pulvérisations.

Les épreuves de résistance aux insecticides sur *A. pulcherrimus* et *A. coustani* var. *tenebrosus* ont montré que ces deux espèces étaient devenues résistantes à la dieldrine, la CLM de cet insecticide variant de 0,4 à plus de 8%. Par contre, les deux espèces étaient sensibles au DDT. Avant et pendant l'emploi du DDT on trouvait rarement *A. pulcherrimus*, mais depuis l'emploi de la dieldrine il est de plus en plus répandu et l'on peut se demander s'il n'est pas susceptible de transmettre le paludisme dans la région en question.

Quelques spécimens de *A. fluviatilis* et de *A. sergenti* ont été soumis aux épreuves de résistance: ces deux espèces étaient fortement sensibles et au DDT et à la dieldrine. La CLM de DDT était inférieure à 0,5%, celle de dieldrine était inférieure à 0,05%.

Les mensurations de plusieurs séries d'œufs d'*A. stephensi* recueillis sur place (et les caractères des pontes) correspondaient aux données connues pour *A. stephensi mysorensis*, qui n'avait pas été signalé jusqu'à présent hors de l'Inde.

REFERENCES

Boyd, M. F. (1949) *Malariology*, Philadelphia, Saunders, pp. 427, 434, 435, 494, 674

Busvine, J. R. (1956a) *Bull. Wld Hlth Org.*, **15**, 389

Busvine, J. R. (1956b) *Bull. Wld Hlth Org.*, **15**, 787

Busvine, J. & Nash, R. (1953) *Bull. ent. Res.*, **44**, 371

Davidson, G. (1958) *Bull. Wld Hlth Org.*, **18**, 579

Daggy, R. H. (1957) *Oasis malaria.* In: *Industry and tropical health*, Cambridge, Mass., Harvard School of Public Health, vol. 3, p. 42

Daggy, R. H. (1959) *Amer. J. trop. Med. Hyg.* (in press)

Horsfall, W. R. (1955) *Mosquitoes ; their bionomics and relation to disease*, New York, Ronald, pp. 75, 274

Krishnan, K. S. (1954) *Bull. nat. Soc. India Malar.*, **2**, 98

Leeson, H. S., Lumsden, W. H. R., Yofe, J., & Macan, T. T. (1950) *Anopheles and malaria in the Near East* London, Lewis, pp. 151, 155, 177

Litchfield, J. T. & Wilcoxon (1949) *J. Pharmacol. exp. Ther.*, **96**, 99

Mattingly, P. F. & Knight, K. L. (1956) *Bull. Brit. Mus. (Nat. Hist.) Ent.*, **4**, 91

Mohan, B. N. (1955) *Indian J. Malar.*, **9**, 287

Sharma, M. I. D., Krishnamurthy, B. S., Khamre, J. S. & Singh, N. N. (1957) *Bull. nat. Soc. India Malar.*, **5**, 198

Smart, J. (1956) *Insects of medical importance*, London, British Museum (Natural History), p. 185

Sweet, W. C., Rao, R. A., & Shubha Rao, A. M. (1938) *Jour. Malar. Inst. India*, **1**, 149